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Reducing inputs and losses of nitrogen and energy on dairy farms

Final Report Project AIR3 CT92-0332

H.G. van der Meer (co-ordinator)

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Organisations involved:

1. Research Institute for Agrobiology and Soil Fertility (AB-DLO)
2. The Danish Institute of Plant and Soil Science
3. Ente Regionale per la Promozione e lo Sviluppo dell'Agricoltura
4. Research Station for Cattle, Sheep and Horse Husbandry
5. Scottish Agricultural College
6. Centro de Investigaciones Agrarias Mabegondo – Xunta de Galicia -

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Executive Summary

Background and contents of the project

The need to reduce nitrogen (N) and phosphorus (P) losses as well as the (direct and indirect) use of fossil energy on intensive dairy farms in Europe to ecologically acceptable levels, requires radical changes of the management of these farms. A strong reduction in the use of artificial fertilisers, and on some farms also in N and P inputs in purchased feeds will be necessary. In general, this will decrease farm output. However, at least a part of this decrease may be compensated by an improvement of N and P utilisation on the farm. Analysis of the N and P cycles on dairy farms showed that the main opportunities to improve N and P utilisation within the production system are (1) improvement of the conversion of feed N and P into N and P in animal products, and (2) improvement of the re-utilisation of N and P in animal manure by grass and forage crops.

Based on these considerations, the project ‘Reducing inputs and losses of nitrogen and energy on dairy farms’ has been formulated in 1992 and started in 1993 with financial support of the European Community Specific Programme for Research, Technological Development and Demonstration in the field of Agriculture and Agro-industry, including Fisheries (AIR). The objective of the project was to develop sustainable dairy farming systems with minimum inputs and losses of nitrogen, phosphorus and energy. The following results were anticipated:

1. A better knowledge of the practical possibilities to improve the utilisation of dietary N by the dairy herd and to reduce N excretion in urine in different forage production systems.
2. Information about the suitability of some crops, crop rotations and feeding systems in different regions of the European Community to replace a part of the purchased concentrates on dairy farms.
3. Efficient slurry utilisation systems on grass/clover swards and maize.
4. A better understanding of the effectiveness and interactions of different measures to decrease losses of nitrogen, phosphorus and energy on dairy farms.

The project comprised the following research tasks and sub-tasks:

A. Evaluation of rations and feeding strategies to improve the utilisation of N in the feed of the dairy herd in order to minimise the production of urinary N.
    1. Determine the minimum levels of N intake by dairy cows in early and mid lactation without reduction in milk yield (6 feeding experiments in Denmark).
    2. Study the importance of synchronisation of the intake of rumen-degradable protein and fermentable carbohydrates (this sub-task has been cancelled to avoid overlap with research in other programmes in which the need for this synchronisation has been shown. The research capacity planned for sub-task 1.2 has been employed in sub-task 1.1).
    3. Determine the effects of supplementing dairy cows zero-grazing N fertilised grass with ground maize-ear silage (low-N/high-energy supplement) (2 feeding experiments with variations in herbage allowance and supplement level in The Netherlands).
    4. Study herbage intake and N utilisation of dairy cows on grass/white clover swards with minimum supplementation (1 grazing experiment in Scotland).
    5. Investigate strategic supplementation of cows on mixed swards to maximise N utilisation (1 grazing experiment in Scotland).
    6. Study strategic protein supplementation of cows on grass/clover silage (1 feeding experiment in Scotland (sub-task A6-1) and 2 in Spain (sub-task A6-2).

B. Comparison of crops, cropping systems and feeding systems to increase the proportion of home-grown feed protein, phosphorus and energy in dairy nutrition, thus reducing the consumption of purchased feeds and the related input of plant nutrients and indirect use of fossil energy.
    1. Study the agronomic potentials and environmental constraints of 2 extreme forage production systems (1 farm-scale field experiment in Denmark).
    2. Evaluate the quality of ground maize-ear silage as a supplement for dairy cows zero-grazing N fertilised grass (the same experiments as for sub-task A3).
    3. Evaluate the quality of ground maize-ear silage and fodder beet to supplement grass silage for dairy cows (2 feeding experiments in The Netherlands).
    4. Evaluate the quality of barley as a supplement for dairy cows grazing grass/clover swards (the same experiment as for sub-task A5).
    5. Evaluate the quality of barley and fodder beet to supplement grass/clover silage for dairy cows (1 feeding experiment in Scotland).
    6. Study the interactions between fertiliser N and white clover on herbage yield and quality and nitrate leaching under grazing and cutting (1 grazing and 1 cutting experiment in Spain).

C. Quantification of the effectiveness of different slurry utilisation systems on low-input dairy farms, and in particular or grass/clover swards and silage maize.
    1. Study the effects of different slurry application techniques on the utilisation of slurry N, P and K by a grass/clover sward (1 field experiment with annually repeated slurry application in The Netherlands).
    2. Study the effects of low-emission slurry application techniques and periods of application on the performance of a grass/clover sward and the clover component in particular (1 field experiment with annually repeated slurry application in The Netherlands).
    3. Study utilisation of slurry N by maize, as affected by period, method and rate of slurry application and additional fertiliser N (1 annually repeated field and lysimeter experiment in Italy).
    4. Study utilisation of slurry N by combinations of maize and *Lolium multiflorum* as a cover crop (1 field experiment in 1993 and 1994 in Italy).

D. Evaluation and integration of the results of the experimental work to contribute to the development of dairy farming systems at the project sites with more efficient utilisation of N, P and fossil energy.
    1. Search for information about the effect of reduced excretion of urine N on N losses.
    2. Identify possible consequences of replacing purchased concentrates by home-grown feeds.
    3. Formulate essences of effective slurry utilisation systems on low-input dairy farms.

Six Research Organisations participated in this project:
The *Research Institute for Agrobiology and Soil Fertility* (Participant 1, Wageningen, The Netherlands) conducted the research related to the sub-tasks C1 and C2 and Task D.
The *Danish Institute of Plant and Soil Science* (Participant 2, Foulum, Denmark) conducted the research related to the sub-tasks A1 and B1.
The *Ente Regionale per la Promozione e lo Sviluppo dell'Agricoltura* (Participant 3, Pordenone, Italy) conducted the research related to the sub-tasks C3 and C4.
The *Research Station for Cattle, Sheep and Horse Husbandry* (Participant 4, Lelystad, The Netherlands) conducted the research related to the sub-tasks A3 (B2) and B3.
The *Scottish Agricultural College* (Participant 5, Dumfries, Scotland) conducted the research related to the sub-tasks A4, A5 (B4), A6-1 and B5.
The *Centro de Investigaciones Agrarias Mabegondo (CIAM) – Xunta de Galicia* – (Participant 6, La Coruña, Spain) conducted the research related to the sub-tasks A6-2 and B6.

Most of the experimental research was adapted to the local conditions of the participants involved. The following section summarises the experimental work carried out.

Experimental work

Sub-task A1

The overall objective of the 6 feeding experiments conducted in Denmark under sub-task A1, was to determine the minimum intake of N and the minimum excretion of N in urine that could be obtained from dairy cows in different stages of lactation without a considerable reduction in milk yield. In other words, the objective was to maximise the efficiency of feed N use under various feeding conditions. New principles of protein evaluation were used with the aim to optimise microbial capture of N and synthesis of protein in the rumen. In addition, the aim was to challenge the cow's ability to recycle N from the body to the rumen and use it again for protein synthesis. In both situations the level of protein was reduced below standard levels, and efforts were made to increase the efficiency of utilisation of feed N. The effects were studied by measuring feed intake, milk production, and milk composition in feeding experiments. The studies were based on feeds from two different cropping systems, a rotation with protein-rich forage crops, and a rotation with energy-rich crops (studied under sub-task B1). With feeds from the protein rotation, *viz.* grass/clover silage and pea silage, the aim was to stimulate the microbial capture of easily degradable home-grown forage protein. With feeds from the energy rotation, *viz.* whole-crop wheat or barley silage and fodder beet, the cow's ability to use recycled N was tested. Both feeding strategies were studied in experiments with productive cows in early lactation (yield of energy-corrected milk on the best diets averaged >30 kg cow^{-1} day^{-1} during the experimental period of 17 weeks). The ability of the cow to recycle N was also studied in late lactation (yield of energy-corrected milk still averaged >20 kg cow^{-1} day^{-1} in almost all the experimental groups). The studies comprised six feeding experiments, each with three treatments and with a total of 158 cows.

Unfortunately, the crude protein (CP) content of the grass/clover silage from the protein rotation was lower than expected, *viz.* 130 g CP (kg DM)$^{-1}$, and thus the amount of rumen-degraded protein possibly was close to a minimum level for optimal fermentation. But based on other studies, it is assumed that the protein balance of the rumen was high enough to test the efficiency of utilisation of surplus degradable protein.

In the experiments with feeds from the protein rotation and varying AAT (absorbed amino acids from the small intestine) contents, AAT content of the diets varied from 81 to 98 g FU^{-1}, and CP content from 148 to 183 g (kg DM)$^{-1}$. In these experiments, the yield of milk protein decreased significantly when the AAT content was lower than 95 g FU^{-1} and the CP content lower than 175 g (kg DM)$^{-1}$. This reduction was approximately linear between 95 and 81 g AAT FU^{-1} and amounted to about 9 g milk protein per unit reduction in AAT content.

In the experiments with feeds from the energy rotation and varying PBV (protein balance in the rumen) values, PBV intake varied from –81 to –660 g cow^{-1} day^{-1} for the cows in early lactation and from –1 to –449 g cow^{-1} day^{-1} for the cows in late lactation. The corresponding CP content of the diets varied from 145 to 111 g (kg DM)$^{-1}$ and from 144 to 113 g (kg DM)$^{-1}$, respectively. Milk yields of cows in early lactation were lower in these experiments than in the experiment with feeds from the protein rotation. This was probably caused by the lower digestibility of the 'energy' feeds, whereas a sub-optimal protein supply to the cows cannot be excluded. Over the whole range of PBV intakes, feed intake and yields of milk and milk protein were negatively affected by a reduction in PBV intake. The reduction in milk protein yield in early lactation was about 25 to 30 g for each 100 g reduction in PBV intake. Results in late lactation indicate a small effect on milk protein yield of a reduction in PBV intake to *ca.* –200 g cow^{-1} day^{-1}, but also a reduction of about 30 g milk protein for each 100 g further reduction in PBV intake.

In the feeding experiments with cows in early lactation, it was intended to estimate the efficiency of protein synthesis in the rumen by measuring the excretion of purine derivatives in urine. In general, no clear relationships were observed between diet composition and excretion of creatinine, allantoin and uric acid. Only in the two experiments with different levels of AAT, significant differences were found in allantoin excretion. In experiment A1-1, allantoin excretion was significantly higher at an AAT content of the diet of 88 g FU^{-1} than at AAT contents of 95 and 81 g FU^{-1}. In experiment A1-4, excretion of allantoin was significantly reduced by reducing the AAT content from 98 to 95 and 92 g FU^{-1}, which does indicate a reduced synthesis of microbial protein. No explanation was found for this difference between both experiments and, in general, for the absence of clear relationships between diet composition and excretion of purine derivatives. It can be concluded that these measurements did not contribute to the interpretation of the results of these experiments.

Protein supply to the cows significantly affected the urea content of the milk. Diets with a sub-optimal protein content for production of milk protein generally gave milk urea contents under 20-25 mg per 100 ml for cows in early lactation and, possibly, under 10-15 mg per 100 ml for cows in late lactation. This offers a possibility for monitoring protein supply of the dairy herd under farming conditions.

The final conclusion of these experiments is that no possibility was found to reduce the estimated levels of AAT or PBV below minimum values of current Danish feeding standards without a reduction in milk protein yield. Thus, there is no immediate solution for reducing intake and excretion of N by dairy cows in practice. But results of this work may be used to predict the response in milk and milk protein yield to different kinds of reductions in the protein or N supply in response-based rationing systems, thereby giving useful solutions in situations in which maximum production might not always be the optimum solution.

Sub-task A3

Two experiments were carried out in The Netherlands to evaluate ground maize ear silage (GMES) as a replacement for a purchased concentrate (beet pulp) (experiment A3-1) or fresh cut herbage (experiment A3-2) in rations for dairy cows. In experiment A3-1, dairy cows fed *ad libitum* fresh cut herbage were supplemented with 5.6 kg DM day^{-1} of beet pulp (treatment BP), or alternatively with 2.5 kg DM day^{-1} of GMES, 0.6 kg DM day^{-1} of soybean meal solvent extract (SMSE) and 2.5 kg DM day^{-1} of beet pulp (treatment BPSM), or with 4.5 kg DM day^{-1} of GMES and 1.2 kg DM day^{-1} of SMSE (treatment SM). In order to obtain equal intakes of DVE (digestible protein available in the intestine), SMSE was used as a protein source in diets containing GMES. Average CP content of the herbage was 183 g kg^{-1} DM.

Partial or full replacement of beet pulp by GMES and SMSE had no effect on intakes of total DM, net energy for lactation (NE_L) and DVE, yields of milk, fat and protein-corrected milk (FPCM) and milk fat, and concentration of milk protein. However, the concentration of milk fat tended to be lower and yield of protein higher. Yield and concentration of lactose and excretion and concentration of milk urea were significantly higher when beet pulp was replaced by GMES and SMSE. Yield of FPCM averaged 23.6 kg cow^{-1} day^{-1} in this experiment. Yields and concentrations of milk solids for BP, BPSM and SM, respectively, were: protein yield (g cow^{-1} day^{-1}) 794, 838 and 832 (s.e.d. = 26); lactose yield (g cow^{-1} day^{-1}) 1003, 1077 and 1084 (s.e.d. = 39); urea excretion in milk (mg cow^{-1} day^{-1}) 4615, 5664 and 6696 (s.e.d. = 325); fat concentration in milk (g kg^{-1}) 42.5, 40.5 and 40.3 (s.e.d. = 1.2); lactose concentration in milk (g kg^{-1}) 44.6, 45.8 and 46.1 (s.e.d. = 0.4); and urea concentration in milk (mg $litre^{-1}$) 205, 241 and 285 (s.e.d. = 9.6). There was little effect on N utilisation, which was 27.2% on the diet with beet pulp and 25.9% on the diet with GMES and SMSE.

In experiment A3-2, a ration based on fresh cut herbage *ad libitum* and 1.8 kg day^{-1} of a concentrate based on beet pulp and SMSE, was supplemented with 0, 2.5, 5 or 7.5 kg DM cow^{-1} day^{-1} of GMES, for treatment M00, M25, M50 and M75, respectively. Average CP content of the herbage in this experiment was only 135 g kg^{-1} DM. Feeding larger amounts of GMES resulted in a higher intake of total DM and NE_L, but did not affect intake of DVE. However, the supply of DVE corrected for a negative degradable protein balance in the rumen (OEB) was lower at higher intake of GMES, resulting in a DVE supply below the requirements in M25, M50 and M75. OEB intake was negative in all the experimental treatments and varied from –221 to –361 g cow^{-1} day^{-1}. The intake of fresh cut herbage decreased with increasing amounts of GMES in the ration. The substitution rate was about 0.8. Replacing fresh cut herbage by GMES resulted in higher yields of milk, fat, protein and lactose. The concentration of both fat and protein was not affected, but the concentration of lactose increased. Excretion and concentration of milk urea were lower with larger quantities of GMES in the ration, related to decreased OEB. Yields and concentrations of milk and milk solids in M00, M25, M50 and M75, respectively, were: milk yield (kg cow^{-1} day^{-1}) 22.4, 24.2, 23.8 and 23.9 (s.e.d. = 0.5); milk fat yield (g cow^{-1} day^{-1}) 979, 1051, 989 and 1014 (s.e.d. = 32.5); milk protein yield (g cow^{-1} day^{-1}) 712, 781, 760 and 778 (s.e.d. = 19.0); lactose yield (g cow^{-1} day^{-1}) 1010, 1117, 1092 and 1105 (s.e.d. = 27.9); urea excretion in milk (mg cow^{-1} day^{-1}) 3577, 3253, 2746 and 2435 (s.e.d. = 127); protein concentration in milk (g kg^{-1}) 31.7, 32.2, 32.0 and 32.5 (s.e.d. = 0.9); lactose concentration (g kg^{-1}) 45.0, 46.1, 46.0 and 46.2 (s.e.d. = 0.5); and urea concentration in milk (mg litre^{-1}) 159, 134, 116 and 102 (s.e.d. = 5.1). Replacing fresh cut herbage by GMES resulted in an improved utilisation of dietary N at animal level, *viz.* from 31.6% on M00 to 36.6% on M75.

Replacing a purchased concentrate like beet pulp by home-grown GMES did not negatively affect milk performance. Substitution of home-grown GMES for fresh cut herbage is an opportunity to increase the energy to N ratio in the diet and, consequently, improve milk performance and N utilisation. Replacing herbage by GMES, thus reducing OEB intake, is likely to be more effective to reduce the losses of N in dairy cows than replacing concentrates by GMES. Home-grown GMES may have some potential to reduce the inputs and losses of N and energy on dairy farms. However, whole-farm studies are necessary to elucidate the effects at farm-scale.

Sub-task A4

The objective of this study was to estimate herbage intake and N utilisation of dairy cows in different stages of lactation grazing grass/clover swards. An experiment was carried out in Scotland involving 30 Holstein Friesian cows of which 15 were autumn-calving and 15 spring-calving. The experiment commenced on 1 May 1993 and lasted until 24 July 1993. Intensive sampling of milk, urine and faeces was performed in the weeks of 17 May and 29 July. During these weeks, herbage intake was estimated using the n-alkane technique. The accuracy of this technique was studied in a small validation experiment carried out between 7 June and 2 July with Holstein Friesian cows fed known amounts of DM. Throughout the experimental period, animal performance was monitored and herbage samples were taken. The collected samples were analysed for chemical composition.

In the validation experiment, the n-alkane technique was very accurate in predicting DM intake and clover content of the diet. This work has already been published in a scientific journal. In the first intensive measurement period of the main experiment, however, estimates of herbage and total DM intake were unlikely low compared to animal performance and N excretion in faeces. Estimated ME intake of the spring-calving cows in this period was only 74% of the calculated ME requirement; for the autumn-calving cows this figure was 86%. As a result of the low DM intake estimates, estimated N intake of the cows also was low and the calculated production of urinary N was very low. As a consequence, the calculated N efficiencies were very high, *viz.* 38% for the spring-calving cows and 29% for the autumn-calving cows. Estimated ME intakes in the second intensive measurement period were more realistic, *viz.* 98 and 96% of the calculated ME requirements of the spring- and autumn-calving cows, respectively.

Average white clover content of the grazed sward was 9.1% in May, 20.9% in June and 46.6% in July. Average CP content of the herbage DM on offer was 153 g kg^{-1} DM in May, 181 g kg^{-1} DM in June and 197 g kg^{-1} DM in July. White clover contents in the intensive measurement periods in May and July were 7 and 44%, respectively, and CP contents of herbage DM on offer 150 and 195 g kg^{-1} DM. Milk yield in July of both spring- and autumn-calving cows was slightly lower than in May, but protein content of the milk was significantly higher. The higher protein content of the diet in July resulted in significantly higher urea and non-protein nitrogen (NPN) contents of the milk that could have important negative consequences for the manufacturing quality of the milk. The urea contents of milk in May were 180 and 206 mg kg^{-1} for the spring- and autumn-calving cows, respectively, and indicate rather low protein supplies to the cows. In July, these values were 261 mg kg^{-1} milk for both groups, indicating a sufficient protein supply.

If ME intakes in May were supposed to equal ME requirements, corrected N efficiencies were 28 and 25% for the spring- and autumn-calving cows, respectively. In July, these values were slightly lower, *viz.* 25 and 23%. This gives rather small differences between the periods as well as between the spring-calving and autumn-calving cows.

Sub-task A5

The objective of this study was to improve N efficiency of dairy cows grazing grass/clover swards with different (low and high) clover contents, using supplementation with different types and/or levels of concentrates. Two experiments were carried out in Scotland with 30 Holstein Friesian cows which were all spring-calving. Experiment A5-1 lasted from 16 May until 17 June 1994 and experiment A5-2 from 11 July until 5 August 1994. The animals grazed grass/clover swards with a clover content of about 7% of DM on offer in experiment A5-1 and grass/clover swards with a clover content of about 38% in experiment A5-2. The concentrates in experiment A5-1 contained soya, cane molasses, minerals and different proportions of sugar beet pulp and urea. They were fed to the experimental groups A, B and C at 0.9, 3.7 and 3.7 kg cow^{-1} day^{-1}, respectively, and contained 276, 230 and 204 g CP kg^{-1} DM. The concentrates in experiment A5-2 contained soya and different proportions of sugar beet pulp. They were fed to the experimental groups A, B and C at 0.4, 3.4 and 5.8 kg cow^{-1} day^{-1}, respectively, and contained 457, 150 and 134 g CP kg^{-1} DM. In both experiments, the CP content of the herbage on offer was lower than expected, *viz.* on average, 126 g CP kg^{-1} DM in experiment A5-1 (May/June) and 155 g CP kg^{-1} DM in experiment A5-2 (July/August).

The supplementation in spring did not improve the N efficiency of the cows, because N efficiency of group A was already very high, *viz.* 38%. Despite the very low CP content of the diet of group A, supplementation with sugar beet pulp + urea had no significant effect on animal performance and non-protein nitrogen (NPN) content of the milk. It only tended to increase the protein content of the milk. The urea contents of the milk of the experimental groups A, B and C were 164, 159 and 145 mg kg^{-1} milk, respectively, indicating a rather low protein supply to the cows in all the groups and no clear effect of the urea supplement in the groups B and C.

Supplementation of the moderately N-rich herbage in summer with sugar beet pulp slightly reduced N intake of the cows and significantly increased yield of milk and milk protein. As a result, it improved N efficiency of the cows from 27.5% in group A to 32.4% in group C. In addition, supplementation significantly decreased NPN and urea content of the milk. Whether this is a cost-effective strategy to improve performance and N efficiency of cows grazing grass/clover swards will depend on the ratio between the milk price and the costs of grazed herbage and concentrate.

Sub-task A6-1

During the winter of 1994/95, a feeding experiment was carried out in Scotland involving 30 autumn-calving cows. The experiment started on 18 December 1994 for a 17-week period. Three treatments were imposed based on a basal diet of grass/cover silage and a mixture of barley and fodder beet. The treatments consisted of different types of under-supply of protein in the diet, *viz.* in diet A: 80% of the calculated requirements of both rumen-degradable protein (ERDP) and metabolisable protein (MP); in diet B: 80% of ERDP and 100% of MP requirement; and in diet C: 100% of both ERDP and MP requirement. Actual ERDP levels were in accordance with the planned levels, but actual MP levels were more than 20% higher than planned and exceeded requirement in all experimental treatments. Despite this, considerable differences in DM intake, animal performance and N efficiency among the experimental groups were recorded. Experimental group B had a significantly higher DM intake than A and C. The yields of fat and protein-corrected milk of the cows on the diets A, B and C were 22.8, 26.6 and 23.8 kg day^{-1}, respectively, and the yields of milk protein 707, 834 and 714 g day^{-1}. These differences could not be explained on the basis of ERDP and MP levels, but, possibly, the quality of the protein supplements was responsible, because group B mainly received soya and group C a mixture of molasses and urea, which apparently was not effective.

Nitrogen contents of the diets A, B and C were 24.3, 27.8 and 30.7 g kg^{-1} DM, respectively, and N efficiencies of the cows on these diets were 28.8, 27.6 and 24.0%. Urea contents of the milk of the groups A, B and C were 141, 170 and 173 mg litre^{-1}, respectively, indicating protein shortage in group A, and a better, although possibly sub-optimal protein supply in the groups B and C.

A comparison of animal performance in this experiment and in group BF of a comparable feeding experiment (diet and cows), conducted in the winter of 1993/94 (sub-task B5), indicate that the supplementation strategies in this experiment, in particular in the groups A and C, had a significantly negative effect on the yields of fat and protein-corrected milk and milk protein. This confirms that animal performance may respond strongly to small differences in protein supply. This should be taken into account in attempts to improve N efficiency of dairy cows.

Sub-task A6-2

In the two years of experimentation under sub-task A6-2 in Spain, 3 supplementation strategies were tested for dairy cows in early lactation fed *ad libitum* grass/clover silage (experiment A6-2.1 in the first year) or a mixture of maize silage, vetch/oats silage and soybean-meal (experiment A6-2.2 in the second year). In both experiments, 4 diets were compared:

A. *Ad libitum* silage + 6 kg cow^{-1} day^{-1} of a conventional dairy concentrate, containing about 230 g CP kg^{-1} DM.
B. *Ad libitum* silage + 1.5 kg cow^{-1} day^{-1} of a concentrate with a high content of protein of a low rumen-degradability. The CP content of the concentrate DM was 479 g kg^{-1} in experiment A6-2.1 and 517 g kg^{-1} in experiment A6-2.2.
C. *Ad libitum* silage + 6 kg cow^{-1} day^{-1} of a high-energy concentrate, containing 157 g CP kg^{-1} DM in experiment A6-2.1 and 130 g kg^{-1} in experiment A6-2.2.
D. *Ad libitum* silage without supplement.

The grass/clover silage in experiment A6-2.1 contained 135 g CP kg^{-1} DM; the mixed silage in experiment A6-2.2 115 g kg^{-1}.

Average silage intake in experiment A6-2.1 was 10.9 kg DM cow^{-1} day^{-1} (6.7 kg NDF cow^{-1} day^{-1}) and this was not significantly affected by supplementation. Animal performance in the only-forage group was poor: mean milk yield in the experimental period (5th to 15th week of lactation) was only 14.2 kg cow^{-1} day^{-1}, *i.e.* 9 kg cow^{-1} day^{-1} lower than in week 3. In addition, protein content of the milk was only 27.7 g kg^{-1} and the cows lost much weight. In the supplemented groups, milk yield was 6-7 kg cow^{-1} day^{-1} higher than in the control group and protein content of the milk was about 30 g kg^{-1}, without significant differences among the supplemented groups. Supplemented cows lost significantly less weight than cows in the control group. However, cows with the protein supplement (group B) tended to loose more body weight and for a longer period than cows in the groups A and C, although this difference was not statistically significant.

Average silage intake of group D in experiment A6-2.2 was 14.7 kg DM cow^{-1} day^{-1} and supplementation decreased this by 1.2 to 1.8 kg DM cow^{-1} day^{-1}. Average milk yield of group D in the experimental period was 20 kg cow^{-1} day^{-1}, but the protein content of the milk was only 27.1 g kg^{-1} and the cows had a moderate loss of body weight. The effect of supplementation was 3.5-5.0 kg milk cow^{-1} day^{-1}, an increase of the protein content of the milk to about 29.0 g kg^{-1} and gain of body weight. Differences among the supplemented groups were not significant, but the cows in group B tended to gain less weight than the cows in A and C.

Both experiments show that rather good performance can be achieved in early lactation with protein supplements with low rumen-degradability. These supplements will improve the energy balance of the farm compared to conventional supplements. However, more attention should be given to the effects of this supplementation strategy on body weight and the consequences of this for long-term cow performance.

Sub-task B1

Authorities in Denmark require a sustainable and environmentally friendly agriculture. Amongst other things, nitrate leaching should be reduced by 50% in the year 2000. Two cropping systems, a 'protein rotation’ and an ‘energy rotation’ were established at Silstrup Research Station in 1992 to study yield, quality and nutrient balances of crops included. The objective of the ‘protein rotation’ was to maximise yield of milk protein per ha with minimum use of fertiliser N in the fields and a minimum of protein supplements in the diets of the cows. The objective of the ‘energy rotation’ was to maximise yield of metabolisable energy per ha. Crops in both rotations were produced in an environmentally sound manner. The ‘protein rotation' contained a large proportion of legumes and the ‘energy rotation’ consisted of cereals, fodder beet and grass and had a larger requirement for N input than the ‘protein rotation’.

Yields of DM and energy were higher, but less stable for crops in the energy rotation. Feeding value, *i.e.* the content of metabolisable energy (ME), was nearly equal in the two rotations, while the CP concentration was higher in the protein rotation, *viz.* on average 122 g kg^{-1} DM compared to 98 g kg^{-1} DM in the energy rotation. Protein yields of the 2 cropping systems were equal, but the proportion of rumen-degradable protein in the protein crops may be too high for optimal utilisation by dairy cows. The energy rotation will be difficult to use in practice because of the limited suitability of the crops for grazing and high-quality silage production. The protein rotation requires about 35% more land then the energy rotation to produce the same amount of DM or ME.

Average leaching of nitrate-N to drainage water in the protein rotation was 36 kg ha^{-1} $year^{-1}$ compared to 42 kg ha^{-1} $year^{-1}$ in the energy rotation. Leaching of nitrate-N at Silstrup from 1988 to 1992 was 30 kg ha^{-1} $year^{-1}$ higher, probably because the input of N was approximately 100 kg ha^{-1} $year^{-1}$ higher. Spring ploughing and optimal timing of slurry and fertiliser application in the present study also have contributed to lower leaching. It is concluded that energy crops yielded more DM, but that protein crops had more stable yields from season to season. The cropping systems will be difficult to use in practice because of high rumen-degradability of CP in the protein crops and insufficient crops for grazing and high-quality silage in the energy rotation. Leaching of nitrate to drainage water was more related to N input and management of crops than to selection of crops *per se.*

Sub-task B3

Two experiments were carried out in The Netherlands to evaluate ground maize ear silage (GMES) and/or fodder beet as replacement for purchased concentrates (experiment B3-1) or grass silage (experiment B3-2) in rations for dairy cows. In experiment B3-1, a control group (treatment BS) received a concentrate supplement, which consisted of 8.7 kg DM day^{-1} of sugar beet pulp and 1.3 kg DM day^{-1} of soybean meal solvent extract (SMSE). These concentrates were replaced by 5 kg DM day^{-1} of fodder beet, 2.7 kg DM day^{-1} of SMSE and 2.3 kg DM day^{-1} of beet pulp (treatment FB), or 2.8 kg DM day^{-1} of SMSE, 0.6 kg DM day^{-1} of sugar beet pulp, 3.3 kg DM day^{-1} of fodder beet and 3.3 kg DM day^{-1} of ground maize ear silage (GMES) (treatment FBMS), or 2.6 kg DM day^{-1} of SMSE, 2.4 kg DM day^{-1} of sugar beet pulp and 5 kg DM day^{-1} of GMES (treatment MS). The rations were formulated to contain equal amounts of DVE (digestible protein available in the intestine). The CP content of the grass silage in this experiment was 189 g kg^{-1} DM.

Replacing part of the purchased concentrates by fodder beet or GMES had no effect on intake of total DM and net energy for lactation (NE$_L$). However, type of concentrate had significant effects on the yields of milk, fat and protein-corrected milk (FPCM), lactose and urea excretion in milk and on the concentrations of protein and urea, but not on the yields of fat and protein and the concentrations of fat and lactose. Yields and concentrations of milk solids in the groups BS, FB, FBMS and MS, respectively, were: milk yield (kg cow^{-1} day^{-1}) 31.0, 29.4, 30.4 and 32.4 (s.e.d. = 0.65); FPCM yield (kg cow^{-1} day^{-1}) 32.2, 31.2, 32.2 and 33.2 (s.e.d. = 0.64); lactose yield (g cow^{-1} day^{-1}) 1458, 1349, 1414 and 1504 (s.e.d. = 28); urea excretion in milk (mg cow^{-1} day^{-1}) 9381, 8493, 9665 and 11273 (s.e.d. 507); protein concentration (g kg^{-1}) 32.3, 34.0, 33.9 and 31.3 (s.e.d. = 0.65); and urea concentration (mg litre^{-1}) 301, 282, 319 and 350 (s.e.d. = 14.4). Larger quantities of SMSE in the rations with either fodder beet or GMES were accompanied by increased intake of OEB (degradable protein balance in the rumen) and N and reduced N utilisation. N utilisation values for BS, FB, FBMS and MS were 27.6, 26.2, 25.4 and 25.3%, respectively.

Experiment B3-2 was conducted to evaluate GMES as a replacement for grass silage at a high or low level of DVE in the ration. Four experimental rations were evaluated: a high-roughage, low-protein ration (treatment HRLP) consisting of *ad libitum* grass silage, 4 kg DM day^{-1} of GMES and 5.0 kg DM day^{-1} of a pelleted concentrate based on beet pulp (BSC); a high-roughage, high-protein ration (treatment HRHP) consisting of *ad libitum* grass silage, 4 kg DM day^{-1} of GMES, 3.6 kg DM day^{-1} of BSC and 1.4 kg DM day^{-1} of a pelleted concentrate based on soybean meal (SCF); a low-roughage, low-protein ration (treatment LRLP) consisting of 8.2 kg DM day^{-1} of grass silage, 8 kg DM day^{-1} of GMES, 4.6 kg DM day^{-1} of BSC and 0.4 kg DM day^{-1} of SCF, and a low-roughage, high-protein ration (treatment LRHP) consisting of 8.2 kg DM day^{-1} of grass silage, 8 kg DM day^{-1} of GMES, 3.2 kg DM day^{-1} of BSC and 1.8 kg DM day^{-1} of SCF. The CP content of the grass silage in this experiment was 140 g kg^{-1} DM. The yields of milk, FPCM, lactose and protein, the excretion of urea in milk and the concentrations of milk fat and urea were affected by the level of DVE, but not by the level of roughage. Yields and concentrations of milk solids in HRLP, HRHP, LRLP and LRHP, respectively, were: milk yield (kg cow^{-1} day^{-1}) 28.9, 31.8, 29.0 and 31.7 (s.e.d. = 0.99); FPCM yield (kg cow^{-1} day^{-1}) 31.4, 33.1, 31.1 and 32.7 (s.e.d. = 0.80); lactose yield (g cow^{-1} day^{-1}) 1330, 1448, 1325 and 1444 (s.e.d. = 49); protein yield (g cow^{-1} day^{-1}) 977, 1042, 977 and 1025 (s.e.d. = 28); urea excretion in milk (mg litre^{-1}) 4538, 5714, 4048 and 5647 (s.e.d. = 340); fat concentration (g kg^{-1}) 47.1, 43.6, 46.2 and 43.1 (s.e.d. = 1.63), and urea concentration (mg litre^{-1}) 157, 180, 140 and 178 (s.e.d. = 8.4). There were no significant differences in protein and lactose concentration. N utilisation of the cows was high for all the diets and differences were small due to the low CP content of the grass silage N utilisation values for HRLP, HRHP, LRLP and LRHP were 34.8, 32.5, 36.0 and 34.5%, respectively.

Sub-task B5

During the winter of 1993/94, a feeding experiment (experiment B5-1) was carried out in Scotland to evaluate the quality of barley and fodder beet as supplements for dairy cows fed grass/clover silage. The experiment started on 13 December for a 17-week period and involved 30 autumn-calving cows. Three treatments were imposed, using 5 kg DM cow^{-1} day^{-1} of barley (group B), of fodder beet (group F), or of a combination of barley and fodder beet (group BF) as energy supplements to *ad libitum* grass/clover silage + 3 kg soya DM cow^{-1} day^{-1}. The feeds for this experiment were produced on the basis of cattle slurry, and no fertiliser N was used. A first-cut silage was produced from 15 ha of grass/white çlover swards and, in addition, 4 ha of barley and 3 ha of fodder beet were grown. The experiment was carried out as planned and the efficiency of utilisation of N was estimated.

Crude protein contents of the diets B, BF and F were 195, 189 and 180 g kg^{-1} DM, respectively. Milk production was not significantly affected by the supplements, but fodder beet tended to increase milk yield and significantly decreased milk protein content. Average milk yields of the groups B, BF and F were 23.6, 26.0 and 26.5 kg cow^{-1} day^{-1}, and fat and protein-corrected milk yields were 25.5, 27.7 and 27.7 kg cow^{-1} day^{-1}, respectively. Average protein contents of the milk of B, BF and F were 35.0, 34.3 and 32.8 g kg^{-1}, and milk protein yields 821, 886 and 863 g cow^{-1} day^{-1}, respectively. Supplementation with fodder beet, compared to barley, slightly reduced N intake of the cows and significantly reduced NPN content of the milk and total N and urea N contents of urine. Urea content of the milk from the PM milking was significantly affected by supplementation; average contents for B, BF and F were 214, 207 and 195 mg litre^{-1}, indicating a satisfactory protein supply in all the groups. N efficiencies of the cows on the diets B, BF and F were 24, 28 and 27%, respectively. The results indicate that feeding a mixture of starch and sugar containing supplements (barley and fodder beet) results in a good performance and N efficiency of the cows.

Sub-task B6

Research was carried out at whole-farm scale in North-West Spain to define the conditions in which high-quality forage can be produced for dairy cows at low inputs of chemical fertilisers. This study included grass/white clover swards for grazing and cutting, and silage maize in rotation with a mixture of vetch and oats. Three experiments were carried out in the period 1993-1995: a grazing experiment to study the effects of fertiliser nitrogen (N) on the productivity of a grass/clover sward (experiment B6-1), farm-scale production of high-quality silages from a grass/clover sward, maize and a mixture of vetch and oats (experiment B6-2), and a small-plot field experiment to study the effect of early application of fertiliser N on the yield of a mixed sward under cutting (experiment B6-3).

Fertiliser N only slightly increased the yield of a grazed grass/clover sward (experiment B6-1). Compared to the sward without applied N, application of 240 kg N ha^{-1} year^{-1} increased gross herbage yield from 11.42 to 12.62 t DM ha^{-1} year^{-1}, net herbage yield (herbage consumed) from 10.37 to 11.37 t DM ha^{-1} year^{-1} and milk yield form 8680 to 9320 kg ha^{-1} year^{-1}. Average milk yield per cow was 4400 kg year^{-1}. This was obtained with only 400 kg concentrates cow^{-1} year^{-1} and not significantly affected by N application. Applied N increased the DM yield of the clover component by about 2 t ha^{-1} year^{-1} and decreased the DM yield of the grass in the mixture by about 1 t ha^{-1} year^{-1}. Applied N had little effect on the quality of the herbage on offer; it increased the mean CP content from 177 (range: 155-213) to 188 (range 167-215) g kg^{-1} DM. Grazing management aimed to supply the cows with high-quality forage with a good proportion of clover.

Silage was made from grass/clover swards fertilised with 50 kg N ha^{-1} to the first cut and 40 kg N ha^{-1} to the second cut. Average DM yields at cutting were slightly over 4000 kg ha^{-1}; clover contents were very low. The silage contained less than 20% DM, and 135 g CP and 467 g acid detergent fibre (ADF) kg^{-1} DM. Maize and a mixture of vetch and oats were grown in rotation for silage production. Maize DM yields were 9 t ha^{-1} in the first two years and 6 t ha^{-1} in the dry third year. Average DM yields of vetch/oats were 4930 kg ha^{-1} with about 100 g CP kg^{-1} DM in the first two years when the seed mixture contained 100 kg oats and 40 kg vetch per ha. In the last two years when the seed mixture contained 40 kg oats and 100 kg vetch per ha, the average DM yield was 4000 kg ha^{-1} and the CP content 160 g kg^{-1} DM. The silages produced were used in the feeding experiments conducted under sub-task A6-2.

Nitrogen applied in early spring to a mixed sward accelerated herbage growth and increased DM yield of the first cut, in particular at a later cutting date. Strong effects of applied N on the yield of this first cut were associated with a reduction of the clover content of the sward. This contributed to a negative residual effect of early N on the DM yield of the second cut and resulted in a small overall effect of applied N on annual herbage yield.

Sub-task C1

A field experiment (experiment C1-1) was conducted in 1993, 1994 and 1995 on a sandy soil near Wageningen, The Netherlands, to study the effects of different slurry application techniques on the utilisation of N, P and K from cattle slurry by a grass/white clover sward. For this purpose, the yields of slurry-treated plots were compared with those of fertiliser-treated plots that received the three main elements (NPK) or only two of them (PK, NK, NP). The rate of slurry application aimed to replace the withdrawal of P in the harvested herbage (P replacement). The rates of fertiliser N, P and K equalled the contents of inorganic N, total P and total K of the slurry applied. Some plots received 50% extra fertiliser P and K to evaluate the adequacy of the (replacement) rates of P and K. The techniques of slurry application were: surface application of untreated slurry, surface application of 1:2 diluted slurry, injection with open slits, and deep injection. Effectivity of slurry N was related to the rate of inorganic N applied and expressed as apparent N efficiency (ANE, in kg DM kg^{-1} inorganic N applied), and apparent N recovery (ANR, as percentage of the amount of inorganic N applied). Similarly, effectivity of slurry P was related to the amount of total P applied, and expressed as APE and APR, and effectivity of slurry K was related to the amount of total K applied, and expressed as AKE and AKR.

Clover content of the sward decreased strongly due to periods with dry and hot weather in the summers of 1994 and 1995. Both slurry and fertiliser N increased the DM yield of the grass component and decreased the DM yield of the clover component of the mixture (compared to the treatment without applied N: the PK treatment). In the first experimental year, the negative effect of slurry on the clover component exceeded the positive effect on the grass component, resulting in a negative effect on total DM yield. In this year, the negative effect of fertiliser N on the clover was smaller and fertiliser N had a slightly positive effect on the yield of the mixture. In 1994 and 1995, the negative effect of both N sources on the clover was much smaller than in 1993. This resulted in an increasing effect of both slurry and fertiliser N on the DM yield of the mixture. In 1994 and 1995, relatively low ANE and ANR values were recorded on the plots with surface-applied untreated slurry, due to N losses by ammonia volatilisation, and on the plots without P or K application.

The efficiency of utilisation of P from slurry or fertiliser depended strongly on other factors determining the yield of the mixture, in particular N supply, but also K supply and damage caused by the slurry or the slurry application technique. This hampers a good comparison of the effectivity of slurry P and fertiliser P and of the slurry application techniques. In 1994 en 1995, the efficiency of utilisation of P from surface-applied diluted slurry was high compared to that from slurry applied by the other techniques and fertiliser. In 1995, the efficiency of utilisation of P from injected slurry (both techniques) was high compared to that from fertiliser. There is no indication that a replacement rate of P application was insufficient.

The rate of slurry application aiming at P replacement did not supply sufficient K to replace the withdrawal of K in the harvested herbage. This led to increasing K shortage on most experimental treatments. The treatment without K application showed a strong negative effect of K shortage on the K content and DM yield of the white clover in the mixture. After 2 years white clover almost had disappeared from the sward on this treatment. The efficiency of utilisation of K from slurry or fertiliser was affected by other factors that determined the yield of the mixture, in particular the damage caused by the slurry or the slurry application technique, the supply of N and, to a lesser extent, also of P. Except in 1993, when slurry had a negative effect on the yield of the sward, the efficiency of utilisation of K from slurry applied by low-emission techniques was similar or higher than the efficiency of utilisation of fertiliser K.

In this experiment, slurry applied by low-emission techniques proved to be an effective source of nutrients for a mixed sward, and the efficiency of utilisation of slurry inorganic N, total P and total K was comparable to or slightly better than the efficiency of utilisation of fertiliser N, P and K. The strongly negative effect of slurry on the yield of white clover in the first experimental year requires more research.

Sub-task C2

A field experiment (experiment C2-1) was conducted in 1993, 1994 and 1995 on a sandy soil near Wageningen, The Netherlands, to study the effects of low-emission slurry application techniques and periods of slurry application on the yield and N economy of a mixed sward of perennial ryegrass and white clover. The slurry application techniques studied were: (1) surface spreading of 1:2 diluted slurry (SAD), (2) injection with open slits (Inj OS), and (3) deep injection (Inj). The rate of slurry application aimed to replace the estimated withdrawal of P in the harvested herbage (P replacement). The effects of slurry were compared with those of the following combinations of artificial fertilisers: NPK, applied to an untreated sward (NPK) as well as to a sward treated with the open-slit injector to quantify effects of the injection equipment on the sward (Inj OS NPK), and PK applied to an untreated sward (PK). The rates of fertiliser N, P and K equalled the contents of inorganic N, total P and total K of the slurry applied. In addition, all the slurry and fertiliser treated plots received 50% extra P and K from artificial fertilisers to assure an ample supply of these nutrients. The 3 periods of application of both slurry and artificial fertilisers were: (1) to the first and second cut, (2) to the third and fourth cut, and (3) to the first and third cut. The extra P and K was applied to the fourth, the first and the fourth cut, respectively.

All the slurry and fertiliser treatments were applied to the mixture and to a monoculture of perennial ryegrass. The differences between the DM and N yields of both swards were considered as the (apparent) contribution of white clover to the yield of the mixed sward. Effectivity of both slurry and fertiliser N was related to the rate of inorganic N applied and expressed as apparent N efficiency (ANE: the increase of DM yield kg^{-1} inorganic N applied), and apparent N recovery (ANR: the increase of N yield, calculated as a percentage of the rate of inorganic N applied).

Both slurry and fertiliser N strongly increased DM and N yield of the grass in monoculture: average (of 3 application periods and 3 years) ANR values of SAD, Inj OS, Inj, Inj OS NPK, and NPK were 51, 71, 84, 71, and 77%, respectively, and average ANE values 22.8, 27.9, 29.9, 29.3, and 32.7 kg DM kg^{-1} inorganic N applied. The differences between the slurry application techniques reflect different N losses through ammonia volatilisation. ANR and ANE values of slurry were relatively low in 1993, possibly as a result of the low N content of the diet of the cows in that year. N applications to the first and second cut, on average, gave slightly higher ANR values than later applications.

Slurry and fertiliser N had a small effect on the N yield of the mixture and a moderate effect on the DM yield (compared to the PK treatment). Average ANR values of the main treatments SAD, Inj OS, Inj, Inj OS NPK, and NPK were 14, 28, 11, 12, and 17%, respectively, and average ANE values 9.7, 12.3, 6.1, 10.0, and 11.6 kg DM kg^{-1} inorganic N. The effects of slurry or fertiliser N on the N and DM yield of the mixture resulted from a strong increase of the N and DM yield of the grass component and a reduction of the N and DM yield of the clover. In the first year, the negative effect of some experimental treatments on the clover exceeded the positive effect on the grass. In 1994 and 1995, applications of slurry or fertiliser N were less harmful to the clover. Averaged over 3 years, SAD, Inj OS, Inj, Inj OS NPK, and NPK decreased clover yield with 6.1, 9.9, 15.0, 13.0 and 10.6 kg DM kg^{-1} inorganic N applied, respectively. Hence, there were large differences between the main experimental treatments, but slurry inorganic N was not more harmful to the clover than fertiliser N. The grass in the mixture nearly absorbed the same proportion of applied N than the grass in monoculture, *viz.* on average 47, 71, 78, 71, and 68% on the main treatments SAD, Inj OS, Inj, Inj OS NPK, and NPK, respectively. The corresponding ANE values for the grass component of the mixture were 15.8, 22.2, 21.1, 22.9, and 22.1 kg DM kg^{-1} inorganic N applied. The period of N application affected the composition of the sward. Late N applications (to the third and fourth cut) were less effectively utilised by the grass component of the mixture and at the same time less harmful to the clover than early N applications, particularly in the first and second experimental year.

In the absence of applied N, white clover increased DM yield on average with 5.66 t ha^{-1} year^{-1}, and N yield with 234 kg ha^{-1} year^{-1}. On the treatments with applied N, *viz.* SAD, Inj OS, Inj, Inj OS NPK, and NPK, white clover contributed 3.69, 3.74, 2.89, 3.50, and 3.30 t DM ha^{-1} year^{-1} and 179, 181, 149, 168, and 167 kg N kg^{-1} year^{-1}, respectively. Hence, Inj had the most negative effect on the contribution of white clover and SAD and Inj OS the least negative effect.

Nitrogen fixation efficiency (NFE) on the treatments without applied N averaged 46.6 kg N t^{-1} clover DM harvested. On the treatments with slurry or fertiliser N, NFE averaged 45.2 kg N t^{-1} clover DM harvested. Differences between N sources and application techniques were negligible, only N applications to the third and fourth cut gave slightly lower NFE values than early N applications. Hence, applications of N decreased the clover content of the sward but did not significantly affect the fixation of N per ton of clover DM harvested.

Sub-task C3

A field experiment of three years (experiment C3-1) was carried out in Italy to study the utilisation of slurry N by maize as affected by period, method and rate of slurry application. Slurry produced by dairy cows was applied at 4 different rates (0, 80, 160 and 240 kg inorganic N ha^{-1}), in winter or spring, and with or without immediate incorporation into the soil by ploughing. Yields and N content of the crop and inorganic N content of the soil were determined.

A lysimeter experiment (experiment C3-1a) was carried out simultaneously to measure nitrate leaching from maize plots fertilised with 0, 160 or 240 kg inorganic N ha^{-1} supplied by slurry.
Although the experimental treatments had significant effects on maize DM and N yields, the efficiency of utilisation of applied N was low. The mean apparent recovery of inorganic N from slurry varied from 16.0% for winter-applied not incorporated slurry to 21.8% for spring-applied incorporated slurry. The effects of the period and method of slurry application were much smaller than expected. This led to the conclusion that large N losses by ammonia volatilisation took place, probably caused by a too large time interval between slurry application and incorporation, and/or poor effectivity of incorporation owing to poor soil structure and high soil pH. The mean apparent recovery of fertiliser N was 31.1% and that of a combination of slurry and fertiliser N 43.2%. Nitrate leaching losses during the growth of the maize crop were small.

Sub-task C4

A field experiment of three years (experiment C4-1) was carried out in Italy to study the utilisation by Italian ryegrass of residual inorganic N in the soil after a maize crop and to investigate the behaviour of the two crops under different periods, methods and rates of organic fertilisation. Italian ryegrass was sown immediately after harvesting silage maize and was cut in spring. Maize plots were fertilised with different rates of slurry application (0, 80, 160 and 240 kg inorganic N ha^{-1}) and different periods and methods of application (winter and spring application and surface spreading with or without immediate incorporation into the soil by ploughing) or with artificial N fertiliser (0, 80, 160 and 240 kg N ha^{-1} at maize sowing). Italian ryegrass DM and N yields were measured. A lysimeter experiment (experiment

C4-1a) was conducted simultaneously to measure nitrate leaching from Italian ryegrass plots and from bare soil in winter.

Applications of slurry or fertiliser N to maize hardly affected the amount of inorganic N in the soil after harvesting the maize. As a consequence, the residual effects of these treatments on Italian ryegrass were small and, expressed as apparent recovery by the Italian ryegrass, never exceeded 6% of inorganic N applied. On average, the residual effects varied from 1.9% of inorganic N applied for winter-applied and not incorporated slurry to 4.6% for spring-applied and incorporated slurry. For spring-applied fertiliser N and slurry + fertiliser N, these values were 2.3 and 3.0%, respectively. Spring-applied slurry had a slightly larger residual effect on the Italian ryegrass than winter-applied slurry; the differences between the methods of slurry application were negligible. Nitrate leaching losses were relatively small in 1994/1995 and moderate in the other years. They were larger from the plots with 240 kg inorganic N ha^{-1} than from the plots with 160 or 0 kg inorganic N ha^{-1}. No differences in nitrate leaching were measured between the plot without slurry and that with 160 kg inorganic N ha^{-1} from slurry and between the plots with the highest rate of slurry with and without Italian ryegrass as a winter crop. Hence, the winter crop Italian ryegrass did not reduce nitrate leaching in this lysimeter experiment.1.

Evaluation and integration

Improve the utilisation of dictary nitrogen integration

A reduction in the rate of N application to grassland and fodder crops is the most effective measure to reduce NH_3 volatilisation and NO_3^- leaching from intensive dairy farms. This measure reduces direct losses of applied N, as well as indirect losses related to N excreted by the animals. The latter is most obvious for grassland, where a lower N rate not only reduces N excretion in faeces and urine, but also increases the capacity of the sward to re-utilise this N. Moreover, the N status of the sward affects the exchange of NH_3 between the sward and the atmosphere and this may partly explain the relatively low NH_3 emission from grazed swards fertilised with low N rates (see Paragraph 5.1.6).

In this project, grass and forage crops for feeding experiments generally were grown with medium or low rates of N application. However, CP contents of these forages were variable and often lower than expected and this affected the protein content of the diets in some experiments, particularly in 1994. The Danish feeding experiments, conducted under sub-task A1, showed a rather strong negative effect of a sub-optimal protein supply on milk protein yield. This should be taken into account in attempts to minimise N excretion of the dairy herd. Therefore, it was concluded that accurate diet preparation is important and that this requires a quick and simple method to determine the protein content and degradability of herbage and other feeds on dairy farms.

The results obtained in this project under sub-task A1, confirm that the urea content of the milk may be used as an indicator of the protein supply of the dairy herd. For productive cows in early lactation, milk urea contents under *ca.* 20-25 mg per 100 ml indicated a sub-optimal protein supply. For cows in late lactation, the critical milk urea content was *ca.* 10-15 mg per 100 ml. This confirms a critical milk urea content at herd level of *ca.* 15-20 mg per 100 ml, as used in Dutch advisory work. In several feeding experiments in this project, milk urea contents were below these critical values, indicating a too low protein supply to the cows. This was generally caused by a lower than expected CP content of the forages used. Based on the milk urea content in different experiments, it was concluded that an N efficiency (N in milk/N intake) of lactating cows of >30% often was associated with a sub-optimal protein supply. This was also the case when the proportion of excreted N in urine was lower than 40-45%, when the mean N content of urine was lower than 6 g $litre^{-1}$, and when the proportion of urea-N in total urinary N was lower than 60%.

It is generally assumed that a reduction in the N content of the diet of the dairy herd causes a corresponding reduction in excretion of urinary N and in excretion and concentration of urinary urea-N. Based on this assumption and on the reported linear relationship between the concentration of urinary urea-N and NH_3 volatilisation, a reduction in the N content of the diet is advocated as an effective measure to reduce NH_3 volatilisation from dairy farms. However, it has been shown in Chapter 5.1 of this report that this popular conception is too simple and possibly misleading. Some studies revealed that a reduction in dietary N content, e.g. by replacing N-rich grass by maize silage or GMES, may also cause a reduction in N excretion in faeces and, as a consequence, a smaller than expected reduction in N excretion in urine. In addition, it has been shown that the concentration of urea in urine not only depends on urea excretion, but also on the volume of urine produced which is strongly affected by K and Na intake. Often, a reduction in N intake is associated with a reduction in K and Na intake and then the effect on the concentration of urinary urea will also be smaller than expected. Finally, NH_3 volatilisation from a dairy farm, particularly from the barn, depends on a series of complicated processes and it appears not sufficiently proven that the amount and concentration of urinary urea always is the most limiting factor for NH_3 emission. The significance of the preceding observations for the relationship between dietary N content and NH_3 emission is not clear and should be studied.

The limited number of scientific publications on the relationship between dietary N content and NH_3 emission unfortunately does not provide a sound basis for practical recommendations in this field (Chapter 5.1 of this report). Some studies did not make clear to which extent the observed reduction in NH_3 volatilisation was caused by the reduction in dietary N, because other factors also were varied. Other studies were carried out under laboratory conditions and from the results it was evident that these conditions were not representative for those on a dairy farm. As a consequence, it must be concluded that more systematic and detailed studies are required on the effects of a reduction in the N content of the diet of the dairy herd on NH_3 volatilisation and on the practical possibilities to reduce NH_3 volatilisation by better animal feeding practices.

The rate and period of N application are the dominant factors determining NO_3^- leaching from grassland and maize land. The rate of N application has a direct effect on NO_3^- leaching that does not depend on the type of N applied (fertiliser N or effective slurry N). A reduction in the rate of N application to grassland also has an indirect effect on NO_3^- leaching, because it reduces the N content of the herbage and the production of faecal and urinary N, and increases the capacity of the sward to absorb this N. Replacement of a part of the N-rich herbage by high-energy and low-protein feeds is also advocated as a measure to reduce NO_3^- leaching. However, it has been shown in Chapter 5.1 of this report that the effects of this measure on NO_3^- leaching may be negative as well as positive, depending on other aspects of farm management. As a consequence, effects of a reduction in dietary N content on NO_3^- leaching should be evaluated at the level of the whole production system.

The uncertain effects of a reduction in dietary N content on NH_3 volatilisation and NO_3^- leaching, in combination with the negative effect of a sub-optimal protein supply to the cows on the production of milk protein, indicates that it is not recommendable to reduce dietary N content below present-day feeding standards.

Increase the proportion of home-grown feeds

Dairy farms with a high animal density and/or on-farm production of forages with low protein and P contents need large N and P inputs in purchased feeds and generally have a (latent) surplus of manure N and P and related environmental problems. These problems can be solved by (1) increasing the on-farm production of feed protein and P, e.g. by growing forages with a higher N and P content, (2) avoiding surpluses of feed protein and P in the diets, and (3) exporting manure to other farms. Economic factors determine the suitability of these measures for a given farm. Increasing the on-farm production of dietary feed protein and P has the advantage that it also increases the capacity to re-utilise manure N and P on the farm. Under good climatic conditions, N-fertilised grasses and grass/clover mixtures may produce a large proportion of dietary protein and P and have a large capacity to re-utilise manure N and P. On the contrary, forage crops like maize, cereals and fodder beet have low N and P contents and require supplements with high N and P contents. If these crops occupy a large part of the farm area, the production of manure N and P easily exceeds their N and P requirement. It has been proposed in Chapter 5.2 of this report to use farm N and P balances to analyse potential pollution from animal feeding practices. In this approach the import of N and P in roughage and concentrates from outside the farm should not exceed the export of N and P in animal products + the ecologically acceptable N and P losses.

On dairy farms with a surplus of roughage or where the feed production capacity is not fully utilised, it may be interesting to grow concentrate replacers, like GMES, barley and fodder beet. These feeds have been tested in a number of feeding experiments. In general, they had a good effect on animal performance and should be further tested at farm level. These feeds may be used on biological dairy farms to increase self-support.

Improve the utilisation of slurry nutrients

Inorganic N, total P and total K from dairy cattle slurry, applied by low-emission techniques, gave approximately the same effects on DM and NPK yields from a mixed sward than N, P and K from chemical fertilisers. Application of slurry or fertiliser N to the mixed sward increased DM and N yield of the grass component and decreased DM and N yield of the clover component. Overall, the effect of applied N on DM yield of a clover-rich sward was small and, as a consequence, biological dairy farmers may consider alternatives for slurry use, like (1) application to non-leguminous forage and food crops, (2) application to mixed swards with a low clover content, (3) application to mixed swards in early spring to accelerate early growth, and (4) application to clover-rich swards to avoid a too high clover content. If a considerable part of the slurry is used on other crops, attention should be given to P and, particularly, K supply to the mixed sward, because this project showed a dramatic effect of K shortage on white clover performance. From an ecological point of view, P fertilisation at replacement (P application = P yield in the harvested crop) appeared to be a good criteria for slurry application (Chapter 5.3 of this report).

1. Introduction

Inorganic nitrogenous fertilisers and concentrates have strongly contributed to the increase of forage and milk production in the European Union. Relatively low prices have stimulated a liberal use of these means of production on many farms. The use of fertiliser nitrogen (N) is highest in The Netherlands, in particular on grassland where it increased from about 50 kg ha^{-1} in 1950 to as much as 315 kg ha^{-1} in 1985 (Van der Meer, 1991). In the same period, the consumption of concentrates increased from *ca.* 450 to 2250 kg cow^{-1} year^{-1} (Korevaar *et al.*, 1988). Although average figures for fertiliser N and concentrate consumption are much lower in other European countries, many individual dairy farms attain comparable levels. According to Wilkins (2000), 12% of grassland in the UK received >300 kg fertiliser N ha^{-1} in 1986.

Since the 1980s, there is increasing concern in many European countries over nutrient losses to the environment from intensively managed ruminant livestock farms. Until now, the pollution of water resources by leaching and run-off of nitrates, phosphorus (P) and organic matter has received most attention. In addition, ammonia volatilisation requires attention, particularly in areas with high livestock densities where emitted ammonia causes high levels of atmospheric N deposition (Van Breemen *et al.*, 1982; ApSimon *et al.*, 1987; The United Kingdom Review Group on Impacts of Atmospheric Nitrogen, 1994; Heij & Schneider, 1995). Nitrogen deposition causes eutrophication of terrestrial ecosystems and associated loss of biodiversity, increased susceptibility of trees to stress, soil acidification and, in some cases, even too much nitrate leaching from affected ecosystems (Heij & Schneider, 1995). Finally, emissions of greenhouse gases from ruminant production systems need attention because of the alleged contribution of these gases to the global climate change induced by human activities (Duxbury *et al.*, 1993). Ruminant production systems emit greenhouse gases, not only from the biological components of the system (N_2O from soils and CH_4 from animals and manure storage facilities), but also through the direct and indirect use of fossil energy (CO_2 from combustion of fuels).

1.1 Nutrient balances to estimate potential losses

It is impossible to quantify all nutrient (N, P) flows and emissions in the various production systems and conditions. Therefore, the use of nutrient balance-sheets was proposed to assess the efficiency of nutrient use on dairy farms and the potential losses to the environment (Van der Meer, 1982). Hence, the N balance sheet or N balance of a farm includes all known N inputs from outside the farm, *i.e.* in fertilisers, manure, roughage and concentrates, and *via* atmospheric deposition and biological N fixation, on the one hand, and all N outputs, *i.e.* in crops, milk, meat (liveweight gain) and manure, on the other (Van der Meer, 1982). The difference between N inputs and outputs is the N surplus, expressed in kg N ha^{-1} year^{-1}. Because only inputs from outside the farm and outputs which leave the farm are considered, these balances are often indicated as farm-gate balances. The N balances presented in 1982, illustrated the poor utilisation of N on intensively managed dairy farms in comparison with that on an extensively managed biodynamic dairy farm: the two times higher yield of milk and beef on the intensive farms had been obtained with a six times higher N surplus (Van der Meer, 1982). Similar figures have been derived from statistical information on fertiliser and concentrate use and milk and beef production of the total dairy sector in The Netherlands in the period 1950-1985 (Van der Meer *et al.*, 1997). They indicate the very negative effect of intensification on the N use efficiency of grassland-based dairy farms.

Since the early 1990s, nutrient balances play an important part in the agriculture/environment issue in The Netherlands. They are drawn up for many farms, e.g. for the farms included in the Farm Accountancy Data Network (FADN). Table 1.1 presents the average N, P and K balances of the specialised dairy farms in FADN in 1992/1993.

Table 1.1. Average N, P and K balances in 1992/1993 of the specialised dairy farms included in the Farm Accountancy Data Network in The Netherlands (calculated from Poppe et al.*, 1994).*

	Element (kg ha^{-1} year^{-1})		
	N	P	K
Inputs			
artificial fertilisers	257	12	10
animal manure [1)]	18	5	12
purchased feeds	168	29	90
miscellaneous	61 [2)]	2	6
Total	504	48	118
Outputs			
milk	62	10	17
animals	29	7	2
crops	3	1	3
animal manure	12	2	8
miscellaneous	5	1	5
Total	111	21	35
Surplus (Inputs - Outputs)	393	27	83

[1)] *imported from other farms*

[2)] *includes atmospheric deposition of 45 kg ha^{-1} year^{-1}*

From Table 1.1 it is evident that artificial fertilisers contributed most to the surplus on the N balance, and purchased feeds to the P and K surpluses. The nutrient outputs presented in Table 1.1 correspond with a production of about 12,000 kg milk and 1000 kg live weight ha^{-1} year^{-1}. This indicates that some of these dairy farms have production side-lines of pigs or poultry. Comparison of the balances presented in Table 1.1 with the balances of a similar group of dairy farms in 1983-1986 (Aarts *et al.*, 1992) shows that the surpluses of N, P and K decreased by *ca.* 20%. This is mainly caused by a 10 to 20% reduction in the use of artificial fertilisers (LEI-DLO/CBS, 1994), indicating both improved utilisation of nutrients in animal slurry and a better awareness amongst farmers of the environmental problems associated with a too liberal use of fertilisers.

The low N efficiency on intensively managed grassland-based dairy farms is mainly caused by:

1. The high rates of N application (from animal manure and artificial fertiliser) on grassland, and the declining response of dry matter yield in combination with the increasing N content of herbage at high N rates. Hence, the economically marginal rate of N application generally produces herbage with a much too high protein/energy ratio.
2. The poor utilisation of dietary N by the animals. In particular when heavily fertilised grass is the main component of the diet, utilisation of dietary N by the herd can be as low as 15% and a large part of ingested N is excreted in urine. But also with a better balanced diet, N utilisation generally does not exceed 25% (Aarts *et al.*, 2000) which implies that at least 75% of ingested N is excreted in faeces and urine.
3. The poor re-utilisation of excreted N by crops owing to ineffective slurry storage and application practices and ineffective utilisation of N excreted in grazed pastures because of the uneven distribution of faeces and urine on swards already treated with high rates of slurry and fertiliser N.

Besides the high inputs of artificial fertilisers, large nutrient inputs through purchased forages and concentrates may cause large nutrient surpluses on livestock farms. This occurs on farms with a high livestock density and, in some cases, also on farms producing forages with low nutrient contents that require supplements with high nutrient contents. Information about the nutrient balances of ruminant livestock farms in other European countries is still limited. Based on information from the Farm Accountancy Data Network, Brouwer *et al.* (1995) calculated net N surpluses for different types of farms in the EC 12. They defined net N surplus as: total supply of N from atmospheric deposition, inorganic fertilisers, and purchased and produced manures, *minus* ammonia N losses from manures, and N yield in the harvested crops (all in kg N ha^{-1} $year^{-1}$). In fact, this is an N balance of the farm-land and the surpluses calculated in this way should not differ from the surpluses on the 'farm-gate balances' (Table 1.1) by more than the ammonia losses from manure. Brouwer *et al.* (1995) estimated that a good 10% of the dairy farms in the EC 12 had a net N surplus of more than 300 kg ha^{-1} $year^{-1}$. However, the official figures used in their study for N excretion by dairy cows in some countries (85 kg $year^{-1}$) and for N uptake by crops (86 kg ha^{-1} $year^{-1}$) are unrealistically low, particularly for grassland-based production systems. It is not clear how these assumptions affect the calculated surpluses. Nevertheless, statistical information of some countries or regions on livestock density, purchased feeds and animal production on the 25% dairy farms with the highest calculated net N surplus, indicates that many of these farms will have high N and P surpluses and, consequently, large losses (Brouwer *et al.*, 1995).

The surplus on a nutrient balance equals the sum of losses to the environment and changes of the content of nutrient stocks (soil, crops, animals, manure) in the production system. In particular for N, it is impossible to estimate the various forms of emission from (the surplus on) the balance-sheet of a farm. For this, quantification of the N flows and losses, as affected by physical conditions, farm structure and farm management is necessary. Results of such studies have been used to construct rather simple simulation models of the production process and related N flows and losses (Scholefield *et al.*, 1991; Van de Ven, 1992; Van der Putten & Van der Meer, 1995). These models have been used to assess the relationships between the N surplus of different types of dairy farms and N losses by ammonia volatilisation, denitrification and nitrate leaching. In addition, they have been used to establish relationships between ecologically acceptable losses of N and the N surplus of dairy farms differing in soil type, land use, grassland utilisation system, livestock density, herd composition and productivity, *etc.* (Van der Putten & Van der Meer, 1995; Van der Meer & Van der Putten, 1995; Van de Ven, 1996). But even without these calculations, the nutrient balance of a farm provides important information. It indicates potential losses and shows the relative importance of the various inputs and outputs (Table 1.1). In addition, it can be used to monitor the effectiveness of measures to improve nutrient management and reduce losses. For instance, measures to reduce losses of a nutrient should result in a lower surplus by a decrease in inputs and/or an increase in outputs.

1.2 Ecologically acceptable nutrient losses

Pollution of drinking water sources by nitrates is of serious concern in most Member States of the European Union. According to the EU drinking water standards, nitrate concentration in ground and surface water that can be used for preparation of drinking water should not exceed 50 mg nitrate per litre, *i.e.* 11.3 mg nitrate-N per litre. In addition, a target concentration of 25 mg nitrate per litre has been recommended. If the nitrate concentration exceeds 50 mg per litre, nitrates have to be removed from the water, which is a very costly process (Van der Meer & Wedin, 1989). The critical nitrate concentration of 50 mg per litre is a difficult target for farms in areas with a small precipitation surplus and freely drained soils with a limited denitrification capacity. For instance, in regions with a precipitation surplus of 300 mm year^{-1}, acceptable nitrate leaching losses are only 34 kg N ha^{-1} year^{-1}. In The Netherlands, the critical value of 50 mg nitrate per litre applies to all groundwater resources that potentially can be used for drinking water, *i.e.* water with less than 150 mg chlorine per litre. Moreover, critical values have been defined for average N and P contents in stagnant surface waters in summer. These are 2.2 and 0.15 mg per litre, respectively. However, knowledge of the effects of land use and farm management on these quality traits is still limited (Goossensen & Meeuwissen, 1990). Finally, international agreements on a reduction of total N and P emissions to the North Sea have to be observed. For both elements, this reduction amounts to 50% of the 1985 level in 1995. For this, it is imperative to reach a P equilibrium in Dutch agriculture and to stop further accumulation of P in Dutch soils.

Ammonia volatilisation contributes considerably to the high rate of atmospheric N deposition in The Netherlands and other West European countries (Van Breemen *et al.*, 1982; ApSimon *et al.*, 1987; Berendse *et al.*, 1988). On average, N deposition in The Netherlands amounted to 38 kg ha^{-1} in 1993; about 72% of this was as ammonia or ammonium salts, together indicated as NH_X (Lekkerkerk *et al.*, 1995). In some areas with high livestock densities, average NH_X deposition was as high as 70 kg N ha^{-1} year^{-1}. Similar values have been reported in the United Kingdom (The United Kingdom Review Group on Impacts of Atmospheric Nitrogen, 1994). After volatilisation, about 30% of the ammonia returns as wet or dry deposition to soils and vegetation within 5 km of the source. A large part of the remaining 70% reacts in the atmosphere with SO_2 and NO_X and is transported over a distance of 5 to about 1000 km (Lekkerkerk *et al.*, 1995). High rates of N deposition cause ecological damage to forests and nutrient-poor natural ecosystems. These vegetation's absorb and accumulate this N very effectively (Heij & Schneider, 1995). The resulting increase of N availability causes undesirable floristic changes, loss of biodiversity, and physiological problems to trees, such as increased susceptibility to abiotic and biotic stress (drought, frost, herbivory, fungal diseases) and deficiencies of other nutrients. Besides, deposition of NH_X potentially contributes to soil acidification which may also affect vegetation and groundwater quality. This acidifying effect only occurs after nitrification of NH_X in the soil, particularly when part of the nitrates produced is lost by leaching because the vegetation was not able to absorb it (United Kingdom Terrestrial Effects Review Group, 1988; Lekkerkerk *et al.*, 1995).

Based on an assessment of ecologically acceptable values for acid and N deposition in different natural ecosystems, the Dutch government aims at a reduction of the average acid deposition from 4280 mol H^+ ha^{-1} in 1993 to 2400 mol H^+ ha^{-1} in 2000 and 1400 mol H^+ ha^{-1} in 2010. Simultaneously, average atmospheric N deposition should be reduced from 38 kg ha^{-1} in 1993 to 22 kg ha^{-1} in 2000 and 14 kg ha^{-1} in 2010 (VROM *et al.*, 1993; Lekkerkerk *et al.*, 1995). Related to this, ammonia volatilisation from the animal production sector has to decrease gradually, and in the year 2000 it should be at least 50% but preferably 70% less than in 1980. A further reduction is envisaged for 2010.
In the United Kingdom, agricultural emissions of ammonia contribute as much to total N deposition throughout the country as NO_X emissions from industry and vehicles. Thus emission control to reduce problems of total N deposition must address emissions from industry, vehicles and agriculture (The United Kingdom Review Group on Impacts of Atmospheric Nitrogen, 1994).

So far, concern about emission of greenhouse gases (CO_2, CH_4 and N_2O) did not lead to the establishment of critical values for these emissions.

1.3 Environmental legislation

In The Netherlands, the targets for environmental quality mentioned in the preceding paragraph and the large nutrient surpluses and losses on farms (Table 1.1), have stimulated environmental legislation. In the first phase, this focused on production, handling and application of animal manure and included the following regulations:

- Farms with an annual 'P production' in manure of more than 55 kg ha^{-1} are not allowed to increase livestock density.
- Gradually increasing P-based restrictions on the rate of manure application (Table 1.2). For grassland, this rate includes P excreted by grazing animals. The very high values for grassland and maize in 1987 reflected the rates of manure application at that time in some regions with a very high livestock density (Van Boheemen, 1987). These restrictions stimulated slurry transport to regions and farms with low livestock densities, as well as the adoption of animal feeding practices aiming at a reduction of P and also of N excretion.
- On most soils, a ban on slurry application in the period from 16 September to 31 January inclusive, and in all cases when the soil is frozen or covered with snow. This means that livestock farms need to have slurry storage capacity for at least 5 months.
- Since 1995, slurry application techniques with low ammonia emission (so-called low-emission techniques) are compulsory on grassland and arable land on almost all soil types. On grassland, deep injection, shallow injection, injection with open slits, and application by trailing-feet machines are officially accepted as low-emission techniques. On arable land, direct incorporation of slurry into the soil is compulsory.
- Slurry storage facilities have to be covered.

Table 1.2. Maximum allowed rates of manure application, in kg P ha^{-1} year^{-1}, for different crops in The Netherlands since the introductrion of environmental legislation (Van Boheemen, 1987; Brouwer et al., 1996).

Year	Grassland	Maize	Arable crops
		(kg P ha^{-1} year^{-1})	
1987	109	153	55
1991	87	109	55
1994	87	66	55
1995	66	48	48
1996	59	48	48

These regulations, determining rate, period and technique of slurry application, are essential to improve the utilisation efficiency of slurry nutrients, in particular of N (Van der Meer, 1994; Van der Meer & Van der Putten, 1995). They make animal slurries a reliable source of plant nutrients and they lead to lower nutrient losses if the rates of application of artificial fertilisers are adjusted properly to the improved availability of slurry nutrients. However, they are not sufficient to limit nutrient losses to the required levels and, therefore, the Dutch government introduced the Nutrient Accounting System (MINAS) in 1997 (Van den Brandt & Smits, 1998). Since 1998, N and P balances of individual farms serve as a basis to stimulate greater nutrient use efficiency and to discourage excessive nutrient use by financial penalties. Consequently, levy-free N and P surpluses have been set for grassland and arable land, which will be lowered gradually up until 2003 (Van der Meer *et al.*, 1997; Van der Meer, 2001).

Many European countries have legislation to reduce nutrient losses from agriculture. This legislation is often focused on the rate and period of application of animal manure. Accepted rates of manure application differ widely among countries and regions (Schröder, 1992; Brouwer & Godeschalk, 1995) and in many cases the main objective of these regulations seems avoidance of severe pollution rather than reduction of nutrient losses to ecologically acceptable levels. The Nitrate Directive (91/676/EEC) issued by the EU in 1991, was an important step towards a more common European approach concerning the protection of surface and groundwaters against pollution by nitrates from agricultural sources (CEC, 1991). The objective of the Nitrate Directive is to reduce water pollution caused, or induced, by nitrates from agricultural sources, as well as to prevent future pollution. It applies to (1) surface freshwaters and groundwater used or intended for the abstraction of drinking water, and (2) natural freshwater bodies and coastal and marine waters which are, or may become, eutrophic. The Directive invites the governments of the Member States to identify zones which drain into waters (potentially) affected by pollution with nitrates, and to establish an action programme for these zones. This action programme must include measures related to period and rate of application of animal manures and chemical fertilisers, and to storage capacity for animal manures. Member States should also establish codes of good agricultural practice, to be implemented by farmers on a voluntary base, and to contain provisions for environmentally friendly storage and application of animal manures and chemical fertilisers. An important feature of the Nitrate Directive is that it specifies the maximum amount of animal manure that can be applied to farmland each year. This should not exceed 210 or 170 kg N ha^{-1} $year^{-1}$ (from 20/12/1998 and 20/12/2002, respectively), including excreted N by grazing animals. This standard has to be met in 1999 unless the goals formulated in the Directive can be achieved by other means. A derogation may be approved for crops with a long growing season and a large capacity for N uptake and for conditions with a large denitrification. In many parts of Western Europe, grass has a long growing season as well as a large capacity for N uptake (Ten Berge *et al.*, 2000). Several Member States, *viz.* Denmark, Germany, Luxembourg and The Netherlands consider the whole country to be vulnerable to nitrate leaching (Brouwer & Hellegers, 1995).

Unfortunately, the Nitrate Directive only quantifies the maximum rate of application of manure N and gives qualitative indications of other measures to reduce leaching and run-off of nitrates. This has resulted in a biased approach to (the reduction of) manure N production and application instead of an integral attitude to the complex problem of nitrate leaching. Another problem is that the mentioned maximum rate of manure N applies to all agro-ecological regions in the European Union. Calculations with a simulation model have shown that the maximum rate of manure N established by the Nitrate Directive is too restrictive for Dutch dairy farms with good nutrient management practices imposed by Dutch legislation (Van der Meer & Van der Putten, 1995).

1.4 Background of the project

The need to reduce N and P losses as well as the (direct and indirect) use of fossil energy on intensive dairy farms to ecologically acceptable levels, requires radical changes of the management of these farms. A strong reduction in the use of artificial fertilisers, and on some farms, in N and P inputs in purchased feeds will be necessary. In general, this will decrease farm output. However, at least a part of this decrease may be compensated by an improvement of N and P utilisation on the farm. Analysis of the N and P cycles on dairy farms showed that the main opportunities to improve N and P utilisation within the production system are (1) improvement of the conversion of feed N and P into N and P in animal products, and (2) improvement of the re-utilisation of N and P in animal manure by grass and forage crops (Van der Meer, 1982).

Sub 1

Standard figures from dairy cattle husbandry in the 1980s showed that on intensively managed farms only between 15 and 20% of the N in the feed was converted into milk protein, whereas the rest (80 to 85%) was excreted in faeces and urine. Research on supplementation of N rich grass-based diets with low-N/high-energy feeds showed that N utilisation by the cows can be improved to *ca.* 25 or 30%, because of a lower N intake and an equal or slightly higher production of milk protein (Van Vuuren &

Meijs, 1987). As a result, N excretion in faeces slightly increased, and excretion in urine decreased by about 45%. This will reduce N losses, particularly on farms with poor manure management and large losses of manure N. The low-N/high-energy supplements increase the production of microbial protein in the rumen and the protein supply of the cow. In fact, the microbes in the rumen need the energy supplement to capture N from degraded grass protein and N recirculating from the body metabolism to the rumen. The introduction of new protein evaluation systems in several countries has increased the possibilities to obtain a better control of the supply and utilisation of feed protein in ruminants, and to reduce urinary N (Vérité *et al.*, 1987; Jarrige, 1989; Hvelplund & Madsen, 1990; AFRC, 1992; Tamminga *et al.*, 1994). Most of the relevant research is done with pelleted compound concentrates. But it is also possible to use suitable by-products from arable crops (e.g. beetpulp) or home-grown forages, concentrates or concentrate replacers. On many dairy farms, partial replacement of purchased feeds by home-grown forages and concentrates is possible due to the reduction in the number of cows as a consequence of the introduction of milk quotas and the increasing milk production per cow. In a workshop organised in 1992 by some research organisations of EC Member States to prepare this research project, it was concluded that research was needed to identify efficient combinations of grass or grass/clover and home-grown concentrates.

Sub 2
Up to about 1990, utilisation of slurry nutrients on dairy farms was inefficient due to inadequate storage and application practices. Most farmers considered animal slurry a waste rather than a valuable source of plant nutrients. Research has shown, however, that the apparent recovery of slurry N in the year of application can be improved from less than 10% of total N in some situations (excessive rates of application to maize, applications in autumn and winter) to 50 or 60% (application by injection techniques in spring) with a corresponding reduction in N losses (Van der Meer *et al.*, 1987; Van der Meer, 1994). This means that slurry N can replace large amounts of fertiliser N. However, the expected future increase of the role of legumes on dairy farms, together with an increase of arable crops for concentrate production, required additional research on slurry utilisation. Low-emission systems of slurry application probably will reduce the clover content of mixed swards and stimulate weeds in pure legume stands. This will motivate farmers to apply too much slurry to the non-leguminous crops and this will cause large nitrate leaching losses and accumulation of P in the soil. Therefore, in the mentioned workshop research was considered necessary to develop a good strategy of slurry application on legume-based dairy farms.

1.5 Objective and contents of the project

Based on the considerations presented in the preceding paragraph, the project 'Reducing inputs and losses of nitrogen and energy on dairy farms' has been formulated in the beginning of 1992 and started in November of that year with financial support of the European Community Specific Programme for Research, Technological Development and Demonstration in the field of Agriculture and Agro-industry, including Fisheries (AIR). The objective of the project was to develop sustainable dairy farming systems with minimum inputs and losses of nitrogen, phosphorus and energy. The following results were anticipated:

1. A better knowledge of the practical possibilities to improve the utilisation of dietary N by the dairy herd and to reduce N excretion in urine in different forage production systems.
2. Information about the suitability of some crops, crop rotations and feeding systems in different regions of the European Community to replace a part of the purchased concentrates on dairy farms.
3. Efficient slurry utilisation systems on grass/clover swards and maize.
4. A better understanding of the effectiveness and interactions of different measures to decrease losses of nitrogen, phosphorus and energy on dairy farms.

The project comprised the following research tasks and sub-tasks:

A. Evaluation of rations and feeding strategies to improve the utilisation of N in the feed of the dairy herd in order to minimise the production of urinary N.

1. Determine the minimum levels of N intake by dairy cows in early and mid lactation without reduction in milk yield (6 feeding experiments in Denmark).
2. Study the importance of synchronisation of the intake of rumen-degradable protein and fermentable carbohydrates (this sub-task has been cancelled to avoid overlap with research in other programmes in which the need for this synchronisation has been shown. The research capacity planned for sub-task 1.2 has been employed in sub-task 1.1).
3. Determine the effects of supplementing dairy cows zero-grazing N fertilised grass with ground maize-ear silage (low-N/high-energy supplement) (2 feeding experiments with variations in herbage allowance and supplement level in The Netherlands).
4. Study herbage intake and N utilisation of dairy cows on grass/white clover swards with minimum supplementation (1 grazing experiment in Scotland).
5. Investigate strategic supplementation of cows on mixed swards to maximise N utilisation (1 grazing experiment in Scotland).
6. Study strategic protein supplementation of cows on grass/clover silage (1 feeding experiment in Scotland (sub-task A6-1) and 2 in Spain (sub-task A6-2).

B. Comparison of crops, cropping systems and feeding systems to increase the proportion of home-grown feed protein, phosphorus and energy in dairy nutrition, thus reducing the consumption of purchased feeds and the related input of plant nutrients and indirect use of fossil energy.

1. Study the agronomic potentials and environmental constraints of 2 extreme forage production systems (1 farm-scale field experiment in Denmark).
2. Evaluate the quality of ground maize-ear silage as a supplement for dairy cows zero-grazing N fertilised grass (the same experiments as for sub-task A3).
3. Evaluate the quality of ground maize-ear silage and fodder beet to supplement grass silage for dairy cows (2 feeding experiments in The Netherlands).
4. Evaluate the quality of barley as a supplement for dairy cows grazing grass/clover swards (the same experiment as for sub-task A5).
5. Evaluate the quality of barley and fodder beet to supplement grass/clover silage for dairy cows (1 feeding experiment in Scotland).
6. Study the interactions between fertiliser N and white clover on herbage yield and quality and nitrate leaching under grazing and cutting (1 grazing and 1 cutting experiment in Spain).

C. Quantification of the effectiveness of different slurry utilisation systems on low-input dairy farms, and in particular or grass/clover swards and silage maize.

1. Study the effects of different slurry application techniques on the utilisation of slurry N, P and K by a grass/clover sward (1 field experiment with annually repeated slurry application in The Netherlands).
2. Study the effects of low-emission slurry application techniques and periods of application on the performance of a grass/clover sward and the clover component in particular (1 field experiment with annually repeated slurry application in The Netherlands).
3. Study utilisation of slurry N by maize, as affected by period, method and rate of slurry application and additional fertiliser N (1 annually repeated field and lysimeter experiment in Italy).
4. Study utilisation of slurry N by combinations of maize and *Lolium multiflorum* as a cover crop (1 field experiment in 1993 and 1994 in Italy).

D. Evaluation and integration of the results of the experimental work to contribute to the development of dairy farming systems at the project sites with more efficient utilisation of N, P and fossil energy.

1. Search for information about the effect of reduced excretion of urine N on N losses.
2. Identify possible consequences of replacing purchased concentrates by home-grown feeds.
3. Formulate essences of effective slurry utilisation systems on low-input dairy farms.

Six Research Organisations participated in this project:
The *Research Institute for Agrobiology and Soil Fertility* (Participant 1, Wageningen, The Netherlands) conducted the research related to the sub-tasks C1 and C2 and Task D.
The *Danish Institute of Plant and Soil Science* (Participant 2, Foulum, Denmark) conducted the research related to the sub-tasks A1 and B1.
The *Ente Regionale per la Promozione e lo Sviluppo dell'Agricoltura* (Participant 3, Pordenone, Italy) conducted the research related to the sub-tasks C3 and C4.
The *Research Station for Cattle, Sheep and Horse Husbandry* (Participant 4, Lelystad, The Netherlands) conducted the research related to the sub-tasks A3, B2 and B3.
The *Scottish Agricultural College* (Participant 5, Dumfries, Scotland) conducted the research related to the sub-tasks A4, A5, A6, B4 and B5.
The *Centro de Investigaciones Agrarias Mabegondo (CIAM) – Xunta de Galicia* – (Participant 6, La Coruña, Spain) conducted the research related to the sub-tasks A6 and B6.

Most of the experimental research was adapted to the local conditions of the participants involved. This is shown in the following introductory sections.

1.6 Experimental work

1.6.1 Sub-task A1

National legislation in Denmark, as well as EC regulations require more efficient utilisation of N and restrict the amount of N from animal manure that may be applied to the fields. These measures are taken to reduce leaching of nitrates from agriculture to ground and surface water. It is difficult for many cattle farmers to cope with these requirements. All efforts to demonstrate possibilities to reduce the flow of N in dairy farming systems, therefore, are required (Van der Meer, 1982; Tamminga & Verstegen, 1991; Tamminga, 1992; Hof & Tamminga, 1994; Williams, 1995).

The efficiency of utilisation of N in dairy herds is relatively low. In a typical Danish dairy system, only 21-24% of the feed N is recovered in milk and liveweight gain, while the rest, *i.e.* 76-79%, is excreted in urine and faeces (Kristensen, 1995). Approximately 30-35% of the feed N is excreted in faeces and 45% in urine. The N in faeces originates mainly from undigested feed N, endogenous N secreted to the digestive tract, and microbial residues from the digestive tract. The possibilities of reducing N concentration in faeces by varying N in the feed source are limited. Nitrogen excretion in urine, on the other hand, is strongly affected by feeding. It originates partly from ammonia absorbed from the rumen, and partly from amino acids that are metabolised in the body of the cow.

Important reasons for the poor utilisation of N by dairy cows are the complexity of N metabolism and the losses of N caused by the microbial metabolism of nitrogenous compounds in the digestive tract. Previous protein evaluation systems were based on 'digestible crude protein'. But 'digestible crude protein' does not consider the extensive microbial metabolism of protein in the digestive tract. New protein evaluation systems have been introduced during the last decade (Hvelplund & Madsen, 1990; INRA, 1989; AFRC, 1992; Tamminga *et al.*, 1994). These systems consider protein metabolism in the digestive tract. The new systems predict the absorption of amino acids from the small intestine (named AAT in the Nordic protein evaluation system). The new systems also evaluate the availability of protein or N for the micro-organisms in the rumen (protein balance in the rumen, called PBV in the Nordic system). These systems still are imprecise in estimating the protein metabolism and the amino acid supply to ruminant animals, but they increase the possibilities to improve N utilisation.

The ability of ruminants to recycle N from the internal metabolism to the rumen could possibly be used to improve the N utilisation of the dairy cow. Recycled N may be used for synthesis of microbial protein in the rumen and once again be made available to the cow. One objective of the experiments conducted under sub-task A1 was to study the cow's ability to recycle N by feeding diets with a low level of rumen-degradable protein relative to the level of rumen-fermentable carbohydrates. The high ratio of fermentable carbohydrates to rumen-degradable protein was expected to maximise the utilisation of N recycled to the rumen. These studies used feed from the energy-cropping system (studied under sub-task B1), as this cropping system provided feeds with low concentrations of proteins and relatively high concentrations of carbohydrates.

The microbial breakdown of feed protein in the forestomachs of ruminants may be a disadvantage for obtaining an efficient N utilisation. This is often the case when home-grown protein-rich feeds, principally grass and legumes, are an important part of the diet. These protein sources normally are easily degraded in the rumen, and there will often be an excess of degraded protein relative to fermentable carbohydrates, *i.e.* a positive N balance in the rumen (PBV). This surplus of N is absorbed as ammonia and mainly excreted as urea in urine. Therefore, another purpose of the experiments conducted under Sub-task A1 was to maximise the utilisation of home-grown plant protein in grass/clover and whole-pea silages. A lowly degradable protein supplement (heat-treated rapeseed meal) was replaced by grain to increase the amount of fermentable carbohydrates in the diet and thus stimulate the microbial capture and utilisation of degraded feed protein. The diets were fed as total mixed rations to synchronise the supply of degradable protein and fermentable carbohydrates and to make optimum conditions for microbial growth. The replacement of the protein supplement by grain reduced the estimated level of AAT in the diet. These studies used feed from the protein-cropping system (studied under sub-task B1).

The main objective of these experiments was to determine the minimum level of N intake and N excretion in urine that could be obtained in different parts of the lactation period without a considerable reduction in milk yield. This was done by reducing the levels of AAT and PBV below recommended minima, when conditions for N utilisation were optimised. The effects were studied by measuring feed intake, milk production and milk composition. The utilisation of recycled protein (low PBV levels) was studied in feeding experiments in early and late lactation. The experiment on the utilisation of easily degradable home-grown protein (low AAT) was carried out in early lactation. All three experiments were repeated in the second year. In the experiments in early lactation, the complete N balance was measured by determining the recovery of N in milk, faeces, and urine during three days in the middle of the experimental period.

The efficiency of protein synthesis in the rumen was estimated by measuring the excretion of purine derivatives in urine. The concentration of urea in milk in individual cows was determined once during the experimental period in order to provide data for development of a possible system for estimation of the protein/energy status of dairy cows in practice.

1.6.2 Sub-tasks A3 and B2

During the last decade, the number of dairy cows per hectare grassland and fodder crops decreased in The Netherlands due to the introduction of the milk quota system and the increased milk production per cow. In some regions, the possibility exists to replace surplus grassland by home-grown concentrates (fodder crops with a high net energy concentration). This may reduce the use of purchased concentrates. Replacing purchased concentrates by these home-grown concentrates may offer scope to reduce the surplus of N at farm level and, hence, its losses to the environment. Losses of N in dairy cows can be reduced by optimising the intake of N relative to energy. This can be achieved by substitution of low-N feeds for high-N forages (Tamminga, 1992). On the other hand, Van Vuuren & Meijs (1987) indicated that the possibilities for an improvement of the efficiency of N utilisation in ruminants with grass as the sole feed are limited. In most cases, the required measures, *viz.* reduction in the rate of N application and in cutting and/or grazing frequency, result in a decline in animal performance due to a decrease in the intake of net energy for lactation (NE_L). A better response seems possible if grazing cows are supplemented with good quality concentrates or forages with a relatively high energy and low protein content (Van Vuuren & Meijs, 1987). An example of such a forage or concentrate replacer is ground maize-ear silage (GMES), a palatable high-energy low-protein feed, consisting of the complete ear of the maize plant, including the grains, the husks and the cob.

In addition to contributing to an improvement of N utilisation, replacing purchased compound concentrates by home-grown concentrates may also result in a reduction of the use of fossil energy in dairy farming. Approximately 30% of the direct and indirect use of fossil energy on dairy farms is associated with the use of compound concentrates. Hageman *et al.* (1995) calculated that replacing grassland by fodder crops such as GMES will result in a considerable reduction in the requirement of fossil energy in dairy farming systems.
The concentration and composition of structural and non-structural carbohydrates in GMES are markedly different from those in conventional concentrates and herbage. The most important energy source in GMES is starch, while a large proportion of the energy in compound concentrates and herbage comes from cell wall components. Therefore, replacing compound concentrates or herbage by GMES will affect the concentration and composition of structural and non-structural carbohydrates in the ration. Sutton (1989) showed that the yield of milk and the concentration of milk constituents are influenced by the composition and concentration of dietary carbohydrates. However, detailed information on the effects of replacing either herbage or compound concentrates by GMES is very scarce. Therefore, two experiments were carried out to evaluate the effects of GMES as a replacement for either purchased concentrates (experiment A3-1) or fresh cut herbage (experiment A3-2) on feed intake, yields of milk and milk constituents and utilisation of dietary N.

1.6.3 Sub-task A4

The use of ryegrass/white clover mixtures is increasingly being proposed in several European countries as an alternative to pure ryegrass swards in dairy production systems. Biological N fixation decreases the use of fossil energy for the production of fertiliser N and, possibly, reduces nitrate leaching to groundwater (Ruz-Jerez *et al.,* 1995). However, clover content of mixed swards and, consequently, protein content of the herbage on offer often varies strongly between sites, between years and within years. Little is known about the animal N efficiency when animals are grazing ryegrass/white clover mixtures. Therefore, an experiment was carried out in Scotland to investigate seasonal fluctuations in herbage intake and N utilisation of dairy cows on grass/white clover swards with minimum supplementation.

1.6.4 Sub-tasks A5 and B4

Initial investigations with dairy cows grazing ryegrass/white clover mixtures in Scotland, conducted in 1993 under sub-task A4, indicated that animal N efficiency is dependent on the clover content of the sward and on the production level of the cows. White clover content of the grazed sward was *ca.* 9% in May and 47% in July and mean CP contents of the herbage DM on offer were 15.3 and 19.7%, respectively. Such differences indicate a need for strategic supplementation of dairy cows on mixed swards in order to maximise N utilisation. Therefore, some concentrate supplementation strategies were evaluated taking clover content into account in order to investigate the possibilities to improve animal N efficiency by supplementation. Because of its low costs, beet pulp, a by-product of the local sugar industry, was used in stead of barley grain as the main supplement ingredient.

1.6.5 Sub-task A6-1

Environmental pressure on agriculture to reduce the use of nitrogenous mineral fertiliser, combined with a trend to more extensive systems of production, has led to renewed interest in white clover for dairy systems (Bax & Thomas, 1992; Bax & Browne, 1994; Frankow-Lindbergh *et al.*, 1995). However, very little information is available on the feeding value of grass/clover silage in dairy cows. Korevaar (1992) suggested that on-farm production of concentrates or concentrate replacers, e.g. fodder beet, could improve N efficiency of the whole dairy system, since these products can be produced with a higher N efficiency than grass silage. However, this of course is only of benefit if these home-produced concentrates can be used effectively within dairy cow feeding systems. Therefore, an experiment was carried out under sub-task B5 in Scotland during the winter of 1993/94 to investigate the potential of barley grain and fodder beet as home-grown concentrates to supplement grass/clover silage. This study showed that grass/white clover silage can be supplemented effectively with these home-grown concentrates, in particular with a mixture of both. There exists, however, a need to investigate the potential for further reducing total N intake to increase N efficiency in the animal, as has been shown to be possible by Van Vuuren *et al.* (1993). An experiment was therefore carried out in the Winter of 1994/95 to investigate the effects on animal performance and animal N efficiency of further reductions in N intake of dairy cows fed grass/clover silage supplemented with barley and fodder beet. Hence, the principal objective of this experiment was to explore the effects of different types of under-supply of protein.

1.6.6 Sub-task A6-2

Two forage systems are used in dairy operations in Galicia, Spain, *viz.* grazed pasture plus grass/clover silage *versus* grazed pasture plus fodder crop silage. These systems differ considerably in their inputs but also in their management flexibility. The main farm-grown forages for silage are grass/clover silage, maize silage and winter crop silages (rye, Italian ryegrass, vetch/oats). Except for maize, the forage is usually harvested with a low dry matter (DM) content and not wilted before ensiling because of the high risk of rain damage during the field period. The resulting silages are characterised by a low DM content, acceptable protein and energy contents and moderate ingestibility. Grass/clover silage, produced under Galician conditions, has usually a moderate crude protein (CP) content (13-15%). Maize silage is a better source of energy, but its CP content is too low for dairy cows, even when fed mixed with silage of winter legumes.

One of the purposes of supplementation with grain is to facilitate the capture of degraded forage N in the rumen and to increase the amount of absorbable protein in the small intestine. This might be the main benefit of supplementation when the rates of concentrate feeding have to be low. If this is true, the use of limited amounts of protein supplements with a low rumen-degradability of the protein could nearly be as beneficial as feeding larger quantities of energy supplements.

No protein concentrates are produced in Galicia and the only concentrate cropped in substantial amounts is maize. Identification of supplementation strategies that require the minimum import of concentrates from outside the farm and the region is important to improve overall nutrient use efficiency. The objective of the experiments, conducted under sub-task A6-2, was to evaluate the response of dairy cows in early lactation to supplementation with different amounts of energy and protein that escapes degradation in the rumen. This was done in the two forage systems mentioned, using grass/clover silage in the first year and a mixture of maize silage and vetch/oats silage in the second year.

1.6.7 Sub-task B1

The current trend in Europe is towards increased milk yields per cow by animal breeding and high inputs of concentrated feeds. Since the introduction of milk quotas, the possibility of producing home-grown concentrates has improved, because the productivity per cow has increased and the number of cows has decreased, thereby making more land available per animal unit.

A greater reliance on grassland with a rigid control of concentrate input should give a more predictable level of profitability, despite some limitations for milk yield per cow imposed by such a system (Leaver, 1988). A combination of efficient grassland use and production of home-grown concentrates is also possible.

On conventional dairy farms in Denmark, N use efficiency, calculated as output of N in milk and meat divided by net input of N in purchased feeds, manure, fertiliser, and atmospheric deposition, is only 16% (Halberg *et al.*, 1995). On these farms, the yearly surplus of N may be as high as 242 kg ha^{-1}. According to Halberg *et al.* (1995), substantial reductions in N loss probably cannot be made without a reduction in production intensity. On Dutch dairy farms in the mid 1980s, N use efficiency was reported to be 14% (Aarts *et al.*, 1992).

Average leaching of nitrate in Denmark is estimated at 74 kg N ha^{-1} $year^{-1}$; this corresponds to 51% of applied fertiliser on sandy soils and 26% of applied fertiliser on loamy soils (Jensen *et al.*, 1994). Use and, possibly, losses of N may be reduced if more crops capable of fixing atmospheric N are grown. Loss of nutrients may be further reduced if minimum tillage is practised or if tillage is practised at a time during the growing season when mineralisation is low (Olsen, 1995). Jensen *et al.* (1994) reported that leaching of nitrate at sites only receiving artificial fertiliser was smaller from cereals under-sown with grass than from crops followed by a winter cereal or winter rape. Leaching was greater when cereals, rape or root crops were grown without a catch crop (Jensen *et al.*, 1994). The use of long-term grass/clover leys should reduce the rate of N application to the following crop in the cropping sequence. One explanation is that the concentration of organic N in the soil increases, which results in a higher net mineralisation. Johnston *et al.* (1994) reported that as much as 230 kg inorganic N ha^{-1} may be found in the soil in the first autumn following ploughing of three to six-year old grass/clover leys. As a consequence, the potential leaching of nitrate is high and Johnston *et al.* (1994) reported that 202 kg nitrate N ha^{-1} was lost from these soils. Olsen (1995) also reported nitrate leaching losses of as much as 190 kg N ha^{-1} after ploughing grass/clover leys. A dry growing season, large amounts of water from irrigation or rain, growing of field pea, or poor crop management also potentially result in high nitrate leaching (Olsen, 1995).

The preceding shows that the type of crop, cropping sequence and crop management affect nitrate leaching. Two contrasting cropping systems, *viz.* a ‘protein rotation’ and an ‘energy rotation’, were established at Silstrup Research Station, Denmark, in 1992, with the aim to study yield, quality and nutrient balances of these systems and the crops included. Both cropping systems had a presumed ability to produce a large proportion of the diet of dairy cows and the crops were produced in an environmentally sound manner.

1.6.8 Sub-task B3

Since the introduction of the milk quota system, many dairy farms in The Netherlands have a surplus of home-grown forages. Nevertheless, large quantities of purchased concentrates are fed to meet the energy and protein requirements of high-yielding dairy cows. In such situations, replacing purchased concentrates by home-grown concentrates and concentrate replacers may offer scope to reduce the surplus of N at farm level and, consequently, the losses of N to the environment. Losses of faecal and urinary N can also be reduced by minimising the intake of N relative to energy. This can be achieved by replacing high-N forages (e.g. grass silage) by low-N feeds (Tamminga, 1992). Another point of concern with respect to the environment is the use of fossil energy. Production of compound concentrates requires large quantities of fuel. At present, approximately 30% of the fossil energy used in dairy farming is for production and transport of compound feeds. Replacing compound concentrates by home-grown concentrates can reduce fossil energy use on dairy farms considerably (Hageman *et al.*, 1995).

Fodder beet and ground maize-ear silage (GMES) are both palatable high-energy and low-N feeds, suitable to replace concentrates or high-N forages. Fodder beet is a well-known fodder crop, but GMES is relatively new. GMES consists of the complete ear of maize, including grains, husks and cob.

The main energy sources in fodder beet are sugars (sucrose, glucose and fructose), that in GMES is starch. In contrast, compound concentrates often contain fibrous by-products from the food industry. Replacing purchased concentrates or grass silage by home-grown concentrates will have a considerable effect on the concentration and composition of structural and non-structural carbohydrates in the ration. The composition of the carbohydrate fraction in the ration, the amount of roughage, the forage/concentrate ratio and intake can have major effects on yield and composition of milk (Sutton, 1989). However, information on the effects of replacing purchased concentrates or grass silage by either fodder beet or GMES is rather sparse. Therefore, two experiments were carried out in The Netherlands to investigate the effects of home-grown GMES and fodder beet as replacements for purchased concentrates or grass silage on feed intake, yield and composition of milk and milk solids, and utilisation of N.

1.6.9 Sub-task B5

Environmental pressure on agriculture to reduce the use of nitrogenous inorganic fertiliser, combined with a trend to more extensive systems of production, has led to renewed interest in white clover for dairy systems (Bax & Thomas, 1992; Bax & Browne 1994; Frankow-Lindbergh *et al.*, 1995). However, very little information is available on the feeding value of grass/clover silage in dairy cows. Korevaar (1992) suggested that the production of concentrates on farm could improve N efficiency of the whole dairy system, since these products can be produced with a higher N efficiency than grass silage. However, this of course is only of benefit if these home-produced concentrates can be used effectively within dairy cow feeding systems. An experiment was therefore carried out in Scotland to investigate the potential of home-grown barley grain or fodder beet as supplements of a grass/clover silage for dairy cows.

1.6.10 Sub-task B6

The use of large rates of fertiliser N on grassland is a normal practice for milk production in maritime parts of Western Europe. Since the introduction of milk quotas, low production costs are achieved by making milk production seasonal and more dependent on grazed pasture and forage crops. Stocking rate, cow quality and early-season performance are the most important factors in determining how effectively pasture is converted into milk (Bryant, 1986). Effective utilisation of pasture may reduce the dependence on concentrates, but this is not observed in practice.

Galicia is the main dairy region of Spain, with small farms and small milk quota. The mean size of the farms is about 8 ha with 35,000 litres of quota. Barbeito (1995) describes a farm of 14 ha with a milk quota of 148,000 litres, using pastures and forage crops and, in addition, as much as 2000 kg of concentrates cow^{-1} year^{-1}. There is a tendency among leading farmers to increase the use of N and concentrates. However, grass/clover swards can be successfully used, provided management succeeds to maintain a high clover content and high-quality herbage on offer. In this project the aim is to assess the potential of grass/white clover pastures for milk production and to study the interaction between fertiliser N and white clover on herbage yield and quality. For comparison, forage yield and quality of a rotation of maize and a mixture of vetch and oats were studied (see also sub-task A6-2).

1.6.11 Sub-task C1

Environmental legislation for agriculture in The Netherlands first focused on improvement of animal manure management (Van der Meer *et al.*, 1997). This included the following regulations:

- A gradually increasing restriction on the rate of manure application, related to its P content. The admitted rate of application to grassland decreased from 109 kg P ha^{-1} in 1987 to 59 kg P ha^{-1} in 1996. A further reduction in the admitted rate of application to strict P replacement (where the rate of P application equals the removal of P in the harvested herbage) has been advocated, but has not yet been introduced because of concern about negative long-term effects on the P status of the soil and on herbage yield and quality.
- On most soils, a ban on slurry application in the period between 16 September and 1 February, and in all cases when the soil is frozen or covered with snow.
- Since 1995, slurry application techniques with low ammonia emission (so-called low-emission techniques) are compulsory on grassland and arable land on all soil types. On grassland, deep injection, shallow injection, injection with open slits, and application by trailing-feet machines are officially accepted as low-emission techniques.

These regulations, determining rate, period and technique of slurry application strongly improve the utilisation of slurry N by pure grass swards (Van der Meer *et al.*, 1987; Van der Meer, 1994; Geurink & Van der Meer, 1995; Van der Meer & Van der Putten, 1995). However, there is still little information about the effects of the mentioned slurry application techniques on the utilisation of slurry P and K, and in general, about their effects on mixed swards. Therefore, the effects of different slurry application techniques on the utilisation of slurry N, P and K by a mixed sward of grass and white clover have been studied in a field experiment of 3 years.

1.6.12 Sub-task C2

Improvement of manure management generally is the most effective way to reduce nutrient losses from dairy farms. Nutrient losses from applied manure strongly depend on the rate, time and method of application (Van der Meer, 1994; Brandjes *et al.*, 1995). The rate of manure application should not exceed the capacity of crops to absorb the nutrients in order to avoid (temporal) accumulation in the soil and subsequent leaching losses (inorganic N, P and K). The most convenient time of manure application is the growing season, just prior or during vigorous crop growth. Reduction of N losses by ammonia volatilisation requires rapid incorporation of the manure into the soil, e.g. by injection techniques or immediate cultivation after surface application. Environmental legislation for agriculture in The Netherlands includes regulations for the rate, time and method of slurry application (Van der Meer *et al.*, 1997). For grassland, these regulations prescribe to apply animal slurries between early spring and September by low-emission techniques. Experiments on pure grass swards have shown that this gives efficient utilisation of slurry N (Van der Meer *et al.*, 1987; Geurink & Van der Meer, 1995).

Mixtures of grass and white clover do not need N from external sources because of symbiotic N fixation by the association of the legume and Rhizobium. However, a mixed sward does require P and K applications and on many farms animal slurry may supply a large proportion of its requirement. This means that considerable amounts of slurry N will also be applied to the mixture. There are some reports in literature that slurry N is harmful to white clover (e.g. Schechtner *et al.*, 1980) and many biological farmers and farm advisers are concerned about this. This may be a special problem with regulations prescribing techniques and periods of slurry application that strongly improve the effectivity of slurry N. Therefore, a field experiment has been carried out to study the effects of low-emission slurry application techniques and periods of application on the performance of a mixed sward of perennial ryegrass and white clover.

1.6.13 Sub-task C3

Slurry is often considered by farmers as a waste product and incorrectly used on fields, causing atmospheric pollution by volatilisation of ammonia and water pollution by nitrate leaching. Environmental problems caused by slurry often are not caused by the characteristics of slurry but rise from improper methods of utilisation. When it is managed in the right way, however, slurry is an important and economical source of N and other nutrients for field crops. For a favourable agronomic exploitation of slurry, it is important to define the quantity to be used for each crop and the method and time of application.

The aim of the experiments, conducted under sub-task C3 in Italy, was to clarify the conditions that influence the efficiency of slurry as a source of N for maize, and to determine the best combination of period, method and rate of slurry application to obtain the maximum utilisation of slurry N by maize.

1.6.14 Sub-task C4

Maize has an important role in intensive land use systems to buffer seasonal variations in grass growth and herbage supply to grazing livestock. Fields with silage maize are often fertilised with up to 300 kg inorganic N ha^{-1}, and even more in very intensive systems; in such conditions a serious risk of nitrate leaching and consequent water pollution occurs.

In order to reduce nitrate leaching in Winter, it is possible to grow a winter-hardy crop after maize to keep the soil covered during Winter and to take up part of the residual inorganic N. In North Italy such a ‘two crops per year’ system frequently consists of maize and Italian ryegrass. Italian ryegrass is sown in September, after harvesting silage maize, and cut the following Spring for hay or silage. Immediately afterwards, the land is cultivated and sown again to maize.

A field experiment of three years was carried out to determine the efficiency of Italian ryegrass in utilising residual inorganic N after maize and to verify the feasibility of such a year-round cropping system in intensive farming.

2. Materials and Methods

2.1 Sub-task A1

In the two experiments with diets based on protein-rich home-grown feeds (various levels of AAT in the diet) in early lactation (experiments A1-1 and A1-4), cows were fed the diets *ad libitum* as total mixed rations (Table 2.1). The levels of AAT were planned to vary from approximately 95 to 85 g per Scandinavian Feed Unit (FU). The minimum requirement in the Danish feeding standards for cows in early lactation is 90 g per FU (Madsen *et al.*, 1995). The concentrate ingredients of these diets were mixed and then pelleted. The composition of these mixtures is shown in Table 2.2.

Experiments with low levels of PBV (feeds from the energy-cropping system) were conducted in early lactation (experiments A1-2 and A1-5) and late lactation (experiments A1-3 and A1-6). Planned levels of PBV were approximately 0, -250 and -500 g cow^{-1} day^{-1}. The Danish standard for early lactation is a minimum of 0, and for late lactation a minimum of -100 g cow^{-1} day^{-1}. Cows in the experiments A1-2 and A1-5 were fed the diets shown in Table 2.3, and in the experiments A1-3 and A1-6 the diets shown in Table 2.4. Feeding in experiment A1-3 differed somewhat from feeding in experiment A1-6, as wheat silage and oat were used in experiment A1-3, while barley silage and barley grain were given in experiment A1-6. For unknown reasons, voluntary intake of whole-crop silage in experiment A1-5 was relatively low. Therefore, the amount of oat was increased from 3.0 to 3.6 kg DM cow^{-1} day^{-1} and 1 litre of beet molasses cow^{-1} day^{-1} was poured on to the silage. In the experiments A1-2, A1-3, A1-5 and A1-6, feeds were fed separately and twice daily; silage and fodder beet were fed on the floor, and concentrates in a separate trough.

Table 2.1. Experiments A1-1 and A1-4. Composition of total mixed rations fed ad libitum, *g (kg DM)$^{-1}$.*

	High	Mean	Low
Grass/clover silage	277	277	277
Pea silage	277	277	277
Concentrate mixture 1	446		
Concentrate mixture 2		446	
Concentrate mixture 3			446

Table 2.2. Composition of pelleted concentrate mixtures used in experiments A1-1 and A1-4, g (kg DM)$^{-1}$

	Mixture		
	1	2	3
Rapeseed cake[1)]	247	124	
Rapeseed[2)]		43	85
Fish meal[3)]	48	40	32
Barley grain	705	793	883

[1)] *Double-low, heat-treated, high-fat (150 g kg^{-1})*

[2)] *Double-low*

[3)] *Low rumen-degradability of protein*

Table 2.3. Experiments A1-2 and A1-5. Daily amounts of feed per cow.

	g PBV cow^{-1} day^{-1} [1]		
	0	-250	-500
Whole-crop wheat silage	*Ad lib.*	*Ad lib.*	*Ad lib.*
Fodder beet, kg DM	4.8	4.8	4.8
Rolled oats, kg DM	3.0	3.0	3.0
Rapeseed cake[2], kg DM	2.5	2.5	2.5
Chalk, g	150	150	150
Mineral + vitamin mixture, g	150	150	150
Urea, g	160	80	0
$Na_2SO_4.10\ H_2O$, g	60	60	60

1) *Planned levels*

2) *Double-low, heat-treated, high-fat (150 g kg^{-1})*

Table 2.4. Experiments A1-3 and A1-6. Daily amounts of feed per cow.

	g PBV cow^{-1} day^{-1} [1]		
	0	-250	-500
Whole-crop silage	*Ad lib.*	*Ad lib.*	*Ad lib.*
Fodder beet, kg DM	3.6 or 2.3[3]	3.6 or 2.3[3]	3.6 or 2.3[3]
Rolled barley or oats, kg DM	1.8 or 3.4[3]	1.8 or 3.4[3]	1.8 or 3.4[3]
Rapeseed cake[2], kg DM	1.6 or 1.8[3]	1.6 or 1.8[3]	1.6 or 1.8[3]
Chalk, g	100	100	100
Mineral + vitamin mixture, g	100	100	100
Urea, g	160	80	0
$Na_2SO_4.10\ H_2O$, g	60	60	60

1) *Planned levels*

2) *Double-low, heat-treated, high-fat (150 g kg^{-1})*

3) *In experiments A1-3 and A1-6, respectively*

Five out of six feeding experiments were carried out by using 27 cows per experiment of the Black and White Danish breed, nine cows in each of three treatments. Experiment A1-3, with cows in late lactation in the first experimental year, was carried out with eight cows per treatment. One cow in experiment A1-2 was excluded because of illness. The experiments were continuous trials with randomised complete blocks, a standard feeding period and an experimental period. Cows were blocked before the start of the experiment according to parity (first, second, and later), expected date of calving, live weight, pedigree, and yield index (first lactation), or level of yield in the previous lactation (for cows in second and later lactations). Three or four cows in each treatment group were in first lactation. In early lactation, a standard feeding period of three weeks (0-3 weeks *post partum*), followed by an experimental period of 17 weeks (3-20 weeks *post partum*) was used. In late lactation, a standard feeding period of two weeks followed by an eight (first year) or nine (second year) weeks experimental period at 31 to 39 weeks *post partum* was used. Cows were tied in a stanchion barn and fed individually.

Individual voluntary feed intake generally was determined by feeding about 10 per cent in excess of intake capacity and weighing the refusals back once every morning.

Milk yield was recorded with Tru-Test Milk Meters (New Zealand), once weekly and the concentrations of protein, fat and lactose were determined with Milko-Scan 104 19900 (N. Foss Electric, Denmark). Cows were weighed once every fortnight and the change in weight was determined by a quadratic regression of weight over time.

Digestibility of the diets and excretion of N in faeces in the experiments with cows in early lactation (experiments A1-1, A1-2, A1-4 and A1-5) were determined by using four cows per treatment in the middle of the experimental period. Chromic oxide was used as marker, of which 10 g doses were supplied orally twice daily (06:00 h and 17:00 h) for 12 days. Faeces were grab-sampled twice daily during the last three days of this period, thoroughly mixed and analysed for dry matter (DM), ash, chromic oxide (spectrophotometrically), N and Acid Detergent Fibre (ADF) (Anonymous, 1992). Quantitative collection of urine from the same four cows was made by using an external urine cup mounted on the cow during the same three days as faeces were sampled. A 17% (v/v) sulphuric acid solution was added to the container before urine was collected to keep pH below 3. Samples of urine were analysed for N, allantoin, uric acid and creatinine. Allantoin, uric acid and creatinine were determined in the laboratory at Viborg Hospital. A composite sample for all three days was analysed in experiments A1-1 and A1-2, while three daily samples were analysed separately in experiments A1-4 and A1-5.

Concentration of urea in milk was determined during the days of sampling faeces and urine (spectrophotometric determination of ammonia) (Anonymous, 1992). Potassium dichromat was added to the milk samples. Milk then was frozen until analysis.

All feedstuffs were sampled once weekly. Four to five weekly samples then were pooled and analysed by using the proximate feedstuff analysis completed by determinations of Acid Detergent Fibre (ADF) in the first year and both ADF and Neutral Detergent Fibre (NDF) in the second year (Anonymous, 1992). The digestibility of organic matter (DOM) in grains and concentrates was determined by using an enzymatic method (Weisbjerg & Hvelplund, 1993). Digestibility of forage was determined by using an *in vitro* technique in which samples were incubated in buffered rumen fluid (Møller *et al.*, 1989). DOM was calculated from regressions of laboratory values on digestibility determined in sheep. Effective rumen-degradability of protein and intestinal digestibility of undegraded feed protein were determined in all feedstuffs in one composite sample representing the whole experimental period. Effective rumen-degradability of protein was determined by incubating samples in nylon bags in the rumen of fistulated cows. Rumen-degradability was based on a supposed outflow rate of 5% per hour and the results adjusted for particle losses from the nylon bags (Madsen *et al.*, 1995). Intestinal digestibility of undegraded feed protein was determined by using the mobile-bag technique (Madsen *et al.*, 1995). Determinations of degradability and digestibility were made in three ruminally and intestinally cannulated cows.

The results of determinations of degradability of 10 feedstuffs from experiments A1-4, A1-5 and A1-6 deviated somewhat from normal values. Therefore, the analyses of these feeds were repeated. Results from the two analyses did not differ considerably. Therefore, averages of all determinations were used in further calculations. The AAT and PBV values were calculated as described by Hvelplund & Madsen (1990). The energy values, expressed in Feed Units (FU), were calculated according to Weisbjerg & Hvelplund (1993).

Average individual data on feed intake, milk yield and composition were analysed by using a covariance analysis in the GLM procedure of the SAS system. The following model was used:

$$Y_{ijk} = m + BLOCK_i + bX_{ijk} + TREATMENT_j + RESIDUAL_{ijk}$$

where:
Y_{ijk} = observed data for the individual cow
m = overall average
$BLOCK_i$ = effect of block i
b = regression coefficient
X = covariable
$TREATMENT_j$ = effect of treatment j
$RESIDUAL_{ijk}$ = random variation

2.2 Sub-task A3

General

Experimental procedures were similar in experiment A3-1 as well as in experiment A3-2. Before the start of the experiment, the cows were blocked according to age, stage of lactation, live weight, milk production and milk composition in the actual lactation. Within each block, the animals were randomly assigned to one of the experimental treatments. Experiment A3-1 was carried out between May 17 and August 28, 1993, and experiment A3-2 between May 21 and September 3, 1994. Previous to the start of each experiment, the cows were fed a ration consisting of *ad libitum* fresh cut herbage, supplemented with a commercial compound concentrate according to the recommendations of the Central Bureau for Livestock Feeding at Lelystad (CVB, 1995).

Housing

Experiment A3-1 was carried out at experimental farm Aver Heino, Heino, and experiment A3-2 at experimental farm De Waiboerhoeve, Lelystad. The cows were housed in cubicle sheds with alleys of concrete slats and herringbone milking parlours with automatic cluster removal and electronic milk weight recording units. Both sheds were equipped with electronic feed access doors (Calan gates). Dry concentrate pellets were fed using computer-controlled feeders. The cows had free access to drinking water and stone salt.

Grassland and fodder crop management

In both experiments, herbage was cut twice a day (morning and afternoon) with a disc mower and harvested with a pick-up loader from swards which predominantly consisted of *Lolium perenne*. Herbage was cut at a sward height of 15 to 18 cm, corresponding to a DM yield of 1400 to 2000 kg ha^{-1}. To maintain a constant quality, herbage was rejected for the experiment when the duration of regrowth was longer than 30 days and/or when DM yield exceeded 2000 kg ha^{-1}.

Prior to the first cut, the grassland was fertilised in March or early April with 80 kg effective inorganic N ha^{-1} from cattle slurry and calcium ammonium nitrate (CAN, 27 per cent N). The second cut was fertilised with 60 kg N ha^{-1} and subsequent cuts with 40 kg N ha^{-1} from CAN up to a maximum of 250 kg N ha^{-1} yr^{-1}. Irrigation was applied during periods of drought.

GMES (variety Anjou 09) was harvested with a precision field chopper equipped with a picker head, and ensiled in ride-over silage clamps and sealed with plastic sheets. GMES was harvested in October 1992 (experiment A3-1) and November 1993 (experiment A3-2).

Animals and treatments

Experiment A3-1

Thirty six Red Holstein Friesian dairy cows (27 multiparous and 9 primiparous) were used. The cows selected for the experiment were on average 86 days *post partum* (range: 22-181 days) and their milk yield averaged 29.4 kg day^{-1} (range: 16.4-37.3 kg day^{-1}) at the start of the experiment. During the first four weeks of the experimental period, all cows received the same ration which consisted of *ad libitum* fresh cut herbage and 6.0 kg DM day^{-1} of sugar beet pulp. From the fifth experimental week onwards, the animals received the experimental rations. These rations were based on *ad libitum* fresh herbage. The basal ration was supplemented with 5.6 kg DM day^{-1} of beet pulp (treatment BP), or 2.5 kg DM day^{-1} of GMES, 0.6 kg DM day^{-1} of soybean meal solvent extract (SMSE) and 2.5 kg DM day^{-1} of beet pulp (treatment BPSM), or 4.5 kg DM day^{-1} of GMES and 1.2 kg DM day^{-1} of SMSE (treatment SM). The rations were formulated to contain equal amounts of DVE (digestible protein available in the intestine) according to the Dutch protein evaluation system, the DVE/OEB system (Tamminga *et al.*, 1994).

Fresh herbage was fed at 8:00, 14:00 and 22:00 h. *Ad libitum* intake of herbage was achieved by offering enough herbage at each meal to ensure a refusal weight of at least 10 per cent of the fresh weight offered. During one hour at each milking, the cows of the groups BPSM and SM were allowed access to 1.25 and 2.25 kg DM of GMES, respectively. Beet pulp and SMSE were available in small portions throughout the day. The refusals of herbage were removed and weighed at 6:00 and 17:00 h. Refusal weights of GMES were determined once a day at 19:00 h.

The cows were milked twice a day between 6:00 and 7:00 h and between 17:00 and 18:00 h.

Experiment A3-2

Forty eight Holstein Friesian dairy cows (40 multiparous and 8 primiparous) were used. At the start of the experiment, the cows were on average 68 days *post partum* (range: 2-141 days) and average milk yield was 27.9 kg day^{-1} (range: 17.5-33.7 kg day^{-1}). During the first four experimental weeks, all cows received a ration consisting of *ad libitum* fresh cut herbage supplemented with 3.8 kg DM day^{-1} of GMES and 1.8 kg DM day^{-1} of a pelleted concentrate based on beet pulp and SMSE (BSF). In the fifth experimental week, the cows were switched over to their experimental rations (M00, M25, M50 and M75). These rations were based on *ad libitum* fresh herbage and 1.8 kg DM day^{-1} of BSF (M00), supplemented with 2.5 kg DM day^{-1} of GMES (M25), 5 kg DM day^{-1} of GMES (M50), or 7.5 kg DM day^{-1} of GMES (M75).

Fresh cut herbage was offered twice a day at 9:00 and 18:00 h. Enough herbage was permanently available to ensure *ad libitum* herbage intake. The refusals of herbage were removed and weighed once a day at 7:00 h. The cows in M25 and M50 were fed two portions of GMES per day of 1.25 and 2.5 kg DM, respectively, at 7:00 and 18:00 h, and the cows in M75 were fed three portions of 2.5 kg DM per day at 7:00, 13:00 and 18:00 h. The refusals of GMES were removed and weighed 1 hour after feeding. The beet pulp and concentrate BSF were fed in small portions during the day.

Milking was scheduled between 6:00 and 7:00 h and between 17:00 and 18:00 h.

Measurements and data collection

Milk production and composition

Milk weights were recorded each milking. Weekly, during four consecutive milkings, milk was sampled for analysis of fat, protein, lactose and urea content. Milk fat concentration was determined according to the method of Röse-Gottlieb described in procedure IDF1C of the International Dairy Federation (IDF, 1987). Milk protein concentration was derived from Kjeldahl-N analysis in accordance with procedure IDF20B (IDF, 1993). High-Performance Liquid Chromatography was applied for determination of lactose concentration (MCS, Zutphen, The Netherlands, unpublished). Milk urea concentration was determined with the segmented flow technique (De Jong *et al.*, 1992). Analysis of the milk samples was carried out at the Milk Control Station, Zutphen, The Netherlands.

Blood and urine

In the 4th, 10th and 15th week of the experiments, spot samples of blood of each cow were taken from the tail vein. In week 10 of the experiments, a urine sample of each cow was taken at 8.00 and 19.00 h by using catheters. Immediately after collection, the samples of blood and urine were transported to the laboratory of the Public Health Service for Animals (GD, Deventer, The Netherlands) for chemical analysis.

Blood was analysed for ß-hydroxybutyrate (ß-HB) and urea content (BU), and urine for urinary N (UN) and urinary urea N (UUN) content. The concentration of ß-HB was used as an indicator of energy supply.

Feed intake, feed composition and feeding value

The weights of herbage, GMES and concentrates offered and refused, were recorded for each cow on 5 consecutive days each week. Herbage and GMES offered were sampled at feeding, and herbage and GMES refused were sampled at removal for determination of the DM content after oven-drying at 104 °C for 48 h. All feeds were also sampled for analysis of composition, *in vitro* digestibility and nutritive value. Samples of herbage were taken each feeding, while GMES was sampled daily, and sugar beet pulp, SMSE and BSF weekly. The daily herbage samples were oven-dried at 70 °C for 48 h. A composite per week of the daily herbage samples was made for chemical analysis. The samples of GMES were frozen and those of beet pulp and SMSE stored in air-tight bottles and pooled to composite samples per five-week period.

The feed samples were analysed for air-dried DM, DM, crude protein, crude fibre, crude ash, crude fat, starch and sugar, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ammonium, potassium, calcium, magnesium, phosphorus, sodium and *in vitro* digestibility. Chemical analysis of the feeds was carried out at the Laboratory for Soil and Crop Testing (BLGG), Oosterbeek, The Netherlands. Air-dried DM content was determined gravimetrically after oven-drying for 48 hours at 70 °C. Dry matter, crude protein, crude fibre, crude ash, crude fat, starch and sugar content were determined according to procedures NEN3332, NEN3145, NEN3327, NEN3329, NEN3576, NEN3574 and NEN3571, respectively, published by the Netherlands Normalisation Institute (NNI). The cell wall constituents NDF and ADF were determined gravimetrically after boiling in a neutral detergent (pH 7) or an acid detergent, respectively, followed by calcination (BLGG, unpublished). ADL was determined gravimetrically after boiling in an acid detergent, extraction with a 72 per cent H_2SO_4 solution, and calcination (BLGG, unpublished). Potassium, calcium, magnesium, nitrate, phosphorus and sodium content were analysed photo-spectrometrically (BLGG, unpublished). *In vitro* digestibility of organic matter (DOM) was determined according to Tilley & Terry (1963). The fermentation characteristics of the GMES (*i.e.* pH, ammonia, alcohol, butyric acid, acetic acid and lactic acid content) were determined to assess the quality of silage preservation. Composition of the experimental rations for individual cows was calculated from the chemical composition of the feeds and the proportion of each feed in the ration. The nutritive value of the feeds, expressed as NE_L (net energy for lactation), DVE (available digestible protein in the small intestine), OEB (degradable protein balance in the rumen) and FOM (fermentable organic matter) were derived from the chemical composition according to the calculation methods of CVB (1996). Daily intake of nutrients for individual cows was calculated as the concentration of the nutrients in the feeds multiplied by the intake of DM of the feeds, assuming that the chemical composition of the refusals equalled the composition of the feeds offered.

Body weight was measured on 3 consecutive days after morning milking in weeks 1, 5, 10 and 15 of the experiments. Full records of animal health were kept throughout the experiments.

Statistical analysis

In both experiment A3-1 and A3-2, a Randomised Block Design was used. The first four weeks of the experiments were used as a covariate-period. Data on milk yield, milk composition and feed intake recorded during the covariate-period, were used as covariates. The data on milk production, milk composition, and feed intake were analysed using the ANOVA procedure. Statistical analysis was carried out with Genstat 5 (1993). Treatment means were compared using Students t-test.

2.3 Sub-task A4

Objective

The objective of this study was to estimate herbage intake and N utilisation-combined indicators of the efficiency of rumen function in dairy cows at different stages of lactation, grazing grass/white clover swards and using minimum levels of supplementation.

Outline of experiment A4-1

On 1 May 1993 at the Acrehead Clover Unit, Crichton Royal Farm, 30 Holstein Friesian cows were allocated to a grazing trial. The group of dairy cows consisted of 15 autumn-calving and 15 spring-calving cows. The 15 autumn-calving cows were on average 200 days in lactation (s.e. = 2.9 days), while the spring-calving cows were on average 95 days in lactation (s.e. = 3.7 days). The animals grazed grass/clover swards continuously at a grass height of 5-9 cm. During the first 5 weeks of the experimental period the animals received 1 kg concentrate day^{-1}. Thereafter no concentrate was fed.

Sample collection

Grass height measurements and collection of herbage and concentrate samples were carried out weekly and samples were stored at -20 °C until chemical analyses. The animals were weekly weighed and condition-scored. Milk recording was carried out at 2 consecutive milkings once weekly. During two intensive recording periods of 12 days in May and July, herbage intake was estimated using the n-alkane technique, described by Mayes *et al.* (1986) and Dove & Mayes (1991). The animals were dosed twice daily with paper pellets containing C_{32} and C_{36}. During the last 5 days of the 12-day period a faecal sample was taken every morning. These samples were frozen at -20 °C. Additionally, milk, faecal and urine samples were collected during this 5-day period in order to assess N efficiency and rumen function more accurately.

Sample analyses

The herbage samples collected were split into grass and clover fractions and these were analysed for crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), *in vitro* digestibility (*in vitro* D) and ash content. During periods of herbage intake estimation, daily herbage samples were collected and analysed for n-alkane content. Concentrate samples were analysed for DM, CP, NDF, ADF, neutral cellulase gaminase digestibility (NCGD) and acid hydrolysis ether extract (AHEE). Weekly milk samples were analysed for fat, protein and lactose content and additional samples, taken during the intensive recording periods, were also analysed for non-protein nitrogen (NPN) and urea. Faeces collected were analysed for DM, total N and n-alkane content. Urine samples were analysed for total N and urea.

N balance calculations

The n-alkane technique allows faecal output to be calculated. This together with the N content of the faeces allows total faecal N excretion to be calculated. N intake was calculated from the estimated DM intake and N content of the herbage consumed. N excretion in milk was calculated from the quantity of milk produced and N content of the milk. N excretion in urine was then calculated by balance:

Urine N = N intake - N in faeces - N in milk - N in liveweight gain

The surpluses of effective rumen-degradable protein (ERDP) and metabolisable protein (MP) were calculated according to AFRC (1993), using the known intakes of grass, clover and concentrate.

The validation experiment (experiment A4-2)

Additionally, a validation experiment was carried out from 7 June until 2 July 1993. Eight Holstein cows were fed known amounts of herbage DM, varying from 8-14 kg day^{-1}. Half of the animals was supplemented with 2 kg DM day^{-1} of barley. The herbage fed was cut daily from a grass/clover sward. Measurements and analyses were as described for the main experiment; additionally, faeces were collected twice daily, from each animal after the morning (AM) and afternoon (PM) milkings. These samples were bulked over the 5 days to give one AM and one PM sample for each animal. In addition, these samples were then subsampled to obtain an AM + PM sample for each animal (see Hameleers & Mayes (1998) for a detailed report on the validation experiment).

2.4 Sub-task A5

Objective

The objective of this study was to improve N efficiency of dairy cows grazing grass/clover swards with different (low and high) clover contents using supplementation with different types and/or levels of concentrates.

Outline of the experiments

Two experiments were carried out at Crichton Royal Farm in the summer of 1994. Experiment A5-1 was carried out on a grass/clover sward with a low clover content (7.1%) from 16 May until 17 June, with a covariance data collection week before 16 May. Thirty multiparous Holstein Friesian cows, which calved in February-March were allocated to the experiment. Experiment A5-2 was carried out on a grass/clover sward with a moderately high clover content (38%) from 11 July until 5 August, with a covariance week before 11 July. Also this experiment employed thirty multiparous Holstein Friesian cows, which calved in February-March. Both experiments consisted of three treatments, as shown in Table 2.5.

Table 2.5. Design of the supplementation experiments on a grass/clover sward. Target protein supplies.

Experiment	Treatment	ERDP % of requirement	MP % of requirement	Total concentrate (kg cow^{-1} day^{-1})
Spring (A5-1)	A	93	100	0.9
	B	101	108	3.7
	C	93	101	3.7
Summer (A5-2)	A	119	113	0.4
	B	101	111	3.4
	C	87	100	5.8

ERDP, effective rumen-degradable protein; MP, metabolisable protein.

The different target levels of protein supply in terms of ERDP and MP were achieved by feeding different types and levels of concentrate supplements as shown in Table 2.6. The concentrate supplements were fed in two equal portions during milking at approximately 7:00 h and 16:00 h.

Table 2.6. Composition of the concentrate supplements fed in the two experiments.

Experiment	Treatment	Soya (kg day^{-1})	Cane molasses (kg day^{-1})	Sugar beet pulp (kg day^{-1})	Urea (kg day^{-1})	Minerals (kg day^{-1})
Spring (A5-1)	A	0.4	0.3	-	-	0.2
	B	0.4	0.3	2.7	0.110	0.2
	C	0.4	0.3	2.7	0.055	0.2
Summer (A5-2)	A	0.4	-	-	-	-
	B	0.4	-	3.0	-	-
	C	0.4	-	5.4	-	-

Sample collection

Grass height measurements and collection of herbage and concentrate samples were carried out weekly. Samples were stored at -20 °C until chemical analyses. The animals were weighed and condition-scored on a weekly basis. Milk recording was carried out at 2 consecutive milkings once weekly. During the last 12 days of each experiment, herbage intake was estimated using the n-alkane technique, described by Mayes *et al.* (1986), Dove & Mayes (1991) and Hameleers & Mayes (1998). The animals were dosed twice daily with paper pellets containing C_{32} and C_{36}. During the last 5 days of the 12-day period a faecal sample was collected every morning. These samples were stored at -20 °C. Additionally, milk and urine samples were collected during this 5-day period in order to assess N efficiency and rumen function more accurately.

Sample analyses

The herbage samples collected were split into a grass and a clover fraction and these were analysed for crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), *in vitro* digestibility and true protein. During periods of herbage intake estimation, daily herbage samples were collected and analysed for n-alkane content. Concentrate samples were analysed for DM, CP, NDF, ADF, neutral cellulase gaminase digestibility (NCGD), starch, water soluble carbohydrates (WSC), *in vitro* organic matter digestibility (IVOMD), magnesium (Mg), calcium (Ca), phosphorus (P), sodium (Na), potassium (K) and acid hydrolysis ether extract (AHEE). Weekly milk samples were analysed for fat, protein and lactose content and additional samples were also analysed for non-protein nitrogen (NPN) and urea. Faeces collected were analysed for DM, total N and n-alkane content. Urine samples were analysed for total N and urea N.

N balance calculations

The n-alkane technique allows faecal output to be calculated. This together with the N content of the faeces allows faecal N to be calculated. N intake was calculated from the estimated DM intake and N content of the herbage consumed. N excretion in milk was calculated from the CP of the milk and the quantity of milk produced. N excretion in urine was then calculated by balance:

Urine N = N intake – N in faeces – N in milk – N in liveweight gain

The surpluses of effective rumen-degradable protein (ERDP) and metabolisable protein (MP) were calculated according to AFRC (1993), using the determined intakes of herbage and concentrate supplement.

2.5 Sub-task A6-1

Objective

The objective of this study was to evaluate the effects of a reduction in the protein supply of dairy cows below actual feeding standards on animal performance and N efficiency. The cows received a diet consisting of *ad libitum* grass/clover silage, supplemented with barley, fodder beet and different quantities and types of protein-rich concentrates.

Outline of experiment A6-1.1

During the growing season of 1994, barley, fodder beet and grass/white clover silage were produced at Crichton Royal Farm without the use of mineral fertiliser. These feeds were used in a winter-feeding experiment (experiment A6-1.1) involving 30 multiparous Holstein/Friesian autumn-calving cows. The animals were fed individually through Calan Broadbent gates for a covariate period of one week and a treatment period of 16 weeks. The experiment employed a continuous design and the treatments are shown in Table 2.7.

Table 2.7. Experimental diets in the winter-feeding experiment of 1994/1995 (experiment A6-1.1).

	Experimental diets (kg DM cow^{-1} day^{-1})		
	A	B	C
Grass/clover silage	*Ad lib*	*Ad lib*	*Ad lib*
Barley	3.0	2.5	2.4
Fodder beet	3.0	2.5	2.4
Soybean meal	0.05	0.4	0.40
Soypass$^{1)}$	0.05	1.0	0.05
Regumaize$^{2)}$	0.65	0.4	1.6
Total concentrate	6.75	6.80	6.85

$^{1)}$ *Soypass: soybean meal containing protected protein;*

$^{2)}$ *Regumaize: a mixture of molasses and urea.*

The feeding treatments were planned to give the following combinations of effective rumen-degradable protein (ERDP) and metabolisable protein (MP), as shown in Table 2.8.

Table 2.8. Planned protein levels in the winter-feeding experiment of 1994/1995 (experiment A6-1.1).

Diet	ERDP % of requirement	MP % of requirement	CP content of the diet (g kg^{-1} DM)
A. 80:80	80	80	140
B. 80:100	80	100	160
C. 100:100	100	100	170

ERDP, effective rumen-degradable protein; MP, metabolisable protein; CP, crude protein.

Agronomy of feed production

The silage was produced from grass/clover swards established in 1988. The clover varieties used were medium and broad-leafed types and the perennial ryegrass varieties used were tetraploid and diploid of the intermediate type. These swards received approximately 50 m^3 of slurry ha^{-1} and 50 kg P_2O_5 ha^{-1} in Spring. The grass/clover crop was cut on 24 May 1994 and ensiled on 25 May 1994. An area of 16.2 ha was ensiled yielding 2.9 t DM ha^{-1} with an average DM content of 200 g kg^{-1}. The low yield was due to a drought during the spring growth period. The total amount produced would not be sufficient for a winter feeding trial. It was therefore decided to take a second cut from an 8.3 ha area. The herbage was cut on 11 July 1994 and ensiled on 12 July. Average yield was 2.8 t DM ha^{-1}.

The fodder beet was sown on 11 May 1994. The field received 75 m^3 of slurry ha^{-1}. The variety used was Bolero, which was sown at 135,000 seeds ha^{-1} to achieve a population of 75,000 plants ha^{-1}. Weeds were controlled using chemicals pre and post emergence. The crop was harvested on 3 November 1994 and stored in a silage clamp. A yield of 11.8 t DM ha^{-1} was achieved.

The spring barley used in this experiment was grown in a field which in the previous year had a fodder beet crop. The barley was sown on 16 April 1994 by which time the field had received 80 m^3 of slurry ha^{-1}. No fungicides were used but weeds were controlled chemically. The crop was harvested on 10 August 1994 and yielded 5 t of grain ha^{-1} with 850 g DM kg^{-1}.

Sample collection

All feeds used were sampled twice a week and bulked to a fortnightly sample. The animals were weighed, condition scored and milk recorded on the last day of every week of the experimental period. Intakes were measured every second week of the experiment during the last 3 days of that week. Faecal samples (AM and PM) were collected during the last 2 days of weeks 4, 8, 12 and 16. Urine samples were taken 5 times a day on one of the 2 faecal sampling days. During this period additional milk samples were also taken for non-protein nitrogen (NPN) and urea analysis.

Sample analyses

The silage samples were analysed for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid hydrolysis ether extract (AHEE), acid detergent insoluble nitrogen (ADIN), *in vitro* organic matter digestibility (IVOMD), starch, water soluble carbohydrates (WSC) and ash content. The concentrate samples were analysed for DM, CP, neutral cellulase gaminase digestibility (NCGD), AHEE, ADIN, IVOMD, starch, WSC, NDF and ADF. All milk samples were analysed weekly for fat, protein and lactose and monthly samples were analysed for NPN and urea. Faeces collected were analysed for DM and total N. Urine samples were analysed for total N and urea. Additionally, all feeds used in the trial were assessed for their protein degradability characteristics using fistulated sheep.

2.6 Sub-task A6-2

Supplementation of grass/clover silage (experiment A6-2.1)

Experiment A6-2.1 was conducted with 48 cows calving between December 1993 and April 1994. All animals were fed the same grass/clover silage *ad libitum* and a fixed amount of concentrates of different compositions.

Four experimental diets were compared:

A. *Ad libitum* silage + 6 kg cow^{-1} day^{-1} of a conventional dairy concentrate with barley, beet pulp, soybean-meal, vitamins and minerals, containing 23% CP.
B. *Ad libitum* silage + 1.5 kg cow^{-1} day^{-1} of a concentrate with a high protein content of low rumen-dagradability. This concentrate was a mixture of fish meal, barley, vitamins and minerals, and contained 47.9% CP.
C. *Ad libitum* silage + 6 kg cow^{-1} day^{-1} of a high-energy concentrate, consisting of barley, beet pulp, soybean-meal, vitamins and minerals and containing 15.7% CP.
D. *Ad libitum* silage without supplement.

The composition of the grass/clover silage (averages and standard deviations of weekly composites throughout the experiment) is given in Table 2.9. Yields and harvest conditions are reported under subtask B6 (Table 3.87).

Table 2.9. Chemical composition of the grass/clover silage fed ad libitum *during the first 15 weeks of lactation in experiment A6-2.1 (in % of DM, unless indicated otherwise).*

Component[1]	Average	s.d.
DM (% of fresh)	19.8	±1.5
OM	86.3	±3.0
CP	13.5	±1.0
UIP (% of CP)	25.4	±2.9
NDF	59.2	±3.9
ADF	46.7	±4.6
Ca	0.8	±0.1
P	0.3	±0.0
Acetic acid	0.5	±0.3
Lactic acid	0.9	±0.8
NEL (Mcal kg^{-1} DM)	1.3	±0.1
pH	4.2	±0.3
IVOMD (%)	71.0	±3.0
NH_3-N (% of total N)	9.5	±5.6

[1] *DM, dry matter; OM, organic matter; CP, crude protein; UIP, rumen-undegradable protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; Ca, calcium; P, phosphorus; NEL, net energy for lactation; IVOMD,* in vitro *organic matter digestibility.*

Concentrates were fed once daily, after the morning milking. Table 2.10 shows the chemical composition of the concentrate ingredients and the composition of the concentrate. The actual chemical composition of the concentrates is given in Table 2.11.

Table 2.10. Chemical composition of the concentrate ingredients (in % of DM, unless indicated otherwise) and concentrate composition (in %) in experiment A6-2.1.

Component[1]	SBM	Barley	Fish meal	Beet pulp	Vit. mix	Dical. phos.
DM (% of fresh)	86.7	95.9	95	88.3		
OM	92.6	97.4	78.4	91.4		
CP	53.6	12.2	68.1	10.7		
UIP (% of CP)	37.4	45.1	58	40		
NDF	15.4	36.6	27.3	44.4		
ADF	15.4	9.42	10.2	31.4		
Ca	0.27	0.05	6.72	0.89		
P	0.74	0.32	4.32	0.15		
EE	1.64	1.9	6.44	0.82		
Concentrate composition						
A	30.0	48.1	-	19.2	0.2	2.5
B	-	30	67.3	-	0.2	2.5
C	7.0	64.5	-	25.8	0.2	2.5

[1] *see Table 2.9 for the meaning of the abbreviations; EE, ether extract.*

Table 2.11. Chemical composition of the concentrates (in % of DM, unless indicated otherwise) in experiment A6-2.1.

	Concentrate					
Component[1)]	A		B		C	
DM (% of fresh)	90.9	±1.6	91.2	±2.8	91.6	±1.2
OM	92.8	±1.7	83.4	±4.6	93.5	±0.9
CP	23.0	±1.7	47.9	±7.4	15.7	±1.9
UIP (% of CP)	37.4	±2.6	57.6	±1.9	36.1	±2.4
NDF	48.0	±6.9	32.2	±11.4	56.9	±10.5
ADF	15.3	±1.6	12.8	±4.1	15.0	±1.8
Ca	1.0	±0.5	4.6	±1.1	0.9	±0.2
P	0.7	±0.1	2.7	±0.5	0.6	±0.1
EE	1.8	±0.3	5.9	±1.9	1.7	±0.3

1) *see Table 2.9 for the meaning of the abbreviations.*

Milk weights were recorded on six consecutive milkings and milk samples were taken from two consecutive milkings every week and analysed for fat and protein. Body weights were recorded once a week after the morning milking. During the first three weeks after calving, all cows were fed together in the same pen. Milking performance in the third week of lactation was used as a covariate for measurements during the experimental period.

Supplementation of fodder crop silage (experiment A6-2.2)

The silage fed in experiment A6-2.2 was a mixture of maize silage and vetch/oats silage. The two forages were rotated on the same fields as summer and winter crops, respectively, and data on sowing and harvesting conditions and yields are presented under sub-task B6 (Chapter 3.10). The two forages were mixed in a 1:1 ratio (DM basis) although yield of maize was more than two times as high as that of vetch/oats. Table 2.12 shows the composition of the two forages. Because of their low CP content and in order to avoid a severe protein deficiency in the control group (group D, see below) and to bring the protein content of the forage mixture closer to that of the grass/clover silage used in experiment A6-2.1, 10% soybean meal (SBM) was added to the forage mixture. The third column of Table 2-12 refers to the mixture of the silages and SBM.

The experimental treatments and the rates of concentrate feeding and their ingredients were the same as in experiment A6-2.1, conducted in the first year. The average chemical composition of the concentrate ingredients and the concentrates, given in the Tables 2.13 and 2.14, shows minor differences with those in experiment A6-2.1. Concentrate A had a CP content of 22.9%, concentrate B of 51.7% and concentrate C of 13.0% of the DM (Table 2.14).

Table 2.12. Chemical composition of vetch/oats silage, maize silage and the mixture of these silages + 10% soybean meal (SBM) fed ad libitum *during the first 15 weeks of lactation in experiment A6-2.2. Chemical composition in % of DM, unless indicated otherwise.*

Component1)	Vetch/oats silage		Maize silage		Silage mixture + SBM	
DM (% of fresh)	23.0	±0.6	26.5	±0.6	26.4	±0.4
OM	90.9	±0.6	95.5	±0.1	92.2	±0.3
CP	9.7	±0.4	6.5	±0.2	11.5	±0.3
UIP (% of CP)	27.2	±2.7	32.5	±1.6	34.3	±2.3
NDF	75.8	±1.5	55.3	±0.8	61.0	±3.9
ADF	38.3	±0.8	33.9	±0.5	38.4	±0.8
Ca	0.42	±0.05	0.21	±0.02	0.32	±0.03
P	0.14	±0.02	0.15	±0.01	0.19	±0.01
Acetic acid	0.34	±0.03	0.74	±0.13		
Lactic acid	3.1	±0.3	0.53	±0.06		
NEL (Mcal kg^{-1} DM)	1.12	±0.04	1.29	±0.04	1.28	±0.04
pH	3.6	±0.1	4.0	±0.1		
IVOMD (%)	61.6	±1.7	67.6	±1.3	68.5	±1.7
NH$_3$-N (% of total N)	4.47	±0.48	6.25	±0.60		

1) *see Table 2.9 for the meaning of the abbreviations.*

Table 2.13. Chemical composition of the concentrate ingredients (in % of DM, unless indicated otherwise) and concentrate composition (in %) in experiment A6-2.2.

Component1)	SBM	Barley	Fish meal	Beet pulp	Vit. mix	Dical. phos.
DM (% of fresh)	90.3	92.8	93.0	92.0		
OM	93.5	97.6	79.7	92.2		
CP	48.5	12.4	56.7	9.9		
UIP (% of CP)	35.3	31.0	61.2	42.7		
NDF	19.6	17.2	9.3	35.6		
ADF	9.9	7.7	0.3	24.9		
Ca	0.37	0.13	3.33	0.76		
P	0.60	0.25	5.35	0.36		
EE	2.03	1.74	7.33	0.98		
Concentrate composition						
A	30.0	48.1	-	19.2	0.2	2.5
B	-	30	67.3	-	0.2	2.5
C	7.0	64.5	-	25.8	0.2	2.5

1) *see Table 2.9 for the meaning of the abbreviations.*

Table 2.14. Chemical composition of the concentrates (in % of DM, unless indicated otherwise) in experiment A6-2.2.

Component[1]	Concentrate A		Concentrate B		Concentrate C	
DM (% of fresh)	92.3	±0.5	94.1	±0.2	94.8	±0.4
OM	93.8	±0.3	87.7	±1.1	95.3	±0.5
CP	22.9	±0.8	51.7	±0.3	13.0	±1.3
UIP (% of CP)	35.4	±2.8	56.8	±3.4	32.2	±3.5
NDF	27.3	±0.9	19.5	±1.9	30.1	±2.5
ADF	11.4	±0.5	8.6	±1.2	13.1	±1.5
Ca	0.71	±0.05	2.45	±0.28	0.56	±0.08
P	0.62	±0.05	2.55	±0.18	0.51	±0.15
EE	1.4	±0.1	4.5	±0.8	1.4	±0.1

1) *see Table 2.9 for the meaning of the abbreviations.*

Experiment A6-2.2 included 32 cows calving between February and April of 1995. Milk and body weight measurements and samples were taken as described for the first experiment.

2.7 Sub-task B1

Silstrup Research Station is situated 4 km outside of Thisted in Northern Jutland. Silstrup has approximately 60 ha of not irrigated clay soil and 74 animal units (AU), of which 54 AU are dairy cows. Approximately 2100 tons of slurry are produced annually corresponding to 95 kg total N AU^{-1}.

Two cropping systems were compared in experiment B-1.1, a 'protein rotation' and an 'energy rotation'. The objective of the protein rotation was to maximise yield of milk protein per ha with minimum use of fertiliser N in the fields and a minimum of protein supplements in the diets of the cows. The objective of the energy rotation was to maximise yield of metabolisable energy per ha. Crops in the two rotations were established in the Autumn of 1992 and Spring of 1993 and studied until the Spring of 1996. All crops were treated according to 'best management practices'.

On average, protein crops occupied 28 ha and energy crops 25 ha. Field size within each cropping system ranged from 0.6 to 5.5 ha. Each crop in both cropping systems was more closely studied for nutrient leaching in 10 individually drained experimental fields of approximately 0.6 ha each (Tables 2.15 and 2.16).

Water was sampled weekly at 120 cm depth from a drainage well in each experimental field for determination of the concentration of nitrate N. Water samples were frozen immediately after sampling. Before sending samples to the laboratory for chemical analysis, four weekly samples were pooled.

All crops, except winter wheat, were spring-ploughed and spring-sown. The sowing date ranged from 13 April to 11 May. Sowing dates for winter wheat ranged from 18 to 21 September. All crops, except oat and fodder beet, were under-sown with grass or grass/clover (Tables 2.15 and 2.16).

Table 2.15. Lay-out of crops in the protein rotation at Silstrup Research Station. Each experimental field was approximately 0.6 ha and individually drained.

Year	Experimental fields in the protein cropping system				
	1	2	3	4	5
1992	Barley + Grass/clover[1)]	Whole pea + Ital. ryegrass	Whole barley + Ital. ryegrass	Grass/clover, 2nd production year	Grass/clover, 1st production year
1993	Grass/clover, 1st production year	Barley + Grass/clover	Whole pea + Ital. ryegrass	Whole barley + Ital. ryegrass	Grass/clover, 2nd production year
1994	Grass/clover,2nd production year	Grass/clover,1st production year	Barley + Grass/clover	Whole pea + Ital. ryegrass	Whole barley + Ital. ryegrass
1995	Whole barley + Ital. ryegrass[2)]	Grass/clover, 2nd production year	Grass/clover, 1st production year	Barley + Grass/clover	Whole pea + Ital. ryegrass

[1)] *Grass/clover is a mixture of 20 kg perennial ryegrass, 3 kg red clover and 3 kg white clover per hectare.*

[2)] *Italian ryegrass.*

Table 2.16. Lay-out of crops in the energy rotation at Silstrup Research Station. Each experimental field was approximately 0.6 ha and individually drained.

Year	Experimental fields in the energy cropping system				
	1	2	3	4	5
1992	Barley + per. ryegrass[1)]	Fodder beet	Whole wheat + Ital. ryegrass	Oat	Per. ryegrass, 1st production year
1993	Per. ryegrass, 1st production year	Barley + per. ryegrass	Fodder beet	Whole wheat + Ital. ryegrass	Oat
1994	Oat	Per. ryegrass, 1st production year	Barley + per. ryegrass	Fodder beet	Whole wheat + Ital. ryegrass
1995	Whole wheat + Ital. ryegrass[2)]	Oat	Per. ryegrass, 1st production year	Barley + per. ryegrass	Fodder beet

[1)] *Perennial ryegrass.*

[2)] *Italian ryegrass.*

Slurry was stored in two uncovered tanks. Rain and wastewater were allowed to enter the tanks. Slurry contained approximately 620 g NH_4^+-N per kg total N, but the concentration of total N varied, for unknown reasons, from 3.2 kg ton^{-1} in 1993 to 4.8 kg ton^{-1} in 1994 and 1995. Based on N contents in feed, milk and meat, it was decided that 3.2 kg total N ton^{-1} was the most realistic value to use. The dry matter (DM) concentration of slurry at sampling ranged from 17 to 51 g kg^{-1} depending on length of storage and time of sampling. The problem of a reliable determination of the concentration of N in slurry was greater in this study than in other Danish experiments, but, in general, variation is so high that Danish authorities no longer accept chemical determination of nutrient composition of slurry when calculating N balances at farms.

Cattle slurry or mineral fertiliser were applied alone or in combination to standing crops or to ploughed fields, beginning on 1 to 10 April each year. Slurry was applied by a tractor-pulled 20-ton wagon with hoses spaced at 20 cm and dragging on the ground. Slurry was stirred in the storage tank for approximately two days before bringing it out in the slurry wagon. Sampling of slurry for chemical analysis was done when pumping it into the slurry wagon. Concentrations of DM, total N, ammonia N, P and K were determined.

Application of slurry had priority over application of mineral fertiliser. Nitrogen application to crops was based on the estimated need and followed recommendations of the Danish Ministry of Agriculture. Application rates of slurry in a certain year were based on the chemical composition of the slurry in the previous year. The length of time from sampling of slurry to determination of its composition was too long to be able to use the data within one season. Nitrogen application and composition of slurry are presented in the Annexes B1-7, B1-8, B1-9, B1-10, B1-11 and B1-12.

Actual evaporation and leaching of water from the root zone were estimated by using EVACROP, a model developed at the Danish Institute of Plant and Soil Science (Olesen & Heidmann, 1990). Input data to the model were temperature, precipitation, and evaporation, measured at Silstrup Research Station. Furthermore, drainage water in each of 10 separately drained experimental fields was collected once weekly in drainage wells at 120 cm depth throughout the year. Four weekly samples were then pooled before analysis of nitrate N.

Spring cereals were harvested for grain between 15 August and 6 September, winter wheat for whole-crop silage between 19 and 31 July, pea for whole-crop silage between 22 and 28 July, and barley for whole-crop silage between 23 July and 3 August. Harvest of fodder beet normally commenced on 20 September and was completed between 28 October and 3 November. The first cut of grass/clover and perennial ryegrass was taken between 18 May and 1 June. Thereafter, these forages were cut two or three times per year. In some fields, one to two cuts were exchanged for grazing. Because of the difficulty to estimate amounts of grazed grass or grass/clover, these amounts are not included in the DM yields presented.

All harvested feed was weighed and sub-samples were taken for quality analyses before being ensiled or dry-stored at the research station. All samples were analysed for concentration of DM, *in vitro* organic matter disappearance, total N, water-soluble carbohydrates and crude fibre. Silage samples were also analysed for fatty acids, pH, ammonia N, starch and crude fat. Ensiled crops were cored after 90 days and concentrations of DM, ash, crude protein, sugar, starch, crude fibre, lactic acid, acetic acid, butyric acid, as well as *in vitro* digestibility of organic matter and pH were determined. Crops from the two rotation systems were used in the feeding experiments described under sub-task A1.

It was not possible to separate the slurry from cows that either were fed protein crops or energy crops. Therefore, the concentration of N in slurry from either ‘protein cows’ or ‘energy cows’ was estimated from N in feed and N in milk and meat. These estimated values are used in the Tables 3.70 and 3.73.

From 1 June until 31 July in 1994 and 1995, it was unusually warm and dry compared with a 10-year average at Silstrup Research Station.

2.8 Sub-task B3

General

Experimental procedures and herd management were similar in experiments B3-1 and B3-2. Before the start of the experiment, the cows were blocked according to variation in milk production and composition in the previous lactation, age, calving date and body weight. Within each block, the animals were randomly assigned to one of the experimental treatments. The experiment started the first week after calving and lasted 15 weeks. In the dry period previous to the experiment, the cows were fed a mixture of straw and *ad libitum* grass silage supplemented with minerals and 1 kg of concentrate according to the net energy requirements as recommended by the Central Bureau for Livestock Feeding and Feedstuffs (C.V.B., 1995).

Housing

Both experiments were executed at Dairy Unit 3 of the experimental farm De Waiboerhoeve, Lelystad, the Netherlands. The cows were housed in a cubicle shed with slatted concrete alleys. Forages and home-grown concentrates were fed individually, using electronic feed access doors (Calan). Pelleted concentrates were offered with a computer-controlled concentrate dispenser (NEDAP). The cows had free access to drinking water and stone salt. They were milked twice a day at 6:00 and 16:00 h in a herringbone milking parlour with automatic cluster removal and electronic milk weight recording units.

Grassland and fodder crop management

Grass silages were made from the first cut of swards that predominantly consisted of *Lolium perenne* in experiment B3-1, or *Lolium perenne* (*ca.* 75%) and *Phleum pratense* (*ca.* 25%) in experiment B3-2. The pastures were fertilised in March or early April with 80 kg effective inorganic N ha^{-1} from cattle slurry and calcium ammonium nitrate (CAN). The grass was cut with a disc mower-conditioner combination, tedded twice and wilted under good weather conditions for 24 to 36 hours. Harvest dates were April 27 and 29 and May 5, 1993, in experiment B3-1 and May 4, 5 and 7, 1994, in experiment B3-2. The wilted grass was collected with a precision-chop harvester and ensiled in ride-over silage clamps and sealed with plastic sheets. Average DM yield was approximately 3000 kg ha^{-1}.

Fodder beet (variety Kyros) was harvested on November 3, 1993, with a fodder beet harvester and stored frost-free covered with straw in a bunker.

GMES (variety Anjou 09) was harvested on November 2, 1993, (experiment B3-1) and November 3, 1994, (experiment B3-2) with a precision-chop harvester equipped with a picker head. The theoretical chop size was adjusted at 4 mm to ensure the maize kernels were damaged. GMES was ensiled in ride-over silage clamps and sealed with plastic sheets.

Animals and diets

Experiment B3-1

Fifty six Holstein Friesian dairy cows (40 multiparous and 16 primiparous) were used. During the first four weeks after calving, all animals received the same ration consisting of *ad libitum* grass silage, 1.3 kg DM day^{-1} of a pelleted concentrate based on soybean meal solvent extract (SMSE) and 8.7 kg DM day^{-1} of pelleted beet pulp. From the fifth week onwards, the animals received their experimental rations. These rations contained *ad libitum* grass silage, supplemented with 1.3 kg DM day^{-1} of SMSE and 8.7 kg DM day^{-1} of beet pulp (treatment BS); 2.7 kg DM day^{-1} of SMSE, 2.3 kg DM day^{-1} of beet pulp and 5 kg DM day^{-1} of fodder beet (treatment FB); 2.8 kg DM day^{-1} of SMSE, 0.6 kg DM day^{-1} of beet pulp, 3.3 kg DM day^{-1} of fodder beet and 3.3 kg DM day^{-1} of GMES (treatment FBMS); 2.6 kg DM day^{-1} of SMSE, 2.4 kg DM day^{-1} of beet pulp and 5 kg DM day^{-1} of GMES (treatment MS). The rations were formulated to contain equal amounts of DVE (digestible protein available in the intestine) according to the Dutch protein evaluation system, the DVE/OEB system (Tamminga *et al.*, 1994).

During 1 hour after each milking, the cows on FB, FBMS and MS were allowed access to either GMES or fodder beet. Prior to feeding, the fodder beet were washed and chopped. Grass silage was fed at 9:00 and 18:00 h. To ensure *ad libitum* intake of grass silage, refusal weight of grass silage was at least

10 per cent of the amount offered. Refusals of grass silage, GMES and fodder beet were removed once a day at 6:00 h.

Experiment B3-2

Fifty two Holstein Friesian dairy cows (44 multiparous and 8 primiparous animals) were used. During the first four weeks after calving, all animals received the same ration consisting of *ad libitum* grass silage, supplemented with 6 kg DM day^{-1} of GMES and 4.1 kg DM day^{-1} of a pelleted concentrate based on beet pulp (BSC) and 0.9 kg DM day^{-1} of a pelleted concentrate based on soybean meal (SCF). From the fifth week onwards, four experimental rations were fed: 1) a high-roughage, low-protein diet (treatment HRLP) consisting of *ad libitum* grass silage, 4 kg DM day^{-1} of GMES and 5.0 kg DM day^{-1} of BSC; 2) a high-roughage, high-protein diet (treatment HRHP) consisting of *ad libitum* grass silage, 4 kg DM day^{-1} of GMES, 3.6 kg DM day^{-1} of BSC and 1.4 kg DM day^{-1} of SCF; 3) a low-roughage, low-protein diet (treatment LRLP) consisting of 8.2 kg DM day^{-1} of grass silage, 8 kg DM day^{-1} of GMES, 4.6 kg DM day^{-1} of BSC and 0.4 kg DM day^{-1} of SCF; 4) a low-roughage, high-protein diet (treatment LRHP) consisting of 8.2 kg DM day^{-1} of grass silage, 8 kg DM day^{-1} of GMES, 3.2 kg DM day^{-1} of BSC, and 1.8 kg DM day^{-1} of SCF.

Grass silage was fed at 10:00 and 19:00 h and GMES was fed immediately after each milking. The refusals of grass silage and GMES were removed once a day. The refusal weight of grass silage in the treatments HRLP and HRHP was at least 10 per cent of the amount offered to ensure *ad libitum* intake of grass silage.

Measurements and data collection

Milk production and composition

Milk weights were recorded each milking. Weekly during four consecutive milkings, milk samples were taken for analysis of fat, protein, lactose and urea concentration at the laboratory of the Milk Control Station (MCS), Zutphen, The Netherlands. The method of Röse-Gottlieb was used for analysis of milk fat in accordance with procedure IDF1C of the International Dairy Federation (IDF, 1987). Milk protein concentration was derived from Kjeldahl-N analysis (IDF, 1993). Lactose concentration was determined by High-Performance Liquid Chromatography (MCS, unpublished). Milk urea content was determined using the segmented flow technique described by De Jong *et al.* (1992).

Blood and urine

In the 4th, 10th and 15th week after calving, spot samples of blood of each cow were taken from the tail vein. In week 10 of the experiment, a urine sample of each cow was taken at 8:00 and 19:00 h using catheters. Immediately after collection, the samples of blood and urine were transported to the laboratory of the Public Health Service for Animals (GD), Deventer, The Netherlands, for chemical analysis. Blood was analysed for ß-hydroxybutyrate (ß-HB) and urea content (BU), and urine for urinary N (UN) and urinary urea N (UUN) content. The concentration of ß-HB was used as an indicator of energy supply.

Feed intake, feed composition and feeding value

The weights of grass silage, fodder beet, GMES, sugar beet pulp, SMSE, offered and refused, were recorded for individual cows during 5 consecutive days each week. Daily, samples of fodder beet, GMES and grass silage, offered and refused, were taken for estimation of DM content by oven-drying at 104 °C for 48 hours. Daily DM intake of individual cows was calculated from fresh weight consumed and DM content.

In addition, samples of the feeds were taken for chemical analyses. Grass silage, fodder beet and GMES were sampled daily and sugar beet pulp, SMSE, BSC and SCF weekly. Samples of grass silage, fodder beet and GMES were stored frozen. A weekly composite of proportional samples of grass silage, and five-weekly composites of proportional samples of fodder beet, GMES, sugar beet pulp and SMSE were used for chemical analyses at the Soil and Crop Testing Laboratory (BLGG), Oosterbeek, The Netherlands. Air-dried DM content was determined gravimetrically after oven-drying during 48 hours at 70 °C. DM content was determined gravimetrically after oven-drying of air-dried material during 12 hours at 104 °C according to procedure NEN3332 of the Netherlands Normalisation Institute (NNI). Crude protein was determined as 6.25 × Kjeldahl-N minus NH_3-N (NEN3145). Crude fibre was analysed gravimetrically after calcination of the non-soluble residues, which remain after boiling in 0.26N H_2SO_4 and 0.23N NaOH, respectively (NEN3327). Crude ash was determined gravimetrically after calcination during 4 hours at 550 °C (NEN3329). Crude fat was determined as ether extract (NEN3576). Starch was analysed by enzymatic hydrolysis with amyloglucosidase (NEN3574). Sugar was determined after boiling in a $BaCO_3$ solution, protein extraction, and Luff-Schoorl titration, respectively (NEN3571). The cell wall constituents neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined gravimetrically after boiling in a neutral detergent (pH 7) and an acid detergent, respectively, followed by calcination of the residues according to the procedures of BLGG, Oosterbeek, The Netherlands (unpublished). Acid detergent lignin (ADL) was determined gravimetrically after boiling in an acid detergent and extraction with a 72 per cent H_2SO_4 solution, and calcination of the residues, respectively (BLGG, unpublished). Ammonia, potassium, calcium, magnesium, phosphorus and sodium content were analysed spectro-photometrically (BLGG, unpublished). *In vitro* digestibility was determined according to the method of Tilley & Terry (1963).

Fermentation characteristics (pH and content of ammonia, alcohol, butyric acid, acetic acid and lactic acid) of grass silage and GMES were determined to assess the quality of preservation.
Composition of the experimental ration for each cow was calculated from the chemical composition of each feed and the quantity consumed. The values of NE_L (net energy for lactation), DVE, OEB (degradable protein balance in the rumen) and FOM (fermentable organic matter) were derived from the chemical composition (CVB, 1995). Daily intake of nutrients by individual cows was calculated as DM intake of the feeds multiplied by the concentration of nutrients, assuming that the chemical composition of the refusals was not different from the composition of the feeds offered. Body weight was measured on 3 consecutive days after morning milking in the 1st, 5th, 10th and 15th week after calving. Full records of animal health were kept throughout the experiment.

Statistical analysis

In both experiments a randomised block design was used. The first four weeks after calving were used as a covariate period. Data on milk yield, milk composition and feed intake, recorded during this period, were used as covariates. In both experiment B3-1 and B3-2, data on milk production, milk composition and feed intake were analysed using the ANOVA procedure of statistical package Genstat 5 release 3 (Rothamsted Experimental Station, 1993). Means of the treatments were compared using Students t-test.

2.9 Sub-task B5

Objective

The objective of this experiment (B5-1) was to evaluate the quality of barley and fodder beet as supplements to dairy cows fed grass/clover silage.

Outline of experiment B5-1

During the growing season of 1993, barley, fodder beet and grass/white clover silage were produced at Crichton Royal Farm without the use of mineral fertiliser. These feeds were used during a winter-feeding experiment involving 30 multi-parous Holstein/Friesian autumn-calving cows. The animals were fed individually through Calan Broadbent gates for a period of 17 weeks: a covariance period of one week and a treatment period of 16 weeks. The experiment employed a continuous design and the treatments were:

B *ad libitum* grass/clover silage + 5 kg DM day^{-1} of barley + 3.0 kg DM day^{-1} of soya

BF *ad libitum* grass/clover silage + 2.5 kg DM day^{-1} of barley + 2.5 kg DM day^{-1} of fodder beet + 3.0 kg DM day^{-1} of soya

F *ad libitum* grass/clover silage + 5 kg DM day^{-1} of fodder beet + 3.0 kg DM day^{-1} of soya

Agronomy of feed production

The grass/clover silage was produced from grass/clover swards established in 1988 by direct drilling of white clover into a perennial ryegrass sward. The clover varieties used were medium and broad-leafed types and the perennial ryegrass varieties used were tetraploid and diploid of the intermediate type. These swards received approximately 50 m^3 of slurry ha^{-1} and 50 kg P_2O_5 ha^{-1} in Spring. The grass/clover crop was cut on 27 May 1993 and ensiled on 28 May 1993. An area of 15.1 ha was ensiled yielding 4.2 t DM ha^{-1} with an average DM content of 178 g kg^{-1}.

The fodder beet was sown in early May. The field received 80 m^3 of slurry ha^{-1}. The variety used was Bolero, which was sown at 135,000 seeds ha^{-1} to achieve a population of 75,000 plants ha^{-1}. Weeds were controlled using chemicals pre and post emergence. The crop was harvested in October and stored in a silage clamp. A yield of 12.6 t DM ha^{-1} was achieved.

The spring barley used in this experiment was grown in a field which in the previous year had a fodder beet crop. The crop was sown on 14 April 1993, by which time the field had received 80 m^3 of slurry ha^{-1}. No fungicides were used, but weeds were controlled chemically. The crop was harvested on 17 August and yielded 4.2 t DM of grain ha^{-1} at 850 g DM kg^{-1}.

Sample collection

All feeds used were sampled twice a week and bulked to a fortnightly sample. The animals were weighed, condition scored and milk recorded on the last 4 days of each week of the experimental period. Intakes were measured every second week of the experiment during the last 3 days of that week. Faecal samples (AM and PM) were collected during the last 2 days of weeks 4, 8, 12 and 16. Urine samples were taken 5 times a day on one of the 2 faecal sampling days. During this period, additional milk samples were taken for non-protein nitrogen (NPN) and urea analysis.

Sample analyses

The silage samples were analysed for DM, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid hydrolysis ether extract (AHEE), *in vitro* organic matter digestibility (IVOMD), starch, water soluble carbohydrates (WSC), pH, ammonia and organic matter (OM) content. The concentrate samples were analysed for DM, CP, neutral cellulase gaminase digestibility (NCGD), AHEE, acid detergent insoluble nitrogen (ADIN), IVOMD, starch, WSC, NDF and ADF. All milk samples were analysed weekly for fat, protein and lactose and some additional samples were analysed for NPN and urea. Faeces collected were analysed for DM and total N. Urine samples were analysed for total N, urea and purine derivatives.

2.10 Sub-task B6

Three experiments were carried out, one under grazing and two under cutting on the performance of mixed swards of grass and white clover:

Experiment B6-1. A grazing experiment comparing:
  a. a grass/clover sward without fertiliser N,
  b. a grass/clover sward with 240 kg N ha^{-1} year^{-1}.

Experiment B6-2. A farm-scale experiment on silage production comparing:
  a. grass/clover sward,
  b. maize,
  c. a vetch/oats mixture grown after the maize.

Experiment B6-3. A small plot cutting experiment to study the response of grass/clover to N and management:
  Nitrogen rates: 0, 40, 80 and 120 kg ha^{-1}, applied in Mid February.
  Date of the first cut: 30, 45 and 60 days after N application.

Experiment B6-1. Grazing experiment

Grassland

All treatments were applied in the same way in 1993, 1994 and 1995. The grazing trial was located on 12 ha, 7 ha for grazing and 5 ha for silage on the same mixed sward, established in the Autumn of 1992. The grazing area was divided into two independent areas, one of 3.7 ha, which received no N (group A) and the other one of 3.3 ha with 240 kg N ha^{-1} year^{-1} (group B). Fertiliser N was split into 4 applications of 40 kg ha^{-1} in Spring and 2 in Autumn.

Sampling

Yield of the grazed fields was determined by cutting five plots of 1 m^2 pre-grazing and post-grazing. The difference between the 2 values was considered as herbage intake by the cows. One sample was taken each time for manual determination of botanical composition, the other one for determination of dry matter (DM) content and chemical composition. Milk yield was recorded every day.

Animal management

Two groups of 14 cows (7 primiparous + 7 second-lactation Friesian cows) were used for rotational grazing of each area. Each area was divided into 12 paddocks. Grazing management aimed to maintain clover and quality of herbage. Grazing rotations were flexible trying to maintain the same height of pasture in both treatments: 15-20 cm at the start and 6 cm at the end of a grazing period. When paddocks accumulated too much herbage, herbage was cut for silage. Four or five grazing rotations were made in Spring and two in Autumn at each level of N. Cows were turned out to pasture after calving, starting in January and ending in March. Concentrate feeding was suppressed in early April. Normal summers in Galicia are with early drought and in most years cows have to be housed at the end of August.

Experiment B6-2. Farm-scale experiment on silage production

Silage production was monitored at farm scale, weighing the whole production (all loads) of the fields, and sampling for DM content and chemical composition, in order to evaluate the potential of different crops with low fertiliser inputs. This study included three crops: 1) grass/clover swards, 2) maize, and 3) vetch/oats grown after maize.

Fertiliser N applied to grass/clover silage was 50 kg ha^{-1} for the first cut and 40 kg ha^{-1} for the second cut in both treatments of experiment B6-1. P and K were applied in Winter at a rate of 75 kg ha^{-1}. During the growing season, grazing always had priority and the area for silage was flexible. The whole area of maize, from 9 to 12 ha, was cropped with a mixture of vetch and oats during Winter. Maize was fertilised with 50 kg N ha^{-1}; the mixture of vetch and oats was not fertilised at all. In the first 2 years, the seed mixture contained 40 kg ha^{-1} of vetch, variety Jaga, and 100 kg ha^{-1} of oats, variety Previsión. In the last 2 years, the same varieties were used, but the sowing rate was 100 kg ha^{-1} of vetch and 40 kg ha^{-1} of oats. Formic acid was added to the harvested forage at 3 l ton^{-1} to improve conservation. Information on sowing and harvesting dates of maize and the vetch/oats mixture is presented in Annex B6-5.

Experiment B6-3. Grass/clover response to nitrogen and management

The small-plot experiment to study the effects of fertiliser N, applied in early Spring, and cutting regime on the yield and botanical composition of a mixed sward, was established every year at a new site on a resown sward. This experiment included 12 treatments: 4 rates of N application *viz.* 0, 40, 80 and 120 kg ha^{-1}, combined with 3 cutting regimes. The experiment had a split-plot design with 4 replications. Fertiliser N was applied in February in accordance with the results of previous experiments on the use of N for early grazing (González, 1996a). The first cut was taken about 30, 45 or 60 days after N application (T1, T2 and T3, respectively; Table 2.17). The second cut was taken 25 to 45 days after the first one and the dates of following cuts depended on growth.

Table 2.17. Cutting dates in the small-plot experiment (B6-3) to study the response of a grass/clover sward to applied N and management.

	First cut			Second cut			Third cut		
Year	T1	T2	T3	T1	T2	T3	T1	T2	T3
1993	12/3	25/3	7/4	7/4	20/4	3/5	3/5	-	-
1994	21/3	5/4	19/4	5/5	19/5	2/6	22/6	5/7	18/7
1995	4/4	18/4	2/5	16/5	2/6	16/6	27/6	-	-

2.11 Sub-task C1

An experiment (C1-1) was established in 1993 on a young grass/clover sward at the experimental farm Droevendaal near Wageningen, The Netherlands, to study the effects of different slurry application techniques on the utilisation of slurry N, P and K. The sward consisted of *Lolium perenne* cv. Magella and *Trifolium repens* cv. Retor. It was sown in August 1992 on a field where arable crops had been grown during several years (peas in 1992). Establishment of the sward was slow due to a lack of rain in the latter part of the Summer of 1992. As a consequence, the start of the experiment had to be postponed to May 1993, after taking a light first cut. The first growth in 1993 received a uniform dressing of 30 kg P and 150 kg K ha^{-1}.

The soil of the experimental field was a sandy loam with 3.6% organic matter in the top layer, and a pH-KCl of 6.1 in November 1992. The P status of the soil was rather low (P-AL: 27 mg P_2O_5 per 100 g air-dry soil) and the K status was sufficient (K index: 25).

The experiment had a randomised-blocks design with 11 treatments and 3 replications. Cattle slurry was applied by 4 different techniques and its effects were compared with the effects of different combinations of artificial fertilisers (Table 2.18).

The total rate of slurry application was approximately 45 metric tons ha^{-1} year^{-1}. This rate was chosen to replace the estimated withdrawal of P in the harvested herbage (P replacement). In this experiment, the treatments 5, 10 and 11 can be considered as references that did not receive N, K or P, respectively (Table 2.18). The treatments 7 and 8 received the 3 main elements (N, P and K) contained in artificial fertilisers, and the treatments 1, 2, 3 and 4 received them in cattle slurry, applied by different techniques. The treatments 6 and 9 received additional P and K to evaluate the adequacy of the replacement rates of P and K in the treatments 5 and 8.

Table 2.18. Experimental treatments of experiment C1-1.

Treatments (code)	Periods and rates of application [1]		
	before cut 1	after cut 2	after cut 4
1. Cattle slurry (CS), surface application (SA)	CS	CS	
2. CS, diluted 1:2, surface application (SAD)	CS	CS	
3. CS, injection with open slits (Inj OS)	CS	CS	
4. CS, deep injection (Inj)	2CS		
5. Artificial fertilisers ($N_0P_2K_2$)	PK	PK	
6. Artificial fertilisers ($N_0P_3K_3$)	PK	PK	PK
7. Artificial fertilisers ($N_1P_2K_2$)	(½N)PK	(½N)PK	
8. Artificial fertilisers ($N_2P_2K_2$)	NPK	NPK	
9. Artificial fertilisers ($N_2P_3K_3$)	NPK	NPK	PK
10. Artificial fertilisers ($N_2P_2K_0$)	NP	NP	
11. Artificial fertilisers ($N_2P_0K_2$)	NK	NK	

[1] *rates of application:*
CS: about 22.5 metric tons of cattle slurry ha^{-1} ;
NPK: the same quantities of inorganic N, total P and total K as CS contains.

Slurry was obtained from dairy cattle fed a low-N diet based on maize silage in 1993, or a moderately N-rich diet based on grass silage in 1994 and 1995. The slurry was stored in a large container and within a year the same slurry was used at each application time (also in the other experiment conducted under sub-task C2). Slurry was applied with the Schepan MMM application unit of the former DLO Institute for Soil Fertility Research at Haren (Scheepers, 1978). This machine can be equipped with different application systems and has an accurate dosing unit that registers the rate actually applied. The surface spreader of the Schepan application unit distributes the slurry at a height of about 50 cm above the soil surface. The open-slit injector places the slurry in V-shaped slits of about 6 cm depth; the distance between these slits is 20 cm. The deep-injection unit places the slurry completely covered at a depth of 15 to 18 cm in the soil; the distance between injection tines is 50 cm. Artificial fertilisers used were calcium ammonium nitrate (27% N), triple superphosphate (45% P_2O_5) and potassium chloride (60% K_2O). The rates of application were planned to be similar to the contents of inorganic N, total P and total K in the slurry applied. As the rates of K application did not completely replace the withdrawal of K in the herbage harvested, an additional 75 kg fertiliser K ha^{-1} was applied to all the experimental plots in 1995. Artificial fertilisers were broadcasted with an adapted sowing-machine.

The size of the experimental plots was 10 m x 2.70 m. Herbage yields were determined by harvesting 15 m^2 from the centre of each plot with a Haldrup plot harvester at a cutting height of about 5 cm. Two samples of 500–700 g fresh herbage were taken from each plot, one for direct analysis of the DM, N, P and K contents of the mixture, and the other one for determination of the clover content and the DM, N, P and K contents of the separated grass and clover components of the mixture. Dry matter content was determined after drying the fresh herbage overnight at 70/105 °C under forced ventilation. Total N content of the dried and ground herbage (1 mm sieve) was assessed according to Dumas (Macro N, Foss Heraeus) and the P content was assessed colorimetrically (Starrcol) after destruction with H_2SO_4/HNO_3.

At each slurry application time, 3 or 4 samples, both of the untreated and the diluted slurry, were taken for analysis of DM, total N, inorganic N, total P and total K. Dry matter content was determined by pre-drying the slurry overnight under reduced pressure at 50 °C and drying the residue at 105 °C. Total N was determined according to Deys (1961) with a KJELTEC AUTO 1030 Analyzer (Tecator) after destruction with sulfuric and salicylic acid. For the determination of inorganic N, the slurry was mixed with demineralised water (w : v = 1 : 20) and NH_4^+-N was determined colorimetrically in the filtrate (TRAACS 800 system of Bran & Luebbe).

The composition of the slurry samples hardly differed within a year. Mean compositions are shown in Table 2.19.

Table 2.19. Composition (kg per metric ton) of the cattle slurries applied in experiment C1-1. Means of 6 samples, 3 at each period of application.

Year	Total N	Inorganic N	Total P	Total K	Dry matter
Untreated slurry					
1993	4.51	2.18	0.68	5.05	94.7
1994	5.47	2.94	0.82	6.75	81.9
1995	5.79	3.11	0.93	5.69	102.2
Diluted slurry					
1993	1.61	0.83	0.24	1.69	30.3
1994	1.52	0.84	0.22	1.88	19.2
1995	1.69	0.94	0.24	1.63	25.8

The rates of application of inorganic N, total P and total K are presented in Annex C1-1. For slurry, these rates have been calculated by multiplying the rates of slurry actually applied by the mean concentrations at each time of application.
In February 1995 and 1996, soil samples of the layer 0-20 cm have been taken for analysis of the P and K status. The samples were composed of 8 cores with a diameter of about 4 cm. They were analysed by the Laboratory for Soil and Crop Testing at Oosterbeek, The Netherlands.

2.12 Sub-task C2

Experiment C2-1 was established in 1993 on a young grass/clover sward at the experimental farm Droevendaal near Wageningen, The Netherlands. The sward consisted of *Lolium perenne* cv. Magella and *Trifolium repens* cv. Retor. It was sown in August 1992 on a field where arable crops had been grown during several years (peas in 1992). Establishment of the sward was slow due to a lack of rain in the latter part of the Summer of 1992. Consequently, the sward was not sufficiently firm in the Spring of 1993 to carry the slurry application equipment, and the start of the experiment had to be postponed to May, after taking a light first cut. The first growth in 1993 received a uniform dressing of 17.5 kg P and 66 kg K per ha. The soil of the experimental field was a sandy loam with 3.6% organic matter in the top layer of 0-20 cm, and a pH-KCl of 6.1 in November 1992. The P status of the soil was rather low according to the standards (P-AL: 27 mg P_2O_5 per 100 g air-dry soil) and the K status was sufficient (K-index: 25).

The experiment had a split-plot design with the type of sward as the main plots and the slurry and artificial fertiliser treatments as the sub-plots. The experiment had 3 replications. The types of sward were a mixed sward of 'Magella' perennial ryegrass and 'Retor' white clover, and a pure grass sward. The pure grass sward was obtained by herbicide treatment of the mixed sward during early growth in 1993. White clover completely disappeared by this treatment.

This experiment compared the effects on herbage yield and composition of 3 techniques of low-emission slurry application and artificial fertilisers. The low-emission slurry application techniques were: surface application of diluted slurry (slurry:water = 1:2), injection with open slits and deep injection. The rate of slurry application aimed to replace the estimated withdrawal of P in the harvested crop (P replacement). Given the composition of available cattle slurry, this rate was about 45 metric tons ha^{-1} $year^{-1}$. With the techniques surface application of diluted slurry and injection with open slits, the slurry was applied in 2 equal rates, and with deep injection in 1 rate, both according to common practice.

The effects of slurry were compared with the effects of the following combinations of nutrients from artificial fertilisers: NPK, applied to an untreated sward as well as to a sward treated with the open-slit injector to quantify effects of the injection equipment on the sward, and PK applied to an untreated sward. The rates of fertiliser N, P and K equalled the contents of inorganic N, total P and total K of the slurry applied. In addition, all the slurry and fertiliser-treated plots in this experiment received 50% extra P and K from artificial fertilisers to assure an ample supply of these nutrients. The 3 periods of application of both slurry and fertiliser NPK were: (1) to the first and second cut, (2) to the third and fourth cut, and (3) to the first and third cut.

Table 2.20 presents the slurry and fertiliser treatments applied to the mixed and the pure grass sward. The treatments 15, 16 and 17 did not receive slurry or fertiliser N and can be considered as controls.

Slurry was obtained from dairy cattle fed a low-N diet based on maize silage in 1993, or a moderately N-rich diet based on grass silage in 1994 and 1995. The slurry was stored in a large container and within a year the same slurry was used at each application time and also in the other experiment conducted under sub-task C1. Slurry was applied with the Schepan MMM application unit of the former DLO Institute for Soil Fertility Research at Haren (Scheepers, 1978). This machine can be equipped with different application systems and it has an accurate dosing unit that registers the rate actually applied. The surface spreader of the Schepan application unit distributes the slurry at a height of about 50 cm above the soil surface. The open-slit injector places the slurry in V-shaped slits of about 6 cm depth; the distance between these slits is 20 cm. The deep-injection unit places the slurry completely covered at a depth of 15 to 18 cm in the soil; the distance between injection tines is 50 cm. Artificial fertilisers used were calcium ammonium nitrate (27% N), triple superphosphate (45% P_2O_5) and potassium chloride (60% K_2O). Artificial fertilisers were broadcasted with an adapted sowing-machine.

The size of the experimental plots was 10 m x 2.70 m. Herbage yields were determined by harvesting 15 m^2 from the centre of each plot with a Haldrup plot harvester at a cutting height of about 5 cm. Two samples of 500-700 g fresh herbage were taken from each plot, one for direct analysis of the dry matter and N contents of the mixture, and the other one for determination of the clover content and the dry matter and N contents of the separated grass and clover components of the mixture. Dry matter content was determined after drying the fresh herbage overnight at 70/105 °C under forced ventilation. Total N content of the dried and ground herbage (1 mm sieve) was assessed according to Dumas (Macro N, Foss Heraeus).

At each slurry application time, 3 or 4 samples, both of the untreated and the diluted slurry, were taken for analysis of dry matter, total N, inorganic N, total P and total K. Dry matter content was determined by pre-drying the slurry overnight under reduced pressure at 50 °C and drying the residue at 105 °C. Total N was determined according to Deys (1961) with a KJELTEC AUTO 1030 Analyzer (Tecator) after destruction with sulfuric and salicylic acid. For the determination of inorganic N, the slurry was mixed with demineralised water (w:v = 1:20) and NH_4^+-N was determined colorimetrically in the filtrate (TRAACS 800 system of Bran & Luebbe). The composition of the slurry samples hardly differed within a year. Mean compositions are shown in Table 2.21.

Table 2.20. Slurry and fertiliser application treatments in experiment C2-1.

Treatments (code)	Type and period of application 1)			
	before cut 1	2	3	4
1. CS, diluted 1:2, surface application (SAD 1,2)	CS	CS		PK
2. CS, diluted 1:2, surface application (SAD 3,4)	PK		CS	CS
3. CS, diluted 1:2, surface application (SAD 1,3)	CS		CS	PK
4. CS, injection with open slits (Inj OS 1,2)	CS	CS		PK
5. CS, injection with open slits (Inj OS 3,4)	PK		CS	CS
6. CS, injection with open slits (Inj OS 1,3)	CS		CS	PK
7. CS, deep injection (Inj 1)	2CS			PK
8. CS, deep injection (Inj 3)	PK		2CS	
9. Cutting with open-slit injector + NPK (Inj OS NPK 1,2)	NPK	NPK		PK
10. Cutting with open-slit injector + NPK (Inj OS NPK 3,4)	PK		NPK	NPK
11. Cutting with open-slit injector + NPK (Inj OS NPK 1,3)	NPK		NPK	PK
12. Artificial fertilisers, NPK (NPK 1,2)	NPK	NPK		PK
13. Artificial fertilisers, NPK (NPK 3,4)	PK		NPK	NPK
14. Artificial fertilisers, NPK (NPK 1,3)	NPK		NPK	PK
15. Artificial fertilisers, PK (PK 1,2)	PK	PK		PK
16. Artificial fertilisers, PK (PK 3,4)	PK		PK	PK
17. Artificial fertilisers, PK (PK 1,3)	PK		PK	PK

1) *types of application:*
CS: about 22.5 metric tons of cattle slurry per ha
NPK: the same quantities of inorganic N, total P and total K as CS contains.

Table 2.21. Composition (kg per metric ton) of the cattle slurries applied in experiment C2-1. Means of at least 12 samples, 3 or 4 at each period of application.

Year	Total N	Inorganic N	Total P	Total K	Dry matter
Untreated slurry					
1993	4.50	2.27	0.75	5.20	97.0
1994	5.40	3.09	0.80	6.78	79.2
1995	5.65	3.10	0.89	5.77	99.6
Diluted slurry					
1993	1.51	0.76	0.23	1.62	29.0
1994	1.49	0.88	0.22	1.79	19.0
1995	1.61	0.96	0.23	1.66	24.9

The rates of application of inorganic N, total P and total K are presented in Annex C2-1. For slurry, these rates have been calculated by multiplying the rates of slurry actually applied by the mean concentrations at each time of application.

Inorganic N in the soil was measured on some experimental treatments in the autumn of each year. In 1993 and 1994, one sample composed of 8 cores was taken from each experimental treatment sampled, and subdivided into the layers 0-20, 20-40 and 40-60 cm. In 1995, when the amount of residual inorganic N was expected to be higher than in 1993 and 1994, the treatments were sampled in triplicate. For the determination of inorganic N, the soil sample was extracted with 1 N KCl solution for 1 hour using a soil : water ratio of 1 : 2.5 (w : v), and nitrate N and ammonium N were determined colorimetrically in the filtrate (TRAACS 800 system of Bran & Luebbe).

Definitions

The efficiency of utilisation of slurry or fertiliser N can be expressed as apparent N efficiency (ANE) and apparent N recovery (ANR) (Van der Meer *et al.*, 1987). In this report, ANE is the increase of harvested herbage DM per kg inorganic N applied in slurry or fertiliser. ANR is the increase of the amount of N contained in the harvested herbage, expressed as a percentage of the amount of inorganic N applied in slurry or fertiliser. In this experiment, slurry N and fertiliser N were compared on the basis of inorganic N. In other experiments, it has been shown that for low-emission techniques of slurry application, the effectivity of slurry N is well characterised by its content of inorganic N. In other words: equal rates of slurry inorganic N, applied by low-emission techniques, and fertiliser N have approximately the same effect on herbage DM and N yield (Geurink & Van der Meer, 1995). Effects of slurry or fertiliser N on the components of the mixture are also assessed and compared on the basis of ANE and ANR.

The contribution of white clover to the N yield in the harvested herbage can be expressed as the apparent N fixation (ANF). ANF is the difference between the N yields of the mixed sward and the grass in monoculture at a given rate of N application. ANF can be expressed per metric ton of clover DM harvested; this is called the N fixation efficiency (NFE).

2.13 Sub-task C3

A maize experiment of three years, experiment C3-1, was carried out at 'F. Ricchieri' experimental farm in Fiume Veneto (PN), Italy. The treatments of this experiment were obtained combining 4 rates, 2 periods and 2 methods of slurry application. The rates of slurry application were: 0, 80, 160 and 240 kg inorganic N ha^{-1}; the periods of slurry application were Winter and Spring; the methods of application were surface spreading without incorporation and surface spreading with immediate incorporation by ploughing. In addition, 4 treatments with artificial N fertiliser (0, 80, 160 and 240 kg ha^{-1}, applied at maize sowing) were considered and another treatment was obtained combining slurry and fertiliser N according to the EC Directive on Nitrates (91/676/EC) and the National disposition of the Italian Ministry (DPR No. 13 of 20/01/92). In the combined treatment, slurry (95 kg inorganic N ha^{-1}) was applied during the winter period and immediately incorporated, while fertiliser N (145 kg ha^{-1}) was applied at the seven-leaves stage. Corrections with inorganic P and K were made in order to give all the treatments the same rate of P and K as the treatment with the highest rate of slurry.

The field experiment had a split-plot design and 4 replicates, giving a total of 88 sub-plots. Simultaneously with the field trials, a lysimeter experiment was carried out to measure nitrate leaching from the following treatments: 0, 160 and 240 kg inorganic N ha^{-1} from slurry applied on 3 February 1993, 18 May 1994 and 5 May 1995 and immediately incorporated into the soil. The slurry was produced by 100 cows (Italian Simmenthal and Holstein-Friesian) in the herd of 'De Munari' at San Vito.

Soil characteristics of the experimental site are summarised in Table 2.22.

Table 2.22. Characteristics of the soil in experiment C3-1.

Sand (%)	33	P (ppm)	24
Silt (%)	22	K (ppm)	113
Clay (%)	45	Mg (ppm)	328
pH-H_2O	8.1	Fe (ppm)	19.4
pH-KCl	7.5	Mn (ppm)	7.6
Limestone (%)	12.3	Zn (ppm)	0.93
Organic C (%)	1.07	Cu (ppm)	4.2
Total N (%)	0.09	B (ppm)	0.2

Table 2.23 presents the chemical characteristics of the slurries used.

Table 2.23. Chemical composition of the slurries applied in experiment C3-1 (in % of fresh weight).

Component	1993	Winter 1994	Spring 1994	Winter 1995	Spring 1995
Total N	0.42	0.28	0.29	0.34	0.43
Inorganic N	0.21	0.13	0.14	0.17	0.22
Dry matter	9.2	5.8	6.5	7.4	9.5
Organic matter	6.9	4.3	4.6	5.4	6.7
P_2O_5	0.23	0.17	0.16	0.21	0.26
K_2O	0.55	0.35	0.30	0.37	0.55

Table 2.24 presents the dates of slurry application and of sowing and harvesting the maize.

Table 2.24. Dates of slurry application and of sowing and harvesting the maize in experiment C3-1.

	1st year	2nd year	3rd year
Slurry application	03/02/93 (Winter)	23/11/93 (Winter)	30/11/94 (Winter)
	13/04/93 (Spring)	27/04/94 (Spring)	13/04/95 (Spring)
Sowing	23/04/93	03/05/94	24/04/95
Harvesting (silage stage)	16/09/93	06/09/94	26/09/95

Geodesinfestation with Carbosulfan (500 g ha^{-1}) at sowing and weeding with Metholaclor + Terbutilazina (1800 g ha^{-1} + 900 g ha^{-1}) were made each year on all the plots.

Irrigation was carried out as follows: 13/5/93: 20 mm, 21/5/93: 30 mm, 26/5/93: 20 mm, 9/6/93: 20 mm; 8/6/94: 25 mm, 1/7/94: 25 mm, 7/7/94: 25 mm, 15/7/94: 25 mm, 23/7/94: 25 mm, 30/7/94: 25 mm, 6/8/94: 25 mm, 11/8/94: 25 mm, 18/8/94: 25 mm; 9/5/95: 25 mm, 12/7/95: 25 mm, 26/7/95: 25 mm, 2/8/95: 25 mm.

The maize hybrid 'Costanza' (FAO 600) was used. In 1993, due to an unusual dry period, maize had to be re-sown using the hybrid 'Messicano' (FAO 500). The final density of maize was 75,000 plants ha^{-1}. Yields were registered at silage stage, harvesting the 4 central rows of each sub-plot and samples were taken for chemical analyses. Soil samples were taken immediately before maize sowing and after harvest. Soil samples were immediately frozen and stored at –18 °C.
N content of maize DM and inorganic N content of the soil profile were determined.
Crop and soil analyses were carried out at the ERSA laboratory; official methods were employed.

2.14 Sub-task C4

Maize treatments have been described in Paragraph 2.13. They were obtained combining 4 rates, 2 periods and 2 methods of slurry application. The rates of slurry application were: 0, 80, 160 and 240 kg inorganic N ha^{-1}; the periods of application were Winter and Spring, and the methods of application were surface spreading without incorporation and with immediate incorporation by ploughing. Each year, Italian ryegrass was sown after the harvest of silage maize to study utilisation of residual soil inorganic N (experiment C4-1). Soil preparation was done by ploughing and harrowing. No fertiliser or slurry was applied to the Italian ryegrass.
Dates of sowing and harvesting Italian ryegrass are reported in Table 2.25. The varieties used were Lirasand in 1993 and 1994 and Asso in 1995. The seeding rate was 50 kg ha^{-1} in 1993 and 40 kg ha^{-1} in 1994 and 1995.

Table 2.25. Dates of sowing and harvesting Italian ryegrass in experiment C4-1.

	1993-94	1994-95	1995-96
Sowing	31/10/93	13/09/94	04/10/95
Harvesting	09/05/94	08/05/95	23/05/96

Herbage yield was determined and soil samples were taken immediately after harvest to evaluate the content of inorganic N. Herbage and root samples were taken from each plot to determine N content.

Root samples were obtained by washing root-soil lumps of 30 cm x 30 cm x 15 cm. Simultaneously with the field trials, lysimeter experiments were carried out to measure nitrate leaching. Lysimeter plots with 0, 160 and 240 kg inorganic slurry N ha^{-1}, applied to maize in Spring, were sown to Italian ryegrass. A fourth lysimeter test plot with 240 kg of inorganic N ha^{-1} was uncultivated after maize harvest to measure nitrate leaching from bare soil as a reference for the determination of the nitrate catching capacity of Italian ryegrass.

3. Results

3.1 Sub-task A1

The energy and protein values of all feedstuffs are shown in the Tables 3.1, 3.2 and 3.3. The results from the experiments with different AAT levels, testing the utilisation of home-grown feed protein, are shown in Table 3.1. Rumen-degradability of protein in grass/clover was lower in experiment A1-4 than in experiment A1-1. A high rumen-degradability of protein generally means a low true digestibility of undegraded protein in the small intestine (Hvelplund *et al.*, 1992). Both factors are thus influencing the AAT value in the same direction. There was a clear difference in AAT values of grass/clover silage between experiments A1-1 and A1-4 (Table 3.1). In pea silage, the AAT value tended to be higher in experiment A1-4 than in experiment A1-1.

Protein degradability of the pelleted feed mixtures was calculated from degradability determined in single ingredients of the mixtures. The difference in AAT values of the three concentrate mixtures was much larger in experiment A1-1 than in experiment A1-4, because the rumen-degradability of protein in heat-treated rapeseed cake was essentially lower in experiment A1-1 (410 g kg^{-1} crude protein) than in experiment A1-4 (550 g kg^{-1} crude protein). Barley grain, on the other hand, had an exceptionally high concentration of protein (150 compared with 138 g kg^{-1} DM) with a low degradability (620 g kg^{-1} crude protein compared with 770 g kg^{-1} crude protein) in experiment A1-4 compared with experiment A1-1. Thus, replacement of rapeseed cake by barley resulted in much larger differences in AAT values of the concentrate mixtures in experiment A1-1 (from 137 to 105 g AAT kg^{-1} DM) than in experiment A1-4 (from 132 to 120 g AAT kg^{-1} DM).

Results from the early-lactation experiments with varying PBV values are shown in Table 3.2. The degradabilities of protein in wheat silage and fodder beet were lower in experiment A1-5 than in experiment A1-2. Thus, AAT values were higher and PBV values lower in experiment A1-5 than in experiment A1-2. In heat-treated rapeseed cake the opposite was the case. The protein concentration of oat was extremely low in experiment A1-5.

The results in Table 3.3 are from the experiments with low PBV values in late lactation. The AAT and PBV values of the feedstuffs fed in both experiments were similar.

Table 3.1. *Energy and protein values of feeds used in the experiments A1-1 and A1-4.*

	Grass/clover silage		Pea silage		Feed mix. no. 1		Feed mix. no. 2		Feed mix. no. 3	
	A1-1	A1-4	A1-1	A1-4	A1-1	A1-4	A1-1	A1-4	A1-1	A1-4
Dry matter, g kg^{-1}	400	310	250	310	863	891	868	907	862	880
Crude ash, g kg^{-1} DM	100	85	60	67	35	37	30	32	24	27
Crude protein, g kg^{-1} DM	130	133	156	176	219	221	190	203	160	175
RDP1), g kg^{-1} crude protein	860	780	860	830	530	550	590	570	670	590
TDAA2), g kg^{-1} undeg. feed AA	360	590	500	660	840	820	830	840	800	850
AAT3), g kg^{-1} DM	68	75	72	76	137	132	121	126	105	120
PBV4), g kg^{-1} DM	5	-3	24	40	10	16	0	8	-9	-11
DOM5), g kg^{-1} organic matter	810	770	800	800	840	850	840	840	850	850
SFU6), kg^{-1} DM	0.91	0.84	0.97	0.94	1.19	1.21	1.20	1.21	1.20	1.21

1) *Rumen-degradability of feed protein*

2) *True intestinal digestibility of undegraded feed amino acids (AA)*

3) *Amino acids absorbed from the small intestine*

4) *Protein balance in the rumen*

5) *Sheep digestibility of organic matter predicted from* in vitro *digestion either enzymatically or in rumen liquor*

6) *Scandinavian feed units*

Table 3.2. Energy and protein values of feeds used in the experiments A1-2 and A1-5.

	Wheat silage			Fodder beet		Oat grain		Rapeseed cake	
	A1-2	A1-5 (silo 6)	A1-5 (silo 7)	A1-2	A1-5	A1-2	A1-5	A1-2	A1-5
Dry matter, g kg^{-1}	380	513	478	176	160	852	867	895	897
Crude ash, g kg^{-1} DM	55	52	61	89	60	28	28	68	67
Crude protein, g kg^{-1} DM	89	78	78	72	65	129	89	348	315
RDP1), g kg^{-1} crude protein	800	650	680	850	800	660	680	410	580
TDAA2), g kg^{-1} undeg. feed AA	550	720	670	530	490	850	860	830	760
AAT3), g kg^{-1} DM	69	80	76	87	91	93	87	179	122
PBV4), g kg^{-1} DM	-37	-62	-57	-79	-94	-18	-50	85	121
DOM5), g kg^{-1} organic matter	700	710	700	900*)	900*)	720	730	800	810
SFU6), kg^{-1} DM	0.74	0.75	0.73	0.99	1.03	0.92	0.91	1.27	1.31

1) *Rumen-degradability of feed protein*

2) *True intestinal digestibility of undegraded feed amino acids (AA)*

3) *Amino acids absorbed from the small intestine*

4) *Protein balance in the rumen*

5) *Sheep digestibility of organic matter predicted from* in vitro *digestion either enzymatically or in rumen liquor*

6) *Scandinavian feed units*

*) *Tabulated value*

Table 3.3. *Energy and protein values of feeds used in the experiments A1-3 and A1-6.*

	Wheat silage	Barley silage		Fodder beet		Oat grain	Barley grain	Rapeseed cake	
	A1-3	A1-6 (silo 4)	A1-6 (silo 5)	A1-3	A1-6	A1-3	A1-6	A1-3	A1-6
Dry matter, g kg^{-1}	371	426	428	164	167	884	854	897	910
Crude ash, g kg^{-1} DM	52	59	59	144	88	26	21	63	62
Crude protein, g kg^{-1} DM	87	86	111	72	83	122	105	327	341
RDP[1], g kg^{-1} crude protein	820	780	710	780	750	690	670	580	570
TDAA[2], g kg^{-1} unde. feed AA	500	660	700	680	460	840	840	790	790
AAT[3], g kg^{-1} DM	70	71	77	85	88	94	103	125	130
PBV[4], g kg^{-1} DM	-38	-39	-27	-75	-76	-29	-62	134	141
DOM[5], g kg^{-1} organic matter	710	690	720	900[*]	900[*]	770	840	810	810
SFU[6], kg^{-1} DM	0.75	0.72	0.80	0.91	1.00	1.00	1.11	1.35	1.35

[1] *Rumen-degradability of feed protein*

[2] *True intestinal digestibility of undegraded feed amino acids (AA)*

[3] *Amino acids absorbed from the small intestine*

[4] *Protein balance in the rumen*

[5] *Sheep digestibility of organic matter predicted from* in vitro *digestion either enzymatically or in rumen liquor*

[6] *Scandinavian feed units*

[*] *Tabulated value*

Results concerning voluntary feed intake, protein supply and performance of cows fed home-produced forage protein in diets with different calculated AAT values are shown in Tables 3.4 and 3.5. The differences in AAT values of feedstuffs between the two years resulted in differences in the range of AAT concentration per FU. In experiment A1-1, there was a large difference, from 95 to 81 g AAT FU^{-1} (Table 3.4). In the following year, the range between the highest and lowest level was only 6 g AAT FU^{-1} (Table 3.5). The level of PBV varied slightly with the level of AAT, but was positive in all diets and at a rather low level (50-350 g day^{-1}).

At the same level of AAT (95 g FU^{-1}), the levels of feed intake and milk yield were nearly equal in the two years. Within experiments, feed intake increased significantly with an increasing level of AAT. In experiment A1-1, the level of AAT varied from 81 to 95 g FU^{-1}, while the daily yield of milk and energy-corrected milk (ECM) increased by approximately 2 and 3 kg cow^{-1}, respectively, although the effect on milk yield was not statistically significant. At the higher level of AAT in experiment A1-4 (between 92 and 98 g FU^{-1}), milk yield did not vary with AAT level. The protein concentration in the milk decreased at the lowest level of AAT in experiment A1-1. In experiment A1-4, a similar tendency was observed at 92 g AAT FU^{-1}, but the difference was not significant. The effect of AAT on milk protein yield was highly significant in experiment A1-1. In experiment A1-4, protein yield also tended to be lower at 92 g AAT FU^{-1} than at higher levels. Fat content of milk was relatively low in these experiments and the protein to fat ratio rather high, in most cases between 0.9 and 1.0.

Table 3.4. *Experiment A1-1. Daily feed intake, protein intake, milk yield, and concentration and yield of milk constituents of cows fed diets with different levels of AAT.*

	AAT, g FU^{-1}			SEM[1]	p
	95	88	81		
No. of cows	9	9	9		
AAT, g cow^{-1}	1880	1701	1454		
PBV, g cow^{-1}	217	129	50		
Crude protein, g kg^{-1} DM	174	161	148		
Feed units	19.8	19.4	17.9		
Total feed intake, kg DM	19.0	18.5	17.1	0.5	0.016
Milk, kg	33.9	32.6	31.8	0.8	0.21
Fat, g kg^{-1}	32.7	33.2	30.4	1.6	0.27
Protein, g kg^{-1}	32.0	31.7	30.2	0.4	0.012
Lactose, g kg^{-1}	50.4	51.0	50.8	0.3	0.66
Fat, g	1110	1081	967	50	0.15
Protein, g	1086	1033	961	18	0.0009
ECM[2], kg	31.1	30.1	27.9	0.7	0.048

[1] *Standard error of mean*

[2] *Energy-corrected milk (Sjaunja et al., 1990)*

Table 3.5. Experiment A1-4. Daily feed intake, protein intake, milk yield, and concentration and yield of milk constituents of cows fed diets with different levels of AAT.

	AAT, g FU^{-1}			SEM[1)]	p
	98	95	92		
No. of cows	9	9	9		
AAT, g cow^{-1}	2000	1897	1797		
PBV, g cow^{-1}	346	268	103		
Crude protein, g kg^{-1} DM	183	175	162		
Feed units	20.5	20.0	19.5		
Total feed intake, kg DM	20.1	19.5	19.1	0.3	0.051
Milk, kg	33.1	33.8	33.5	0.9	0.83
Fat, g kg^{-1}	36.1	35.5	37.2	1.5	0.67
Protein, g kg^{-1}	32.9	32.5	31.2	0.5	0.15
Lactose, g kg^{-1}	48.8	49.4	49.2	0.5	0.49
Fat, g	1196	1201	1246	33	0.52
Protein, g	1090	1097	1046	17	0.11
ECM[2)], kg	31.7	32.1	32.2	0.5	0.83

[1)] and [2)] see Table 3.4

Results from experiments in which cows in early lactation received feed from the energy-cropping system and with different PBV levels in the diets are shown in Tables 3.6 and 3.7. Silage intake in experiment A1-5 was lower than in experiment A1-2. This difference can not simply be explained by differences in feeding value. The concentration of butyric acid was rather high (7 g kg^{-1} DM), particularly in one of the two silos used in experiment A1-5. But the difference in quality alone probably can not explain the difference in feed intake. Energy intake in the experiments A1-2 and A1-5 was lower than in the experiments A1-1 and A1-4, primarily because the energy value of the wheat silage (Table 3.2) was much lower than that of the grass/clover and pea silage (Table 3.1) . Total DM intake was at the same level in the two sets of experiments.

In experiment A1-2, the levels of PBV intake were close to the intended levels, while they were much lower than intended in experiment A1-5 (-295 to -660 g day^{-1}). The reason of the low values in experiment A1-5 was that the rumen-degradability of protein in both wheat silage and fodder beet, and the protein concentration in oat were considerably lower than standard values. The level of AAT was rather high in experiment A1-2, primarily because of a lower than expected rumen-degradability of protein in heat-treated rapeseed cake.

Table 3.6. Experiment A1-2. Daily feed intake, protein intake, milk yield, and concentration and yield of milk constituents of cows given different levels of PBV in early lactation.

	PBV, g cow^{-1}			SEM1)	p
	-81	-314	-527		
No. of cows	9	8	9		
AAT, g cow^{-1}	1746	1752	1705		
AAT, g FU^{-1}	102	102	103		
Crude protein, g kg^{-1} DM	145	132	120		
Total intake, kg DM	19.8	19.9	19.3		
Feed units	17.1	17.2	16.6		
Voluntary silage intake, kg DM	9.0	9.1	8.5	0.3	0.47
Milk, kg	29.6	26.7	27.1	0.8	0.043
Fat, g kg^{-1}	43.1	42.9	43.7	1.2	0.81
Protein, g kg^{-1}	32.3	32.9	31.6	0.3	0.11
Lactose, g kg^{-1}	49.4	49.9	50.5	0.4	0.041
Fat, g	1277	1146	1185	42	0.14
Protein, g	955	879	857	25	0.037
ECM2), kg	30.8	27.9	28.4	0.9	0.097

1) and 2) see Table 3.4

Table 3.7. Experiment A1-5. Daily feed intake, protein intake, milk yield, and concentration and yield of milk constituents of cows given different levels of PBV in early lactation.

	PBV, g cow^{-1}			SEM1)	p
	-295	-483	-660		
No. of cows	8	8	8		
AAT, g cow^{-1}	1686	1631	1555		
AAT, g FU^{-1}	95	95	94		
Crude protein, g kg^{-1} DM	131	121	111		
Total intake, kg DM	18.3	17.6	16.5		
Feed units	17.7	17.2	16.5		
Voluntary silage intake, kg DM	7.5	6.8	5.7	0.4	0.015
Milk, kg	27.7	25.5	24.3	0.8	0.024
Fat, g kg^{-1}	35.6	36.2	34.5	2.1	0.77
Protein, g kg^{-1}	30.6	30.8	30.2	0.6	0.25
Lactose, g kg^{-1}	49.2	48.1	49.1	0.5	0.97
Fat, g	987	924	839	54	0.19
Protein, g	848	785	734	20	0.0042
ECM2), kg	25.8	23.8	22.6	0.9	0.068

1) and 2) see Table 3.4

Only in experiment A1-5, silage intake was significantly reduced by a reduction in PBV level, while differences in intake in experiment A1-2 were small. There was a clear effect of low PBV on milk yield. The yields of milk and ECM decreased by approximately 2.5 kg cow^{-1} day^{-1} from the highest to the lowest level of PBV in experiment A1-2, and by approximately 3 kg cow^{-1} day^{-1} in experiment A1-5. Protein concentration in milk tended to decrease at the lowest level of PBV, but the differences were not statistically significant. The yield of milk protein decreased significantly in both experiments.

Tables 3.8 and 3.9 show the results of similar experiments carried out in late lactation (experiments A1-3 and A1-6). In these experiments, there was a reduction in PBV levels from nearly zero to -405 and -449 g cow^{-1} day^{-1}, respectively. The AAT values were 93 and 95 g FU^{-1}. Energy intake and milk yield were higher in experiment A1-6 than in experiment A1-3. As in experiment A1-2, there were only minor effects on silage intake by reducing the PBV to -400 or -450 g cow^{-1} day^{-1}. Yield of milk protein and ECM were negatively affected by a reduction in PBV, but the effect on protein concentration in milk was not clear.

Table 3.8. Experiment A1-3. Daily feed intake, protein intake, milk yield, and concentration and yield of milk constituents of cows given different levels of PBV in late lactation.

	PBV, g cow^{-1}			SEM[1)]	p
	-1	-219	-405		
No. of cows	8	8	8		
AAT, g cow^{-1}	1336	1314	1232		
AAT, g FU^{-1}	93	93	93		
Crude protein, g kg^{-1} DM	139	126	113		
Total intake, kg DM	16.7	16.8	15.9		
Feed units	14.4	14.2	13.3		
Voluntary silage intake, kg DM	9.3	9.4	8.5	0.2	0.045
Milk, kg	20.6	19.7	18.4	0.6	0.041
Fat, g kg^{-1}	44.8	46.1	45.1	0.8	0.40
Protein, g kg^{-1}	32.4	32.6	31.7	0.3	0.69
Lactose, g kg^{-1}	50.6	50.2	50.0	0.3	0.35
Fat, g	923	909	829	29	0.092
Protein, g	667	642	583	15	0.0058
ECM[2)], kg	22.0	21.4	19.6	0.6	0.028

1) and 2) see Table 3.4

Table 3.9. Experiment A1-6. Daily feed intake, protein intake, milk yield, and concentration and yield of milk constituents of cows given different levels of PBV in late lactation.

	PBV, g cow^{-1}			SEM[1)]	p
	-30	-262	-449		
No. of cows	8	8	8		
AAT, g cow^{-1}	1545	1580	1513		
AAT, g FU^{-1}	95	95	95		
Crude protein, g kg^{-1} DM	144	131	121		
Total intake, kg DM	18.0	18.6	17.6		
Feed units	16.2	16.6	15.9		
Voluntary silage intake, kg DM	10.1	10.7	9.7	0.3	0.19
Milk, kg	23.2	22.6	21.8	0.6	0.23
Fat, g kg^{-1}	41.6	43.4	41.4	0.9	0.24
Protein, g kg^{-1}	31.6	32.8	31.7	0.2	0.057
Lactose, g kg^{-1}	50.9	48.2	48.5	0.2	0.12
Fat, g	964	981	903	21	0.072
Protein, g	734	742	691	16	0.090
ECM[2)], kg	23.8	23.6	22.0	0.4	0.058

[1)] and [2)] see Table 3.4

Crude protein concentration in the diets in these experiments was 148 to 174 g kg^{-1} DM in experiment A1-1, 162 to 183 g kg^{-1} DM in experiment A1-4, and as low as 111 to 145 g kg^{-1} DM in experiments A1-2, A1-5, A1-3 and A1-6.

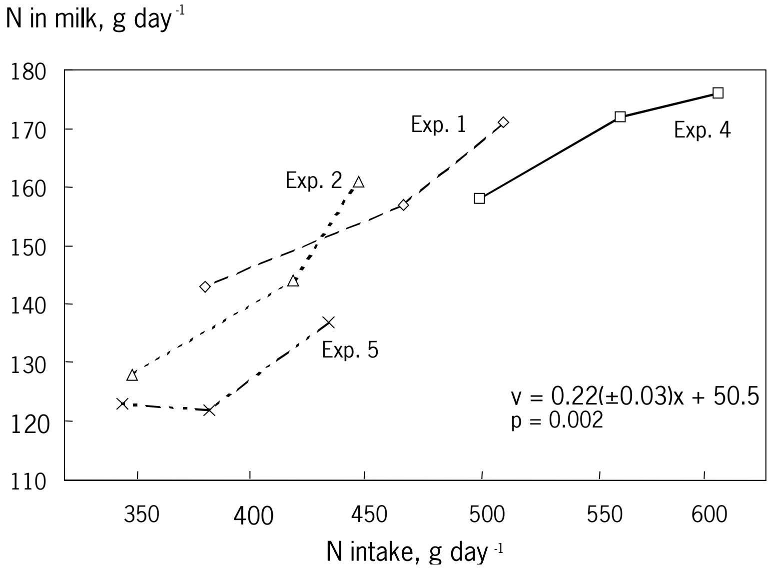

Figure 3.1. Relationship between total N intake and N retention in milk in the feeding experiments with cows in early lactation.

Tables 3.10 and 3.11 show the N balances of cows fed diets with different AAT levels (experiments A1-1 and A1-44) and different PBV contents (experiments A1-2 and A1-5), respectively. The excretion of N in urine ranged from 55 to 88 g cow^{-1} day^{-1} in the experiments A1-2 and A1-5 at low PBV values to between 132 and 190 g cow^{-1} day^{-1} in experiment A1-4 at high levels of AAT and total N intake. Also retention of N in milk increased with increasing N intake. Within experiments, approximately 220 g kg^{-1} of an increase in N intake was retained in milk, and this relationship seemed to be much the same whether the variation in N intake was caused by varying AAT or PBV levels (Figure 3.1). The proportion of N retained in milk was high in all experiments, ranging from about 300 to 360 g kg^{-1} ingested N. It was higher in experiment A1-1 than in experiment A1-4, probably because of lower N intakes in experiment A1-1. There was a similar difference in N utilisation between the experiments A1-2 and A1-5. There is no obvious explanation of this latter difference. The proportion of N intake retained in milk tended to increase with a reduction in the concentration of AAT within experiments (Table 3.10). There was no clear relationship between the level of PBV and the proportion of N retained in milk (Table 3.11). However, the proportion of N excreted in urine was 240 to 260 g kg^{-1} ingested N in experiment A1-1 and 270 to 320 g kg^{-1} ingested N in experiment A1-4. N excreted in urine was as low as 160 to 200 g kg^{-1} ingested N in the experiments A1-2 and A1-5. Excretion of N in faeces ranged between 400 and 500 g kg^{-1} ingested N in the experiments A1-2 and A1-5, but with the higher N intake in experiment A1-4, only 320 to 350 g kg^{-1} ingested N was excreted in faeces.

Table 3.10. Experiments A1-1 and A1-4. Nitrogen balances in cows given diets with different levels of AAT; 4 cows per treatment.

Experiment A1-1	g N cow^{-1} day^{-1}				
g AAT FU^{-1}	Feed	Milk	Faeces	Urine	Balance
95	508	171	183	131	23
88	465	157	202	122	-16
81	380	143	155	91	-9
	Per cent of ingested N				
95	100	33.7	36.0	25.8	4.5
88	100	33.8	43.4	26.2	-3.4
81	100	37.6	40.8	23.9	-2.4
Experiment A1-4	g N cow^{-1} day^{-1}				
g AAT FU^{-1}	Feed	Milk	Faeces	Urine	Balance
98	600	176	192	190	42
95	558	172	184	163	39
92	498	158	174	132	34
	Per cent of ingested N				
98	100	29.3	32.0	31.7	7.0
95	100	30.8	33.0	29.2	7.0
92	100	31.7	34.9	26.5	6.8

Table 3.11. Experiments A1-2 and A1-5. Nitrogen balances in cows given diets with different levels of PBV; 4 cows per treatment.

Experiment A1-2	g N cow^{-1} day^{-1}				
g PBV day^{-1}	Feed	Milk	Faeces	Urine	Balance
-81	446	161	196	88	1
-314	418	144	211	68	-5
-527	349	128	158	63	0
	Per cent of ingested N				
-81	100	36.1	43.9	19.7	0.2
-314	100	34.4	50.5	16.3	-1.2
-527	100	36.7	45.3	18.1	0
Experiment A1-5	g N cow^{-1} day^{-1}				
g PBV day^{-1}	Feed	Milk	Faeces	Urine	Balance
-295	433	137	183	73	40
-483	382	122	154	74	32
-660	345	123	161	55	6
	Per cent of ingested N				
-295	100	31.6	42.3	16.9	9.2
-483	100	31.9	40.3	19.4	8.4
-660	100	35.7	46.7	15.9	1.7

The results of the determinations of the digestibility of the diets in dairy cows are presented in the Tables 3.12 and 3.13. In experiments with varying AAT levels, based on grass/clover and whole-pea silage, digestibility of DM was 680 to 730 g kg^{-1}, and that of organic matter 700 to 750 g kg^{-1}. Digestibility of cell wall components was higher at the high level of AAT in experiment A1-4, and the same tendency was seen in experiment A1-1. There was a tendency to a reduction in the digestibility of DM and organic matter at the lower levels of dietary N in these experiments. A tendency to a reduction in N digestibility at the lower levels of dietary protein was only shown in experiment A1-1.

The digestibility of the diets with different PBV levels and based on whole-crop cereals and fodder beet (Table 3.13) was much lower than the digestibility of the diets with grass/clover and pea silage (Table 3.12). Digestibility of DM in the experiments A1-2 and A1-5 in most cases was as low as 600 to 650 g kg^{-1}. In particular, the digestibility of the cell wall components, which mainly originated from cereal straw in whole-crop silage, was very low. The variability of the digestibility of cell wall components in these experiments was also very large. No clear effect of the level of N on digestibility was found.

Table 3.12. Experiments A1-1 and A1-4. Per cent digestibility of diet components in dairy cows fed diets with different levels of AAT; 4 cows per treatment.

	Level of AAT FU^{-1}			$SEM^{1)}$	p
	High	Mean	Low		
Experiment A1-1					
DM	71	68	69	1.1	0.14
Organic matter	74	70	72	1.0	0.10
Nitrogen	65	57	59	1.8	0.069
ADF	53	46	48	2.1	0.13
Experiment A1-4					
DM	73	72	70	1.3	0.34
Organic matter	75	74	72	1.2	0.29
Nitrogen	68	67	65	1.9	0.49
ADF	60	53	53	1.3	0.016
NDF	63	55	53	1.3	0.045

1) *Standard error of mean*

Table 3.13. Experiments A1-2 and A1-5. Per cent digestibility of diet components in dairy cows fed diets with different levels of PBV; 4 cows per treatment.

	g PBV cow^{-1} day^{-1}			$SEM^{1)}$	p
Experiment A1-2	-89	-314	-527		
DM	62	60	64	1.6	0.25
Organic matter	65	64	68	1.5	0.24
Nitrogen	56	50	55	1.6	0.062
ADF	25	22	27	4.4	0.75
Experiment A1-5	-295	-483	-660		
DM	66	71	63	2.5	0.20
Organic matter	68	73	65	2.4	0.20
Nitrogen	60	63	52	2.7	0.11
ADF	30	43	24	7.8	0.37
NDF	34	46	29	7.0	0.39

1) *Standard error of mean*

The results of the measurements of excretion of creatinine and purine derivatives are shown in Tables 3.14 and 3.15. In the experiments A1-1 and A1-4, significant differences were found in the excretion of allantoin. In experiment A1-1, no clear relationship was found between diet composition and excretion of allantoin, but in experiment A1-4, excretion of allantoin was lower with a decreasing level of AAT. Variability of allantoin excretion was much larger in the experiments A1-2 and A1-5 than in the experiments A1-1 and A1-4, and there was no indication of any treatment effect in the experiments A1-2 and A1-5.

Table 3.14. Experiments A1-1 and A1-4. Excretion of purine derivatives and creatinine (mmol day^{-1}) in cows fed diets with different levels of AAT.

	Level of AAT FU^{-1}			SEM[1)]	p
	High	Mean	Low		
Experiment A1-1					
Creatinine	122	144	126	4	0.012
Allantoin	243	281	216	14	0.044
Uric acid	24	25	24	3	0.95
Experiment A1-4					
Creatinine	110	103	99	7	0.20
Allantoin	264	192	177	16	0.0001
Uric acid	25	25	22	4	0.52

[1)] *Standard error of mean*

Table 3.15. Experiments A1-2 and A1-5. Excretion of purine derivatives and creatinine (mmol day^{-1}) in cows fed diets with different levels of PBV.

	g PBV cow^{-1} day^{-1}			SEM[1)]	p
Experiment A1-2	-89	-314	-527		
Creatinine	114	122	105	4	0.046
Allantoin	223	233	231	39	0.98
Uric acid	24	22	24	2	0.62
Experiment A1-5	-295	-483	-660		
Creatinine	91	99	89	10	0.40
Allantoin	184	188	194	27	0.89
Uric acid	18	19	20	4	0.64

[1)] *Standard error of mean*

The concentration of urea in milk in experiment A1-4 was close to average values found in practice (Bang & Strudsholm, 1993), but particularly in the experiments with varying levels of PBV the concentrations of urea in milk were very low (Table 3.16). The concentration of urea was generally reduced at a low level of protein in the diet.

Table 3.16. Concentration of urea in milk, mg (100 ml)$^{-1}$.

Experiment	Level of AAT			SEM[1)]	p
	High	Mean	Low		
A1-1	20.6	12.6	10.0	1.3	0.0001
A1-4	28.7	27.6	22.9	1.1	0.005
	Intended level of PBV, g cow^{-1} day^{-1}				
	0	-250	-500		
A1-2	13.3	12.8	8.5	1.2	0.0185
A1-3	8.9	7.2	4.4	0.5	0.0001
A1-5	11.7	9.5	7.1	1.5	0.14
A1-6	13.8	10.5	5.6	0.7	0.0001

Standard error of mean

3.2 Sub-task A3

In experiment A3-1, one cow suffered severely from sole ulcer which led to low feed intake and poor milk production. This animal was assigned as a missing value in the analysis of data.

Chemical composition of feedstuffs

Chemical composition and feeding value of the feeds are presented in Table 3.17. The chemical composition and feeding value of the herbage and concentrates fed in experiment A3-1 were similar to the reference values of CVB (1995). However, the NE_L value, and the DVE, OEB and crude protein contents of the herbage fed in experiment A3-2 were low compared with the reference values of CVB (1995).

Dry matter and starch contents, *in vitro* digestibility of organic matter (dOM) and NE_L value of GMES were higher in experiment A3-1 than in experiment A3-2, and the cell wall content (crude fibre, NDF, ADF) was lower. In both experiments, the fermentation characteristics of GMES indicated good preservation.

Table 3.17. Chemical composition and feeding value of the feeds, in g kg^{-1} DM (except where indicated otherwise).

	Experiment A3-1				**Experiment A3-2**		
	Herbage	GMES	Beet pulp	SMSE	Herbage	GMES	BSF
DM (g kg^{-1} fresh)	151	625	938	893	181	481	893
Crude protein	183	101	109	486	135	83	196
Total N	29.3	15.9	17.3	76.6	20.5	14.3	30.0
Nitrate	1.9	-	-	-	0.2	-	-
Crude fibre	219	73	179	63	238	86	115
Crude ash	120	15	75	75	113	27	109
Sand	22	-	-	-	29	-	-
Crude fat	40	37	15	20	45	41	11
Sugar	114	6	119	106	143	4	185
Starch	-	578	-	75	-	529	132
NDF	512	216	453	156	504	428	289
ADF	268	94	241	100	258	103	150
ADL	25	8	14	5	21	12	12
dOM *in vitro* (%)	80.5	84.2	87.3	90.9	78.1	82.1	90.8
FOM	619	574	754	636	601	589	576
NE_L[1] (MJ kg^{-1} DM)	6.53	7.82	7.16	7.89	5.91	7.48	6.74
DVE[2]	93	76	113	255	75	63	151
OEB[3]	28	-30	-64	185	-13	-33	-4
Calcium	4.2	0.1	8.4	3.4	5.4	0.8	9.9
Phosphorus	4.1	2.6	1.3	6.6	3.8	2.9	3.6
Magnesium	2.2	1.1	1.8	3.1	2.2	1.1	5.1
Sodium	1.3	<0.1	1.1	0.3	0.9	<0.1	4.5
Potassium	37.0	4.6	8.9	24.5	34.1	6.4	16.0
Fermentation characteristics[4]							
Ammonia (%)	-	3.7	-	-	-	11.3	-
Butyric acid (%)	-	0.0	-	-	-	0.3	-
Acetic acid (%)	-	8.3	-	-	-	6.7	-
Lactic acid (%)	-	13.5	-	-	-	13.9	-
Alcohol (%)	-	2.7	-	-	-	1.4	-
pH	-	4.1	-	-	-	4.1	-

[1] *Net energy for lactation (Van Es, 1977)*

[2] *Digestible protein available in the small intestine (Tamminga et al., 1994)*

[3] *Rumen-degradable protein balance (Tamminga et al., 1994)*

[4] *As percentage of total volatile fatty acids*

Feed intake

Experiment A3-1

The means of herbage intake and total DM intake (TDMI) were not significantly different among treatments (Table 3.18). Intake of beet pulp in BP and BPSM was slightly lower than expected. It was depressed from week 7 till 9 of the experiment, probably due to hardening of the pellets during storage, but recovered when the batch in store was replaced by a new batch.

Table 3.18. Mean values for daily intake of diet components, total DM (TDMI), NE_L, DVE, OEB and N, and for NE_L-coverage (NE_L-intake/NE_L-requirement) × 100% and DVE-coverage (DVE-intake/DVE- requirement) × 100%, all values in kg DM except where indicated otherwise. For description of the treatments (BP, BPSM, SM, M00, M25, M50 and M75) see paragraph 2.2.

	Experiment A3-1					
	BP	BPSM	SM		sed	Significance[1]
Beet pulp	5.0	2.3	0.0			
GMES	0.0	2.5	4.5			
SMSE	0.0	0.6	1.2			
Herbage	12.8	12.5	11.9		0.5	
TDMI	17.7	18.0	17.6		0.3	
NE_L (MJ)	119.9	122.2	122.3			
DVE (g)	1765	1780	1762		46	
OEB (g)	0	199	375		20	***
N (g)	458	489	508		15	*
NE_L coverage (%)	107	110	109		2.9	
DVE coverage (%)	130	127	125		4.5	
	Experiment A3-2					
	M00	M25	M50	M75	sed	Significance[1]
BSF	1.6	1.6	1.5	1.5		
GMES	0.0	2.4	4.4	6.4		
Herbage	16.4	15.0	12.4	11.1	0.4	***
TDMI	17.7	18.8	18.2	18.8	0.3	**
Forage : concentrate	93:7	80:20	68:32	59:41		
NE_L (MJ)	106.4	115.8	115.5	122.1	2.4	***
DVE (g)	1437	1481	1408	1424	29	
OEB (g)	-221	-276	-310	-361	7	***
N (g)	354	361	339	339	8	**
NE_L coverage (%)	98	100	103	107	1.8	***
DVE coverage (%)	103	96	91	86	2.0	***

[1] *Significance: *** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$; ~ = $p<0.1$*

Experiment A3-2

TDMI was significantly lower in M00 than in the other treatments (Table 3.18). Intake of herbage decreased with larger amounts of GMES in the ration, which resulted in significant differences in herbage intake among treatments. The substitution rates of GMES for herbage were 0.6, 0.9 and 0.8 for M25, M50 and M75, respectively.

Energy and protein intake

Experiment A3-1

Intake of both NE_L and DVE was similar in all treatments (Table 3.18). There were no significant differences in NE_L coverage (NE_L-intake/NE_L-requirement) × 100% and DVE coverage (DVE-intake /DVE-requirement) × 100%, among the treatments. Intake of NE_L and DVE was approximately 9 and 27 per cent above the requirements, respectively. Thus, the coverage of both NE_L and DVE was largely sufficient for all treatment groups. Intake of total N and OEB increased with increasing amounts of SMSE, resulting in significant differences among BP, BPSM and SM.

Experiment A3-2
Intakes of NE_L in M25 and M50 were significantly higher than in M00, whereas in M75 it was significantly higher than in the other treatments (Table 3.18). Intake of DVE was significantly higher in M25 than in M50. NE_L coverage was sufficient in M25, M50 and M75 and almost sufficient in M00. DVE coverage corrected for negative OEB values according to the DVE/OEB-system (Tamminga *et al.*, 1994), was sufficient in M00 but not in M25, M50 and M75. Feeding larger amounts of GMES resulted in higher NE_L coverage, but lower DVE coverage. Intake of OEB and N decreased when the proportion of GMES in the ration increased, resulting in significant differences among treatments.

Composition of the ingested diets

Experiment A3-1
Partial or full replacement of beet pulp by GMES and SMSE increased the concentrations of crude protein, total N and starch and the levels of NE_L and OEB, and reduced the concentrations of sugar and structural carbohydrates (NDF, ADF) in the ration (Table 3.19).

Experiment A3-2
Larger amounts of GMES in the ration resulted in increased concentrations of starch, OM and dOM, a higher level of NE_L and lower concentrations of crude protein, total N, crude fibre, crude ash, sugar and structural carbohydrates (NDF, ADF) and lower levels of DVE and OEB (Table 3.19).

Table 3.19. Chemical composition of the ration, in g kg^{-1} DM (except where indicated otherwise).

	Experiment A3-1			**Experiment A3-2**			
	BP	BPSM	SM	M00	M25	M50	M75
Crude protein	161	171	181	131	125	120	115
Total N	25.7	27.3	28.8	20.0	19.2	18.6	18.0
Crude fibre	175	173	171	237	217	198	184
Crude ash	99	91	85	119	107	96	87
Crude fat	34	37	40	44	44	43	41
Sugar	142	122	106	136	119	104	93
Starch	0	83	153	10	77	139	190
NDF	477	436	402	498	485	471	460
ADF	257	232	212	258	236	217	202
ADL	20	19	17	22	21	20	19
OM	901	909	915	881	893	904	913
dOM	743	745	750	677	693	708	721
FOM	659	630	606	586	586	585	585
NE_L (MJ kg^{-1})	6.72	6.83	6.94	6.01	6.16	6.34	6.49
DVE	99	100	100	81	79	77	76
OEB	0	11	22	-13	-15	-17	-19

Milk yield and composition

Experiment A3-1

There were no significant differences in the yields of milk, fat and protein corrected milk (FPCM), fat and protein. However, protein yield tended to be lower in BP than in SM and BPSM ($p<0.1$) (Table 3.20). The yield of lactose was significantly lower in BP than in SM and BPSM ($p<0.05$). There were no significant differences in fat and protein concentration among the treatments, but the lactose concentration was significantly lower in BP than in SM and BPSM ($p<0.05$). Both urea concentration and urea excretion in milk were higher in SM than in BPSM and BP ($p<0.001$) and higher in BPSM than in BP ($p<0.05$).

Table 3.20. Mean daily yields of milk, FPCM (Fat and Protein-Corrected Milk) and milk constituents, milk composition and N utilisation (N in milk/N intake) × 100%.

	Experiment A3-1					
	BP	BPSM	SM		sed	Significance[1)]
Milk (kg)	22.5	23.5	23.5		0.8	
FPCM (kg)	23.2	24.0	23.7		0.8	
Fat (g)	948	953	944		37	
Protein (g)	794	838	832		26	~
Lactose (g)	1003	1077	1084		39	*
Urea (mg)	4615	5664	6696		325	***
Fat (g kg^{-1})	42.5	40.5	40.3		1.2	~
Protein (g kg^{-1})	35.3	35.7	35.4		0.4	
Lactose (g kg^{-1})	44.6	45.8	46.1		0.4	*
Urea (mg l^{-1})	205	241	285		9.6	***
N utilisation (%)	27.2	27.0	25.9		1.0	
	Experiment A3-2					
	M00	M25	M50	M75	sed	Significance[1)]
Milk (kg)	22.4	24.2	23.8	23.9	0.5	**
FPCM (kg)	23.2	25.0	24.1	24.5	0.6	**
Fat (g)	979	1051	989	1014	32.5	~
Protein (g)	712	781	760	778	19.0	**
Lactose (g)	1010	1117	1092	1105	27.9	***
Urea (mg)	3577	3253	2746	2435	127	***
Fat (g kg^{-1})	43.6	43.4	41.6	42.4	1.4	
Protein (g kg^{-1})	31.7	32.2	32.0	32.5	0.9	
Lactose (g kg^{-1})	45.0	46.1	46.0	46.2	0.5	**
Urea (mg l^{-1})	159	134	116	102	5.1	***
N utilisation (%)	31.6	34.0	35.0	36.6	0.7	***

[1)] *Significance: *** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$; ~ = $p<0.1$*

Experiment A3-2

The yields of milk, FPCM, protein and lactose were significantly lower in M00 than in M25, M50 and M75 (Table 3.20). Fat yield in M00 was significantly lower than in M25 ($p<0.05$). There were no significant differences in milk fat and protein concentration. However, the concentration of lactose was significantly lower in M00 than in M25 and M75 ($p<0.05$) and tended to be lower in M00 than in M50 ($p<0.1$). The concentration and excretion of milk urea decreased gradually with larger amounts of GMES in the ration, resulting in significant differences among the treatments ($p<0.001$).

Nitrogen utilisation

Nitrogen utilisation was calculated as (N excretion in milk/N intake) × 100. In both experiment A3-1 and A3-2, differences in N utilisation followed the differences in OEB content (Tables 3.20 and 3.19).

Live weight

Neither in experiment A3-1 nor in A3-2, significant differences were found in live weight. Mean liveweights in BP, BPSM and SM were 590, 584 and 567 kg (s.e.d. = 20), respectively, and mean liveweight gains during the experimental period were 10, 6 and 6 kg. Mean liveweights in M00, M25, M50 and M75 were 564, 577, 584 and 579 kg (s.e.d. = 18), respectively, and mean liveweight gains during the experimental period were -4, 7, -9 and -12 kg.

Blood and urine analyses

The results of the analyses of blood and urine are given in Table 3.21. In experiment A3-1, urinary nitrogen (UN), urinary urea nitrogen (UUN) and blood serum urea (BU) followed the differences in intake of dietary N. However, in experiment A3-2, only BU followed the differences in intake of dietary N, but not UN and UUN. Further analysis of urine revealed that creatinine concentration, a measure for the quantity of urine produced, gradually increased with larger quantities of GMES in the ration, while concurrently the ratio of N to creatinine decreased. This suggests that urine production decreased with increasing amounts of GMES in the diet. In both experiment A3-1 and A3-2, the levels of plasma ß-HB were low, and the differences in ß-HB among the treatments were negligible.

Table 3.21. Urinary nitrogen, urinary urea nitrogen and creatinine in spot samples collected in the 10th experimental week; serum urea and ß-hydroxybutyrate in the 4th, 10th and 15th experimental week.

	Experiment A3-1				**Experiment A3-2**				
	BP	BPSM	SM	sed	M00	M25	M50	M75	sed
Urinary nitrogen, g l^{-1}									
7.00 AM	6.64	7.41	8.65	0.70	5.44	5.57	4.78	5.69	0.56
7.00 PM	ND	ND	ND	ND	4.20	4.35	5.08	6.08	0.61
Urinary urea nitrogen, g l^{-1}									
7.00 AM	4.62	5.00	5.79	0.44	3.28	3.04	2.40	2.64	0.35
7.00 PM	ND	ND	ND	ND	2.32	2.01	2.28	2.49	0.34
Creatinine, mol l^{-1}									
7.00 AM	ND	ND	ND	ND	3.65	3.98	4.15	6.01	0.67
7.00 PM	ND	ND	ND	ND	2.41	3.25	4.63	6.89	0.69
Serum urea, mmol l^{-1}									
week 4	3.93	3.62	3.95	0.32	2.03	2.11	1.91	1.98	0.14
week 10	4.81	5.49	6.37	0.34	2.28	2.24	2.08	2.23	0.17
week 15	4.85	5.46	6.41	0.30	5.56	5.37	4.40	4.30	0.33
Serum ß-hydroxybutyrate, mmol l^{-1}									
week 4	0.56	0.67	0.58	0.10	0.53	0.50	0.58	0.50	0.10
week 10	0.36	0.43	0.31	0.06	0.61	0.72	0.68	0.73	0.07
week 15	0.48	0.41	0.37	0.04	0.40	0.51	0.55	0.56	0.08

3.3 Sub-task A4

The validation experiment

In Table 3.22 the results of the validation experiment of the n-alkane technique are presented. As shown, the n-alkane technique seems to be very accurate in predicting total DM intake, with errors of less than 2%. The technique predicted clover contents of the diet with a very low variability. The actual clover content of the herbage fed was 43% and as can be seen in Table 3.22, this was predicted with all sampling routines. The results show that the n-alkane technique can be a very useful technique when used in the experiments proposed in this study.

Table 3.22. Results of the validation experiment of the n-alkane technique.

Sampling routine	Predicted - actual intake (kg DM cow^{-1} day^{-1})	Error as proportion of total intake	Predicted clover content of the diet
AM	0.139 ± 0.211	0.004 ± 0.020	0.414 ± 0.006
PM	0.366 ± 0.344	0.020 ± 0.027	0.434 ± 0.008
AM + PM	0.249 ± 0.251	0.013 ± 0.021	0.425 ± 0.006

Grazing trial

The average monthly chemical composition of the herbage during the experiment is given in Table 3.23. The clover content of the sward increased from 9.1% in May to 20.9% in June and 46.6% in July, as would be expected. As can be seen in Table 3.23, the crude protein (CP) content of the clover was high, *viz.* 247-276 g kg^{-1} DM. This, combined with an increasing proportion of clover in the sward during the season resulted in an increase in protein content of the herbage on offer, as calculated in Table 3.25.

Table 3.23. Average monthly herbage composition.

	Clover			Grass		
	May	June	July	May	June	July
DM (g kg^{-1} fresh) 1)	149	156	164	196	194	214
CP (g kg^{-1} DM)	276	247	253	141	163	148
NDF (g kg^{-1} DM)	349	330	340	483	525	502
ADF (g kg^{-1} DM)	252	232	239	255	271	274
OM (g kg^{-1} DM)	895	892	896	903	890	899
In vitro D (%)	67.0	63.8	65.5	68.7	62.1	66.3
ME (MJ kg^{-1} DM)	11.5	10.9	11.2	11.8	10.6	11.3

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; OM, organic matter; ME, metabolisable energy.

In Table 3.24, the chemical composition of the concentrate fed is presented.

Table 3.24. Chemical composition of the concentrate used during the first 5 weeks of the experiment.

DM (g kg^{-1} product)	893
CP (g kg^{-1} DM)	320
NDF (g kg^{-1} DM)	272
ADF (g kg^{-1} DM)	177
EE (g kg^{-1} DM)	61
AHEE (g kg^{-1} DM)	84
NCGD g kg^{-1} DM)	792
ME (g kg^{-1} DM)	13.2
Ash (g kg^{-1} DM)	48.2

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; EE, ether extract; AHEE, acid hydrolysis ether extract; NCGD, neutral cellulase gaminase digestibility; ME, metabolisable energy.

Table 3.25. Calculated chemical composition of herbage on offer.

	May	June	July
DM (g kg^{-1} fresh)	192	186	205
CP (g kg^{-1} DM)	153	181	197
NDF (g kg^{-1} DM)	471	484	426
ADF (g kg^{-1} DM)	255	263	257
OM (g kg^{-1} DM)	902	890	898
In vitro D (%)	68.5	62.4	65.9
ME (MJ kg^{-1} DM)	11.8	10.7	11.3

see Table 3.23 for the meaning of the abbreviations

Table 3.26 presents the average animal performance results over the whole experimental period. Milk fat content was relatively low but milk protein was high.

Table 3.26. Performance of spring- and autumn-calving cows, averaged over the whole experimental period.

	Spring-calving	Autumn-calving	s.e.d.	significance
Milk yield (kg day^{-1})	20.6	15.0	1.41	*
Fat (g kg^{-1})	35.8	38.9	1.32	*
Protein (g kg^{-1})	34.2	35.4	0.58	NS
Lactose (g kg^{-1})	45.9	44.3	0.10	NS
FCM (kg day^{-1})	19.3	14.6	1.33	*
FPCM (kg day^{-1})	19.7	14.9	1.34	*
LW (kg)	549	562	23.0	NS
LWG (kg day^{-1})	0.260	0.290	0.1388	NS
Condition score	2.55	2.80	0.229	NS

$^*p < 0.05$

FCM, fat-corrected milk; FPCM, fat and protein-corrected milk; LW, live weight; LWG, liveweight gain.

Table 3.27 presents the chemical composition of the perennial ryegrass and white clover during the two periods when intensive measurements were carried out. As shown, no major differences in terms of chemical composition due to period did exist in the separate components, ryegrass or white clover. However, due to the large difference in the proportion in which the two herbage components were represented in the sward, differences in chemical composition of herbage on offer did exist between the two periods (Table 3.28). Herbage on offer in the second period had a considerably higher CP content and a lower NDF content.

Table 3.27. Chemical composition of the herbage components during the two intensive measurement periods.

	Perennial ryegrass		White clover	
	Period 1[1]	Period 2[2]	Period 1[1]	Period 2[2]
DM (g kg^{-1} fresh)	190	219	144	167
CP (g kg^{-1} DM)	143	148	280	252
NDF (g kg^{-1} DM)	491	503	357	328
ADF (g kg^{-1} DM)	260	277	263	236
OM (g kg^{-1} DM)	901	898	893	895
IVOMD (%)	75	74	74	73
D value (%)	72.6	70.6	71	70
ME (MJ kg^{-1} DM)	11.6	11.3	11.4	11.2

[1] *Period 1: 17-22 May 1993*

[2] *Period 2: 19-24 July 1993*

see Table 3.23 for the meaning of the abbreviations; IVOMD; in vitro digestibility of organic matter; D value, digestible organic matter in the dry matter.

Table 3.28. Chemical composition of herbage mixtures on offer during the two intensive measurement periods.

	Period 1[1]	Period 2[2]
DM (g kg^{-1} fresh)	188	196
CP (g kg^{-1} DM)	150	195
NDF (g kg^{-1} DM)	484	428
ADF (g kg^{-1} DM)	260	259
OM (g kg^{-1} DM)	900	897
IVOMD (%)	75.2	73
D value (%)	73	70.3
ME (MJ kg^{-1} DM)	11.6	11.2
Clover content (%)	7	44

[1] *Period 1: 17-22 May 1993*

[2] *Period 2: 19-24 July 1993*

see Tables 3.23 and 3.27 for the meaning of the abbreviations.

In Table 3.29, animal performance during the intensive measurement periods is shown. The data indicate that no major changes in performance took place between the two measurement periods. In the second period, milk yields were slightly lower and protein contents of the milk higher than in the first period.

Table 3.29. Performance of spring- and autumn-calving cows during the two intensive measurement periods.

	Spring-calving		Autumn-calving		s.e.d.[2]		
Period[1]	1	2	1	2	Tr	Per	Tr*Per
Milk yield (kg day^{-1})	20.8	19.3	15.2	14.1	1.4*	0.5*	1.5
Fat (g kg^{-1})	40.5	34.9	42.2	40.2	1.4*	0.6*	1.5*
Protein (g kg^{-1})	33.6	35.3	34.9	36.9	0.67*	0.22*	0.70
Lactose (g kg^{-1})	46.3	45.3	44.5	44.1	1.02	0.32*	1.07
LW (kg)	540	549	549	569	24.9	5.8*	25.6
LWG (kg day^{-1})	0.46	0.17	0.58	-0.01	0.314	0.232	0.390
Condition score	2.72	2.61	2.76	3.0	0.24	0.04	0.24*

[1] *Period 1: 17-22 May 1993*
Period 2: 19-24 July 1993

[2] *s.e.d., standard error of the difference between 2 means; Tr, treatment (spring and autumn-calving); Per, period; * $p < 0.05$.*

Average grass height during the first intensive measurement period was 8.2 cm and during the second intensive measurement period 8.7 cm. In Table 3.30, forage and total DM intakes are presented, estimated with the use of the n-alkane technique during the intensive measurement periods. During the first measurement period, no significant difference in intake between the spring and autumn-calving cows was established, whereas in the second measurement period the spring-calving cows had a significantly higher intake. The spring-calving animals also selected a significantly higher proportion of clover in their diet (Table 3.30), but this did not result in major changes of the calculated chemical composition of the forage consumed during the second measurement period.

Table 3.30. Forage and total DM intakes and chemical composition of the diets, selected during the intensive measurement periods.

	Spring-calving		Autumn-calving		s.e.d.[2]		
Period[1]	1	2	1	2	Tr	Per	Tr*Per
Grazed herbage (kg DM day^{-1})	10.5	13.5	10.5	11.5	0.64	0.38*	0.74*
Total intake (kg DM day^{-1})	11.4	13.5	11.4	11.5	0.64	0.38*	0.74*
Proportion clover	0.02	0.46	0.03	0.40	0.01193*	0.01193*	0.61687*
CP in the diet (g kg^{-1} DM)	164	196	163	190	1.2*	1.5*	1.9
D value of the diet (%)	75.5	73.4	75.5	73.4	0.7	0.7*	1.0
NDF in the diet (g kg^{-1} DM)	467	422	468	431	2.1*	2.1*	3.0
ADF in the diet (g kg^{-1} DM)	253	257	254	259	0.5*	0.5*	0.7

[1] *Period 1: 17-22 May 1993*
Period 2: 19-24 July 1993

[2] *see Table 3.29.*

In Table 3.31, the calculated rumen and metabolic protein levels are presented. Both the spring-calving and autumn-calving cows had a shortage of ERDP during the first measurement period and a surplus of ERDP during the second measurement period. This resulted in a shortage of MP for the higher productive spring-calving cows during the first measurement period and a surplus of MP for both spring and autumn-calvers during the second measurement period.

Table 3.31. Calculated rumen protein levels (ERDP; AFRC, 1993) and resulting metabolic protein levels (MP) during the intensive measurement periods.

	Spring-calving		Autumn-calving		s.e.d.2)		
Period1)	1	2	1	2	Tr	Per	Tr*Per
ERDP level (g day^{-1})	-280	127	-205	185	59.8	37.4*	70.5
MP level (g day^{-1})	-193	394	22	269	78.9	68.3*	104.4*

1) *Period 1: 17-22 May 1993*
Period 2: 19-24 July 1993

2) *see Table 3.29.*

At two specific dates in May and July, milk samples were analysed for total N, non protein nitrogen (NPN) and urea N content. The results are shown in Table 3.32.

Table 3.32. Yields of milk and milk N components at 2 days in the intensive measurement periods.

	Spring-calving		Autumn-calving		s.e.d.2)		
Date1)	1	2	1	2	Tr	Per	Tr*Per
Milk yield (kg day^{-1})	20.29	19.75	14.59	14.07	0.994	0.994	1.406
Total N in milk (g day^{-1})	107.9	109.6	80.2	80.9	5.25*	5.25	7.43
NPN (g day^{-1})	5.13	11.05	3.45	6.28	0.828*	0.828*	1.172
Urea N (g day^{-1})	3.66	5.16	3.01	3.67	0.258*	0.258*	0.364

1) *Date 1: 19 May 1993*
Date 2: 21 July 1993

2) *see Table 3.29*

As can be seen in Table 3.32, sampling date had a major effect on the N components in the milk. On the second sampling date, the proportion of NPN in total N doubled from 5 to almost 10%. This was partly due to an increase in the amount of urea in the milk.

In Table 3.33, the concentrations of urine N are presented. N concentration in the urine of the spring-calving cows almost doubled from sampling date 1 in May to sampling date 2 in July. Additionally, the urea proportion of urine N increased. These results tend to be in line with the calculated values for protein supply (Table 3.31).

Table 3.33. Urine N concentrations as affected by period of sampling and stage of lactation.

	Spring-calving		Autumn-calving		s.e.d.[2)]		
Date[1)]	1	2	1	2	Tr	Per	Tr*Per
Urea N (g l^{-1})	2.37	5.03	2.75	4.67	0.377	0.377*	0.533
Total N (g l^{-1})	3.85	5.91	4.31	5.38	0.477	0.447*	0.652

[1)] *Date 1: 19 May 1993*
Date 2: 21 July 1993

[2)] *see Table 3.29.*

In Table 3.34, the N efficiencies (N in milk/N intake, as a percentage) are presented. N intake was significantly affected by period as would be expected from the chemical composition of the diet and the difference in herbage intake. Milk N output was only different between the two calving groups, while faecal N production was not affected by any of the treatments. This resulted in a significant difference in urine N production between the two measurement periods. Overall N efficiency was, therefore, significantly higher in the first measurement period than in the second one for both spring- and autumn-calving cows. In addition, the spring-calving cows were significantly higher in N efficiency than the autumn-calving cows.

Table 3.34. Nitrogen balances and efficiencies of the cows.

	Spring-calving		Autumn-calving		s.e.d.[2)]		
Period[1)]	1	2	1	2	Tr	Per	Tr*Per
N intake (g day^{-1})	296	424	296	350	19.0	11.9*	22.4*
N in milk (g day^{-1})	112	108	85	82	7.4*	3.1	8.0
N in faeces (g day^{-1})	149	153	159	131	14.5	7.7	16.4
N in urine (g day^{-1})	19	153	38	126	21.1	14.6*	25.6
N efficiency (%)[3)]	37.8	25.5	28.7	23.4	2.3*	1.1*	2.5*

[1)] *Period 1: 17-22 May 1993*
Period 2: 19-24 July 1993

[2)] *see Table 3.29*

[3)] *N in milk/N intake.*

In Table 3.35, the energy efficiencies (ME requirement/ME intake) are presented. ME efficiency was significantly higher in the first measurement period than in the second one.

Table 3.35. Energy efficiencies of the cows.

	Spring-calving		Autumn-calving		s.e.d.[2]		
Period[1]	1	2	1	2	Tr	Per	Tr*Per
ME intake (MJ day^{-1})	131	152	131	129	7.3	4.3*	8.4*
ME requirement (MJ day^{-1})	177	155	152	134	11.4*	7.4*	13.6
ME efficiency[3]	1.38	1.02	1.16	1.04	0.085	0.072*	0.112

[1] *Period 1: 17-22 May 1993*
Period 2: 19-24 July 1993

[2] *see Table 3.29*

[3] *ME requirement/ME intake.*

3.4 Sub-task A5

Experiment A5-1. Spring-grazing experiment

The chemical composition of the separate herbage fractions during experiment A5-1 is given in Table 3.36. Mean clover content during the experiment was 7.1% on basis of DM. As shown in Table 3.36, the CP content of the mixture was low but digestibility was high. The mean herbage height was 9.6 cm, ranging from 12.7 cm at the beginning of the experiment to 6.8 cm at the end.

Table 3.36. Average chemical composition of the herbage in experiment A5-1 (Spring of 1994).

Component	Grass	Clover	Mixture
DM (g kg^{-1} fresh)	253	203	250
CP (g kg^{-1} DM)	117	250	126
NDF (g kg^{-1} DM)	411	284	402
ADF (g kg^{-1} DM)	206	176	204
OM (g kg^{-1} DM)	924	903	923
In vitro D (%)	79.8	77.8	79.6
ME (MJ kg^{-1} DM)	12.8	12.1	12.8
% of CP as true protein	80.6	83.4	80.8

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; OM, organic matter; D, digestibility; ME, metabolisable energy.

In Table 3.37, the chemical composition of the three concentrates which were fed is presented. During the first week of the experiment, a standard sugar beet pulp was used. It was observed that in the experimental groups B and C not all the concentrate was consumed. Hardness of the sugar beet pulp pellet was suspected. From week 2 onwards, a softer oil-coated sugar beet pulp pellet was used which solved the problem of concentrate rejection.

Table 3.38 presents the animal performance results for the different treatments. These results are the means over the last two weeks of the experimental period, corrected for covariance except for liveweight gain which was not corrected.

Table 3.37. Composition of the 3 concentrates (A, B and C) in experiment A5-1.

Component	A	B	C
DM (g kg^{-1} product)	813	845	846
CP (g kg^{-1} DM)	276	230	204
AHEE (g kg^{-1} DM)	21.8	19.9	20.2
NCGD (g kg^{-1} DM)	705	827	821
ADIN (mg kg^{-1} DM)	398	762	775
Starch (g kg^{-1} DM)	34.0	12.5	13.3
WSC (g kg^{-1} DM)	159.5	200.7	200.4
NDF (g kg^{-1} DM)	72.5	218.5	218.3
ADF (g kg^{-1} DM)	49.5	137.7	137.3
OM (g kg^{-1} DM)	713	857	855
ME-E3 (MJ kg^{-1} DM)	10.4	12.1	12.0
Ca (g kg^{-1} DM)	43.9	17.8	19.7
P (g kg^{-1} DM)	14.2	3.4	3.8
Mg (g kg^{-1} DM)	26	5.1	5.4
K (g kg^{-1} DM)	34.3	25.3	25.7
Na (g kg^{-1} DM)	26.9	7.5	8.3

DM, dry matter; CP, crude protein; AHEE, acid hydrolysis ether extract; NCGD, neutral cellulase gaminase digestibility; ADIN, acid detergent insoluble nitrogen; WSC, water soluble carbohydrates; NDF, neutral detergent fibre; ADF, acid detergent fibre; OM, organic matter; ME-E3, metabolisable energy calculated using the E3 equation of Thomas et al. (1988); Ca, calcium; P, phosphorus; Mg, magnesium; K, potassium; Na, sodium.

Table 3.38. Animal performance during the last 2 weeks of experiment A5-1.

	Experimental group				
	A	B	C	s.e.d.	Significance[1)]
Milk yield (kg day^{-1})	23.0	22.5	25.1	1.08	NS
Fat content (g kg^{-1})	45.1	44.2	41.8	1.36	*
Protein content (g kg^{-1})	32.6	33.7	33.3	0.59	NS
Lactose content (g kg^{-1})	45.9	44.9	45.9	0.29	*
Fat yield (kg day^{-1})	1.030	0.998	1.052	0.0570	NS
Protein yield (kg day^{-1})	0.752	0.756	0.834	0.0390	NS
Lactose yield (kg day^{-1})	1.052	1.012	1.153	0.0560	NS
FCM (kg day^{-1})	24.3	23.8	25.8	1.28	NS
FPCM (kg day^{-1})	24.3	23.6	25.7	1.23	NS
Live weight (kg)	579	593	596	5.59	*
Liveweight gain (kg day^{-1})	0.13	-0.20	0.51	0.346	NS
Condition score	2.67	2.63	2.66	0.140	NS

[1)] *NS = not significant ($p > 0.05$); * $p < 0.05$*

FCM, fat-corrected milk; FPCM, fat and protein-corrected milk.

Milk yield was lowest on treatment B and highest on treatment C, but these differences were not significant (Table 3.38). Only fat and lactose content were significantly affected by treatment. Protein content of the milk tended to be higher on the diets B and C than on A.

In Table 3.39, forage intakes, estimated by the n-alkane technique, are presented. Forage intake was not significantly affected by treatment. The proportion of clover in the diet was lower than in the sward. This indicates that the animals did not or could not select for clover. CP contents, D values and NDF contents of the diets differed significantly.

Table 3.39. Forage intake and diet composition during the intensive measurement period of experiment A5-1 (Spring of 1994), as affected by type and quality of supplement (see Table 2.5).

	Experimental group				
	A	B	C	s.e.d.	Significance[1)]
Grazed herbage (kg DM day^{-1})	15.6	15.5	13.8	1.58	NS
Total DM intake (kg day^{-1})	16.4	18.7	17.0	1.58	NS
Proportion clover in the diet (fraction)	0.03	0.04	0.01	0.017	NS
CP in the diet (g kg^{-1} DM)	123	141	133	2.52	*
D value of the diet (%)	77.3	81.1	75.1	2.7	*
NDF in the diet (g kg^{-1} DM)	385	371	374	3.4	*
ADF in the diet (g kg^{-1} DM)	195	192	193	1.3	NS

[1)] *NS = not significant ($p > 0.05$); *$p < 0.05$*

CP, crude protein; D value, digestible organic matter in the dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre.

In Table 3.40, the calculated rumen and metabolic protein levels are presented. In all the treatments there was a shortage of ERDP and this resulted in a small MP shortage in the treatments A and C, while in treatment B an MP surplus existed due to supplementation. The ERDP and MP levels of group B were significantly higher than those of A and C. ERDP levels were much lower than planned, whereas MP levels were in agreement with the planning (compare with Table 2.5).

Table 3.40. Calculated rumen protein levels (ERDP; AFRC, 1993) and resulting metabolic protein levels (MP) in Experiment A5-1.

	Experimental group				
	A	B	C	s.e.d.	Significance[1)]
ERDP level (g day^{-1})	-843	-635	-948	96	*
MP level (g day^{-1})	-23	351	-14	126	*

[1)] *$p < 0.05$

In Table 3.41, milk N components, which were not covariate-corrected, are presented. The values found are similar to those observed in the previous trial during Spring (Paragraph 3.3, Table 3.32). Addition of urea to the diet (diets B and C, Table 2.6) did not increase NPN content of the milk and significantly reduced the fraction of urea N in total N. The urea content of the milk of the experimental groups A, B and C was 164, 159 and 145 mg kg^{-1} milk (calculated from the Tables 3.38 and 3.41).

This indicates a rather low protein supply to the cows: a urea content of 200-250 mg kg^{-1} milk is considered desirable.

Table 3.41. Yield of milk N components in experiment A5-1 (1 day of the intensive measurement period).

	Experimental group				
	A	B	C	s.e.d.	Significance[1)]
Total N in milk (g day^{-1})	115.9	116.5	126.0	5.7	NS
NPN in milk (g day^{-1})	5.18	5.03	5.49	0.311	NS
Urea N in milk (g day^{-1})	3.78	3.57	3.65	0.203	NS
NPN, % of total N	4.53	4.35	4.34	0.144	NS
Urea N, % of total N	3.37	3.10	2.93	0.129	*
Urea N, % of NPN	74.2	70.8	68.2	3.25	NS

[1)] *NS = not significant ($p > 0.05$); * $p < 0.05$*

In Table 3.42, urine N concentrations are presented. No significant differences were observed. Urine N concentrations tended to be lower on treatment C. The proportion of urea N in total N was low in all the experimental groups.

Table 3.42. Urine N concentrations in experiment A5-1 (intensive measurement period).

	Experimental group				
	A	B	C	s.e.d.	Significance[1)]
Urea N in urine (g l^{-1})	2.81	2.82	2.31	0.402	NS
Total N in urine (g l^{-1})	5.18	5.03	4.45	0.726	NS

[1)] *NS = not significant ($p > 0.05$); * $p < 0.05$*

In Table 3.43, the N efficiencies (N in milk/N intake) are presented. As a consequence of the different N contents of the diets and total DM intakes, a significant difference in N intake did exist. N excretion in milk and faeces was not significantly affected by type and level of supplement. Urine N excretion as well as N efficiency were significantly affected by treatment. A small excretion of urine N was associated with a high N efficiency.

Table 3.43. N balances and N efficiencies in experiment A5-1 (intensive measurement period).

	Experimental group				
	A	B	C	s.e.d.	Significance[1]
N intake (g day^{-1})	323	418	361	31.6	*
N in milk (g day^{-1})	122	121	132	6.0	NS
N in faeces (g day^{-1})	122	143	149	15.0	NS
N in urine (g day^{-1})	70	154	81	17.0	*
N efficiency (%)	38	29	37	2.4	*

[1] *NS = not significant ($p > 0.05$); * $p < 0.05$*

In Table 3.44, the energy efficiencies (ME requirement/ME intake) are presented. As shown, no significant differences in energy efficiency could be detected. However, the calculated energy efficiency of group B was considerably lower than those of A and C, and this again questions the accuracy of the n-alkane technique for DM and N intake estimates and, consequently, for determination of the N balance of grazing cows.

Table 3.44. Energy efficiencies in experiment A5-1.

	Experimental group				
	A	B	C	s.e.d.	Significance[1]
ME intake (MJ day^{-1})	203	236	215	20.2	NS
ME requirement (MJ day^{-1})	196	180	207	9.2	*
ME efficiency	0.97	0.76	0.96	0.089	NS

[1] *NS = not significant ($p > 0.05$); * $p < 0.05$*

Experiment A5-2. Summer grazing experiment

The chemical composition of the mixture and the separate herbage fractions during experiment A5-2 is presented in Table 3.45. Mean clover content during the experiment was 38% on basis of DM. As shown in Table 3.45, the CP content of the grass in the mixture was 127 g kg^{-1} DM, but the overall CP content of the herbage was 155 g kg^{-1} DM due to the rather high proportion of clover. The quality of the herbage on offer was low with particularly low ME and CP contents of the clover component.

Table 3.45. Average chemical composition of the herbage in experiment A5-2 (Summer of 1994).

Component[1]	Grass	Clover	Mixture
DM (g kg^{-1} fresh)	234	184	215
CP (g kg^{-1} DM)	127	200	155
NDF (g kg^{-1} DM)	553	394	493
ADF (g kg^{-1} DM)	289	284	287
OM (g kg^{-1} DM)	912	914	913
In vitro D (%)	67.7	64.7	66.6
ME (MJ kg^{-1} DM)	10.4	9.9	10.2
% of CP as true protein	83.1	83.3	83.2

[1] *see Table 3.36 for the meaning of the abbreviations.*

In Table 3.46, the chemical composition of the three concentrates is given. The CP content of the concentrates B and C was only slightly lower than that of the herbage. No intake problems were experienced with the concentrates in experiment A5-2. The softer sugar beet pulp pellet was used.

Table 3.46. Chemical composition of the 3 concentrates in experiment A5-2.

Component[1]	A	B	C
DM (g kg^{-1} product)	861	865	865
CP (g kg^{-1} DM)	457	150	134
AHEE (g kg^{-1} DM)	78.8	31.2	28.6
NCGD (g kg^{-1} DM)	882	861	859
ADIN (g kg^{-1} DM)	572	838	852
Starch (g kg^{-1} DM)	18.5	16.2	16.1
WSC (g kg^{-1} DM)	88	198	204
NDF (g kg^{-1} DM)	123	249	256
ADF (g kg^{-1} DM)	85	154	158
OM (g kg^{-1} DM)	896	895	894
ME-E3 (MJ kg^{-1} DM)	14.3	12.8	12.8
Ca (g kg^{-1} DM)	4.1	6.9	7.0
P (g kg^{-1} DM)	6.2	1.4	1.1
Mg (g kg^{-1} DM)	8.3	2.3	2.0
K (g kg^{-1} DM)	24.6	23.5	23.4
Na (g kg^{-1} DM)	0.13	2.5	2.7

[1] *see Table 3.37 for the meaning of the abbreviations.*

Table 3.47 presents the animal performance results. These results are the means of the last two weeks of the experimental period corrected for covariance with the exception of liveweight gain.

Table 3.47. Animal performance during the last 2 weeks of experiment A5-2 (Summer of 1994), as affected by type and quantity of supplement (see Tables 2.5 and 2.6).

	Experimental groups				
	A	B	C	s.e.d.	Significance[1)]
Milk yield (kg day^{-1})	20.3	22.2	23.6	0.578	*
Fat content (g kg^{-1})	43.1	41.6	42.5	1.33	NS
Protein content (g kg^{-1})	32.6	33.1	33.7	0.32	*
Lactose content (g kg^{-1})	45.8	46.0	45.6	0.35	NS
Fat yield (kg day^{-1})	0.874	0.921	0.995	0.0404	*
Protein yield (kg day^{-1})	0.662	0.729	0.784	0.0207	*
Lactose yield (kg day^{-1})	0.926	1.017	1.085	0.0285	*
FCM (kg day^{-1})	21.2	22.6	24.3	0.789	*
FPCM (kg day^{-1})	20.9	22.5	24.2	0.704	*
Live weight (kg)	587	598	594	4.77	NS
Liveweight gain (kg day^{-1})	0.25	-0.04	-0.19	0.284	NS
Condition score	2.56	2.67	2.60	0.080	NS

[1)] *NS = not significant ($p > 0.05$); *$p < 0.05$*

All animal performance indicators, except milk fat content, lactose content, live weight, liveweight gain and condition score were significantly affected by treatment. Feeding sugar beet pulp (treatments B and C) significantly increased milk yield, milk protein content and yields of milk fat, milk protein and lactose.

In Table 3.48, forage intake estimates by the n-alkane technique are presented. Forage intake was significantly affected by treatment as would be expected when feeding different levels of concentrate supplements. Total DM intake was not significantly affected by treatment. The proportion of clover in the diet selected was not significantly affected by treatment and was higher than in the herbage on offer. The clover content of the sward was 38%, indicating a preference of the cows for clover. Supplementation with sugar beet pulp (treatments B and D) significantly decreased the CP, NDF and ADF contents of the diet and increased the D value.

Table 3.48. Forage intake and diet composition during the intensive measurement period of experiment B5-1 in the summer of 1994 (see Table 3.39 for the meaning of the abbreviations).

	Experimental group				
	A	B	C	s.e.d.	Significance[1)]
Grazed herbage (kg DM day^{-1})	15.2	12.3	11.4	0.944	*
Total DM intake (kg day^{-1})	15.5	15.2	16.5	0.945	NS
Proportion clover in the diet (fraction)	0.48	0.49	0.48	0.020	NS
CP in the diet (g kg^{-1} DM)	161	154	149	0.96	*
D value of the diet (%)	67.0	70.8	73.6	0.31	*
NDF in the diet (g kg^{-1} DM)	467	431	410	3.7	*
ADF in the diet (g kg^{-1} DM)	318	290	274	3.1	*

[1)] *NS = not significant ($p > 0.05$); *$p < 0.05$*

In Table 3.49, the calculated rumen and metabolic protein levels are shown. Unexpectedly in all treatments a shortage of ERDP existed. The ERDP shortage still resulted in an over-supply of MP. Comparison with Table 2.5 shows that the actual levels of ERDP and MP in this experiment often differed from the planned levels.

Table 3.49. Calculated rumen protein levels (ERDP; AFRC, 1993) and resulting metabolic protein levels (MP) in experiment A5-2.

	Experimental group				
	A	B	C	s.e.d.	Significance[1)]
ERDP level (g day^{-1})	-210	-284	-420	40.3	*
MP level (g day^{-1})	372	259	229	107.7	NS

[1)] *NS = not significant ($p > 0.05$); *$p <0.05$*

In Table 3.50, milk N components, which were not covariate-corrected, are presented.

Table 3.50. Yield of milk N components in experiment A5-2 (1 day of the intensive measurement period).

	Experimental group				
	A	B	C	s.e.d.	Significance[1)]
Total N in milk (g day^{-1})	110.7	109.9	117.1	4.35	NS
NPN in milk (g day^{-1})	6.8	6.2	5.8	0.36	*
Urea N in milk (g day^{-1})	4.3	3.8	3.5	0.21	*
NPN, % of protein N	6.16	5.58	4.90	0.208	*
Urea N, % of protein N	3.92	3.42	2.95	0.167	*
Urea N, % of NPN	63.8	61.6	60.4	2.58	NS

[1)] *NS = not significant ($p > 0.05$); *$p < 0.05$*

As shown in Table 3.50, both the NPN and the urea content of the milk were reduced significantly by the supplements B and C. This indicates that a higher efficiency of N within the animal was achieved.

Table 3.51 presents urine N concentrations. No significant differences were observed, but urine N concentrations tended to be lower on treatment A, *i.e.* on the diet with the highest CP content (Table 3.48).

Table 3.51. Urine N concentrations in experiment A5-2 (intensive measurement period).

	Experimental group				
	A	B	C	s.e.d.	Significance[1]
Urea N in urine (g l^{-1})	4.10	4.61	4.36	0.440	NS
Total N in urine (g l^{-1})	5.59	6.61	6.52	0.621	NS

[1] *NS = not significant ($p > 0.05$)*

In Table 3.52, the N balances and the N efficiencies (N in milk/N intake) of the cows are presented. The different levels of supplementation which resulted in a reduced forage intake lead to a small reduction in N intake. This combined with an increased level of milk N output resulted in a significant difference in N efficiency. N balances were calculated. After subtraction of N in faeces and N in milk from N intake, the remainder was assumed to be excreted in urine. Urine N excretion, as well as N efficiency, were significantly affected by treatment. The lowest N efficiency values were associated with the highest levels of excretion of urine N.

Table 3.52. Nitrogen balances of the cows and N efficiencies (N in milk/N intake) in experiment A5-2.

	Experimental group				
	A	B	C	s.e.d.	Significance[1]
N intake (g day^{-1})	400	377	389	24.3	NS
N in milk (g day^{-1})	110	113	126	4.5	*
N in faeces (g day^{-1})	143	145	160	9.3	NS
N in urine (g day^{-1})	147	119	104	14.7	*
N efficiency (%)	27.5	30.0	32.4	1.64	*

[1] *NS = not significant ($p > 0.05$); * $p < 0.05$*

In Table 3.53, the energy efficiencies (ME requirement/ME intake) are presented. As shown, no significant differences in energy efficiency could be detected but supplementation with increasing amounts of sugar beet pulp tended to decrease the calculated energy efficiencies.

Table 3.53. Energy efficiencies in experiment A5-2 (Summer of 1994).

	Experimental group				
	A	B	C	s.e.d.	Significance[1]
ME intake (MJ day^{-1})	159	163	179	11.7	NS
ME requirement (MJ day^{-1})	176	169	176	9.21	NS
ME efficiency	1.11	1.04	0.98	0.074	NS

[1] *NS = not significant ($p > 0.05$)*

3.5 Sub-task A6-1

The chemical composition of the two silages fed during experiment A6-1.1, conducted in the Winter of 1994/1995, is presented in Table 3.54. The first-cut silage was fed during the first 10 weeks of the experiment, while the second-cut silage was fed during the weeks 11 to 16.

In Table 3.55, the chemical composition of the concentrate replacers and the concentrate components is presented.

Table 3.54. Chemical composition of the grass/clover silages (g kg^{-1} DM, unless otherwise stated) used in experiment A6-1.1 (Winter of 1994/1995).

Component	First cut	Second cut
DM (g kg^{-1} product)	284	232
CP	160	144
OM	910	893
IVOMD (%)	82	70
ME (MJ kg^{-1} DM)	11.7	9.9
pH	4.0	4.0
Ca	7.7	7.9
P	4.1	3.6
Mg	2.8	2.5
K	31.8	29.1
Na	2.2	1.7
EE	37.4	31.1
NDF	382	476
ADF	243	334
AHEE	44.2	37.6
ADIN (mg kg^{-1} DM)	556	844
WSC	89	23.9

DM, dry matter; CP, crude protein; OM, organic matter; IVOMD, in vitro *organic matter digestibility; ME, metabolisable energy; Ca, calcium; P, phosphorus; Mg, magnesium; K, potassium; Na, sodium; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; AHEE, acid hydrolysis ether extract; ADIN, acid detergent insoluble nitrogen; WSC, water soluble carbohydrates.*

Table 3.56 presents the parameters of protein degradability of the diet components in this experiment (Ørskov & McDonald, 1979). Protein degradability is calculated with the equation:

$dg = a + b\,(1 - e^{-ct})$, where:

dg: is protein degradability in the rumen (% of total protein),
a: is a protein fraction (% of total protein) which disappears very rapidly from the rumen (‘water soluble protein’),
b: is a second protein fraction which disappears at a constant fractional rate (c) per unit time,
t: is incubation time (hours).

The reported values were used to calculate rumen and metabolic protein supply.

Table 3.55. Chemical composition of the concentrate replacers and the concentrate components (g kg⁻¹ DM, unless otherwise stated) in experiment A6-1.1.

Component	Fodder beet	Barley	Soypass	Soya	Regumaize
DM (g kg^{-1} product)	172	836	862	875	616
CP	65	110	479	491	615
OM	899	974	921	929	950
IVOMD (%)	91	86	87	91	-
Ca	1.2	0.5	5.1	3.2	9.6
P	2.3	4.4	6.5	6.7	1.2
Mg	1.8	1.5	3.6	3.2	4.4
K	21.4	5.3	22.5	24.1	38.6
Na	0.9	0.1	0.7	0.1	1.2
EE	2.4	22.3	19.8	20.2	4.3
ME-E3 (MJ kg^{-1} DM)	12.4	13.5	13.1	13.7	10.7
NDF	129	119	225	124	-
ADF	70.6	54.5	60.8	91.3	-
Starch	39	538	59.3	51.8	0
AHEE	7	32.7	32.7	33.0	5
NCGD	877	902	879	919	-
ADIN (mg kg^{-1} DM)	454	346	835	619	-
WSC	-	-	-	-	550

see Table 3.54 for the meaning of the abbreviations; ME-E3, metabolisable energy calculated using the E3 equation of Thomas et al. (1988); NCGD, neutral cellulase gaminase digestibility.

Table 3.56. Parameters of protein degradability of the diet components of experiment A6-1.1.

	Parameters of protein degradability[1]		
Diet component	a	b	c
Silage first cut	63.1	34.8	0.093
Silage second cut	66.6	26.8	0.247
Fodder beet	632[2]	30.3	0.031
Barley	1.2	23.7	0.020
Soya	19.8	79.5	0.067
Soypass	6.4	62.4	0.013
Molasses	80	0.10	0.08

[1] *see the text for an explanation of the parameters*

[2] *improbable value*

Table 3.57 presents the chemical composition of the 3 experimental diets. As shown, significant differences did exist in terms of CP, starch, WSC, AHEE, NDF and ADF content of the diet.

Table 3.57. Chemical composition of the diets (g kg^{1} DM) in experiment A6-1.1.

Composition[2]	Experimental diet[3] A	B	C	s.e.d.	Significance[1]
CP	152	174	192	0.85	*
Starch	129	109	101	2.7	*
WSC	135	112	157	2.6	*
AHEE	32	33	31	0.2	*
NDF	287	312	294	4.0	*
ADF	190	198	190	3.0	*

[1] *$*p = 0.05$*

[2] *see Table 3.54 for the meaning of the abbreviations*

[3] *see Tables 2.7 and 2.8.*

The animal production results achieved during the experimental period are presented in Table 3.58. No animals had to be withdrawn from the experiment and no health problems were encountered. Experimental group B had a significantly higher silage intake than group A and a significantly higher total DM intake than A and C. As a result, group B had significantly higher yields of milk, fat-corrected milk and fat and protein-corrected milk. Group C had the highest milk fat content. Milk protein content, lactose content, live weight, liveweight gain and condition score were not affected by the diet.

Table 3.58. Animal performance results in experiment A6-1.1 (Winter of 1994/1995).

	Experimental diet A	B	C	s.e.d.	Significance[1]
Milk yield (kg day^{-1})	21.35	24.83	21.44	0.844	*
Fat content (g kg^{-1})	46.4	46.2	49.5	1.39	*
Protein content (g kg^{-1})	33.1	33.6	33.3	0.62	NS
Lactose content (g kg^{-1})	45.6	45.4	45.4	0.53	NS
FCM (kg)	23.2	27.1	24.4	1.00	*
FPCM (kg day^{-1})	22.8	26.6	23.8	0.95	*
Live weight (kg)	580	603	582	11.6	NS
Liveweight gain (kg day^{-1})	-0.099	0.270	0.037	0.171	NS
Condition score	2.54	2.62	2.49	0.698	NS
Silage intake (kg DM day^{-1})	9.4	10.7	10.1	0.43	*
Total DM intake (kg day^{-1})	15.9	17.2	16.2	0.42	*

[1] *NS = not significant ($p > 0.05$); $*p < 0.05$*

FCM, fat-corrected milk; FPCM, fat and protein-corrected milk

Table 3.59 presents the calculated rumen and metabolic protein supplies. As shown, the planned difference in ERDP content was achieved, however, the shortage in MP supply to be generated in treatment 80:80 was not achieved. An over-supply of MP was generated in all treatments.

Table 3.59. Calculated rumen protein levels (ERDP; AFRC, 1993) and resulting metabolic protein levels (MP) in experiment A6-1.1. ERDP and MP levels are presented in relation to the requirements.

	Experimental diet				
	A	B	C	s.e.d.	Significance[1)]
ERDP level (g day^{-1})	-243	-281	244	22.2	*
MP level (g day^{-1})	143	367	336	50	*

*1) *$p < 0.05$*

Table 3.60 presents the urine N contents. Urea and total N contents in the urine of the groups B and C were significantly higher than in group A.

Table 3.60. Urine N concentrations in experiment A6-1.1.

	Experimental diet				
	A	B	C	s.e.d.	Significance[1)]
Urea N in urine (g l^{-1})	2.87	4.30	4.30	0.294	*
Total N in urine (g l^{-1})	4.63	6.28	6.22	0.411	*

*1) *$p < 0.05$*

In Table 3.61, the milk N components are presented. The cows on experimental diet B had a significantly higher yield of total N, NPN and urea in the milk than the cows on diet A. Although group C had lower yields of NPN and urea than group B, the concentrations were slightly higher.

Table 3.61. Milk N components in experiment A6-1.1.

	Experimental diet				
	A	B	C	s.e.d.	Significance[1)]
Total N in milk (g cow^{-1} day^{-1})	109	126	110	6.9	*
NPN in milk (g cow^{-1} day^{-1})	5.24	6.27	5.69	0.396	*
Urea N in milk (g cow^{-1} day^{-1})	3.02	4.21	3.71	0.261	*

*1) *$p < 0.05$*

In Table 3.62, the N and energy efficiency coefficients are shown. Total N intake of group B was 27% higher than that of group A, but N output in milk was also 22% higher and, consequently, N efficiency of B was only slightly lower. Because of the relatively low milk yield of group C, N efficiency of this group was significantly lower than that of A and B.

*Table 3.62. Nitrogen efficiency (N output/N intake * 100) and energy efficiency (ME requirement/ME intake) in experiment A6-1.1.*

	Experimental diet				
	A	B	C	s.e.d.	Significance[1)]
Total N intake (g day^{-1})	378	480	491	11.4	*
N output in milk (g day^{-1})	109	133	118	7.8	*
N efficiency (%)	28.8	27.6	24.0	1.55	*
ME requirement (MJ day^{-1})	172	204	186	4.8	*
ME intake (MJ day^{-1})	184	203	187		
Energy efficiency	0.93	1.00	0.99	0.0469	NS

[1)] *NS = not significant ($p > 0.05$); *$p < 0.05$*

3.6 Sub-task A6-2

Feed intake

Daily nutrient intakes in experiment A6-2.1 are shown in Table 3.63. Values reported are means and s.d.'s of the weekly values per group. Although no strong conclusions can be drawn from this data set because of the group feeding design, it appears that no major substitution effect of concentrate was present, despite the fact that forage intake was high in terms of forage NDF intake. Crude protein intakes in the groups B and C were similar, and approximately 500 g cow^{-1} day^{-1} lower than in group A. Intake of rumen-undegradable protein in group C was 15% lower than in group B.

Table 3.63. Silage intake and total intake per cow in experiment A6-2.1, the experiment with grass/clover silage (average s.d.'s of weekly values).

	Experimental group[2)]							
Component[1)]	A		B		C		D	
Silage intake								
DM (kg day^{-1})	10.9	±1.9	11.0	±1.7	10.5	±1.8	11.1	±1.5
NDF (kg day^{-1})	6.78	±1.3	6.79	±1.1	6.53	±1.2	6.86	±0.9
NDF (% of body-weight)	1.27	±0.2	1.30	±0.2	1.18	±0.2	1.39	±0.2
CP (kg day^{-1})	1.67	±0.3	1.67	±0.2	1.61	±0.3	1.69	±0.2
UIP (kg day^{-1})	0.42	±0.1	0.42	±0.1	0.41	±0.1	0.43	±0.1
Total intake								
DM (kg day^{-1})	16.3	±1.9	12.3	±1.7	16.0	±1.8	11.1	±1.5
CP (kg day^{-1})	2.89	±0.3	2.33	±0.3	2.36	±0.3	1.69	±0.2
UIP (kg day^{-1})	0.88	±0.1	0.80	±0.1	0.68	±0.1	0.43	±0.1
NEl (Mcal day^{-1})	23.9	±2.7	16.3	±2.7	23.4	±2.5	14.1	±2.3

[1)] *see Table 2.9 for the meaning of the abbreviations*

[2)] *see Paragraph 2.6 for the description of the experimental groups*

Intake data from experiment A6-2.2 are given in Table 3.64. Intake of forage DM in group D was more than 1 kg cow^{-1} day^{-1} higher than in the supplemented groups (A, B and C). Forage intake was higher than expected. Even if the intake of SBM is subtracted, intake in group D was considerably higher than that recorded for the grass/clover silage in the previous year. Despite the relatively low CP content of the forage mixture, total CP intake was similar in both experiments.

Table 3.64. Intake of the silage mixture and total intake per cow in experiment A6-2.2, the experiment with the mixture of maize silage, vetch/oats silage and SBM (averages and s.d.'s of weekly values).

	Experimental group[2]							
Component[1]	A		B		C		D	
Silage mixture intake								
DM (kg day^{-1})	13.3	±0.4	13.5	±0.3	12.9	±0.2	14.7	±0.3
NDF (kg day^{-1})	7.33	±0.46	7.48	±0.47	7.15	±0.43	8.11	±0.49
NDF (% of bodyweight)	1.40	±0.05	1.40	±0.03	1.33	±0.04	1.48	±0.03
CP (kg day^{-1})	1.55	±0.08	1.55	±0.08	1.54	±0.07	1.70	±0.07
UIP (kg day^{-1})	0.49	±0.03	0.49	±0.02	0.48	±0.02	0.53	±0.02
Total intake								
DM (kg day^{-1})	18.1	±0.8	15.0	±0.8	18.0	±0.7	14.7	±0.3
CP (kg day^{-1})	2.8	±0.1	2.3	±0.1	2.3	±0.1	1.7	±0.1
UIP (kg day^{-1})	0.94	±0.03	0.90	±0.02	0.72	±0.03	0.53	±0.02
NEl (Mcal day^{-1})	26.4	±1.0	19.1	±1.0	26.2	±0.9	17.8	±1.1

1) *see Table 2.9 for the meaning of the abbreviations*

2) *see Paragraph 2.6 for the description of the experimental groups*

Milk yield

Milk yields in the third week of lactation were not significantly different among groups in both experiments (experiments A6-2.1 and A6-2.2 in Tables 3.65 and 3.66, respectively). During the experimental period (week 5-15) in experiment A6-2.1 (Table 3.65), the animals fed only silage (group D) had significantly lower milk yields than the animals in the other groups, but no significant differences among the other groups were detected, either when expressed as kg uncorrected milk cow^{-1} day^{-1} or as kg 3.5% fat-corrected milk cow^{-1} day^{-1}. Mean milk yield of the only forage group (group D) in the experimental period was 9 kg cow^{-1} day^{-1} lower than in the covariate week (Table 3.65). In the supplemented groups, this difference was only 2 to 3.5 kg cow^{-1} day^{-1}. Milk fat content was not different among treatments, but a significant, severe depression in milk protein content was observed in group D. Protein content of milk from the groups A, B and C were also relatively low, just above 3.0%, and not different among the three treatments. The effects of the dietary treatments on the yields of milk fat and protein were similar to those on milk yield and composition.

Efficiency of N utilisation for milk production was calculated as milk N yield/total N intake * 100. Efficiency of N utilisation was 23.7% for the all forage diet and not significantly affected by the supplementation with concentrates (Table 3.65). The efficiency of N utilisation tended to be highest for the cows in the treatments B and C and, hence, was not impaired by the protein supplement with a low rumen-degradability fed at a low rate.

Table 3.65. Milk yield, milk composition and efficiency of N utilisation for milk production in the experiment with grass/clover silage (experiment A6-2.1), as affected by supplementation.

	Experimental group					
	A	B	C	D	s.e.m.	p
Yield (kg cow^{-1} day^{-1})						
Covariate period						
Uncorrected milk	24.3	21.9	23.7	23.2	0.8	0.2
Experimental period						
Uncorrected milk	21.2a	19.9a	20.2a	14.2b	0.7	0.001
3.5% fat-corrected milk	20.3a	19.3a	19.0a	13.4b	0.8	0.001
Protein	0.63a	0.60a	0.60a	0.40b	0.02	0.001
Fat	0.69a	0.66a	0.63a	0.45b	0.03	0.001
Milk composition (%)						
Protein	2.98a	3.08a	3.02a	2.77b	0.05	0.001
Fat	3.23	3.3	3.23	3.13	0.12	0.7
N efficiency (%)	22.5	25.8	25.0	23.7	0.9	0.12

a, b: numbers in the same row with different superscript differ significantly.

In experiment A6-2.2, mean milk yield of the only-forage group (group D) was 4 kg cow^{-1} day^{-1} lower than in the covariate week (Table 3.66). In the supplemented groups, this difference was approximately 1 kg cow^{-1} day^{-1}. Hence, the persistency of milk production in this experiment was much better than in the first experiment. Supplemented groups outyielded the negative control group on average by 4 kg cow^{-1} day^{-1}. Milk fat content ranged from 3.38 to 3.56%, but the difference was probably not significant. Differences between supplemented and unsupplemented groups in protein content were more pronounced, due to a severe reduction of the protein content of the milk in group D.

Table 3.66. Milk yield and milk composition in the experiment with the mixture of maize silage, vetch/oats silage and SBM (experiment A6-2.2), as affected by supplementation.

	Experimental group							
	A		B		C		D	
Yield (kg cow^{-1} day^{-1})								
Covariate period								
Uncorrected milk	26.0	±1.6	25.9	±1.3	23.9	±1.5	23.8	±1.2
Experimental period								
Uncorrected milk	25.0	±1.0	24.5	±1.6	23.5	±0.6	19.9	±0.5
Protein	0.75	±0.03	0.73	±0.02	0.70	±0.04	0.57	±0.02
Fat	0.92	±0.06	0.87	±0.04	0.81	±0.06	0.71	±0.03
Milk composition (%)								
Protein	2.94	±0.10	2.87	±0.07	2.92	±0.09	2.71	±0.06
Fat	3.56	±0.13	3.50	±0.12	3.38	±0.12	3.41	±0.05

Body weight

During experiment A6-2.1, cows in the control group D had a severe weight loss (Table 3.67). Cows receiving the high-protein concentrate (group B) tended to loose more body weight and for a longer period than cows in the groups A and C, but this difference was not statistically significant (Table 3.67, Figure 3.2).

Table 3.67. Body weight changes throughout experiment A6-2.1 (grass/clover silage).

		Experimental group					
		A	B	C	D	s.e.m.	p
Body weight							
Covariate period	(kg)	543.9	522.3	537.9	541.9	17.0	0.8
Week 7	(kg)	529.1	510.8	535.5	504.4	17.3	0.5
Week 15	(kg)	553.6a	529.9a	556.6a	480.8b	9.4	0.001
Minimum	(kg)	514.8	489.5	511.3	465.2	15.7	0.1
Maximum loss	(kg)	-29.1a	-38.8a	-25.8a	-69.0b	5.6	0.001

a, b: numbers in the same row with different superscript differ significantly.

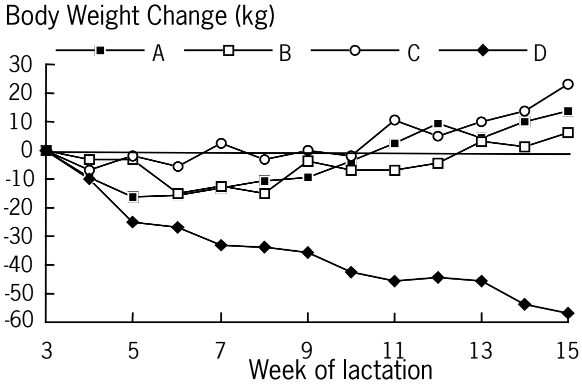

Figure 3.2. Body weight changes throughout the experiment with grass/clover silage (experiment A6-2.1).

In experiment A6-2.2, body weight changes were moderate in the supplemented group (Table 3.68). In week 7 all three groups were already in a positive energy balance. On the contrary, cows in group D had a severe weight loss (up to 5% of body weight), and had not recovered in week 15 of lactation. Cows on the high-protein concentrate (group B) had lower rates of body weight gain than cows on the diets A or C.

Table 3.68. Body weight changes throughout the second experiment (experiment A6-2.2).

		Experimental group							
		A		B		C		D	
Body weight									
Covariate period	(kg)	528	±14	544	±17	540	±20	585	±22
Week 7	(kg)	540	±12	556	±19	557	±24	574	±24
Week 15	(kg)	568	±17	566	±15	579	±28	579	±13
Minimum	(kg)	510	±13	539	±17	531	±23	553	±22

3.7 Sub-task B1

Yields and quality of crops in experiment B1-1 will be discussed in addition to N use, consequences of using the two crop rotations, and need for future work.

Normal yields at Silstrup

The warm and dry weather in 1994 and 1995 was a good test of yield level and yield stability of crops. However, the unusual weather conditions also made a long-term interpretation of results difficult. Particularly yields of grass and grass/clover were negatively affected by the warm and dry weather. These crops yielded 2000 to 3000 kg DM ha^{-1} year^{-1} less than in the preceding 10 years at Silstrup, although in the past they also received more N. Cereals harvested at full maturity or for whole-crop silage also yielded less, but the difference was not as evident as for grass or grass/clover. Fodder beet had unusually high yields in 1993, but yields in 1994 and 1995 were not as negatively influenced by adverse weather compared with the other crops. One reason is that fodder beet has a longer growing season than other crops and also grows in the autumn when water and temperature stress are not as high as in summer. Detailed information on DM yields of the crops in both cropping systems is given in the Annexes B1-1 to B1-6.

Yield and quality

Production results for the two crop rotations in experiment B1-1 are shown in Table 3.69. Crops in the energy rotation had on average 37% higher DM yields than crops in the protein rotation. This result is partly related to the fact that energy crops received 95 kg N ha^{-1} year^{-1} more than protein crops (Table 3.70). There was only a small difference in metabolisable energy concentration between the protein crops and the energy crops. Consequently, differences in metabolisable energy yield, expressed as MJ ha^{-1} year^{-1}, were more a reflection of differences in DM yield than of differences in energy concentration. Crops in the protein rotation had a 24% lower metabolisable energy yield than crops in the energy rotation. Because of the larger proportion of leguminous crops in the protein rotation, the protein concentration was 24 g kg^{-1} DM higher than that of energy crops (Table 3.69). Protein crops also had a higher protein concentration per unit of metabolisable energy than energy crops. Total DM yield of protein crops related to 37 AU, resulted in 4650 kg DM AU^{-1}, or 1200 kg less than the annual requirement of cows receiving feed from the protein rotation (see Paragraph 3.1). To be able to cover this deficiency, the area of protein crops either should be extended from 28 to 35 ha, or the DM yield should be increased by 1600 kg ha^{-1} year^{-1}. If one desired the same amount of DM from the protein crops than from the energy crops, the area of protein crops should be extended from 28 to 38 ha.

Table 3.69. Yield and feeding value of crops in the protein and energy crop rotations in 1993 to 1995 (experiment B1-1). Dry matter yields of grazed fields, straw, and fodder beet tops are not included in the figures. The protein and energy rotations are shown in the Tables 2.15 and 2.16, respectively, and yields of the individual crops in the Annexes B1-1 to B1-6.

Cropping system	Area	Yield	Crude Protein		Metabolisable energy	
	(ha)	(kg DM ha^{-1})	(kg ha^{-1})	(g kg^{-1})	(x 1000 MJ ha^{-1})	(MJ kg^{-1} DM)
Protein crops						
1993	28.8	6149	768	126	67.3	10.95
1994	28.5	5983	711	115	70.1	11.72
1995	27.0	6250	774	125	62.2	9.95
Average	**28.1**	**6125**	**751**	**122**	**66.6**	**10.89**
Energy crops						
1993	27.2	9994	1012	107	105.7	10.57
1994	23.9	6861	549	96	75.8	11.04
1995	24.1	8017	637	89	78.3	9.77
Average	**25.1**	**8365**	**745**	**98**	**87.4**	**10.46**

Utilisation and leaching of N

Utilisation of N in the energy cropping system, estimated as harvested N as a proportion of applied N, was 85% (Table 3.70). A comparable calculation for protein crops is more difficult because the contribution of fixed N by legumes was not directly measured. However, if standard values for N fixation by grass/clover and field pea, presented by Kristensen & Kristensen (1992) are used, N fixation by the protein crops was approximately 60 kg ha^{-1} year^{-1}. N utilisation of crops in this study was within the expected range (Olsen, 1992).

Another method of studying N utilisation is to relate DM yield to the rate of N application. On average, protein crops produced 91 kg DM kg^{-1} N applied, while energy crops produced 52 kg DM kg^{-1} N applied. (Tables 3.69 and 3.70). These calculations indicate that the requirement for slurry or fertiliser N was about 1.8 times lower for protein crops than for energy crops. These calculations do not indicate, however, that protein crops are more environmentally friendly than energy crops.

Table 3.70. Nitrogen balances of the two crop rotations at Silstrup Research Station in 1993 to 1995 (experiment B1-1). The concentration of total N in slurry was 3.2 kg ton^{-1} and ammonium-N 620 g kg^{-1} total N. In harvested N, all plant parts are included. All values are kg N ha^{-1}.

Cropping system	Applied N¹⁾			Harvested N	N balance²⁾
and year	Slurry	Fertiliser	Total		
Protein crops					
1993	44	4	48	127	-79
1994	49	11	60	118	-58
1995	65	27	92	126	-34
Average	**53**	**14**	**67**	**124**	**-57**
Energy crops					
1993	37	124	161	192	-31
1994	71	66	137	108	29
1995	93	94	187	117	70
Average	**67**	**95**	**162**	**139**	**23**

1) *values for slurry are NH_4^+-N*

2) *N balance is the difference between N in the harvested crop and N applied in slurry and fertiliser.*

Concentration of nitrate-N, run-off to drainage pipes and leaching of N are shown in the Tables 3.71 and 3.72. Leaching of nitrate-N was, on average, 36 kg ha^{-1} year^{-1} for protein crops and 42 kg ha^{-1} year^{-1} for energy crops. These values are half as large as average N leaching values in Denmark (Jensen *et al.*, 1994). We had anticipated a larger difference in leaching between crop rotations because energy crops received 95 kg ha^{-1} year^{-1} more N. In the energy cropping system, fields with winter wheat were autumn-ploughed and fields with fodder beet left uncovered throughout the winter, factors that may contribute to N leaching (Olsen, 1995). Fields that were ploughed less than one time per year had lower nitrate concentrations in the drainage water and lower N leaching than fields that were ploughed once yearly. Other crops leached 38 to 50 kg nitrate N ha^{-1} year^{-1}. The variation in leaching is difficult to explain, but other studies have reported that pea may leach 90 kg nitrate-N ha^{-1} year^{-1} and fodder beet as much as 143 kg nitrate-N ha^{-1} year^{-1} (Simmelsgaard *et al.*, 1994).

Table 3.71. Modelled values of nitrate leaching and run-off for crops in the protein rotation. Calculations based on nitrate concentrations in samples taken from drainage wells in individually drained fields at Silstrup Research Station. Modelling began on 30 September 1992 and ended on 31 March 1996. All figures are mean annual values.

Protein crops	Leaching (kg nitrate-N ha^{-1})	Run-off (mm)	Nitrate-N (mg litre^{-1})
Barley + grass/clover	40	382	10.4
Grass/clover, 1st production year	24	381	6.3
Grass/clover, 2nd production year	24	383	6.3
Whole barley + Italian ryegrass	44	379	11.5
Whole pea + Italian ryegrass	50	379	13.1
Average	**36**	**381**	**9.5**

Table 3.72. Modelled values of nitrate leaching and run-off for crops in the energy rotation. Calculations based on nitrate concentrations in samples taken from drainage wells in individually drained fields at Silstrup Research Station. Modelling began on 30 September 1992 and ended on 31 March 1996. All figures are mean annual values.

Energy crops	Leaching (kg nitrate-N ha^{-1})	Run-off (mm)	Nitrate-N (mg $litre^{-1}$)
Barley + per. ryegrass	48	382	12.6
Per. ryegrass, 1st production year	37	367	10.0
Oat	38	372	10.3
Whole wheat + Italian ryegrass	42	372	11.2
Fodder beet	47	396	11.8
Average	**42**	**378**	**11.2**

It is evident from the N balances presented in Table 3.70 and Annex B1-14 that the N deficit of the protein rotation became smaller with the progression of time. In the energy rotation, N accumulated with progression of time (Table 3.70 and Annex B1-15). Concentration of N in the soil was inconclusive with regard to N accumulation (data not shown). But the study probably was too short to see any long-term effects on soil N. Nitrogen flow in this experiment was compared with previous studies at Silstrup Research Station. In this trial, average application of inorganic N to crops in both crop rotations was approximately 115 kg ha^{-1} $year^{-1}$, while 132 kg N ha^{-1} $year^{-1}$ was harvested in the crops (Table 3.70) and 39 kg N ha^{-1} $year^{-1}$ was leached to the drainage water (Tables 3.71 and 3.72). Consequently, the balance of inorganic N was –56 kg ha^{-1} $year^{-1}$. Comparable calculations for trials conducted at Silstrup in 1988 to 1991 showed that 224 kg N ha^{-1} $year^{-1}$ were applied to crops, 182 kg N ha^{-1} $year^{-1}$ were harvested, 70 kg N ha^{-1} $year^{-1}$ were leached to the drainage water, and that the balance of inorganic N was –28 kg ha^{-1} $year^{-1}$ (Simmelsgaard *et al.*, 1994). The difference in inorganic N balance between the two studies was 28 kg ha^{-1} $year^{-1}$.

Consequences and future perspectives of the two cropping systems

By using the same yields and N flows as in Table 3.70, a theoretical calculation of N flows in the protein and the energy cropping system was made and is presented in Table 3.73. The estimated production of ammonium-N of the cows consuming the crops from the protein rotations corresponded to 77 kg ha^{-1} $year^{-1}$, or 10 kg more than the crops required. A comparable calculation for the energy crops resulted in a need to purchase 110 kg of inorganic N ha^{-1} $year^{-1}$.

The selection of crops in both crop rotations should be changed under practical conditions. The area of crops suitable for grazing or high-quality silage production is too small in the energy cropping system. It is also difficult to maintain a production of 9000 kg of energy-corrected milk cow^{-1} $year^{-1}$ when relying on silage from whole-crop winter wheat (see experiments A1-2 and A1-5 in Paragraph 3.1). DM production of crops in the protein rotation was too low to supply cows with sufficient conserved feed throughout the lactation period. Furthermore, it may be necessary to supply cows with energy supplements to be able to efficiently use the protein from the leguminous crops. Consequently, there is a need for silage with a greater feeding value (metabolisable energy concentration) than the winter wheat in the energy rotation. Furthermore, there was a shortage of crops in the protein rotation with a low N degradation in the rumen and a high energy concentration. Hence, a protein crop with a larger proportion of by-pass protein would be desirable.

Table 3.73. Nitrogen balances of the cropping systems in experiment B1-1 at Silstrup Research Station. It is assumed that either crops in the protein cropping system or crops in the energy cropping system are used solely at the farm. All values are kg N ha^{-1} $year^{-1}$.

Cropping system	Measured N in harvested crops[1]	Measured application of inorganic N[1]	Theoretical application of NH_4^+-N in slurry[2]	Requirement for inorganic fertiliser
Protein (P)	124	67	77	-10
Energy (E)	139	162	57	105
Difference E – P	**15**	**95**	**-20**	**115**

[1] *Measured values are taken from Table 3.70.*

[2] *Slurry from cows fed protein crops or energy crops was not separated in the slurry tank. Therefore, concentration of ammonium N in slurry originating from both cropping systems was estimated from the N content of the feed consumed, and milk and liveweight produced.*

Fodder beet and forage maize are examples of crops with a high energy value and low protein concentration. Rape has a large proportion of by-pass protein if it is heat-treated. The role of these crops in sustainable and environmentally friendly agriculture is questionable, however. If these crops are used with some restraint as supplemental feeds, the problem with over-supply of easily degradable N to grazing animals may be reduced or eliminated. However, the need for crops with lower concentration of crude protein or less protein degraded in the rumen, is still valid. Birdsfoot trefoil that contains tannin and red clover that contains polyphenol oxidase have the potential of increasing microbial protein synthesis during grazing. Plant breeding may also play an important role in improving the utilisation of N on dairy farms.

3.8 Sub-task B3

Chemical composition of feedstuffs

Chemical composition and feeding value of most of the feedstuffs, presented in Table 3.74, were similar to the standard values of CVB (1995). However, concentrations of crude protein, N, sugar, DVE and OEB of the grass silage in experiment B3-2 were rather low compared to these standards. Digestibility of organic matter (dOM) and NE_L value of the fodder beet were relatively high compared to the standards of CVB (1995). DM content, starch concentration, dOM and NE_L values of GMES were lower in experiment B3-1 than in experiment B3-2, but the concentrations of crude fibre, NDF and ADF were higher. The fermentation characteristics of grass silage and GMES indicated that these silages were well preserved.

Table 3.74. Chemical composition of feedstuffs, g kg^{-1} DM (except where indicated otherwise).

	Experiment B3-1					Experiment B3-2			
	Grass silage	Fodder beet	GMES	Beet pulp	SMSE	Grass silage	GMES	BSC	SCF
DM (g kg^{-1} fresh)	500	141	478	908	884	435	532	900	894
Crude protein	189	61	88	98	435	140	85	216	419
Total N	30.4	9.8	14.6	15.0	68.1	22.0	14.0	33.0	66.0
Crude fibre	229	67	89	177	66	237	74	134	62
Crude ash	120	77	25	114	94	116	23	118	132
Crude fat	48	8	8	7	20	40	41	11	20
Sugar	107	563	40	102	112	84	5	127	115
Starch	-	-	525	-	29	-	573	30	45
NDF	412	163	329	498	162	429	222	367	195
ADF	242	80	106	241	96	258	98	183	88
ADL	16	8	11	16	8	17	11	17	8
dOM *in vitro* (%)	77.9	91.8	81.5	88.3	90.4	78.7	83.6	88.3	89.7
FOS	571	578	830	746	615	583	607	578	356
NE_L[1] (MJ kg^{-1} DM)	6.21	7.60	7.43	7.25	7.09	6.21	7.78	6.89	6.94
DVE[2]	76	79	64	107	230	67	66	120	342
OEB[3]	54	-77	-28	-71	159	13	-35	7	47
Calcium	6.7	2.5	0.7	8.4	8.3	5.7	0.5	11.8	12.9
Phosphorus	3.8	2.3	3.1	1.0	7.9	3.9	2.8	5.3	10.2
Magnesium	1.7	1.3	1.1	2.0	16.1	1.3	1.0	11.6	16.1
Sodium	1.4	1.6	0.1	0.6	0.5	0.6	0.2	2.2	3.6
Potassium	37.5	24.0	6.1	6.4	23.9	35.4	5.4	13.5	23.0
Fermentation characteristics[4]									
Ammonia	5.5	-	5.4	-	-	4.2	9.1	-	-
pH	5.2	-	4.1	-	-	5.2	4.1	-	-
Butyric acid (%)	<0.1	-	0.2	-	-	<0.1	<0.1	-	-
Acetic acid (%)	7.5	-	5.5	-	-	8.9	5.7	-	-
Lactic acid (%)	25.6	-	13.2	-	-	28.1	13.7	-	-
Alcohol	2.4	-	1.1	-	-	2.3	1.0	-	-

[1] *Net energy for lactation (Van Es, 1978)*
[2] *Digestible protein available in the intestine (Tamminga et al., 1994)*
[3] *Degradable protein balance in the rumen (Tamminga et al., 1994)*
[4] *Percentage of total volatile fatty acids*

Feed intake

Experiment B3-1

Results on feed intake are given in Table 3.75. The means of total DM intake (TDMI) were not significantly different among treatments. However, intakes of beet pulp, GMES and fodder beet were less than expected. The animals in BS consumed only 6.2 kg DM of beet pulp while 8.7 kg was offered. The animals in MS consumed on average 2.9 kg DM of GMES while 5 kg was offered. The means of silage intake were not significantly different among the treatments. However, intake of grass silage tended to be lower in FB than in BS ($0.05<p<0.10$).

Experiment B3-2

Intakes of grass silage and GMES did not differ significantly, neither between the high-roughage treatments (HRLP and HRHP) nor between the low-roughage treatments (LRLP and LRHP). In addition, intake of compound feed (BSC plus SCF) was similar in all treatments. There were no significant differences in TDMI among the treatments (Table 3.75). However, TDMI in LRHP tended to be lower than in HRHP (0.05<p<0.1). Although DM intake in LRLP and LRHP was restricted, the animals consumed less than allowed. Therefore, feeding strategy could be defined as virtually *ad libitum*.

Table 3.75. Mean values of daily intake of diet components, TDMI, NE_L, DVE, OEB, NE_L coverage (NE_L intake/NE_L requirement) × 100% and DVE coverage (DVE intake/DVE requirement) × 100%; all values in kg DM except where indicated otherwise (see paragraph 2.8 for the description of the treatments and abbreviations).

	Experiment B3-1					
	BS	FB	FBMS	MS	sed	Significance[1]
Beet pulp	6.2	2.3	0.6	2.2		
SMSE	1.3	2.2	2.7	2.6		
GMES	-	-	1.6	2.9		
Fodder beet	-	3.8	3.1	-		
Grass silage	13.0	11.9	12.3	12.4	0.64	
TDMI	20.5	20.2	20.3	20.1	0.65	
NE_L (MJ)	134.2	136.7	138.1	132.5	4.5	
DVE (g)	1948	2007	2023	1947	58	
OEB (g)	552	645	897	931	56	***
NE_L coverage (%)	96	101	100	93	2.4	**
DVE coverage (%)	113	118	115	113	2.6	

	Experiment B3-2						
						Significance	
	HRLP	LRLP	HRHP	LRHP	sed	DVE[2]	S[3]
BSC	4.6	4.2	3.3	3.1			
SCF	-	0.4	1.5	1.8			
GMES	3.6	7.6	3.7	7.0			
Grass silage	12.1	7.9	12.4	8.0			
Forage/concentrate ratio	60:40	39:61	59:41	40:60			
TDMI	20.3	20.1	20.9	19.9	0.51		~
NE_L (MJ)	132.5	136.8	136.0	134.8	3.3		
DVE (g)	1533	1614	1863	1882	33	***	*
OEB (g)	95	-66	113	-41	23		***
NE_L coverage (%)	96	100	97	95	2.7		
DVE coverage (%)	87	90	101	101	3.2	***	

[1] *Significance: *** = p<0.001; ** = p<0.01 ; * = p<0.05; ~ = p<0.1*

[2] *DVE = effect of level of DVE*

[3] *S = effect of grass silage allowance*

Energy and protein intake

Experiment B3-1

Intake of NE_L was similar in all treatments. NE_L coverage (NE_L intake/NE_L requirement) was sufficient in FB and FBMS, but not in MS and BS. NE_L coverage in MS was significantly lower than in FB and FBMS ($p<0.05$) (Table 3.75). NE_L coverage in BS was significantly lower than in FB ($p<0.05$). There were no significant differences in DVE intake and DVE coverage (DVE intake/DVE requirement) among treatments. DVE coverage was sufficient for all treatment groups. OEB intake was significantly higher in FBMS and MS than in FB and BS ($p<0.05$).

Experiment B3-2

There were no differences in NE_L and DVE intake between the high-protein treatments (HRHP and LRHP) (Table 3.75). In the low-protein treatments, DVE intake in HRLP was significantly lower than in LRLP ($p<0.05$). DVE coverage was sufficient in HRHP and LRHP, but not in HRLP and LRLP. In all treatments, intake of DVE was less than targeted. DVE coverage in HRLP and LRLP was 87 and 90 per cent, respectively, and in both LRHP and HRHP 101 per cent, thus close to the calculated requirements. OEB was significantly higher in HRLP and HRHP than in LRLP and LRHP ($p<0.05$).

Composition of the ingested diets

Experiment B3-1

Partial replacement of purchased concentrates by either fodder beet or GMES changed the concentration of structural and non-structural carbohydrates in the diet (Table 3.76). Replacing purchased concentrates by fodder beet (treatment FB) increased the sugar content and decreased the concentration of crude fibre and cell wall constituents (NDF and ADF), whereas replacement of purchased concentrates by GMES (treatment MS) increased the starch content and reduced the concentration of crude fibre, sugar, NDF and ADF.

OM content was similar in all rations, but dOM was higher in FBMS and FB. FOM was highest in treatment FB and lowest in treatment MS. NE_L was highest in the rations containing fodder beet. The concentrations of crude protein, total N and OEB were similar in MS and FBMS and higher in these rations than in BS and FB.

Table 3.76. Chemical composition of the ingested rations, g kg^{-1} *DM (except where indicated otherwise).*

	Experiment B3-1				Experiment B3-2			
	BS	FB	FBMS	MS	HRLP	LRLP	HRHP	LRHP
Crude protein	179	188	199	199	142	137	154	151
Total N	28.2	29.7	31.4	31.3	21.9	21.4	23.8	23.5
Crude fibre	206	176	172	185	183	149	178	146
Crude ash	114	109	105	104	95	78	96	81
Crude fat	34	34	36	39	33	33	33	34
Sugar	106	194	173	90	71	56	72	62
Starch	2	3	44	79	117	229	116	213
NDF	418	347	334	376	369	323	358	321
ADF	230	196	188	202	206	173	199	169
ADL	16	12	12	15	15	14	15	13
OM	886	891	895	896	905	922	904	919
dOM	729	750	747	728	721	742	721	741
FOM	628	652	630	597	587	584	572	571
NE_L (MJ)	6.56	6.78	6.80	6.61	6.52	6.80	6.52	6.78
DVE	95	100	100	97	76	80	90	95
OEB	27	32	44	46	5	-3	5	-2

Experiment B3-2

Restricting the intake of grass silage and doubling the allowance of GMES (LR versus HR) almost doubled the concentration of starch in the ingested ration and reduced concentrations of sugar, crude fibre, NDF and ADF (Table 3.76). However, the changes in the concentration of sugar and structural carbohydrates were less dramatic than that in the concentration of starch. In addition, OM content, dOM and NE_L values were higher in LR than in HR rations. OEB was lower in LR than in HR rations. A higher level of DVE in HRHP and LRHP was accompanied by higher concentrations of N and crude protein.

Milk yield and composition

Experiment B3-1

Milk yield in FB was significantly lower than in BS ($p<0.05$) and in MS it was significantly higher than in the other treatments ($p<0.05$) (Table 3.77). FPCM yield was significantly higher in MS than in FB ($p<0.05$). There were no significant differences among treatments in fat and protein yield, but lactose yield was significantly higher in MS than in FB and FBMS ($p<0.05$) and in BS than in FB ($p<0.05$). There were no significant differences in milk fat concentration among treatments, but lactose concentration was significantly lower in FB than in BS, and milk protein concentration was significantly lower in MS and BS than in FB and FBMS ($p<0.05$). Both, milk urea concentration and urea excretion in milk were significantly higher in MS than in the other treatments ($p<0.05$), as well in FBMS than in FB ($p<0.05$).

Experiment B3-2

Milk yield, lactose yield and urea excretion in milk were significantly higher for cows on HP rations than on LP rations ($p<0.05$) (Table 3.77). Protein yield in HRHP was significantly higher than in HRLP and LRLP, and there was a tendency for a higher protein yield in LRHP than in HRLP and LRLP ($0.05<p<0.1$). Milk fat concentration in HRLP was significantly higher than in HRHP and LRHP. Protein concentration in the milk tended to be higher in the LP treatments than in the HP treatments.

Milk urea concentration was higher in the HP treatments than in the LP treatments ($p<0.05$). FPCM yield was higher in HRHP than in HRLP and LRLP ($p<0.05$). FPCM yield in LRHP was significantly higher than in LRLP ($p<0.05$) and tended to be higher than in HRLP ($0.05<p<0.1$).

Nitrogen utilisation

Nitrogen utilisation was calculated as (N excretion in milk/N intake) × 100. In both experiment B3-1 and B3-2, differences in N utilisation followed the differences in intake of dietary N. Lower intake of dietary N resulted in an improved N utilisation (Tables 3.75 and 3.77).

Table 3.77. Average daily yields of milk, FPCM (fat and protein-corrected milk = milk yield × (0.337 + fat conc. × 0.0116 + protein conc. × 0.006) and milk constituents, milk composition and N utilisation (N in milk/N intake) × 100%).

	Experiment B3-1				sed	Significance[1]
	BS	FB	FBMS	MS		
Milk (kg)	31.0	29.4	30.4	32.4	0.65	***
FPCM (kg)	32.2	31.2	32.2	33.2	0.64	**
Fat (g)	1366	1320	1360	1393	39	
Protein (g)	1003	993	1030	1014	21	
Lactose (g)	1458	1349	1414	1504	28	***
Urea (mg)	9381	8493	9665	11273	507	***
Fat (g kg^{-1})	43.9	45.1	44.8	43.1	1.18	
Protein (g kg^{-1})	32.3	34.0	33.9	31.3	0.65	***
Lactose (g kg^{-1})	47.0	46.0	46.6	46.5	0.37	***
Urea (mg l^{-1})	301	282	319	350	14.4	***
N utilisation (%)	27.6	26.2	25.4	25.3	0.8	*

	Experiment B3-2				sed	Significance	
	HRLP	LRLP	HRHP	LRHP		DVE[2]	S[3]
Milk (kg)	28.9	29.0	31.8	31.7	0.99	***	
FPCM (kg)	31.4	31.1	33.1	32.7	0.80	**	
Fat (g)	1362	1338	1387	1366	46		
Protein (g)	977	977	1042	1025	28	***	
Lactose (g)	1330	1325	1448	1444	49	***	
Urea (mg)	4538	4048	5714	5647	340	***	
Fat (g kg^{-1})	47.1	46.2	43.6	43.1	1.63	**	
Protein (g kg^{-1})	33.8	33.7	32.8	32.4	0.81		
Lactose (g kg^{-1})	46.0	45.8	45.6	45.6	0.36		
Urea (mg l^{-1})	157	140	180	178	8.4	***	
N utilisation (%)	34.8	36.0	32.5	34.5	0.8	*	*

[1] *Significance: *** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$; ~ = $p<0.1$*

[2] *DVE = effect of level of DVE*

[3] *S = effect of grass silage allowance*

Live weight

Neither in experiment B3-1 nor in B3-2, significant differences were found in live weight. Mean live weights in BS, FB, FBMS and MS were 590, 604, 602 and 593 kg (s.e.d. = 20), respectively, and mean liveweight gains during the experimental period were -4, 15, 18 and 3 kg, respectively. Mean live weights in HRLP, HRHP, LRLP and LRHP were 596, 594, 592 and 593 kg (s.e.d. = 12), respectively, and mean liveweight gains during the experimental period were 10, 29, 17 and 16 kg, respectively.

Blood and urine parameters

Experiment B3-1
UN, UUN and BU followed the differences in intake of dietary N and OEB (Table 3.76). However, the differences in UUN at 19:00 h were less pronounced than at 8:00 h, suggesting diurnal variation. Plasma ß-HB concentration followed the differences in NE_L coverage. Except for the 4th week after calving, there were no indications of insufficient energy supply (Table 3.78).

Experiment B3-2
As in experiment B3-1, UN, UUN and BU concentration followed the differences in intake of dietary N and OEB. Differences in UN and UUN between 8:00 h and 19:00 h suggest that there was diurnal variation in N excretion in urine. Plasma ß-HB concentrations were low, so there were no indications of insufficient energy supply (Table 3.78).

Table 3.78. Urinary nitrogen (UN) and urinary urea nitrogen (UUN) 10 weeks post partum*; serum urea (BU) and ß-hydroxybutyrate 4, 10 and 15 weeks* post partum.

	Experiment B3-1					**Experiment B3-2**				
	BS	FB	FBMS	MS	sed	HRLP	HRHP	LRLP	LRHP	sed
Urinary nitrogen, g l^{-1}										
8:00 h	6.31	6.31	7.56	7.62	0.60	4.60	4.30	5.34	5.22	0.60
19:00 h	6.56	7.17	7.55	8.95	0.67	5.33	6.33	5.61	6.50	0.74
Urinary urea nitrogen, g l^{-1}										
8:00 h	3.89	4.27	5.03	5.49	0.46	2.56	3.59	2.31	3.14	0.50
19:00 h	4.44	4.93	5.00	6.60	0.45	3.22	5.14	2.73	4.16	0.59
Serum urea, mmol l^{-1}										
4 wks pp.	4.03	4.05	3.69	4.07	0.49	3.35	3.45	2.95	3.15	0.29
10 wks pp.	4.63	4.85	5.08	5.79	0.34	3.12	3.62	3.14	3.53	0.38
15 wks pp.	4.40	5.31	4.88	5.51	0.36	2.97	3.44	3.02	3.6	0.36
Serum ß-hydroxybutyrate, mmol l^{-1}										
4 wks pp.	1.63	1.01	1.09	1.36	0.37	0.84	0.76	0.79	1.03	0.17
10 wks pp.	0.73	0.53	0.54	0.74	0.08	0.55	0.54	0.68	0.92	0.17
15 wks pp.	0.63	0.54	0.65	0.64	0.06	0.86	0.57	0.71	0.66	0.07

3.9 Sub-task B5

The results of the chemical analyses of the feeds used in experiment B5-1 are given in Table 3.79. The DM content of the grass/clover silage was low; the ammonia fraction rather high. Other figures were normal.

Table 3.79. Chemical composition of the feeds in experiment B5-1 (Winter of 1993/1994). Figures are in g kg^{1} DM, unless stated otherwise.

Component[1)]	Grass/clover silage	Fodder beet	Barley	Soya
DM (g kg^{-1} product)	177	182	820	871
OM	920	891	970	935
IVOMD (%)	75.4	89.5	84.7	90.3
ME (MJ kg^{-1} DM)[2)]	11.0	12.8	13.1	13.7
CP	145	61.6	104	486
NDF	478	118	142	125
ADF	320	66	56	86
Starch	23	22.1	550	49
AHEE	46.5	7.7	34.2	34.2
WSC	7.03	425	24.7	88.6
pH	4	-	-	-
Ammonia (g N kg^{-1} total N)	98	-	-	-
NCGD	-	-	887	921

[1)] *DM, dry matter; OM, organic matter; IVOMD,* in vitro *organic matter digestibility; ME, metabolisable energy; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; AHEE, acid hydrolysis ether extract; WSC, water soluble carbohydrates; NCGD, neutral cellulase gaminase digestibility.*

[2)] *ME, calculated from IVOMD for silage and fodder beet and from NCGD for barley and soya*

In Table 3.80, silage and total DM intakes are presented. The highest intakes were achieved on diet F but the differences with the other treatments were not statistically significant ($p < 0.05$).

Table 3.80. Intakes of silage and total DM in experiment B5-1. See paragraph 2.9 for a description of the diets.

	Diet			
	B	BF	F	s.e.d.
Silage DM (kg day^{-1})	8.97	8.31	9.14	0.340
Total DM (kg day^{-1})	16.96	16.48	17.34	0.340

Table 3.81 presents the calculated composition of the experimental diets. CP and starch content were highest in diet B and lowest in F, while WSC content was lowest in diet B and highest in F. NDF and ADF content did not differ.

Table 3.81. Diet composition in experiment B5-1 (in g kg^{-1} DM).

	Diet		
Component[1]	B	BF	F
CP	195	189	180
Starch	183	107	3
WSC	27	93	147
NDF	315	302	307
ADF	199	195	202

[1] *see Table 3.79 for the meaning of the abbreviations.*

Table 3.82 presents the animal performance results. None of the production variables was significantly different except milk protein content, which was significantly lower on diet F. Milk production tended to be higher on BF and F than on B.

Table 3.82. Animal production results in experiment B5-1 (Winter of 1993/1994).

	Diet			
	B	BF	F	s.e.d.[1]
Milk yield (kg day^{-1})	23.6	26.0	26.5	1.35
Fat content (g kg^{-1})	45.4	44.4	44.1	1.59
Protein content (g kg^{-1})	35.0	34.3	32.8	0.80*
Lactose content (g kg^{-1})	45.1	46.0	46.0	0.80
Fat (kg day^{-1})	1.073	1.150	1.169	0.081
Protein (kg day^{-1})	0.821	0.886	0.863	0.044
Lactose (kg day^{-1})	1.067	1.201	1.219	0.072
FCM (kg day^{-1})	25.2	27.7	28.1	1.69
FPCM (kg day^{-1})	25.5	27.7	27.7	1.69
LW (kg)	638	624	634	13.4
LWG (kg day^{-1})	0.507	0.279	0.443	0.177
Condition score	2.87	2.56	2.40	0.20

[1] *$*p < 0.05$

FCM, fat-corrected milk; FPCM, fat and protein-corrected milk; LW, live weight; LWG, liveweight gain.

Table 3.83 presents the concentration of N components in the milk. NPN content was significantly lower on treatment F than on B. Milk urea content was only significantly affected at PM milking, while urea contents in the milk at the AM milking were almost equal. Although the concentrations of NPN were significantly different, total outputs of NPN components were not.

Table 3.83. Milk N components in experiment B5-1.

Component	Treatment B	 BF	 F	s.e.d.[1)]
NPN – AM (% protein eq.)	0.1923	0.1856	0.1788	0.0052 *
NPN - PM (% protein eq.)	0.1837	0.1761	0.1669	0.0049 *
NPN (g day^{-1})	6.81	7.32	7.24	0.384
Urea - AM (mg l^{-1})	198.8	198.3	196.1	6.46
Urea - PM (mg l^{-1})	213.5	207.3	194.7	7.24 *
Urea N (g day^{-1})	4.70	5.15	5.18	0.283

[1)] ** $p < 0.05$*

NPN, non-protein nitrogen; AM, morning; PM, afternoon.

Table 3.84 presents the concentrations of total N and urea N in urine. Both total N and urea N concentration in the urine were significantly lower (p < 0.01) in F than in B.

Table 3.84. Concentrations (g l^{-1}) of total N and urea N in the urine of the cows in experiment B5-1.

Component	Treatment B	 BF	 F	s.e.d.[1)]
Mean urea N	6.24	5.23	4.18	0.318 **
Mean total N	8.75	7.31	6.01	0.418 **
Urea N – 08:00	5.87	5.09	4.09	0.329 **
Urea N – 12:00	6.55	5.95	4.32	0.444 **
Urea N – 16:00	5.79	5.25	4.22	0.460 **
Urea N – 19:00	6.65	4.84	3.80	0.395 **
Urea N – 23:00	6.33	5.03	4.43	0.485 **
Total N – 08:00	8.24	6.98	5.70	0.418 **
Total N – 12:00	9.17	8.13	6.23	0.586 **
Total N – 16:00	8.34	7.65	6.28	0.660 **
Total N – 19:00	8.73	6.59	5.37	0.527 **
Total N – 23:00	9.25	7.18	6.43	0.580 **

[1)] *** $p < 0.01$*

Table 3.85 presents the DM and N contents of the faeces. Faecal N concentration was significantly lower in B than in BF.

Table 3.85. DM and total N contents of the faeces produced in experiment B5-1.

	Treatment			
	B	BF	F	s.e.d.[1)]
DM (g kg^{-1})	112	117	113	5.81
Total N (g N kg^{-1} DM)	36.4	38.4	37.9	0.69 *

[1)] *$*p < 0.05$*

All urine spot samples were analysed for purine derivatives. Purine derivatives should be a good indicator of microbial protein produced, as shown by Chen *et al.* (1990). Chetal *et al.* (1975) suggested that urinary creatinine is a good indicator for estimating urine volume. The analysis for purine derivatives concentration together with creatinine should therefore result in a value for total purine output. However, after preliminary analysis it became clear that purine output, calculated with the above-mentioned method did not bare any relation to potential microbial protein yield. An additional study was therefore initiated (Shingfield, 1996) of which the abstract is presented in Appendix B5-I. Since good estimates of microbial protein production are only possible with total urine collection (Shingfield, 1996), purine analysis was abolished since total urine collection was not possible within the available experimental facilities.

Table 3.86 presents the efficiency coefficients of N and ME for the 3 experimental groups.

Table 3.86. Efficiency coefficients of N (N output in milk/N intake) and ME (ME requirement/ME intake) and ERDP and MP levels of the cows in experiment B5-1 (Winter of 1993/1994).

	B	BF	F	s.e.d.[1)]
Total N intake (g day^{-1})	525	495	501	9.18
N output in milk (g day^{-1})	126	138	136	7.27
N efficiency (%)	24	28	27	1.31*
ERDP surplus (g cow^{-1} day^{-1})	252	84	-31	47.5*
MP surplus (g cow^{-1} day^{-1})	701	624	620	81.2
ME efficiency	0.950	1.009	1.000	0.037

[1)] *$*p < 0.05$*

The results show that the home-grown concentrates fodder beet and the combination of fodder beet and barley can be effective supplements to grass/clover silage. Supplementation with fodder beet (F) resulted in the highest milk yield. This was, however, accompanied with a decrease in protein content of the milk. Milk N (protein) output was not significantly affected by the diet but tended to be higher on BF and F than on B (Table 3.86). Nitrogen efficiency (N in milk/N intake) was highest in the treatments BF and F. This resulted from a slightly lower N intake and a higher N output in the milk.

3.10 Sub-task B6

Experiment B6-1. Grazing experiment

The main results about herbage production and quality from the mixed swards of grass and white clover are presented in the Tables 3.87 and 3.88. The terms used in the tables are the same as described by Smethan (1994). Results of the individual years are presented in the Annexes B6-1, B6-2 and B6-3.

Table 3.87. Productivity of a grazed grass/clover sward, as affected by the rate of N application (averages of 3 years). Experiment B6-1.

		No N	240 kg N ha^{-1}
DM (t ha^{-1})		11.42	12.62
Clover (t ha^{-1}) (%)		5.61 (49)	4.76 (38)
Grazing pressure (cows ha^{-1})		2.91	3.11
Stocking rate (cows ha^{-1})		2.00	2.11
Intake	(t DM ha^{-1})	10.37	11.37
	(kg DM cow^{-1} day^{-1})	13.50	14.15
Milk yield	(kg cow^{-1})	4350	4450
	(kg ha^{-1})	8680	9320

Table 3.88. Crude protein (CP) and acid detergent fibre (ADF) contents and clover contents of the herbage on offer in the grazing rotations in Spring and Autumn in experiment B6-1.

	Spring					Autumn	
	1	2	3	4	5	1	2
CP (%)							
No N	17.2	21.3	17.3	16.4	15.5	16.8	19.1
240 N	17.6	20.3	16.7	19.3	17.4	19.1	21.5
ADF (%)							
No N	22.1	21.8	23.2	25.1	29.2	30.0	25.2
240 N	21.9	24.1	26.8	27.6	32.1	28.3	23.5
Clover (t ha^{-1})							
No N	0.30	1.00	1.20	1.50	1.10	1.00	0.50
240 N	0.25	0.80	1.10	1.35	1.00	0.60	0.40

Table 3.87 shows small effects of applied N on gross herbage yield, herbage intake and milk production per ha. This effect of applied N on gross herbage yield was only 5 kg DM kg^{-1} N applied. The effect of applied N on herbage yield resulted from a moderate increase of the yield of the grass component of the mixture, *viz.* 8.5 kg DM kg^{-1} N applied, and a decrease of the yield of the clover component, *viz.* 3.5 kg DM kg^{-1} N applied. Grazing management, in particular grazing pressure and rotation of the cows, were adjusted to maintain leafy herbage and to avoid flowering stems in the second half of Spring and Summer. Consequently, herbage quality was high throughout the grazing season (Table 3.88) and milk yield of the cows was maintained at a satisfactory level without supplementation. In addition, this management gives efficient utilisation of herbage produced and good clover persistence. Applied N had little effect on the quality of the herbage on offer: it increased the mean CP content from 177 (range:

155-213) to 188 (range 167-215) g kg^{-1} DM and the mean ADF content from 252 (range: 218-300) to 263 (range: 219-321) g kg^{-1} DM (Table 3.88).

Experiment B6-2. Silage production

Conservation cuts from grass/clover swards

Mixed swards were used for large-scale silage making at normal dates in this area, *i.e.* around 10 May. The average DM yield was 4100 kg ha^{-1}. A later cut for hay was obtained around the end of June, with an average DM yield of 4500 kg ha^{-1}. Very low clover contents were found in these cuts. Detailed results are presented in Annex B6-4. Silage from these mixed swards was used in the feeding experiment A6-2.1, reported in Paragraph A6-2. It contained 13.5% CP and 46.7% ADF in the DM. The early silage, made in the first year, had a better quality, but a DM yield of only 3000 kg ha^{-1}. Cutting for silage at the end of April can be recommended if high-quality silage is required.

Rotation of maize and vetch/oats

Maize DM yields were 9000 kg ha^{-1} in the first 2 years and only 6000 kg ha^{-1} in the third year owing to early drought in summer. The crop contained 25-30% DM at ensiling and 6.5% CP in the DM. The mixture of vetch and oats was harvested in late April or early May and yielded 4900 kg DM ha^{-1} in the first 2 years and about 4000 kg DM ha^{-1} in the last 2 years. The higher proportion of vetch in the seed mixture in the last 2 years apparently increased the proportion of vetch in the harvested forage. It was 32 and 33% of DM harvested in the first 2 years and 42 and 50% in the last 2 years. This may in part explain the increase of CP content of the harvested DM from 10% in the first years to 16% in the last years. Detailed results are presented in Annex B6-5. A mixture of maize silage and vetch/oats silage was used in the feeding experiment A6-2.2 reported in Paragraph A6-2.

Experiment B6-3. Grass/clover response to nitrogen and management

Total herbage and clover DM yields are shown in Table 3.89. The results of the individual years are presented in Annex B6.6. Delaying the first cut to 60 days after N application, increased the response to N, obtaining on average 5.83 t DM ha^{-1} with 120 kg N ha^{-1} applied. Clover yield was low at the first cut and inversely affected by N application. The response to N at the highest N rate was 10, 19 and 25 kg DM kg^{-1} N applied, for T1, T2 and T3, respectively.

N application directly increased CP content of the herbage, from 13% to 22% at T1 and from 11% to 17% at T2. There was no significant effect on the silage cut (T3).

After the early first cut (T1), there was a positive residual effect of applied N on the DM yield of the second cut, whereas after T2 there was no clear residual affect and after T3 there was a negative residual effect (Table 3.89). In the first cut, the clover content was small. At T1 and T2, there was no clear effect of applied N on clover yield, whereas at T3, applied N reduced clover yield. Applied N had a negative residual effect on clover yield in the second cut. This was stronger as the first cut was taken later (Table 3.89). Table 3.89 also shows the accelerating effect of applied N on herbage growth in spring. For instance, in the first cut, the DM yield obtained at T1 with 40 kg N ha^{-1} was the same as at T2 without applied N.

The effects of applied N on total and clover DM yields of the first two cuts are reflected in the annual yields. Applied N in spring moderately increased total DM yield and strongly decreased clover DM yield. These effects were not clearly affected by the cutting regime (Table 3.89).

Table 3.89. Total herbage and clover DM yield and CP content (% of DM) of a mixed sward as affected by the rate of N application to the first cut and the date of the first cut (mean of three years). Experiment B6-3.

	T1: Mid March				T2: End of March				T3: Mid April			
N applied (kg ha^{-1})	0	40	80	120	0	40	80	120	0	40	80	120
Total (t DM ha^{-1})												
1st cut	1.19	1.82	2.03	2.45	1.75	2.69	3.59	4.08	2.78	4.04	4.86	5.83
2nd cut	1.47	1.84	2.17	2.60	2.03	1.91	1.79	2.00	2.79	2.26	2.14	1.70
Clover (t DM ha^{-1})												
1st cut	0.19	0.15	0.11	0.17	0.25	0.30	0.32	0.32	0.55	0.42	0.09	0.08
2nd cut	0.61	0.42	0.24	0.22	1.21	0.75	0.45	0.34	1.64	0.94	0.83	0.22
Crude protein (%)												
1st cut	12.9	16.0	19.5	22.2	11.7	12.7	14.4	17.4	12.1	12.8	12.7	13.7
Annual DM yield												
Total (t ha^{-1})	9.04	9.64	9.93	10.4	8.94	9.97	10.2	10.9	10.1	10.7	11.5	11.8
Clover (t ha^{-1})	4.55	4.11	3.59	2.77	3.87	3.27	2.62	2.20	4.71	3.81	3.14	2.21
Clover (%)	50	42	36	26	43	32	25	20	46	35	27	18

3.11 Sub-task C1

Table 3.90 presents the DM yields of the mixture and of the grass and the white clover in the mixture (experiment C1-1). The most striking point in Table 3.90 is the decrease of white clover yields over the years. In 1993, the sward contained a large proportion of white clover, in particular on the experimental treatments without applied N. In 1994, white clover yield decreased on all the experimental treatments, except on the plots with surface-applied diluted slurry where we observed severe damage to the clover (scorched plants) after slurry application in 1993 and an increase of the clover content of the sward in the first months of the growing season of 1994. A long period of dry and hot weather in the summer of 1994 (late June, July and August) reduced the clover content of the sward on all the plots. The extent of the damage was variable, which caused a rather large heterogeneity of herbage growth and sward composition in the last cuts of 1994. Dry and hot weather in the summer of 1995 further reduced clover content of the sward. On most experimental treatments, the decrease of clover yield was compensated for by an increase of grass yield (Table 3.90). This was not the case on the plots that received only 2 of the 3 main elements, *viz.* the experimental treatments 5, 6, 10 and 11.

Effects of slurry or fertiliser N on DM yield

In 1993, slurry had a significant negative effect on the DM yield of the mixture (compared to treatment 5, the treatment without applied N) at all techniques of application, except injection with open slits (Table 3.90). This effect resulted from a moderate increase of grass yields, *viz.* 1300-3210 kg DM ha^{-1}, and a strong reduction of clover yields, *viz.* 2000-4650 kg DM ha^{-1} (Table 3.90). Damage to the white clover, *viz.* serious scorching of leaves and stolons was only observed after surface application of diluted slurry in May 1993. The other application techniques did not cause visible symptoms of damage.

Table 3.90. Dry matter yields (t ha^{-1}) of the mixture and the mixture components in 1993, 1994 and 1995. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SA	8.65	9.49	9.51	4.15	6.14	7.94	4.50	3.35	1.57
2. SAD	7.92	11.09	11.67	6.06	6.74	10.35	1.85	4.35	1.31
3. Inj OS	9.29	10.28	10.59	5.54	6.97	10.08	3.75	3.32	0.51
4. Inj	8.17	10.09	11.15	4.62	7.53	10.87	3.56	2.56	0.28
5. $N_0P_2K_2$	9.34	8.94	6.88	2.85	4.82	5.58	6.50	4.12	1.30
6. $N_0P_3K_3$	9.32	10.07	8.47	3.13	4.67	6.35	6.19	5.39	2.13
7. $N_1P_2K_2$	9.85	9.97	8.13	4.33	5.96	7.19	5.52	4.01	0.95
8. $N_2P_2K_2$	9.89	10.48	10.03	5.37	7.02	9.34	4.51	3.46	0.68
9. $N_2P_3K_3$	10.55	10.64	10.14	5.16	6.72	9.04	5.39	3.92	1.10
10. $N_2P_2K_0$	9.84	9.17	8.46	5.84	6.62	8.33	4.00	2.55	0.13
11. $N_2P_0K_2$	9.95	9.68	9.03	5.81	6.03	8.54	4.14	3.65	0.50
Grand mean	9.33	9.99	9.46	4.82	6.29	8.51	4.50	3.70	0.95
Significance [1]	***	*	***	***	***	***	***	***	***
L.S.D. [2]	0.62	1.20	1.60	0.89	0.84	1.47	1.00	0.84	0.70

[1] *F pr.: ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$*

[2] *L.S.D.: least significant differences of means (at 95%)*

In 1994 and 1995, there was an increasing effect of applied slurry on the DM yield of the mixture. In 1994, treatments 2 and 3 gave a significantly higher DM yield than treatment 5, whereas in 1995 all experimental treatments that received slurry gave significantly higher DM yields than treatment 5. In both years, only injected slurry (treatments 3 and 4) negatively affected the DM yield of the clover. The effect of slurry on the yield of the grass in the mixture was moderate in 1994 and strong in 1995, *viz.* 1320-2710 kg DM ha^{-1} in 1994 and 2360-5290 kg DM ha^{-1} in 1995.

In 1993, fertiliser N tended to increase the DM yield of the mixture, but, in general, this effect was not statistically significant. However, in 1994 and 1995, DM yields of the mixture on experimental treatment 8 were significantly higher than on treatment 5 (Table 3.90). Fertiliser N had a similar positive effect on the DM yield of the grass in the mixture than slurry applied by low-emission techniques (experimental treatments 2, 3 and 4). In 1993, the negative effect of fertiliser N on clover content tended to be smaller than that of slurry applied by the low-emission techniques. In 1994 and 1995, fertiliser N caused a smaller reduction of clover content than slurry applied by deep injection and a similar or larger reduction than slurry applied by the other techniques.

Effects of slurry or fertiliser N on N yield

In the first year, slurry had a significantly negative effect on the N yield of the mixture at all techniques of application (compared to experimental treatment 5), whereas fertiliser N did not significantly affect N yield (Table 3.91). In the second year, both slurry and fertiliser N tended to increase N yield of the mixture. In the third year, these effects were highly significant, even for the treatments without application of P or K. The effects of slurry or fertiliser N on the N yield of the mixture resulted from significantly positive effects on the N yield of the grass component (36-184 kg N ha^{-1}) and, particularly on the treatments with injected slurry (treatments 3 and 4) and fertiliser N, negative effects on the N yield of the clover component. The N yields of the mixture, presented in Table 3.91, generally are lower than the sum of the N yields of the grass (+ herbs) and white clover. This difference can probably be explained by the fact that different samples were used for (1) analysis of DM, N, P, and K contents of the mixture, and (2) analysis of the grass and clover contents of the mixture and the chemical composition of these components, and that treatment of both samples was slightly different. The first sample was immediately dried after harvest and stored in air-tight glass jars until chemical analysis, whereas the second sample lost some sand and inert DM during separation of the mixture components and, possibly, also some DM by respiration in the period between harvest and drying, despite the fact that the samples were stored at about 2 °C. This means that N yields of grass and clover, presented in Table 3.91, are slightly over-estimated. No attempt has been made to correct the figures.

Table 3.91. Nitrogen yields (kg ha^{-1}) of the mixture and the mixture components in 1993, 1994 and 1995. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SA	303	284	223	122	164	196	197	141	59
2. SAD	240	328	298	158	189	269	81	184	53
3. Inj OS	335	312	253	174	207	268	167	140	19
4. Inj	312	313	290	152	245	316	163	113	11
5. $N_0P_2K_2$	368	273	162	86	127	132	288	169	46
6. $N_0P_3K_3$	361	334	214	88	137	154	277	225	80
7. $N_1P_2K_2$	364	299	177	129	164	164	247	166	33
8. $N_2P_2K_2$	347	300	229	161	188	234	197	141	25
9. $N_2P_3K_3$	389	310	236	171	187	231	234	162	40
10. $N_2P_2K_0$	346	268	209	171	188	221	176	112	5
11. $N_2P_0K_2$	357	281	210	182	161	210	182	149	18
Grand mean	337	300	227	146	178	218	199	155	35
Significance 1)	***	n.s.	***	***	***	***	***	***	***
L.S.D. 2)	28	49	42	22	27	36	45	35	27

1) and 2): see Table 3.90

The N concentration in the mixture strongly decreased in the course of the 3 years and averaged 36.2, 30.0 and 24.0 g kg^{-1} DM in 1993, 1994 and 1995, respectively (Annex C1-2). This decrease was caused by the decrease of the clover content of the sward and the decrease of the mean N contents in the mixture components. It is not clear why the mean N contents of the grass and the clover decreased in the course of the experiment. Effects of the experimental treatments on the N content of the mixture generally were small, except on the plots with surface-applied diluted slurry in 1993, where the relatively low N content reflected the low clover content (Table 3.90 and Annex C1-2).

Effects of slurry or fertiliser P on DM and P yield

Compared to the treatment that did not receive P (treatment 11), slurry had a significantly negative effect on the DM yield of the mixture in 1993 (Table 3.90). In 1994 and 1995, surface-applied untreated slurry gave similar DM yields than the treatment without P, whereas slurry applied by low-emission techniques gave higher yields. This yield increase was statistically significant on treatment 2 in both years and on treatment 4 in 1995. Fertiliser P (treatment 8) tended to increase DM yield of the mixture in 1994 and 1995, but this effect was not statistically significant.

The effect of slurry or fertiliser P on the DM yield of the mixture, resulted to a large extent from their effect on the grass component. In 1993, DM yield of the grass on the treatment without P was relatively high, but in 1994 and 1995, it was lower than on the treatments with slurry applied by low-emission techniques (treatments 2, 3 and 4) or with fertiliser P (treatment 8). In most cases, these differences were statistically significant. The effect of slurry or fertiliser P on DM yield of the clover was relatively small and in some cases even negative, *viz.* on treatment 2 in 1993 and on treatment 4 in all the years but particularly in 1994. Only in 1995, there was a significantly positive effect on clover yield of surface-applied untreated and diluted slurry compared to treatment 11.

Compared to the treatment that did not receive P, slurry and fertiliser P had an increasing effect on the P yield of the mixture in the course of the years (Table 3.92). In 1993, differences were small, and effects of slurry were even significantly negative on the treatments 2 and 4. In 1994, P yield of the mixture was significantly higher on the treatments 2, 3 and 8 and in 1995 on the treatments 1, 2, 3, 4 and 8 than on treatment 11.

Application of slurry or fertiliser P generally caused a significant increase of P yield of the grass, except on the treatments 1, 4 and 8 in 1993 and treatment 1 in 1995 (Table 3.92). Surface application of diluted slurry caused a significant decrease of the P yield of the clover in 1993 and a significant increase in 1994 and 1995. In 1995, there was also a significant increase of the P yield of the clover on treatment 1. Injected slurry tended to decrease the P yield of the clover.

The mean P contents of the mixture and of the grass component were rather constant in the 3 experimental years, whereas the mean P content of the clover was considerably lower in 1995 than in the preceding years (Annex C1-3). Compared to the treatment without applied P (treatment 11), slurry and fertiliser P increased the mean P contents of the mixture and the grass component, but hardly affected the P content of the clover. The mean P contents of the mixture and of the grass from the slurry-treated swards tended to be higher than from the sward that received fertiliser P (Annex C1-3).

Table 3.92. Phosphorus yields (kg P ha^{-1}) of the mixture and the mixture components in 1993, 1994 and 1995. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SA	37.0	37.0	39.0	20.2	27.3	35.7	15.8	11.3	4.4
2. SAD	36.2	42.3	46.3	28.2	30.7	44.3	6.6	15.3	4.1
3. Inj OS	40.8	40.7	42.7	27.3	31.3	43.0	13.4	11.3	1.4
4. Inj	33.0	38.3	42.0	21.4	30.3	45.3	12.4	9.3	0.9
5. $N_0P_2K_2$	37.2	34.3	28.7	13.7	21.0	25.7	23.5	14.0	3.9
6. $N_0P_3K_3$	37.2	40.7	35.3	15.9	22.3	29.3	21.7	19.3	6.7
7. $N_1P_2K_2$	41.0	38.3	32.0	20.4	27.0	30.3	20.2	14.3	2.8
8. $N_2P_2K_2$	40.6	39.3	39.3	23.4	29.3	38.7	16.6	12.0	2.0
9. $N_2P_3K_3$	43.5	41.0	40.7	21.7	29.3	38.7	20.8	14.3	3.4
10. $N_2P_2K_0$	40.6	36.7	35.0	24.5	29.0	36.0	14.5	10.0	0.4
11. $N_2P_0K_2$	39.1	34.0	30.0	22.2	23.0	30.3	14.5	11.7	1.3
Grand mean	38.8	38.4	37.4	21.7	27.3	36.1	16.4	13.0	2.8
Significance 1)	***	*	***	***	***	***	***	***	***
L.S.D. 2)	2.5	4.5	7.0	3.5	3.3	7.4	4.2	3.0	2.1

1) and 2): see Table 3.90

Effects of slurry or fertiliser K on DM and K yield

On the treatment that did not receive K (treatment 10), there was a rapid decline of the K yield in the harvested crop, in particular in the clover component (Table 3.93). This caused a rapid decrease of the clover content of the sward, that was already evident before the dry and hot period in 1994. The general decrease of the clover content of the sward in 1994 and 1995 masked this effect of K deficiency in this experiment (Table 3.90), but the strong effect of no K application on clover content was very evident in a similar experiment with a rather constant clover content of the sward on the other experimental treatments (unpublished information).

Table 3.93. Potassium yields (kg K ha^{-1}) of the mixture and the mixture components in 1993, 1994 and 1995. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SA	364	306	265	177	210	242	185	109	45
2. SAD	305	366	354	232	242	329	74	148	40
3. Inj OS	384	336	312	233	255	333	146	96	14
4. Inj	331	347	350	200	290	387	142	80	8
5. $N_0P_2K_2$	393	294	196	124	164	173	263	132	41
6. $N_0P_3K_3$	384	364	269	137	180	207	245	196	75
7. $N_1P_2K_2$	422	335	232	192	221	225	226	129	29
8. $N_2P_2K_2$	420	344	310	229	256	319	184	109	21
9. $N_2P_3K_3$	461	371	317	233	251	323	228	137	37
10. $N_2P_2K_0$	359	190	141	228	170	151	125	43	1
11. $N_2P_0K_2$	437	315	278	256	219	275	171	124	16
Grand mean	387	324	257	204	224	270	181	118	30
Significance 1)	***	***	***	***	***	***	***	***	***
L.S.D. 2)	34	45	60	35	33	56	50	29	24

1) and 2): see Table 3.90

Compared to the treatment that did not receive K (treatment 10), slurry had a negative effect on the DM yield of the mixture in 1993 (Table 3.90). This effect was statistically significant on the treatments 1, 2 and 4. In this first year, fertiliser K did not affect the yield of the mixture. In 1994 and 1995, both slurry and fertiliser K increased the DM yield of the mixture. In 1994, this increase was significant on the treatments 2 and 8, and in 1995 on the treatments 2, 3 and 4
(Table 3.90).

Slurry and fertiliser K affected both the grass and the clover component of the mixture (compared to treatment 10; Table 3.90). In 1993, there were significantly negative effects of the treatments 1 and 4 on the DM yield of the grass in the mixture, and of treatment 2 on the clover yield in the mixture. In 1994 and 1995, slurry applied by low-emission techniques increased the DM yield of the grass. On the treatment with injected slurry, this effect was statistically significant in both years. On the treatments 2 and 3, it was only significant in 1995.

Both slurry and fertiliser K increased the DM yield of the clover component in 1994 and 1995 (Table 3.90). This effect was strongest on the treatments with surface-applied untreated and diluted slurry and smallest on the treatment with injected slurry.

Compared to the treatment that did not receive K (treatment 10), surface-applied diluted slurry significantly decreased and fertiliser K significantly increased the K yield of the mixture in 1993 (Table 3.93). Both effects were caused by significant effects on the K yield of the clover component. Surface-applied untreated slurry significantly increased the K yield of the clover. In 1994 and 1995, slurry as well as fertiliser K significantly increased the K yield of the mixture and of both components (Table 3.93).

The K content of the harvested herbage was high in the first year, *viz.* on average 41.5 g kg^{-1} DM, and decreased to 32.4 and 29.1 g kg^{-1} DM in 1994 and 1995, respectively (Annex C1-4). A similar decrease was observed for both components of the mixture. The causes of this decrease are not completely clear, but it should be taken into account that in the first 2 years the rate of K application was lower than K yield on all the treatments, except on treatment 6. This was caused by the higher than expected K contents of the harvested herbage, in particular in 1993. Hence, the rate of slurry application aiming at P replacement did not supply sufficient K to replace the withdrawal of this element in the harvested herbage. In 1995, however, the rate of K application was higher than K yield on most treatments (except on treatment 4), and despite this, K contents, particularly of the grass continued to decrease.

On the treatment that did not receive K, K contents of the mixture and the mixture components declined rapidly after the start of the experiment (Annex C1-4). Slurry and fertiliser K had a similar effect on K content. The additional K of the treatments 6 and 9 increased the K content of the herbage compared to the treatments 5 and 8 (Annex C1-4). This in part may explain the relatively high DM yields of the mixture and the clover on treatment 6 and to a lesser extent on treatment 9 (Table 3.90).

Effects of the experimental treatments on the P and K status of the soil

Table 3.94 presents the results of soil analyses in February 1995 and 1996. Even after 2 or 3 years, there was no clear effect of the experimental treatments on the P status of the soil. The P-AL values of the experimental treatment that did not receive P (treatment 11) were not significantly lower than the P-AL values of the treatments that received a replacement rate of slurry or fertiliser P (treatments 1, 2, 3, 4 and 8). This indicates that the P-AL values are well buffered by P reserves of lower availability. In 1996, the experimental treatment with extra P (treatment 9) and those without N and K application (treatments 5 and 10, respectively) tended to have higher P-AL values than the other treatments. Most of the P-AL values in 1995 and 1996 were slightly lower than the value of 27.0 measured at the start of the experiment.

The K-index of the soil was strongly affected by K application level, both after 2 and 3 experimental years (Table 3.94). This illustrates the small K reserves in this sandy soil and the importance of a good K supply from slurry or fertiliser. Both in 1995 and 1996, the K-index on the experimental treatment that received fertiliser K (treatment 8) was significantly higher than on most of the treatments that received slurry K. We have no explanation for this difference.

The K-indices measured in February 1996 were high compared to those of 1995 and before the start of the experiment when it was 25.0. In 1995, only the K-index of treatment 10 was significantly lower than at the start of the experiment, despite the fact that in 1993 and 1994 all the other experimental treatments also had a negative K balance (K yield in the harvested crop exceeded K application). This suggests K uptake by the crop from below the sampled soil layer. In 1995, K balances were slightly to moderately positive, except those on the treatments 4 and 10, that were slightly negative (compare Annex C1-1 and Table 3.93). This may explain the increase of the K indices in February 1996 (Table 3.94).

Table 3.94. The P and K status of the soil in 1995 and 1996, as affected by the experimental treatments. The P status is expressed as P-AL (in mg P_2O_5 per 100 g air-dry soil) and the K status as K-index. Experiment C1-1.

Experimental treatment	P-AL 1995	P-AL 1996	K-index 1995	K-index 1996
1. SA	23.7	22.7	24.6	33.2
2. SAD		24.3		32.5
3. Inj OS	25.3	25.0	27.0	36.5
4. Inj	26.0	24.3	23.1	30.2
5. $N_0P_2K_2$		26.0		55.6
6. $N_0P_3K_3$				
7. $N_1P_2K_2$				
8. $N_2P_2K_2$	26.3	25.0	30.2	41.3
9. $N_2P_3K_3$	24.3	29.0	38.9	64.3
10. $N_2P_2K_0$	27.7	27.3	11.1	15.1
11. $N_2P_0K_2$	23.3	23.7	31.8	44.4
F. pr.	0.076	0.060	<0.001	<0.001
L.S.D.	3.0	3.7	4.6	7.1

3.12 Sub-task C2

Effects of slurry or fertiliser N on the grass in monoculture

Table 3.95 presents the DM and N yields of the grass in monoculture in experiment C2-1. In the absence of applied N (treatments 15, 16 and 17), N and DM yields were very low, indicating a small N supply from mineralisation of soil organic N and atmospheric N deposition. Both slurry and fertiliser N significantly increased N and DM yield. In 1993, the yields of the plots that received fertiliser N were slightly higher than those of the slurry-treated plots. In 1994 and 1995, the DM and N yields of the slurry-treated plots were similar or slightly higher than the yields of the fertiliser-treated plots.

Cutting the sward with the open-slit injector had a small negative effect on the DM and N yields in 1993 and 1994, and no effect in 1995 (compare treatments 9, 10 and 11 with treatments 12, 13 and 14 in Table 3.95). Time of slurry or fertiliser N application often had a significant effect on DM yield. Generally, the applications to the first and second cut gave the highest yields and applications to the third and fourth cut the lowest. The effects on N yield were less pronounced. Often, N yields on the plots with early applications of slurry or fertiliser tended to be higher than on the plots with later applications. The fact that the period of N application had a larger effect on DM yield than on N yield probably was caused by differences in DM yield at harvest and, hence, N content of the harvested herbage between the periods of application.

Table 3.95 *Dry matter and N yields (t ha^{-1} and kg ha^{-1}, respectively) from the pure grass sward as affected by the experimental treatments in 1993, 1994 and 1995. Experiment C2-1.*

Experimental treament 1)	Dry matter (t ha^{-1}) 1993	Dry matter (t ha^{-1}) 1994	Dry matter (t ha^{-1}) 1995	Nitrogen (kg ha^{-1}) 1993	Nitrogen (kg ha^{-1}) 1994	Nitrogen (kg ha^{-1}) 1995
1. SAD 1,2	6.76	6.86	9.72	146	142	211
2. SAD 3,4	5.67	4.71	7.72	133	123	175
3. SAD 1,3	6.23	5.68	8.68	137	121	180
4. Inj OS 1,2	6.02	6.34	9.34	146	143	212
5. Inj OS 3,4	5.26	5.23	8.52	133	146	190
6. Inj OS 1,3	5.56	6.16	9.56	129	147	212
7. Inj 1	5.55	7.00	9.49	148	163	229
8. Inj 3	3.77	5.67	8.90	84	163	223
9. Inj OS NPK 1,2	6.92	5.90	9.34	167	114	204
10. Inj OS NPK 3,4	6.16	4.57	7.98	151	137	180
11. Inj OS NPK 1,3	6.59	5.04	8.02	154	113	165
12. NPK 1,2	7.75	6.20	9.28	179	120	204
13. NPK 3,4	6.86	5.21	8.44	167	148	188
14. NPK 1,3	7.03	5.38	7.80	161	117	160
15. PK 1,2	3.18	2.56	4.93	65	60	102
16. PK 3,4	3.21	2.26	4.69	65	56	98
17. PK 1,3	3.43	2.37	4.52	71	61	95
Grand mean	5.64	5.13	8.05	132	122	178

1) *see Table 2.20 for the meaning of the codes*

Table 3.96 presents the ANR and ANE values for fertiliser N and slurry inorganic N, as affected by application technique. The values for the individual treatments are presented in Annex C2-2.

Table 3.96. *Apparent efficiencies (ANE) and apparent recoveries (ANR) of slurry and fertiliser N applied by different techniques to the grass in monoculture. ANE is expressed in kg DM per kg inorganic N applied; ANR in % of inorganic N applied. The values are averages of the 3 periods of application. The ANE and ANR values have been calculated using the average DM and N yields of the 3 treatments without applied N as references. Experiment C2-1.*

N source and application technique 1)	ANE 1993	ANE 1994	ANE 1995	ANE Mean	ANR 1993	ANR 1994	ANR 1995	ANR Mean
SAD	21.3	23.4	23.7	22.8	52	49	54	51
Inj OS	21.4	29.6	31.6	27.9	63	73	76	71
Inj	19.1	36.0	31.5	29.9	68	90	89	84
Inj OS NPK	32.1	26.6	29.2	29.3	88	60	66	71
NPK	38.5	30.7	29.7	32.7	100	66	67	77

1) *SAD: surface application of 1:2 diluted slurry; Inj OS: injection with open slits; Inj: deep injection; Inj OS NPK: a passage with the open-slit injector + fertiliser N, P and K; NPK: fertiliser N, P and K.*

ANR values for fertiliser N were very high in 1993 and rather low in 1994 and 1995 (Table 3.96). High ANR values, as observed in 1993, are rather common for grass on well-drained soils in

The Netherlands (Van der Meer & Van Uum-van Lohuyzen, 1986; Van der Meer *et al.*, 1987). We have no explanation for the rather low values in 1994 and 1995. In 1993, the ANRs from slurry were significantly lower than those from fertiliser, whereas in 1994 and 1995 the ANRs from injected slurry (Inj OS and Inj) were higher than those from fertiliser. The origin of the slurries may explain the rather low ANRs in 1993 and the higher values in 1994 and 1995. In 1993, the slurry was obtained from dairy cows fed a diet based on maize silage, with an N content well below feeding standards (Ketelaars, unpublished results). Probably, this slurry had a high C to inorganic N ratio and, consequently, a reduced availability of slurry inorganic N after application to the field. In 1994 and 1995, the slurry was obtained from cows on a grass silage diet with a considerably higher N content.

ANR values for slurry application techniques mainly differ because of differences in ammonia volatilisation. Although dilution of slurry reduces ammonia volatilisation and improves ANR, injection techniques were much more effective to improve utilisation of slurry N (Table 3.96).

Similar results have been obtained in another experiment with different types of slurry (Geurink & Van der Meer, 1995; Van der Meer & Van der Putten, 1995). In general, high ANR values are attended with high ANE values (Table 3.96). This indicates that uptake of applied N is the main aspect of efficient N utilisation. However, ANE values for slurry applied by deep injection are relatively low compared to ANR. This is caused by the uneven spatial and temporal distribution of slurry applied by this technique owing to the single large rate of application and the large distance between the injection tines.

Table 3.97 presents the ANR and ANE values for the 3 periods of application. On average, ANRs from slurry and fertiliser N applications to the first and second cut were slightly higher than ANRs from later applications, but differences were small. Despite this, application period had a large effect on ANE, due to the fact that the DM yields of the first and the second cut were higher and, hence, the N contents of the harvested herbage lower than DM yields and N contents of later cuts.

Table 3.97. Effect of application period on the ANE and ANR of slurry and fertiliser N applied by different techniques to the grass in monoculture. The values are averages of the 5 combinations of N source and application technique and have been calculated using the average DM and N yields of the 3 treatments without applied N as references. Experiment C2-1.

Application	ANE				ANR			
period [1)]	1993	1994	1995	Mean	1993	1994	1995	Mean
1,2	29.8	35.4	32.6	32.6	81	67	79	76
3,4	22.4	22.2	25.9	23.6	66	70	67	67
1,3	26.3	29.8	28.2	28.1	71	63	64	66

[1)] 1,2: applications to the 1st and 2nd cut; 3,4: applications to the 3rd and 4th cut; 1,3: applications to the 1st and 3rd cut.

Effects of slurry or fertiliser N on the mixture and its components

Table 3.98 presents the DM yields of the mixture and the mixture components in the 3 experimental years. Without applied N (treatments 15, 16 and 17), DM yield of the mixture was about 9 metric tons ha^{-1} $year^{-1}$ and clover content 68, 60 and 36% of the harvested DM in 1993, 1994 and 1995, respectively. Averaged over the 17 treatments, white clover yields were similar in 1993 and 1994 and declined strongly in 1995, probably as a consequence of hot and dry weather in 1994 and 1995.

Table 3.98. Dry matter yields (t ha^{-1}*) of the mixture and the mixture components in 1993, 1994 and 1995. Experiment C2-1.*

Experimental treatment [1)]	Mixture			Grass			White clover		
	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SAD 1,2	9.49	10.99	12.52	5.55	5.66	9.60	3.94	5.33	2.92
2. SAD 3,4	9.84	10.76	11.15	4.51	4.50	8.69	5.33	6.25	2.46
3. SAD 1,3	8.17	11.06	11.22	4.93	5.89	8.87	3.24	5.16	2.34
4. Inj OS 1,2	9.38	10.71	12.07	5.23	6.34	10.32	4.15	4.37	1.75
5. Inj OS 3,4	9.80	10.62	10.64	3.99	5.48	8.86	5.81	5.14	1.78
6. Inj OS 1,3	9.30	10.88	12.24	5.00	6.09	10.13	4.30	4.79	2.11
7. Inj 1	7.84	10.14	10.99	4.32	7.17	9.83	3.52	2.97	1.16
8. Inj 3	9.48	9.29	11.72	2.93	4.31	9.08	6.55	4.98	2.64
9. Inj OS NPK 1,2	9.07	10.45	11.24	5.74	5.90	9.16	3.33	4.54	2.08
10. Inj OS NPK 3,4	9.93	9.26	10.69	5.14	4.91	8.38	4.79	4.35	2.31
11. Inj OS NPK 1,3	9.63	10.05	11.73	5.55	5.62	9.46	4.08	4.43	2.27
12. NPK 1,2	10.26	10.94	11.58	5.92	5.86	9.46	4.34	5.09	2.11
13. NPK 3,4	10.09	9.57	9.91	4.98	4.97	8.05	5.11	4.60	1.86
14. NPK 1,3	10.14	10.30	10.89	5.60	5.34	8.92	4.54	4.96	1.97
15. PK 1,2	8.96	9.34	8.15	2.75	3.94	5.75	6.21	5.40	2.40
16. PK 3,4	8.99	9.66	9.22	2.86	3.45	5.65	6.13	6.21	3.57
17. PK 1,3	8.97	9.44	9.33	2.92	3.88	5.69	6.05	5.56	3.65
Grand mean	9.37	10.20	10.90	4.58	5.25	8.58	4.79	4.95	2.32

[1)] *see Table 2.20 for the meaning of the codes*

With a few exceptions, applied N increased DM yield of the mixture (Table 3.98). In 1993, we observed scorching of clover leaves and stolons after surface application of diluted slurry to the first cut. Later on, no symptoms of damage were observed after application of diluted slurry. In addition to early application of diluted slurry, early injection of slurry (treatment 7) had a significant negative effect on DM yield in 1993, but on this treatment no symptoms of damage were observed. In general, differences between slurry and fertiliser N and between techniques of slurry application were small and partly caused by small differences in the rate of N application (Annex C2-1). The ANE and ANR values allow a better comparison of the experimental treatments (Annexes C2-3 and C2-4).

The period of application often affected DM yield of the mixture (Table 3.98). In 1993, high DM yields were generally recorded on the experimental treatments with late N applications. In 1994 and 1995, late N applications often gave the lowest yields.

The effect of applied N on the DM yield of the mixture always resulted from a strong increase of the DM yield of the grass component and a varying negative effect on the DM yield of the clover component (Table 3.98). This negative effect of N on clover yield was strong in 1993, in particular on the plots with early applied N. It was considerably smaller in 1994 and 1995.

Table 3.99 presents the N yields of the mixture and the mixture components in the 3 experimental years. The N yields of the mixture, presented in this Table, generally are lower than the sum of the N yields of the grass and white clover. This difference can probably be explained by the fact that different samples were used for (1) analysis of DM and N contents of the mixture, and (2) analysis of the grass and clover contents of the mixture and the DM and N contents of these components, and that treatment of both samples was slightly different. The first sample was immediately dried after harvest and stored in air-tight glass jars until chemical analysis, whereas the second sample probably lost some adhering sand and inert DM during separation of the mixture components and, possibly, also some DM by respiration in the period between harvest and drying, despite the fact that the samples were stored at about 2 °C. This means that the N yields of grass and white clover, presented in Table 3.99, are slightly over-estimated. No attempt has been made to correct the figures.

Table 3.99. Nitrogen yields (kg ha^{-1}) of the mixture and the mixture components in 1993, 1994 and 1995. Experiment C2-1.

Experimental	Mixture			Grass			White clover		
treatment [1]	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SAD 1,2	319	376	333	154	177	252	180	228	114
2. SAD 3,4	371	372	294	140	148	230	229	268	92
3. SAD 1,3	266	365	279	142	176	221	145	220	92
4. Inj OS 1,2	349	358	327	160	192	272	190	190	71
5. Inj OS 3,4	386	362	280	130	185	228	256	221	67
6. Inj OS 1,3	331	376	322	156	194	273	192	209	84
7. Inj 1	290	331	298	138	244	279	163	133	47
8. Inj 3	361	350	341	85	154	262	289	231	104
9. Inj OS NPK 1,2	318	342	297	181	175	246	148	192	85
10. Inj OS NPK 3,4	372	316	272	156	155	219	205	188	86
11. Inj OS NPK 1,3	340	336	304	166	177	245	178	183	89
12. NPK 1,2	360	350	307	173	178	257	193	216	86
13. NPK 3,4	379	335	252	154	163	200	225	193	68
14. NPK 1,3	352	329	281	171	162	231	197	205	74
15. PK 1,2	358	326	213	83	117	134	281	228	94
16. PK 3,4	350	338	253	90	105	139	271	265	139
17. PK 1,3	357	332	252	88	114	141	273	234	139
Grand mean	345	347	289	139	166	225	213	212	90

[1] *see Table 2.20 for the meaning of the codes*

Without applied N, average N yields were 355, 322 and 239 kg per ha in 1993, 1994 and 1995, respectively (Table 3.99). In 1993, application of slurry or fertiliser N on average decreased N yield, whereas applied N caused a small increase of N yield in 1994 and a moderate increase in 1995. Just as in case of DM yield, the effect of applied N on the N yield of the mixture resulted from a strong increase of the N yield of the grass component and a varying decrease of the N yield of the clover component. The decrease of the N yield of the clover exceeded the increase of the N yield of the grass on many experimental treatments in 1993 and on a few treatments in 1994, whereas in 1995 the positive effect on grass N yield always was larger than the negative effect on clover N yield.

The N yields of the grass in the mixture (Table 3.99) generally were higher than the N yields of the grass in monoculture (Table 3.95). In the absence of applied N (treatments 15, 16 and 17), the difference averaged 20, 53 and 40 kg N ha^{-1} in 1993, 1994, and 1995, respectively. On the treatments with applied N, it averaged 5, 41 and 49 kg N ha^{-1}. This difference indicates transfer of N from the white clover to the grass, probably by death and decay of plant parts of white clover.

Comparison of the N yields of the grass in the mixture and the grass in monoculture also shows that both N yields responded almost similarly to applied N. The response of the DM yield of the grass in the mixture to applied N was smaller than that of the grass in monoculture, because of the significantly higher N contents of the DM of the grass in the mixture. Table 3.100 presents the ANR and ANE values for slurry and fertiliser N applied by different techniques to the mixed sward.

Table 3.100. Apparent efficiencies (ANE) and apparent recoveries (ANR) of slurry and fertiliser N applied by different techniques to the mixed sward. The values are averages of the 3 periods of application. The ANE and ANR values have been calculated using the average DM and N yields of the 3 treatments without applied N as references. Experiment C2-1.

N source and application technique 1)	ANE (kg DM per kg inorganic N applied)								
	Mixture			Grass			White clover		
	1993	1994	1995	1993	1994	1995	1993	1994	1995
SAD	1.4	10.2	16.2	15.5	11.1	19.9	-14.1	-1.0	-3.8
Inj OS	4.8	10.6	19.6	17.3	18.7	29.1	-12.6	-8.1	-9.5
Inj	-6.7	3.3	16.1	11.5	21.3	26.7	-18.2	-18.0	-10.7
Inj OS NPK	5.6	4.2	18.2	25.7	16.5	25.9	-20.2	-12.3	-7.7
NPK	11.6	7.6	14.8	26.0	15.7	24.4	-14.3	-8.1	-9.6
	ANR (% of inorganic N applied)								
SAD	-26	27	37	42	38	57	-65	-3	-15
Inj OS	0	28	50	56	66	85	-57	-30	-36
Inj	-47	5	50	38	88	93	-80	-66	-40
Inj OS NPK	-11	-1	40	79	55	77	-96	-52	-29
NPK	8	6	32	77	53	72	-68	-36	-38

1) *see Table 3.96 for the meaning of the codes*

The ANR and ANE values of the mixture were much lower than those of the grass in monoculture (compare Tables 3.100 and 3.964). This is because the positive effect of applied N on the DM and N yields of the grass in the mixture to a large extent was undone by the negative effect on the clover yields. Both the ANR and ANE values of the mixture increased in the course of the 3 experimental years. This was caused by the higher than average negative effect of applied N on the clover in 1993, and the higher than average positive effect on the grass in 1995 (Table 3.100).

Comparison of the results of the main experimental treatments shows that N from slurry, applied by open-slit injection, was effectively used by the mixture (Table 3.100). It had a relatively large positive effect on grass DM and N yields and a moderately negative effect on clover yields. Only in 1993, fertiliser N was more effective. Deep injection was less effective than injection with open slits because of its larger negative effect on the clover. Except in 1993, when it caused some scorching, surface-applied diluted slurry had the smallest negative effect on white clover of all the main experimental treatments.

Table 3.100 does not support the concern that slurry N has a stronger negative effect on white clover than fertiliser N. In fact, the most popular low-emission slurry application technique, *viz.* injection with open slits, and fertiliser N (NPK) had similar effects on clover, whereas the combination of cutting the sward with the open-slit injector and fertiliser N (Inj OS NPK) even had a stronger negative effect in 1993 and 1994. This even suggests that slurry N was less harmful than fertiliser N and that the injectors had a significant negative effect on the clover in the first 2 years after establishing the sward.

Table 3.101 presents the effect of application period on the effectivity of slurry and fertiliser N.

Table 3.101. Effect of application period on the ANE and ANR of slurry and fertiliser N applied by different techniques to the mixed sward. The values are averages of the 5 combinations of N source and application technique and have been calculated using the average DM and N yields of the 3 treatments without applied N as references. Experiment C2-1.

Application period [1]	ANE (kg DM per kg inorganic N applied)								
	Mixture			Grass			White clover		
	1993	1994	1995	1993	1994	1995	1993	1994	1995
1,2	2.1	10.1	19.2	22.4	21.1	27.4	-20.3	-11.0	-8.3
3,4	8.5	3.5	13.8	14.5	8.9	21.0	-6.1	-5.4	-7.2
1,3	0.4	8.7	17.7	20.2	19.5	26.4	-19.8	-10.9	-8.7
	ANR (% of inorganic N applied)								
1,2	-25	17	50	66	71	85	-89	-44	-30
3,4	19	12	35	46	40	65	-34	-18	-29
1,3	-35	13	40	61	68	79	-90	-45	-33

[1] *see Table 3.97 for the meaning of the codes*

Table 3.101 shows that late applications of slurry or fertiliser N, *i.e.* to the third and fourth cuts, had a much smaller positive effect on the grass in the mixture than early applications and, in addition, a smaller negative effect on the clover component, particularly in the first year. As a result, late applications had a better effect on the mixture in 1993, whereas early applications were more effective in 1994 and 1995.

Contribution of clover to the DM and N yield of the mixture

The contribution of white clover to the DM and N yield of the mixture can be assessed by comparing the DM and N yields of the mixture and the grass in monoculture. Table 3.102 presents the contribution of the clover to the DM and N yield of the mixture as determined for the main experimental treatments. The results of all the experimental treatments are presented in Annex C2-5.

In the absence of applied N, ANF averaged 288, 273 and 141 kg N ha^{-1} in 1993, 1994 and 1995, respectively (Table 3.102). Average ANF on the treatments with slurry and fertiliser N was 193, 211 and 102 kg ha^{-1}. This indicates a strong negative effect of applied N on ANF in 1993, *viz.* 0.88 kg N per kg inorganic N applied. In 1994 and 1995, this effect was moderately negative, *viz.* 0.54 and 0.28 kg N per kg inorganic N applied, respectively. On average, slurry applied by deep injection had the strongest negative effect on ANF, whereas slurry applied by the two other techniques had the smallest effect (Table 3.102). In general, ANF values were similar or slightly higher than the N yield of the clover component of the mixture (Table 3.99). Higher ANF values indicate transfer of N from the clover to the grass.

Table 3.102. Contribution of white clover to the DM and N yield of the mixture, as affected by the main treatments. The contribution is expressed as extra DM yield (in metric tons ha^{-1}) or extra N yield (apparent N fixation, ANF, in kg ha^{-1}) and calculated as the difference between the DM and N yields of the mixture and the grass in monoculture. The values are averages for the 3 periods of application. Experiment C2-1.

N source and application technique [1]	Extra DM yield				ANF			
	1993	1994	1995	Mean	1993	1994	1995	Mean
SAD	2.95	5.19	2.93	3.69	180	242	114	179
Inj OS	3.88	4.83	2.51	3.74	219	220	105	181
Inj	3.43	3.30	1.94	2.89	187	174	85	149
Inj OS NPK	2.99	4.75	2.77	3.50	186	210	108	168
NPK	2.95	4.67	2.29	3.30	195	210	96	167
PK	5.70	7.08	4.19	5.66	288	273	141	234
Grand mean	3.65	4.97	2.77	3.80	209	222	108	180

[1] *see Table 3.96 for the meaning of the codes*

In the absence of applied N, the effect of white clover on DM yield averaged 5.70, 7.08 and 4.19 metric tons ha^{-1} in 1993, 1994 and 1995, respectively (Table 3.102). In 1993, this increase was slightly smaller than the DM yield of the clover component of the mixture (Table 3.98), indicating a small negative effect of clover on grass DM yield. On the treatments with applied N, the contribution of clover to the DM yield of the mixture was considerably lower than the DM yield of the clover. The negative effect of clover on grass DM yield in this first experimental year was associated with a considerably higher N content of the grass in the mixture than of the grass in monoculture, probably as a result of lower light interception by the grass in the mixture. In 1995, the effect of white clover on the DM yield of the mixture exceeded clover DM yield on all the experimental treatments. In that year, the positive effect of N transfer to the grass exceeded the negative effect of clover competition for light. In 1994, this was only the case on the PK treatment.

Table 3.103 shows the effect of the period of N application on the contribution of white clover to the DM and N yield of the mixture. Applications of slurry or fertiliser N to the third and fourth cut, on average, had the smallest negative effect on the contribution of clover, whereas N applications to the first and second cut had the largest negative effect. Clearly, N applications with a large positive effect on the DM and N yield of the grass in the mixture (Table 3.97) had the largest negative effect on the contribution of clover to the yields of the mixture.

The ANF values presented in the Tables 3.102 and 2.103 can be expressed per ton of clover DM harvested. This is called the N fixation efficiency (NFE). Average NFE values for the main experimental treatments and the periods of N application are presented in Table 3.104. The NFE values of all the experimental treatments are presented in Annex C2-6.

Table 3.103. Effect of the period of slurry or fertiliser N application on the contribution of white clover to the DM and N yield of the mixture. The contribution of the clover is expressed as extra DM yield (in metric tons ha^{-1}) or extra N yield (apparent N fixation, ANF, in kg ha^{-1}) and calculated as the difference between the DM and N yields of the mixture and the grass in monoculture. The values are averages of the 5 combinations of N source and application technique. The values for the control are averages of the 3 treatments without applied N. Experiment C2-1.

Application	Extra DM yield				ANF			
period [1]	1993	1994	1995	Mean	1993	1994	1995	Mean
1,2	2.61	4.19	2.25	3.02	170	215	100	162
3,4	4.28	4.82	2.51	3.87	240	204	97	180
1,3	2.82	4.63	2.70	3.38	170	215	107	164
Control	5.70	7.08	4.19	5.66	288	273	141	234

[1] *see Table 3.97 for the meaning of the codes*

Table 3.104. N fixation efficiencies (NFE, in kg N per ton clover DM harvested) as affected by the main experimental treatments and the period of N application. Experiment C2-1.

Main experimental	NFE (kg N per ton clover DM)			
treatments [1]	1993	1994	1995	Mean
SAD	43.2	43.4	44.4	43.6
Inj OS	46.1	46.1	55.9	47.6
Inj	41.3	47.8	51.5	45.5
Inj OS NPK	45.7	47.3	48.6	47.0
NPK	41.8	43.0	48.5	43.5
Mean of the treatments with N	43.8	44.7	48.9	45.2
PK	47.0	47.7	44.0	46.6
Grand mean	44.5	45.4	48.7	45.4
Application period [1]				
1,2	44.1	48.2	50.1	47.1
3,4	43.5	40.2	43.8	42.2
1,3	43.2	48.2	54.5	47.4

[1] *see Tables 3.96 and 3.97 for the meaning of the codes*

On average, NFE was about 45 kg N per ton clover DM harvested (Table 3.104). Differences between the NFE values for the treatments with applied N and without applied N were remarkably small. Only N applications to the third and fourth cut slightly reduced NFE. The NFE values for the treatments with applied N indicate that white clover practically did not absorb N from early slurry or fertiliser N applications and only a very small part from late applications. In the first experimental year, average NFE was equal to the average N content of the clover, *viz.* 44.5 kg N per ton clover DM. In 1994 and 1995, average N contents of the clover were 42.8 and 38.8 kg per ton DM, respectively. They were lower than the average NFE values in these years, indicating transfer of fixed N to the grass in the mixture.

Residual soil inorganic N in Autumn

Table 3.105 presents the amounts of residual inorganic N in the soil of some experimental treatments after the last cut in Autumn, *i.e.* in late October or early November. In 1993 and 1994, these amounts were very small, probably as a consequence of the high precipitation surplus in September and October: rainfall in these months was about 240 mm in 1993 and 280 mm in 1994. If there was a considerable amount of residual soil inorganic N in late summer, rainfall in September and October of these years was sufficient to remove most of it from the sampled soil layer before the sampling date. Rainfall in September and October of 1995 was only about 120 mm and in this period leaching of nitrate to deeper soil layers most probably was limited or negligable.

The results presented in Table 3.105 do not indicate a positive effect of clover on the amount of residual soil inorganic N in Autumn and, hence, on nitrate leaching. However, the results obtained in the years with the most vigorous clover growth (1993 and 1994) could be misleading due to the considerable precipitation surplus before soil sampling.

Table 3.105. Amounts of residual inorganic N (kg ha^{-1}) in the soil layer of 0-60 cm of selected experimental treatments. Samples were taken after the last cut in Autumn. Experiment C2-1.

Experimental	Mixture			Pure grass		
treament 1)	1993	1994	1995	1993	1994	1995
2. SAD 3,4			25			26
4. Inj OS 1,2			26			27
5. Inj OS 3,4	15	15	26			24
13. NPK 3,4	6	23	21	5	13	30
16. PK 3,4	6	13	22			24

1) *see Table 2.20 for the meaning of the codes*

3.13 Sub-task C3

Field experiment (experiment C3-1)

Dry matter and N yields

Maize DM yields are reported in the Tables 3.106 and 3.107.

Table 3.106. Effects of the period and method of slurry or fertiliser N application on the DM yield of maize (t ha^{-1}). The figures presented are averages of the 4 N levels. Experiment C3-1.

Period and method of application	1993		1994		1995		Average	
Winter	14.3	n.s.	9.6	b	7.3	b	10.4	b
Spring	14.9	n.s.	14.4	a	9.7	a	13.0	a
Incorporated	14.7	n.s.	12.4	a	8.9	a	12.0	a
Not incorporated	14.5	n.s.	11.6	b	8.1	b	11.4	b
Winter, incorporated	14.2	n.s.	10.0	c	7.7	d	10.6	d
Winter, not incorporated	14.4	n.s.	9.3	c	6.9	de	10.2	d
Spring, incorporated	15.1	n.s.	14.9	b	10.2	b	13.4	b
Spring, not incorporated	14.6	n.s.	13.9	b	9.2	c	12.5	c
Spring, artificial fertiliser	14.4	n.s.	18.4	a	12.5	a	15.1	a

In 1993, no significant differences were observed between the periods of slurry application (Winter and Spring). A possible explanation for this may be that, after slurry application on 3 February, rainfall until 13 April was only 50 mm. Average rainfall (30-year period) between November and April is about 500 mm in this region. Also the methods of slurry application (incorporated and not incorporated) did not give significant differences, possibly due to a rather small ammonia volatilisation. The treatment with the highest rate of artificial fertiliser N yielded less than all the treatments with the same rate of inorganic slurry N (Table 3.107).

In 1994 and 1995, significant differences were registered between the periods and between the methods of slurry application. Spring application and immediate incorporation showed to be the most effective treatment (Table 3.106).

Table 3.107. Effects of the rate, period and method of slurry or fertiliser N application (kg inorganic N ha^{-1}) on the DM yield of maize (t ha^{-1}). Experiment C3-1.

Rate of inorganic N	Slurry				Fertiliser
	winter application		spring application		spring application
	incorp.	not incorp.	incorp.	not incorp.	
1993					
0	C 10.5 b	C 11.4 ab	C 11.9 ab	C 11.0 ab	B 12.7 a
80	B 14.7 a	BC 13.7 a	B 14.3 a	B 14.5 a	B 13.3 a
160	B 14.2 a	AB 15.2 a	B 15.7 a	B 15.3 a	A 15.6 a
240	A 17.7 ab	A 17.6 ab	A 18.4 a	A 17.7 ab	A 16.3 b
Slurry + fertiliser N: control (0 N): 12.4; combination (240 N): 18.3					
1994					
0	A 9.4 b	B 8.7 b	A 13.4 a	A 11.7 a	B 13.6 a
80	A 10.1 c	B 8.9 c	A 15.6 b	A 13.7 b	A 18.1 a
160	A 10.1 c	B 9.1 c	A 14.4 b	A 14.4 b	A 20.8 a
240	A 10.4 c	A 10.5 c	A 16.1 b	A 15.8 b	A 21.1 a
Slurry + fertiliser N: control (0 N): 9.3; combination (240 N): 18.6					
1995					
0	B 6.9 bc	A 6.2 c	A 8.7 abc	A 9.1 ab	A 11.0 a
80	AB 7.4 b	A 6.5 b	A 9.8 a	A 9.5 a	A 11.2 a
160	AB 7.9 c	A 7.2 c	A 10.9 ab	A 9.5 bc	A 13.0 a
240	A 8.6 c	A 7.8 c	A 11.3 b	A 8.7 c	A 15.0 a
Slurry + fertiliser N: control (0 N): 8.5; combination (240 N): 16.4					
3-year average					
0	B 8.9 b	B 8.8 b	BC 11.3 a	B 10.6 ab	C 12.4 a
80	AB 10.7 bc	B 9.7 c	B 13.2 a	AB 12.6 b	B 14.2 a
160	AB 10.7 c	B 10.5 c	B 13.7 b	AB 13.1 bc	A 16.5 a
240	A 12.2 d	A 12.0 d	A 15.3 b	A 14.1 c	A 17.5 a
Slurry + fertiliser N: control (0 N): 10.1; combination (240 N): 17.8.					

Capital letters show significance ($p \leq 0.05$) in vertical
Small letters show significance ($p \leq 0.05$) in horizontal

The highest DM yields were obtained with artificial fertiliser and with the combination of artificial fertiliser and slurry. DM yields obtained with the combination of slurry and fertiliser were, averaged over the three years, higher than yields with both artificial fertiliser and 'spring incorporated' slurry (Table 3.107). The effect of the rate of N application on DM yield varied over the main treatments and over the years (Table 3.107). Nevertheless, it was statistically significant for the average values in the 3-year period. A positive influence of spring-ploughing can be noticed comparing the yields on treatments without applied N. This suggests a positive effect of the period of soil tillage on the availability of N to the crop.

Nitrogen yields in the harvested maize are reported in the Tables 3.108 and 3.109.

Table 3.108. Effects of the period and method of slurry or fertiliser N application on the N yield of maize (kg ha^{-1}). The figures presented are averages of the 4 N levels. Experiment C3-1.

Period and method of application	1993		1994		1995		Average	
Winter	149	n.s.	86	b	68	b	101	b
Spring	158	n.s.	119	a	88	a	122	a
Incorporated	155	n.s.	108	a	83	a	115	a
Not incorporated	152	n.s.	97	b	74	b	108	b
Winter, incorporated	153	a	95	c	73	d	107	c
Winter, not incorporated	146	ab	78	d	63	e	96	d
Spring, incorporated	158	a	121	b	92	b	124	b
Spring, not incorporated	158	a	117	b	84	c	120	bc
Spring, artificial fertiliser	135	b	192	a	117	a	148	a

In 1993, no significant differences were registered between periods of slurry application or between methods of slurry application. However, N uptake from spring applications of slurry tended to be higher than from winter applications (Table 3.108). In addition, N yields on the slurry-treated plots were significantly higher than on the plots with artificial fertiliser.

In the second year (1994), significant differences were registered between periods as well as between methods of slurry application. Considering slurry-treated plots, both spring application and immediate incorporation induced a significantly higher N uptake than winter application and surface spreading without incorporation, respectively. In contrast with 1993, the highest N yield was obtained with artificial fertiliser. The positive effect of spring application and immediate incorporation of slurry on maize N yield was confirmed in 1995. As in the previous year, artificial fertiliser N gave the highest N yield.

The rate of slurry application had a significant effect on the yield of N in 1993, whereas in 1994 and 1995 this effect was small and often not statistically significant (Table 3.109). The rate of fertiliser N had a large effect on N yield in 1994 and rather small effects in 1993 and 1995. Averaged over 3 years, both slurry and fertiliser N significantly increased the N yield of the maize.

Table 3.109 shows that in 1994 as well as in 1995, the unfertilised plots on the main treatments 'spring application of slurry or fertiliser' yielded more N than on the main treatments 'winter application of slurry'. There is no clear explanation for this difference, but it may indicate an effect of the period of soil tillage on the availability of N to the crop.

Table 3.109. Effects of the rate, period and method of slurry or fertiliser N application (kg inorganic N ha^{-1}) on the N yield of maize (kg ha^{-1}). Experiment C3-1.

Rate of inorganic N	Slurry												Fertiliser		
	winter application						spring application						spring application		
	incorp.			not incorp.			incorp.			not incorp.					
1993															
0	C	100	a	C	107	a	C	103	a	C	105	a	B	120	a
80	B	160	a	BC	134	ab	B	148	ab	B	156	ab	B	125	b
160	B	158	a	AB	155	a	B	171	a	B	164	a	A	148	a
240	A	192	ab	A	187	b	A	211	a	A	207	ab	A	147	c
Slurry + fertiliser N: control (0 N): 125; combination (240 N): 214															
1994															
0	A	81	c	B	73	c	A	104	ab	A	91	bc	C	112	a
80	A	99	bc	B	70	c	A	129	b	A	112	b	B	171	a
160	A	94	bc	B	76	c	A	113	b	A	123	b	A	240	a
240	A	104	bc	A	94	c	A	138	b	A	140	b	A	245	a
Slurry + fertiliser N: control (0 N): 93; combination (240 N): 236															
1995															
0	C	62	bc	A	55	c	A	75	abc	A	83	ab	A	96	a
80	BC	71	bc	A	60	c	A	92	a	A	84	ab	A	101	a
160	B	73	bc	A	67	c	A	102	ab	A	87	bc	A	122	a
240	A	88	bc	A	69	c	A	102	b	A	82	bc	A	148	a
Slurry + fertiliser N: control (0 N): 78; combination (240 N): 157															
3-year average															
0	C	81	b	B	78	b	C	94	ab	C	93	b	C	109	a
80	B	110	ab	B	88	b	B	123	a	B	117	ab	B	132	a
160	B	108	bc	B	99	c	B	129	b	B	125	b	A	170	a
240	A	128	bc	A	117	c	A	150	b	A	143	b	A	180	a
Slurry + fertiliser N: control (0 N): 99; combination (240 N): 202.															

Capital letters show significance ($p \leq 0.05$) in vertical
Small letters show significance ($p \leq 0.05$) in horizontal

Inorganic N content of the soil

Table 3.110 presents the amounts of inorganic N in the soil layer of 0-40 cm, assuming a soil density of 1.2 kg dm^{-3}.

The experiments were carried out each year on different fields of the experimental farm and this may partly explain the different levels of inorganic N before maize sowing. Besides, the higher temperature and rainfall in the Spring of 1994 (Table 3.111) may have increased mineralisation of organic N in the soil but probably also N losses by denitrification and nitrate leaching. Consequently, it is not clear whether weather conditions caused the high values in this year.

There was a significant effect of the rate of slurry N on the quantity of soil inorganic N before maize sowing in 1993 (Table 3.110). Such an effect was not observed in 1994 and only on the main treatments with spring-applied slurry in 1995. The rate of fertiliser N only affected the amount of soil inorganic N before maize sowing in 1994 and 1995, not in 1993.

The period of slurry application affected the amount of inorganic N in the soil before maize sowing in 1993 and 1995, not in 1994 (Table 3.110). The method of slurry application had a clear effect in 1994, but the presence of this effect on the treatments without slurry points to an effect of soil cultivation rather than of incorporated slurry. In the other years, there was only an effect of the method of slurry application on the amount of inorganic N in the soil before maize sowing on the plots where slurry had been applied in the Spring of 1995.

The amount of inorganic N in the soil after harvesting the maize was moderate in 1993 and small in 1994 and 1995 and not significantly affected by period, method and rate of slurry or fertiliser N application to the maize (Table 3.110).

Table 3.110. Effects of the rate, period and method of slurry or fertiliser N application on the amount of inorganic N in the soil layer of 0-40 cm (kg ha^{-1}) immediately before sowing and after harvesting the maize. Experiment C3-1.

Rate, period and method of application	1993		1994		1995	
	before maize	after maize	before maize	after maize	before maize	after maize
No N						
Winter, incorporated	67.6	54.7	143.7	27.8	43.9	30.4
Winter, not incorporated	53.2	49.4	130.0	29.5	47.0	31.2
Spring, incorporated	64.5	60.2	135.1	22.3	49.2	30.9
Spring, not incorporated	63.6	59.0	117.3	28.3	54.4	28.8
Spring, fertiliser N	46.5	48.4	113.2	23.2	53.0	32.6
80 kg inorganic N per ha						
Winter, incorporated	84.0	48.0	138.7	27.8	44.6	31.9
Winter, not incorporated	71.7	73.7	124.0	24.9	42.7	28.8
Spring, incorporated	103.9	70.3	156.2	24.5	69.3	28.3
Spring, not incorporated	99.1	56.9	132.7	19.9	48.9	36.5
Spring, fertiliser N	53.5	46.1	144.0	26.2	60.7	36.7
160 kg inorganic N per ha						
Winter, incorporated	82.3	60.0	139.4	29.5	44.6	31.4
Winter, not incorporated	90.5	50.9	121.1	30.5	54.2	32.2
Spring, incorporated	104.6	84.9	137.0	21.8	80.9	31.7
Spring, not incorporated	120.7	79.7	134.2	24.0	74.2	29.3
Spring, fertiliser N	56.4	47.0	190.3	29.3	93.8	36.7
240 kg inorganic N per ha						
Winter, incorporated	103.9	51.1	136.1	27.1	46.6	37.9
Winter, not incorporated	101.7	60.2	122.9	27.6	45.3	35.0
Spring, incorporated	158.9	71.3	142.3	31.4	103.9	34.1
Spring, not incorporated	145.9	71.5	131.8	29.3	75.3	30.7
Spring, fertiliser N	54.0	52.5	266.6	30.7	86.9	36.7

Table 3.111. Average temperature and total rainfall before sowing the maize in the three experimental years. Experiment C3-1.

	1/1/93-23/4/93	1/1/94-3/5/94	1/1/95-14/4/95
Average temperature (°C)	5.2	7.9	6.0
Total rainfall (mm)	159	432	113

Lysimeter experiment (experiment C3-1a)

In Table 3.112 nitrate leaching values are reported.

Table 3.112. Nitrate leaching (kg N ha^{-1}) and water percolation (mm) during the growing season of maize, as affected by the rate of slurry application (kg inorganic N ha^{-1}). Experiment C3-1a.

	0		160		240		240*		Rainfall +
Period	N	water	N	water	N	water	N	water	irrigation
1993: from the start of the experiment to maize harvest									
24/05 - 21/06	0.18	6	0.28	2	0.46	4			109
21/06 - 05/07	0.02	2	0.10	6	0.11	1			32
05/07 - 26/07	0.34	2	-	0	0.35	3			114
26/07 - 20/09	-	0	0.11	2	0.17	1			238
Total	**0.54**	**10**	**0.49**	**10**	**1.09**	**9**			**493**
1994: from maize sowing to maize harvest									
09/05 - 06/06	0.10	2	0.14	4	0.74	10	4.83	25	137
06/06 - 04/07	0.09	2	0.21	21	0.11	3	0.34	9	84
04/07 - 18/07	0.10	2	0.08	6	0.06	1	0.05	2	76
18/07 - 16/08	0.28	11	0.14	8	0.16	13	0.46	38	115
16/08 - 12/09	0.69	30	0.21	22	3.18	51	0.28	30	191
Total	**1.25**	**47**	**0.79**	**61**	**4.25**	**78**	**5.96**	**104**	**603**
1995: from maize sowing to maize harvest									
02/05 - 22/05	0.11	28	0.06	12	2.28	60	5.58	36	265
22/05 - 25/09	0.63	105	0.22	96	13.92	194	3.15	144	772
Total	**0.74**	**133**	**0.28**	**108**	**16.20**	**254**	**8.73**	**180**	**1037**

(*) after bare soil in winter

Results of 1993 and 1994 show a small effect of the rate of slurry application on nitrate leaching, but the amounts of N leached during the growing season were low, confirming that most of the leaching occurs during winter (see Paragraph 3.14, Table 3.119).

In 1995, there was almost no leaching from the 0 and 160 N plots, but the quantity of N leached from the 240 N plots was higher than in 1993 and 1994. A possible explanation of these differences may be the much higher rainfall during the growing season of 1995. Besides, it should be taken into account that small plots vary considerably and that the absence of replication does not allow statistical analysis of the data recorded.

3.14 Sub-task C4

Field experiment (experiment C4-1)

Dry matter and N yields

Italian ryegrass yields are presented in the Tables 3.113 and 3.114.

Table 3.113. Effects of the period and method of slurry or fertiliser N application to maize on the DM yield of Italian ryegrass (t ha^{-1}) sown after maize harvest. The figures presented are averages of the 4 N levels. Experiment C4-1.

Period and method of application	1993-94		1994-95		1995-96		Average	
Winter	2.1	b	2.9	n.s.	3.2	n.s.	2.7	b
Spring	2.8	a	3.0	n.s.	3.1	n.s.	3.0	a
Incorporated	2.6	a	3.2	a	3.2	n.s.	3.0	a
Not incorporated	2.3	b	2.7	b	3.1	n.s.	2.7	b
Winter, incorporated	2.3	bc	3.1	a	3.4	n.s.	2.9	b
Winter, not incorporated	2.0	cd	2.8	b	3.0	n.s.	2.6	d
Spring, incorporated	3.0	a	3.3	a	3.0	n.s.	3.1	a
Spring, not incorporated	2.6	b	2.7	b	3.3	n.s.	2.9	bc
Spring, artificial fertiliser	1.7	d	3.1	a	3.3	n.s.	2.7	cd

Averaged over 3 years, the Italian ryegrass yield was significantly affected by both period and method of slurry application to the preceding maize crop. The highest yield was registered on plots with spring application and immediate incorporation of slurry, while the lowest yield was obtained on plots with winter-applied and not incorporated slurry; the difference was about 20% (Table 3.113). In most cases, the Italian ryegrass yield was positively correlated to the rate of N applied to maize (Table 3.114), but averaged over the 3 years, the effect of the rate of N applied to maize on the DM yield of Italian ryegrass was only statistically significant for spring-applied slurry.

Table 3.114. Effects of the rate, period and method of slurry or fertiliser N application to maize on the DM yield of Italian ryegrass sown after maize harvest (t ha^{-1}). Experiment C4-1.

Rate of inorganic N	Slurry												Fertiliser		
	winter application						spring application						spring application		
	incorp.			not incorp.			incorp.			not incorp					
1993															
0	A	1.9	a	A	1.8	a	B	2.4	a	B	1.9	a	A	1.8	a
80	A	2.3	ab	A	1.9	b	AB	3.2	a	AB	2.5	ab	A	1.8	b
160	A	2.3	b	A	2.1	b	AB	2.8	a	A	2.9	a	A	1.7	b
240	A	2.5	bc	A	2.2	c	A	3.6	a	A	3.1	ab	A	1.6	c
Slurry + fertiliser N: control (0 N): 1.9; combination (240 N): 2.9															
1994															
0	A	2.8	a	B	2.3	a	C	2.4	a	C	2.2	a	B	2.3	a
80	A	3.1	a	B	2.7	ab	B	3.1	a	BC	2.4	b	B	2.7	ab
160	A	3.1	a	AB	2.8	a	B	3.3	a	B	2.8	a	A	3.4	a
240	A	3.3	c	A	3.2	c	A	4.2	a	A	3.5	bc	A	4.0	ab
Slurry + fertiliser N: control (0 N): 2.2; combination (240 N): 3.6															
1995															
0	A	3.1	a	A	2.7	a	B	2.7	a	A	2.9	a	A	3.2	a
80	A	3.2	a	A	3.0	a	AB	3.0	a	A	3.2	a	A	3.3	a
160	A	3.6	a	A	3.4	a	A	3.2	a	A	3.5	a	A	3.4	a
240	A	3.8	a	A	3.1	a	A	3.3	a	A	3.5	a	A	3.5	a
Slurry + fertiliser N: control (0 N): 2.4; combination (240 N): 3.7															
3-year average															
0	A	2.6	a	A	2.2	a	B	2.5	a	B	2.3	a	A	2.5	a
80	A	2.9	a	A	2.5	a	AB	3.1	a	AB	2.7	a	A	2.6	a
160	A	3.0	a	A	2.8	a	AB	3.1	a	A	3.1	a	A	2.8	a
240	A	3.2	ab	A	2.8	b	A	3.7	a	A	3.4	a	A	3.0	ab
Slurry + fertiliser N: control (0 N): 2.2; combination (240 N): 3.4															

Capital letters show significance ($p \leq 0.05$) in vertical
Small letters show significance ($p \leq 0.05$) in horizontal

The N yields of Italian ryegrass are reported in the Tables 3.115 and 3.116.

Table 3.115. Effects of the period and method of slurry or fertiliser N application to maize on the N yield of Italian ryegrass (kg ha^{-1}) sown after maize harvest. The figures presented are averages of the 4 N levels. Experiment C4-1.

Period and method of application	1993-94		1994-95		1995-96		Average	
Winter	20.7	b	30.8	n.s.	27.1	n.s.	26.2	b
Spring	28.0	a	30.3	n.s.	27.0	n.s.	28.4	a
Incorporated	26.5	a	32.6	a	26.8	n.s.	28.6	a
Not incorporated	22.3	b	28.4	b	27.3	n.s.	26.0	b
Winter, incorporated	22.8	b	32.6	a	27.6	n.s.	27.6	b
Winter, not incorporated	18.6	c	28.9	b	26.5	n.s.	24.7	c
Spring, incorporated	30.1	a	32.7	a	26.0	n.s.	29.6	a
Spring, not incorporated	25.9	b	27.9	b	28.0	n.s.	27.3	b
Spring, artificial fertiliser	17.1	c	27.4	b	29.2	n.s.	24.6	c

The main treatment 'period of slurry application to maize' had a significant effect on the N yield of Italian ryegrass in 1993, but no effect in 1994 and 1995 (Table 3.115). The main treatment 'method of slurry application to maize' had significant effects in 1993 and 1994 and no effect in 1995. Averaged over the 3 years, N yield of Italian ryegrass was positively affected by the residual effects of both spring application and immediate incorporation of slurry. N yield on the plots with spring-applied and incorporated slurry was almost 20% higher than on the plots with winter-applied not incorporated slurry. On average, N yield of Italian ryegrass was positively affected by the rate of slurry or fertiliser N to the preceding maize crop (Table 3.116).

Root mass values of the Italian ryegrass are presented in Table 3.117. Averaged over the 3 years, the period and method of slurry application to maize did not significantly affect root mass of Italian ryegrass (Table 3.117). Effects in individual years were variable. The rate of N application to maize had a significant positive effect on root mass of Italian ryegrass (results not shown).

Table 3.116. Effects of the rate, period and method of slurry or fertiliser N application to maize on the N yield of Italian ryegrass sown after maize harvest (kg ha^{-1}). Experiment C4-1.

Rate of	Slurry												Fertiliser		
inorganic	winter application						spring application						spring		
N	incorp.			not incorp.			incorp.			not incorp.			application		
1993-94															
0	A	20.0	a	A	16.5	a	B	22.1	a	C	19.3	a	A	18.1	a
80	A	24.5	ab	A	19.6	b	AB	31.6	a	B	25.0	ab	A	17.6	b
160	A	20.6	b	A	19.0	b	AB	29.3	a	AB	28.1	a	A	17.0	b
240	A	25.9	bc	A	19.4	cd	A	37.6	a	A	31.1	ab	A	15.7	d
Slurry + fertiliser N: control (0 N): 20.1; combination (240 N): 29.1															
1994-95															
0	A	30.6	a	B	25.7	ab	C	25.4	ab	B	23.1	b	C	21.8	b
80	A	32.5	a	AB	28.9	ab	B	31.9	a	B	25.7	b	C	23.2	b
160	A	32.0	a	B	28.5	a	B	32.0	a	B	26.7	a	B	29.7	a
240	A	35.5	a	A	32.6	a	A	41.3	a	A	36.2	a	A	35.2	a
Slurry + fertiliser N: control (0 N): 19.8; combination (240 N): 32.3															
1995-96															
0	A	23.7	a	A	24.0	a	B	22.6	a	A	23.7	a	A	26.5	a
80	A	26.5	a	A	24.7	a	B	24.3	a	A	28.6	a	A	30.2	a
160	A	29.4	a	A	29.0	a	A	30.0	a	A	29.4	a	A	27.7	a
240	A	30.9	a	A	28.6	a	AB	27.3	a	A	30.5	a	A	32.6	a
Slurry + fertiliser N: control (0 N): 26.9; combination (240 N): 27.5															
3-year average															
0	C	24.7	a	B	22.0	a	C	23.4	a	C	22.0	a	B	22.1	a
80	B	27.8	ab	A	24.4	b	B	29.3	a	B	26.4	ab	B	23.6	b
160	B	27.3	ab	A	25.5	b	B	30.4	a	B	28.1	ab	B	24.8	b
240	A	30.7	ab	A	26.8	b	A	35.4	a	A	32.6	a	A	27.8	b
Slurry + fertiliser N: control (0 N): 22.3; combination (240 N): 29.6															

Capital letters show significance ($p \leq 0.05$) in vertical
Small letters show significance ($p \leq 0.05$) in horizontal

Table 3.117. Effects of the period and method of slurry or fertiliser N application to maize on the root mass of Italian ryegrass (t ha^{-1}). The figures presented are averages of the 4 N levels. Experiment C4-1.

Period and method of application	1993-94		1994-95		1995-96		Average	
Winter	1.4	b	2.9	n.s.	1.2	n.s.	1.8	n.s.
Spring	1.7	a	2.4	n.s.	1.3	n.s.	1.8	n.s.
Incorporated	1.6	a	2.6	n.s.	1.2	n.s.	1.8	n.s.
Not incorporated	1.5	b	2.7	n.s.	1.3	n.s.	1.8	n.s.
Winter, incorporated	1.5	bc	2.9	ab	1.3	n.s.	1.9	n.s.
Winter, not incorporated	1.4	c	3.0	a	1.0	n.s.	1.8	n.s.
Spring, incorporated	1.8	a	2.4	bc	1.1	n.s.	1.7	n.s.
Spring, not incorporated	1.6	b	2.3	c	1.6	n.s.	1.8	n.s.
Spring, artificial fertiliser	1.5	bc	2.9	a	1.3	n.s.	1.9	n.s.

Inorganic N content of the soil

Table 3.118 presents the inorganic N content of the soil layer of 0-40 cm, assuming a soil density of 1.2 kg dm^{-3}.

Table 3.118. Effects of the rate, period and method of slurry or fertiliser N application to the maize on the amount of inorganic N in the soil layer of 0-40 cm (kg ha^{-1}) immediately after harvesting the maize and after harvesting the Italian ryegrass. Experiments C3-1 and C4-1.

Rate, period and method of application	1993-94 after maize	1993-94 after ryegrass	1994-95 after maize	1994-95 after ryegrass	1995-96 after maize	1995-96 after ryegrass
No N						
Winter, incorporated	54.7	68.2	27.8	9.8	30.5	22.3
Winter, not incorporated	49.4	76.3	29.5	7.9	31.2	19.0
Spring, incorporated	60.2	85.2	22.3	6.2	31.0	21.1
Spring, not incorporated	59.0	83.0	28.3	8.2	28.8	18.5
Spring, fertiliser N	48.5	73.4	23.3	5.8	32.6	21.6
80 kg inorganic N ha^{-1}						
Winter, incorporated	48.0	73.7	27.8	14.6	31.9	23.3
Winter, not incorporated	73.7	81.6	25.0	7.0	28.8	23.5
Spring, incorporated	70.3	74.4	24.5	9.1	28.3	20.9
Spring, not incorporated	56.9	84.2	19.9	7.9	36.5	19.4
Spring, fertiliser N	46.1	86.6	26.2	7.7	36.7	22.8
160 kg inorganic N ha^{-1}						
Winter, incorporated	60.0	74.6	29.5	8.6	31.4	21.1
Winter, not incorporated	50.9	91.9	30.5	7.4	32.2	21.6
Spring, incorporated	85.0	79.0	21.8	7.9	31.7	23.3
Spring, not incorporated	79.7	75.4	24.0	5.5	29.3	21.4
Spring, fertiliser N	47.0	71.0	29.3	6.7	36.7	22.1
240 kg inorganic N ha^{-1}						
Winter, incorporated	51.1	78.7	27.1	9.1	37.9	25.4
Winter, not incorporated	60.2	80.9	27.6	7.0	35.0	20.6
Spring, incorporated	71.3	71.5	31.4	10.3	34.1	22.6
Spring, not incorporated	71.5	77.3	29.3	9.8	30.7	20.9
Spring, fertiliser N	52.6	67.2	30.7	8.2	36.7	25.7

In 1993, there was more residual N in the soil after the maize crop than in the two other years, but this did not result in higher DM and N yields of Italian ryegrass (compare with Tables 3.114 and 3.116). Only in 1993, there was a clear effect of the period and rate of N application on the amount of residual N after the maize. This was not observed in 1994 and 1995.

Values of inorganic N content of the soil after the harvest of Italian ryegrass varied strongly among individual years. In the spring of 1994, these values were even higher than after the harvest of maize in the autumn of 1993, despite the fact that the Italian ryegrass did not receive any N. Apparently the weather conditions in the winter and spring of 1993-94 were unfavourable for grass growth and/or N uptake.

Lysimeter experiment (experiment C4-1a)

Total nitrate leaching (kg N ha^{-1}) and water percolation (mm) between sowing and harvesting of Italian ryegrass are presented in Table 3.119.

Table 3.119. Total nitrate leaching (kg N ha^{-1}) and water percolation (mm) between sowing and harvesting of Italian ryegrass, as affected by the rate of slurry inorganic N applied to the preceding maize crop. Experiment C4-1a.

	0		160		240		240 (bare soil)		Rainfall +
Period	N	water	N	water	N	water	N	water	irrigation
1993-94									
20/09 - 31/10	6.94	65	13.57	74	37.13	75	37.13	75	328
31/10 - 15/11	1.92	20	2.19	17	5.52	23	4.93	21	81
15/11 - 09/05	18.47	162	16.75	164	26.51	183	33.69	186	731
total	**27.33**	**247**	**32.51**	**255**	**69.16**	**281**	**75.75**	**282**	**1140**
1994-95									
12/09 - 26/09	0.23	25	0.19	36	4.47	44	0.25	32	111
26/09 - 02/05	7.96	154	8.16	170	21.87	201	11.63	178	297
total	**8.19**	**179**	**8.35**	**206**	**26.34**	**245**	**11.88**	**210**	**408**
1995-96									
25/09 - 20/05	**21.06**	**282**	**15.23**	**213**	**42.75**	**458**	**47.45**	**292**	**705**

In spite of the good N catching capacity of Italian ryegrass, revealed by the mean N yield in the harvested herbage of about 27 kg ha^{-1} year^{-1}, there was not a clear effect of grass cover on nitrate leaching as measured in these field lysimeters. However, it has to be mentioned that small field lysimeters often are not very precise. The absence of replications prevented a statistical analysis of the data collected.

Table 3.120 presents total nitrate leaching (kg N ha^{-1}) and water percolation (mm) for each production cycle of maize and Italian ryegrass.

Table 3.120. Total nitrate leaching (kg N ha^{-1}) and water percolation (mm) for each production cycle of maize and Italian ryegrass, as affected by the rate of slurry inorganic N. Experiments C3-1a and C4-1a.

	0		160		240		240 (bare soil)		Rainfall +
Period	N	water	N	water	N	water	N	water	irrigation
24/05/93 - 09/05/94	27.87	257	33.00	265	70.25	290	76.84	292	1633
06/06/94 - 02/05/95	9.35	224	9.00	263	29.75	313	13.00	288	874
02/05/95 - 20/05/96	21.80	415	15.51	321	58.95	712	56.18	472	1742

The rate of slurry application produced clear differences in nitrate leaching. No differences were registered between the plots with 0 and 160 kg inorganic N per ha nor between the plots with bare and covered soils. This was not expected and we have no explanation for this.

4. Discussion

4.1 Sub-task A1

A major objective of the experiments conducted under sub-task A1 was to study whether an optimisation of the conditions for microbial growth and capture of degraded feed protein in the rumen could improve the utilisation of the surplus of rumen-degradable protein often found in leafy forages. Such an improvement might increase the AAT value of the feed compared to a standard value, so that the calculated AAT value of the diet could be reduced below a normal minimum level without reducing milk yield of the cows. An increased amount of rumen-fermentable carbohydrates at the expense of protein-rich supplements and an optimum synchronisation of the supply of degradable protein and fermentable carbohydrates obtained by feeding complete mixed diets should form the basis for an increased microbial protein synthesis.

A reduction in the level of AAT in the ration in experiment A1-1 resulted in a significant reduction in the protein concentration of milk and in the production of milk protein and ECM (Figures 4.1 and 4.2). In experiment A1-4, there were no significant differences in milk yield or composition, but a tendency toward a reduction in protein concentration could be seen at the lowest level of AAT. However, in this experiment the estimated level of AAT varied only between 92 and 98 g FU^{-1}. Voluntary feed intake was reduced at the lowest AAT levels in both experiments (Tables 3.4 and 3.5). The results indicate a positive effect on milk protein yield of increasing AAT values up to 95 g FU^{-1}. When the level of AAT was reduced below 90 g FU^{-1}, which is the minimum level in current Danish standards (Madsen *et al.*, 1995), production was certainly reduced. Hence, it was not possible to reduce the protein level below the current recommended minimum without reducing yield of milk and milk protein.

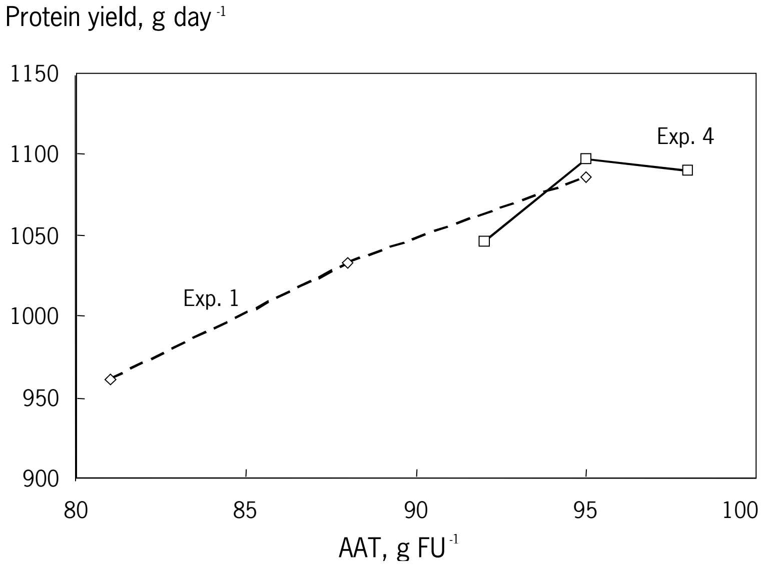

Figure 4.1. Relationship between the concentration of AAT in the diet and milk protein yield per cow.

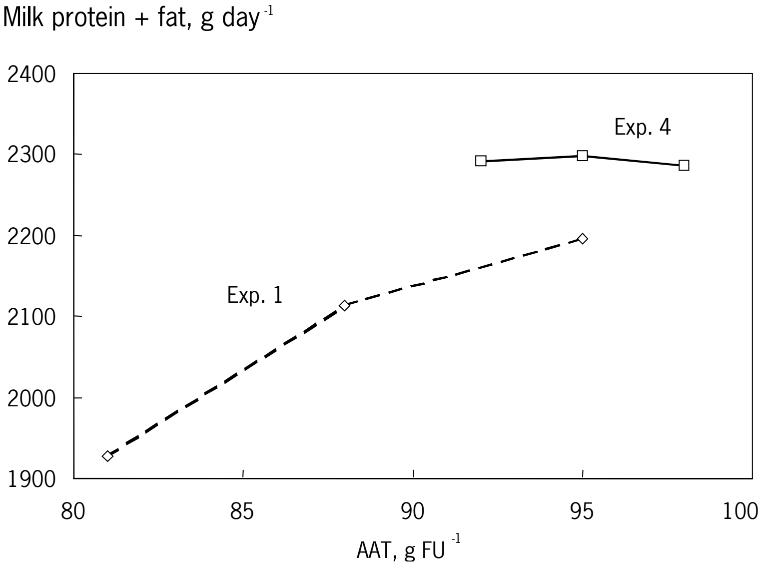

Figure 4.2. Relationship between the concentration of AAT in the diet and yield of milk fat plus protein per cow.

Unfortunately, the protein content of grass/clover was lower than expected and the PBV value of the grass/clover silage was close to zero (Table 3.1), *i.e.* there was no calculated surplus of rumen-degradable protein. The surplus of rumen-degradable protein in pea silage was high enough to give positive PBV values in the total diet in all cases (Tables 3.4 and 3.5), although at the lowest level in experiment A1-1, PBV became as low as 50 g cow^{-1} day^{-1}. Weisbjerg (1997) found no effect on microbial protein synthesis of reducing PBV to -150 g cow^{-1} day^{-1}, but it was reduced by a further reduction in PBV. It is assumed that the calculated level of 50 g PBV day^{-1} was enough for maximum microbial protein synthesis, but it must be admitted that this PBV level was close to the minimum for optimal fermentation.

Another factor that might affect feed intake and milk yield negatively at low AAT levels is the inhibiting effect of starch on fermentation of fibrous carbohydrates in the rumen. Such an effect was indicated by a reduction of the apparent digestibility of fibre in the whole tract (Table 3.12). This effect is possibly caused by a negative effect of starch on the degradation of fibre in the rumen. The concentrate mixture used at the lowest level of AAT in the experiments A1-1 and A1-4 contained 500 g starch per kg DM and the concentration of starch in pea silage was 200 g kg^{-1} DM. Starch from these two sources made up 280 g kg^{-1} DM in the total diet at the lowest AAT concentration. This concentration of starch is close to the maximum limit recommended in Danish standards (Strudsholm *et al.*, 1992). Such negative effects on milk production could be the result of both a poorer energy utilisation and a reduced supply of microbial amino acids.

Analysis of urinary excretion of purine derivatives, especially allantoin, did not show a systematic relationship to treatments in experiment A1-1, but in experiment A1-4, excretion of allantoin was significantly reduced with lower levels of AAT, which does indicate a reduced synthesis of microbial protein (Table 3.14). Several studies have shown a positive correlation between the estimated rumen microbial true protein supply to duodenum and urinary excretion of purine derivatives (Susmel *et al.*, 1993) and between intestinally infused microbial RNA and urinary excretion of allantoin (Antoniewicz *et al.*, 1980; Chen *et al.*, 1990).

In summary, the attempt to maximise protein utilisation in our experiments and reduce the level of AAT below current standards without reducing milk yield was not successful. *Ad libitum* feeding of total mixed diets may thus not show any special advances in the utilisation of feed protein. But an inhibiting effect of high starch levels in the diet on the fermentation of fibre in the rumen possibly affected the results in these experiments. It might be possible to improve the results by replacing a part of the starchy feed by easily digestible fibrous feeds at the low levels of AAT (Istasse *et al.*, 1986).

Low PBV values reduced silage intake in two experiments, one in early (Table 3.7) and one in late lactation (Table 3.8), but in the other two experiments differences were small and insignificant. In treatments with low PBV values, the lack of available N for rumen micro-organisms could be expected to limit the degradation of fibre in the rumen. However, in experiments A1-2 and A1-5 no systematic effects on the digestibility of the diets were found (Table 3.13). The standard errors of apparent fibre digestibility in the experiments A1-2 and A1-5 were extremely high. Urinary excretion of purine derivatives did not differ among PBV treatments (Table 3.15). Also for these determinations, variance was higher than in experiments A1-1 and A1-4. In a basic study with ruminally and intestinally cannulated cows, which at the same time was carried out in our laboratory, a reduction in PBV within the same range as in the present experiments resulted in a reduction of the apparent digestibility of organic matter and fibre in the whole tract, and a reduction of microbial protein synthesis in the rumen (Weisbjerg, 1997).

Milk yield was much lower in the experiments with low PBV than in the experiments with varying AAT levels (Tables 3.4, 3.5, 3.6 and 3.7). This difference was primarily due to the great differences in the digestibility of the diets and energy intake. Yields of milk, milk protein and ECM were reduced by low values of PBV. There was no clear effect on the protein content of the milk. The reduction in daily production of milk protein in early lactation was approximately 25 to 30 g for each 100 g reduction in PBV (Figure 4.3). Daily yield of ECM in early lactation decreased by 0.5 to 0.9 kg per 100 g reduction in PBV below 0. This reduction in yield probably was rather linear.

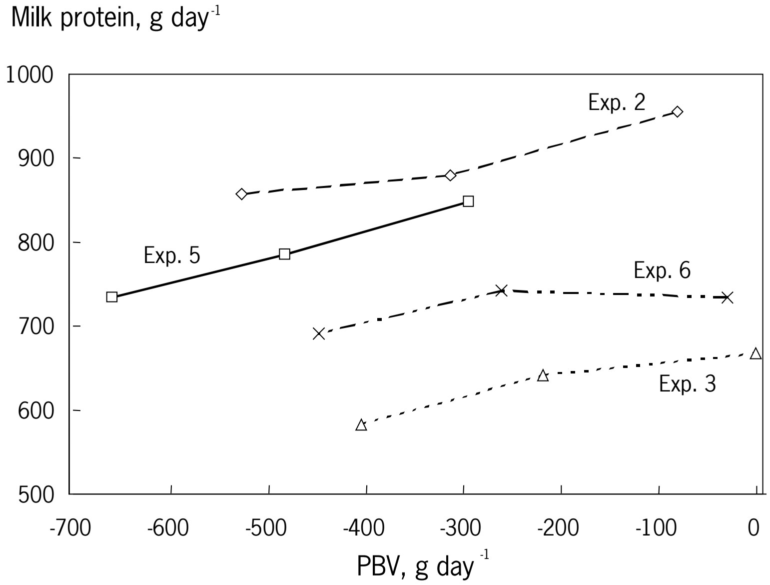

Figure 4.3. Relationship between daily PBV intake and milk protein yield per cow.

Results from late lactation indicated a lower effect on milk yield by reducing PBV to about -200 g cow^{-1} day^{-1} (Figure 4.3). But when PBV was further reduced the effect on milk yield was as large as in early lactation, as the reduction in yield of ECM was about 1 kg for each 100 g further reduction in PBV (Tables 3.8 and 3.9). These results concerning the effects of lowering the level of PBV show, as was the case for AAT, that a reduction below the minimum limits in Danish standards (Madsen *et al.*, 1995) results in a reduced yield of milk protein.

Conclusion

The final conclusion of these experiments is that no possibility was found of reducing the estimated levels of AAT or PBV below minimum Danish standards without a reduction in milk yield and/or milk protein yield. However, the results provide a rather good basis for estimation of the response in milk yield to reductions in the protein supply of cows. This may be useful in future models for feed optimisation in dairy herds, in which a reduction of the protein level in the feed below the point which give maximum production may be the optimum solution under certain conditions. Based on the results of these experiments, it may be shown that by balancing minimum levels of both AAT and PBV, maximum milk yield may be obtained with about 160 g kg^{-1} crude protein in the total diet DM. Based on statistics from the Danish Agricultural Advisory Centre on feed planning in practice, it was estimated that the average crude protein concentration was 170 to 180 g kg^{-1} DM in winter feeding of dairy cows in early lactation (Kristensen, 1995). Presently, no useful laboratory methods are available to determine degradability or intestinal digestibility of protein in home-grown or purchased feedstuffs. Furthermore, the degradability of protein may vary greatly because of climatic influences in home-grown feeds and because of industrial treatments of purchased feed. Therefore, a safety margin is necessary in feed planning. On this background, it must be concluded that there are no real possibilities for reduction in the protein level of Danish winter feeding of dairy cows without risk of reduction in yield. A further development of a more precise estimation of the absorption of both total and individual essential amino acids may be the key to improve the protein utilisation of dairy cows.

4.2 Sub-task A3

Chemical composition of the feeds

The lower concentrations of crude protein, N, DVE and OEB and the higher concentration of sugar in the herbage in experiment A3-2 than in experiment A3-1 (Table 3.17), were probably the result of a limited supply of N from mineralisation of organic N because of cold and wet weather during May and June 1994. In July, weather conditions changed to hot and dry. These conditions also may have had an adverse effect on the uptake of N by the sward resulting in a relatively low concentration of N and a relatively high concentration of water soluble carbohydrates. Such effects of dry and hot weather conditions on the chemical composition of herbage have also been reported by Deinum *et al.* (1968).

DM and starch contents and dOM and NE_L values of GMES were higher in experiment A3-1 than in experiment A3-2. Better weather conditions in 1992 may have resulted in improved maturity at harvest. Harvesting maize at a late stage of maturity results in higher starch and DM contents and higher nutritive value (Deinum & Knoppers, 1979).

Feed intake

The effects of carbohydrate composition of concentrate supplements on the intake of preserved forages are well documented. In contrast, there is only a relatively small number of reports on the effects of concentrate type on intake of dairy cows on fresh herbage-based rations.

In both experiment A3-1 and A3-2, TDMI ranged between 30 and 33 g kg^{-1} live weight, which is in agreement with the standard figures of CVB (1995). In both experiments, TDMI ranged between 17.6 and 18.8 kg day^{-1} (Table 3.18), which is similar to the results of Schwarz *et al.* (1995) for dairy cows fed herbage-based rations supplemented with beet pulp, maize or cereal-based concentrates.

Partial or full replacement of beet pulp by GMES and SMSE (experiment A3-1) had no effect on intake of herbage and total DM (Table 3.18). Valk & Hobbelink (1992) reported similar results in a comparison of beet pulp, GMES and maize-based concentrate as supplements for grazing dairy cows. Van Vuuren *et al.* (1993) found no differences in intake of herbage and total OM when herbage-based diets were supplemented with maize silage or three concentrate mixtures based on corn, beet pulp and corn plus beet pulp. Spörndly (1991) observed no effect of feeding fibrous (beet pulp and wheat bran) or starchy (barley) concentrates on herbage intake and TDMI by grazing dairy cows. However, Schwarz *et al.* (1995) observed a tendency for reduced herbage intake by dairy cows when the concentrate was based on cereals, compared to beet pulp or maize-based concentrates. Valk *et al.* (1990) reported a tendency for higher intake of herbage by dairy cows fed a maize-based concentrate compared to dairy cows fed a beet pulp-based concentrate in a zero-grazing experiment with equal levels of concentrate allowance. In contrast, Meijs (1985) observed a lower herbage intake by grazing cows supplemented with a starchy concentrate, in comparison to those receiving a high-fibre concentrate supplement. Kibon & Holmes (1987) found that intake of herbage by cows grazing at a sward height of 6.5 cm was increased with 0.9 kg OM for animals receiving a pulp-based concentrate, compared to a cereal-based concentrate. However, the same comparison at a sward height of 5.0 cm did not affect herbage intake.

In experiment A3-2, inclusion of GMES in the ration resulted in increased TDMI (Table 3.18). However, intake of herbage decreased with higher levels of GMES supplementation. The substitution rates of GMES for herbage for the treatments M25, M50, M75 were 0.6, 0.9 and 0.8 kg DM, respectively. This is in agreement with the substitution rate of 1 kg DM of GMES for 0.7 kg DM of herbage, calculated by Bruins (1992). Hijink *et al.* (1981) found substitution rates of different pelleted compound feeds for herbage ranging from 0.25 to 0.72 kg DM. Meijs (1985) observed substitution rates of fibrous and starchy concentrates for herbage of 0.21 and 0.45 kg OM, respectively. In contrast, Spörndly (1991) found higher substitution rates of concentrate for herbage for fibrous (beet pulp and wheat bran) than for starchy (barley) concentrates. Hijink *et al.* (1981) and Spörndly (1991) reported that the substitution rate of concentrate for herbage increased with higher levels of concentrate supplementation. Bruins (1992) and Valk & Hobbelink (1992) reported a higher substitution rate of GMES than of compound feeds for herbage. This is possibly related to the physical structure and composition of GMES. GMES contains the complete ear including grains, husks and cob. Due to the presence of coarse particles from the husks and the cob, the digestion characteristics of GMES are probably closer to forage than to compound feed containing milled ingredients.

In experiment A3-2, it can not be ruled out that the negative OEB of the diets has affected the substitution rate of GMES for herbage (Table 3.18). Negative OEB values indicate a shortage of N in the rumen which may have a negative influence on microbial growth and rumen digestion (Tamminga *et al.*, 1994). OEB became more negative with larger amounts of GMES in the ration.

Diet composition

Replacing beet pulp by GMES and SMSE or replacing fresh cut herbage by GMES had a considerable effect on diet composition (Table 3.19). In experiment A3-1, replacing beet pulp by GMES and SMSE resulted in higher concentrations of N, starch, NE_L and OEB, and lower concentrations of crude fibre, sugar, NDF, ADF and FOM. In experiment A3-2, replacing fresh cut herbage by GMES resulted in higher concentrations of starch, OM, dOM, and NE_L, and lower concentrations of N, crude fibre, crude ash, sugar, DVE, OEB, NDF and ADF.

Milk yield and milk composition

The carbohydrate composition of the ration was modified by changing the carbohydrate source of the concentrates (experiment A3-1) or by changing the level of concentrate supplementation (experiment A3-2) (Table 3.19). Modifying the carbohydrate composition of the ration can be expected to affect rumen fermentation and, consequently, production of precursors for milk synthesis (De Visser *et al.*, 1990; 1992).

When considering the effects of feeding starchy or fibrous concentrates on animal performance, it is important to bear in mind that there are major differences in degradation characteristics within the category of starchy concentrate components and within the category of fibrous concentrate components as well (Tamminga *et al.*, 1990).

In experiment A3-1, replacing beet pulp by GMES and SMSE had no significant effect on the yields of milk, fat and protein, and the concentrations of fat and protein (Table 3.20). However, fat concentration tended to decrease when beet pulp was replaced by GMES and SMSE. Valk & Hobbelink (1992) found no differences in yields of milk and milk constituents between treatments with beet pulp or GMES as a supplement for grazing dairy cows. However, supplementation with a maize concentrate resulted in reduced milk fat yield and concentration compared to supplementation with beet pulp or GMES. Replacing compound feed by GMES as supplement to a herbage-based ration for dairy cows in early lactation resulted in a lower milk fat concentration and milk fat yield, but in a slightly higher milk protein yield (Boxem *et al.*, 1994). Bruins *et al.* (1991) found a reduced milk yield and milk fat concentration when grazing cows were supplemented with GMES instead of compound feeds containing 15 or 35% starch and sugars. In addition, Bruins *et al.* (1992) showed that replacing compound feed by GMES in the ration of dairy cows in late lactation resulted in reduced yields of milk, fat and protein. However, replacing compound feed by GMES in the ration of dairy cows in early lactation had no effect on the yields of milk and milk constituents (Bruins *et al.*, 1992). These authors suggest that this differential effect of stage of lactation may be due to hormonal mechanisms. Kibon & Holmes (1987) found no effect of supplementation with a beet pulp-based concentrate or a cereal-based concentrate on milk performance. Spörndly (1991) found no effect of the type of concentrate on milk yield, but milk fat concentration was slightly higher when a starchy concentrate was fed instead of a fibrous concentrate. However, Meijs (1985) observed that supplementation with high-fibre concentrates resulted in higher yields of milk and 4% fat-corrected milk than with high-starch concentrates. Schwarz *et al.* (1995) found higher milk yields when cows received a maize-based or cereal-based concentrate than a beet pulp-based concentrate. Valk *et al.* (1990) found higher milk yields due to improved energy intake when maize replaced beet pulp in the concentrate portion of the ration. However, at equal levels of energy intake, there was no effect of carbohydrate source on milk yield (Valk *et al.*, 1990). In experiment A3-1, NE_L and DVE intakes were similar for all treatments (Table 3.18). However, it should be noted that NE_L and DVE intakes were about 9 and 27 per cent above the requirements, respectively. It is likely that feeding energy and protein above requirements obscured possible differences among the experimental treatments.

In experiment A3-2, supplementation with GMES resulted in increased milk, protein and lactose yields compared to the treatment without supplementation (Table 3.20). The yields of FPCM in M25, M50 and M75 increased with 7.7, 3.9 and 5.6 per cent, respectively, compared to M00. Intake of energy in M25, M50 and M75 was, respectively, 8.8, 8.6 and 14.7 per cent higher (Table 3.18). The higher milk yield can be explained by higher intake of NE_L and NE_L coverage. DePeters & Cant (1992) showed that, in general, increased energy intake will result in increased milk and protein yields. Although NE_L coverage in M50 and M75 was higher than in M25, there was no further increase in milk yield. Intake of DVE was similar in M25, M50 and M75, but coverage of DVE, corrected for a negative OEB, according to the DVE/OEB system (Tamminga *et al.*, 1994), decreased with higher intake of GMES. It is likely that the low DVE coverage in M25, M50 and M75 may have been the limiting factor for milk production. In spite of insufficient DVE coverage in M25, M50 and M75, milk protein yield was higher than in M00. This suggests improved utilisation of protein when herbage is replaced by GMES. It is likely that feeding GMES has improved the supply of starch to the intestine. Nocek & Tamminga (1991) suggest that the exogenous glucose supply from starch may save endogenously synthesised glucose and dietary and tissue amino acids for gut metabolism, allowing more to be directed to the mammary gland.

In experiment A3-2, milk fat concentration was not affected by the amount of GMES in the diet (Table 3.20). Reduced forage to concentrate ratios usually result in lower milk fat concentrations, but the pattern of response varies widely (Sutton, 1989). The effect of reducing the forage to concentrate ratio on milk fat yield and concentration depends on the carbohydrate composition of the concentrates and the proportion of cell wall constituents in the ration (Sutton, 1989). However, in spite of large differences in forage to concentrate ratios in experiment A3-2, the differences in intake of cell wall constituents among treatments were relatively small (Table 3.19), which may explain the small differences in milk yield and fat concentration.

Feeding GMES resulted in increased yield and concentration of lactose (Table 3.20). This may be explained by the fact that feeding slowly degradable starch (e.g. corn starch), which is digested in the rumen and the small intestine, stimulates the production of glucose and glucogenic precursors used for the synthesis of lactose (Nocek & Tamminga, 1991).

The differences in concentration and excretion of milk urea were related to differences in the intake of OEB (Tables 3.20 and 3.19). This is in agreement with the positive correlation between the protein balance in the rumen (OEB) and the concentration of urea in milk found in other studies (Gustafsson & Carlsson, 1993; Gonda & Lindberg, 1994; Schepers & Meijer, in press).

Nitrogen utilisation

In both experiment A3-1 and A3-2, N utilisation, calculated as (N excretion in milk/N intake) × 100 followed the differences in OEB, *i.e.* the N losses from rumen fermentation (Tables 3.20 and 3.19). Differences in OEB in experiment A3-1 were mainly the result of inclusion of SMSE in the concentrate portion of the rations to obtain equal intakes of DVE (paragraph 2.2). In experiment A3-2, replacing herbage by GMES improved energy intake, but resulted in a sub-optimal DVE coverage and a reduced OEB. Consequently, this resulted in an increased energy to N ratio in the diet and, hence, improved utilisation of dietary N.

Blood and urine parameters

In experiment A3-1, the levels of UN and UUN followed the differences in intake of dietary N (Tables 3.21 and 3.19). This is in agreement with the findings of Gonda & Lindberg (1994) and Ciszuk & Gebregziabher (1994). However, there was no such relation in experiment A3-2. The increased concentration of urinary creatinine suggests lower urine production of cows receiving larger amounts of GMES in the diet. Hence, reduced urine production resulted in relatively high concentrations of UN and UUN. Van Vuuren *et al.* (1996) found that UUN excretion was reduced when grass silage was replaced by maize silage or GMES, but concentration of UUN was increased due to a reduced quantity of urine. Table 3.17 shows that the concentration of minerals, in particular potassium, in GMES is low compared to grass. Lower intakes of strong osmotic minerals such as potassium may be responsible for more concentrated urine in cows fed GMES.

In both experiments, the levels of ß-HB were low compared to the reference values of the GD (1994). This confirms that intake of energy was sufficient.

Conclusions

Partial or full substitution of GMES and SMSE for beet pulp, as a concentrate supplement for dairy cows receiving a herbage-based ration (experiment A3-1), had no significant effect on the yield of milk and the yield and concentration of milk protein, but milk fat concentration was slightly reduced and yield and concentration of lactose were increased. No effects on DM intake were observed when beet pulp was replaced by GMES and SMSE. NE_L and DVE intakes, respectively, were about 9 and 27 per cent above the requirements which may have obscured possible effects. Replacing beet pulp by GMES and SMSE resulted in a slightly lower utilisation of N due to a higher N intake.

Replacing fresh cut herbage by GMES (experiment A3-2) resulted in increased total dry matter intake. The substitution rate of GMES for herbage was about 0.8, which appears higher than that of compound concentrates. Increasing the allowance of GMES was accompanied by higher intake of NE_L, but resulted in a ration with a more negative OEB concentration. This caused a shortage of rumen-degradable protein and insufficient DVE coverage. Nevertheless, replacing fresh cut herbage by GMES resulted in increased yields of milk, milk protein and lactose. Concentrations of fat and protein were not affected, but lactose concentration was increased. Replacing herbage by GMES resulted in lower OEB and N intake relative to energy and, consequently, in improved N utilisation at animal level. Replacing herbage by GMES and thus reducing intake of OEB is likely to be more effective to reduce losses of N in dairy cows than replacing concentrates by GMES. However, whole-farm studies are necessary to elucidate the effects of home-grown GMES on the losses of N and energy at farm-scale.

4.3 Sub-task A4

The n-alkane evaluation experiment showed that the n-alkane technique has the potential to be used in dairy cows to give accurate estimates of herbage intake and proportion of clover in it. However, this technique gave unrealistic estimates of DM and N intake in the first intensive measurement period of the main experiment (17-22 May 1993). The estimated total DM intakes of 11.4 kg cow^{-1} day^{-1} (Table 3.30) supplied only 74 and 86% of the ME requirements of the spring-and autumn-calving cows, respectively (Table 3.35). This level of intake is uncommon on good pasture and would result in loss of body weight and a rapid decrease of milk production and these consequences of poor intake were not observed (Table 3.29). The estimated N balances of the cows in the first measurement period, in particular the high proportion of faeces N (more than 50% if the estimated N intake), the very low production of urine N and the very high N efficiencies (Table 3.34), also point to a strong under-estimation of DM and N intakes. Assuming equilibrium between ME intake and requirement and, consequently, 35% higher DM and N intakes of the spring-calving cows and 16% higher intakes of the autumn-calving cows (Table 3.35), both N intake and production of urine N would have been 104 and 47 g cow^{-1} day^{-1} higher for the spring- and autumn-calving cows, respectively. In that case, N efficiencies of the cows would have been 28 and 25%, respectively.

Despite the problems with the n-alkane technique, the results of the main experiment indicated that the increase in clover content accompanied with the increase in CP content of the available herbage in the second half of the herbage growth season and the use of lower productive animals resulted in a decrease in N efficiency of the grazing animal and, thereby, an increase in the potential for environmental pollution. Furthermore, a substantial increase in milk NPN was observed and this would have important consequences for the manufacturing potential of milk produced from high-clover swards.

Future work with dairy cows grazing grass/clover swards throughout the season should therefore concentrate on trying to improve N efficiency of the animal. This should in turn reduce the potential for environmental pollution and increase milk quality.

One of the options to improve N efficiency is to supplement with low-N supplements and, thereby, reduce the N content of the total diet. Supplementation will have to depend on clover content of the sward because this experiment has indicated that this is an important factor determining N efficiency.

4.4 Sub-task A5

The objective of this study was to improve N efficiency of dairy cows grazing grass/clover swards with different clover contents, using supplementation with different types and/or levels of concentrates. In both experimental periods, CP contents of the herbage on offer were lower than expected; on average it was only 12.6% of DM in May/June (experiment A5-1; Table 3.36) and 15.5% DM in July/August (experiment A5-2; Table 3.45). As a consequence, N efficiency of the cows in Spring was already very high with minimum supplementation (group A, Table 3.43), whereas in Summer, it was also rather high in this group (Table 3.52).

Analysis of the results shows that the response to supplementation in Spring (experiment A5-1) was very limited. Increasing ERDP supply by adding urea to the supplement, tended to increase DM intake, as estimated by the n-alkane technique (Table 3.39). However, this did not result in improved animal performance and, therefore, it may be questioned whether this intake estimate as well as the derived N and ME efficiencies (Tables 3.43 and 3.44) are reliable. Supplementation with urea (treatment B) tended to increase the protein content of the milk (Table 3.38), but did not clearly affect the NPN fractions (Table 3.41).

Supplementation in summer with sugar beet pulp (experiment A5-2, Table 2.6) increased both milk and milk protein yield (Table 3.47) and decreased the NPN fractions in the milk produced (Table 3.50). The supplement used tended to increase urine N concentration (Table 3.51). Due to the unexpectedly low CP content of the herbage on offer, this supplement only slightly decreased N intake (Table 3.52). However, the slight reduction in intake and the increase in milk N yield resulted in a considerable increase in N efficiency (Table 3.52). Whether the replacement of moderately N rich herbage by low-N concentrate is a cost-effective method of increasing N efficiency in dairy production systems depends on local economic circumstances. The potential has been indicated by this study.

4.5 Sub-task A6-1

The objective of this experiment was to study the effects of a reduction in ERDP and MP below the recommended levels, on performance and N efficiency of dairy cows fed *ad libitum* grass/clover silage supplemented with barley, fodder beet and protein-rich concentrates. Actual ERDP levels were in accordance with the planned levels, but actual MP levels were higher than planned, and the lowest MP level still exceeded the MP requirement of the cows (Tables 2.8 and 3.59). Nevertheless, considerable differences in animal performance and N efficiency among the groups were observed (Tables 3.58 and 3.62). The cows on diet B, with an ERDP deficit and an MP surplus, outyielded the cows on diet A (ERDP deficit and a small MP surplus) and diet C (ERDP surplus and MP surplus). Because of the difficulty in explaining these results, it was decided to do some additional feed measurements, (*in vivo* degradability of feed N and DM) to enable a more accurate description of the feed and more accurate calculations of the N flows within the animal. However, the cause of the relatively poor performance of the cows on diet C is not clear. It could be that removing excess N from the animal system improves animal performance. In addition, the different types of protein supplement, *viz.* mainly soya for group B and urea/molasses for group C, may explain the better performance of the cows of group B. Supplementation with urea also gave disappointing effects on the performance of cows grazing a grass/clover sward with a low CP content (experimental group B in experiment A5-1, Table 3.38).

In the present experiment, N efficiency of the cows in group A was the highest. However, the extra N intake of the cows in group B resulted in a significantly higher output of milk N and, as a consequence, N efficiency of group B was only slightly lower than that of group A.

The results of experiment A6-1.1 confirm that animal performance may respond strongly to small differences in protein supply (see also results of sub-task A1: paragraphs 3.1 and 4.1) and this should be taken into account in attempts to improve N efficiency of dairy cows.

4.6 Sub-task A6-2

The aim of these experiments was to evaluate whether there is any benefit from feeding low rates of supplements with a high content of protein that escapes degradation in the rumen for high-moisture grass/clover silage (experiment A6-2.1) or a mixture of maize silage and vetch/oats silage (experiment A6-2.2) as the sole forages. Overall, the response of milk yield and quality to 1.5 kg cow^{-1} day^{-1} of a concentrate composed mainly of fish meal (treatment B), was comparable to the response to the other concentrates (treatments A and C, see paragraph 2.6), fed at a higher rate. This result was consistent in the two experiments. We had anticipated a slight improvement in silage DM intake with supplement B, which was not apparent. Total CP intake was similar in groups B and C, but intake of protein that escapes degradation in the rumen was 21% higher and NEL intake was 30% lower in group B. Still, the efficiency of N utilisation for milk production was comparable in both groups and similar to that of the only forage diet (group D). Concentrate C might have improved the capture of N in the rumen, but did not improve the efficiency of transfer of dietary N to milk N. The increase of milk protein yield obtained by the addition of 6 kg of concentrate C was similar to that obtained with only 1.5 kg of concentrate B. Since the amount of N fed and the efficiency of N utilisation were also similar, treatment B does not represent any improvement or deterioration of the N balance of the farm, but it may improve the overall energy efficiency. The rather good performance of the cows in group B was possible because they experienced a more pronounced, longer negative energy balance than the cows in group C. The effects of this on long-term performance of the cows requires further attention. Undoubtedly, the profile of absorbed amino acids was substantially different in group B compared to group C, and this might be one of the basic reasons why diet B, despite its higher CP concentration (same CP intake, but lower DM intake than diet C), maintained a high efficiency of N utilisation.

Crude protein intake in group A was 20% higher than in group B. Intakes of rumen-undegradable protein were planned to be similar in both groups but, because of a slight deviation in concentrate composition, they turned out to be 10 and 4% higher in group A in the first and second year, respectively. The efficiency of transfer of dietary N to milk N in group A tended to be lower than in the groups B and C.

Forage DM intake in experiment A6-2.2 was higher than expected. Even if the 10% intake of the added SBM is subtracted, silage intakes were considerably higher than those recorded for grass/clover silage alone. In general, an improvement of DM intake can be expected from feeding two forages and a concentrate (SBM) as a mixture rather than as separate feeds. Crude protein content of the forage/SBM-mixture was rather low (11.5%), but the high DM intake resulted in CP intakes comparable to those obtained with grass/clover silage (13.5% CP). Despite the low CP content, intake of rumen-undegradable protein from the forage mixture was acceptable and comparable to intakes obtained from grass/clover silage, because of the lower rumen-degradability of maize silage and SBM protein. Still, the projected intakes of energy, CP and rumen-undegradable protein were achieved.

The mixture of the silages and SBM in experiment A6-2.2 supported a daily milk yield of almost 20 kg cow^{-1}, more than 5 kg cow^{-1} higher than the grass/clover silage in experiment A6-2.1, possibly because of the utilisation of a mixed forage and the addition of good quality protein. Crude protein intake by the only forage group in experiment A6-2.2 was the same as in experiment A6-2.1, but energy intake was 3.7 Mcal cow^{-1} day^{-1} higher, enough to account for the milk yield differences. The extra energy came from the protein supplement, 1.3-1.5 kg SBM cow^{-1} day^{-1}, but also from the higher forage intake, which probably was caused by the protein supplement.

4.7 Sub-task B1

See paragraph 3.7.

Conclusions

Yield of crops in the protein rotation was lower, but more stable than in the energy rotation. Energy concentration in harvested DM was slightly higher in the protein rotation, while protein concentration was considerably higher. Nitrate leaching from crops in the energy rotation was slightly higher than in the protein rotation. Nitrate leaching was 30 to 35 kg N ha^{-1} $year^{-1}$ lower than the Danish average, likely as a result of reduced N application rates, better timing of N applications during the year and ploughing of the fields predominantly in Spring.

4.8 Sub-task B3

Chemical composition of feedstuffs

Concentrations of crude protein, DVE and OEB in grass silage were lower in experiment B3-2 than in experiment B3-1 (Table 3.74). These differences could be the result of cold weather in the Spring of 1994 leading to a lower supply of N to the plant. The NE_L and dOM values of fodder beet were relatively high. This may be attributed to removal of soil contamination by washing the fodder beet prior to feeding which resulted in low concentrations of crude ash and sand. DM and starch contents, as well as dOM and NE_L values of GMES were higher in experiment B3-2 than in experiment B3-1, probably because of more favourable climatic conditions during growth and maturation. DM and starch content and nutritive value are higher when maize crops are harvested at a later stage of maturity (Deinum & Knoppers, 1979).

Feed intake

In both experiments, TDMI was not affected by treatment (Table 3.75) and ranged between 3.3 and 3.5 per cent of live weight per day which is in agreement with the standards of CVB (1995). In experiment B3-1, there were no significant differences in the means of silage intake among treatments. Although in experiment B3-2 silage intake was restricted in LRLP and LRHP, TDMI was less than allowed. This suggests that the feeding strategy could be defined as *ad libitum*.

Variations in carbohydrate composition of concentrates are often associated with variations in intake of either forage or total DM. However, the direction of these variations in forage intake and TDMI is variable. De Visser *et al.* (1990) found no effect of the source of carbohydrates in concentrate supplements on TDMI. However, Phipps *et al.* (1987), Sutton *et al.* (1987; 1993) and Aston *et al.* (1994) showed that forage intake was reduced when starch replaced fibre in the concentrates. TDMI of mixed rations was reduced when beet pulp was replaced by corn (Mansfield *et al.*, 1994) and when a high-fibre concentrate (by-products) was replaced by a concentrate based on corn and soybean meal (Weiss, 1995). In contrast, Batajoo & Shaver (1994) found a lower DM intake when rations were fed with less than 30 per cent non-fibre carbohydrates. Mayne & Gordon (1984) reported lower silage intakes with beet pulp-based concentrates than with barley-based concentrates. Fitzgerald (1990) observed no effect on intake of silage and total DM when fodder beet was used to replace concentrates. In contrast, Meijer *et al.* (1994) found a reduced silage intake with fodder beet as concentrate replacer. Including fodder beet in rations for dairy cows decreased silage intake but improved TDMI (Roberts, 1987; Sabri & Roberts, 1988; Fisher *et al.*, 1994).

The conflicting effects of type of contentrate on TDMI may be explained by differences in concentration, degradability and fermentation characteristics of structural and non-structural carbohydrates in the ration. De Visser & De Groot (1980) showed that feeding rations containing large amounts of starch and sugar (>420 g kg^{-1} DM) resulted in lower rumen pH levels and higher concentrations of propionic acid and lactic acid in the rumen fluid, resulting in lower feed intake. Moreover, the rate of *in situ* degradation of structural and non-structural carbohydrates in cereal grains and by-products varies widely (Tamminga *et al.*, 1990), whereas the degradability of carbohydrates influences the fermentation pattern, due to changes in cellulytic and amylolytic activity of the rumen microbes (De Visser, 1993). So, a rough classification of feeds in 'starchy' or 'fibrous' according to the main carbohydrate source in the feed is an over-simplification.

TDMI in LRLP and LRHP was less than allowed (Table 3.75 and paragraph 3.8). The low OEB value of these rations may have had a negative effect on TDMI. A negative value of OEB implies a shortage of N in the rumen, which may impair microbial growth and, consequently, result in a reduced ruminal digestion (Tamminga *et al.*, 1994).

Diet composition

Replacing purchased concentrates or grass silage by home-grown concentrates (GMES, fodder beet) affected the concentration of structural and non-structural carbohydrates in the ration. Sugar content was increased and crude fibre, NDF and ADF contents reduced when beet pulp was replaced by fodder beet (Tables 3.74 and 3.76). Substitution of GMES for beet pulp or grass silage resulted in an increased concentration of starch and reduced concentrations of sugar, crude fibre, NDF and ADF in the ration. Increasing the amount of SMSE in the concentrate portion to obtain equal levels of DVE intake (experiment B3-1 and paragraph 3.8) resulted in higher concentrations of crude protein and OEB in the ration (Table 3.76). Substitution of GMES for grass silage resulted in a lower OEB value of the ration (experiment B3-2).

Milk yield and milk composition

In experiment B3-1, yields of milk, FPCM and lactose and concentrations of protein and lactose were significantly affected by the type of concentrate or concentrate replacer, but fat and protein yields were not influenced (Table 3.77). Previous work on modifying carbohydrate composition of concentrates yielded conflicting results. Replacing fibre by starch in the concentrate portion of the ration by substitution of GMES for beet pulp (treatment MS compared to BS, experiment B3-1) resulted in higher yields of milk and lactose. Sutton *et al.* (1987; 1993) also reported a higher milk yield when starch replaced fibre in the concentrates of low-forage diets containing equal amounts of energy and crude protein. However, the source of carbohydrate had little effect on milk yield at concentrate intakes of up to about 10 kg day^{-1} (Sutton *et al.,* 1987). In various experiments, no effect on milk yield was found of replacing fibrous concentrates by starchy concentrates (Mayne & Gordon, 1984; Phipps *et al.*, 1987; De Visser *et al.*, 1990; Rühle *et al.*, 1992; Aston *et al.*, 1994; Mansfield *et al.*, 1994; Friggens *et al.*, 1995; Weiss, 1995). Gordon *et al.* (1995) found a higher milk yield with a high-fibre concentrate than with a high-starch concentrate. De Visser & De Groot (1980) also found reduced milk yields at higher sugar plus starch contents in the ration. However, the results of the latter experiment were probably associated with a disturbed rumen fermentation due to a too high sugar plus starch concentration in the ration.

The higher milk and lactose yield in MS (experiment B3-1) possibly resulted from an increased supply of glucose. There are indications that feeding slowly degradable starch (e.g. maize starch), that is digested in the rumen and the small intestine, stimulates the production of glucogenic precursors, which may result in a higher lactose yield (Nocek & Tamminga, 1991). Lactose is the most important osmotic constituent of milk and its secretion regulates milk volume.

Replacing fibre by sugar in the concentrate portion of the ration by substitution of fodder beet for beet pulp (treatment FB compared to BS, experiment B3-1) resulted in a lower milk yield (Table 3.77). This is consistent with other experiments where fodder beet was used as a concentrate replacer (Fitzgerald, 1990; Meijer *et al.,* 1994). However, Roberts (1987), Sabri & Roberts (1988) and Fisher *et al.* (1994) observed no significant effect on milk yield. These conflicting results may be due to the difference in function of fodder beet: as a concentrate or as a partial forage replacer (Roberts & Martindale, 1990). An energy balance trial of Schwarz *et al.* (1994) showed that when intake of sugar from either fodder beet or saccharose was increased, yields of milk, protein and lactose decreased, whereas yield of fat was unchanged. This study indicated that the partial efficiency of energy utilisation was 12 per cent lower for sugar than for non-sugar energy. This may be an explanation for the reduced milk yield in treatment FB. Milk yield in FBMS was higher than in FB, whereas the intake of fodder beet was only slightly lower. Probably, in FBMS, the effects of sugar in the ration were partly compensated by the starch from GMES.

In experiment B3-1, milk fat yield and concentration were not affected by the composition of the concentrates (Table 3.77). This is in agreement with the results of experiments of Mayne & Gordon (1984), Rühle *et al.* (1992), Aston *et al.* (1994), Friggens *et al.* (1995) and Gordon *et al.* (1995), where dietary fibre was replaced by starch and with results of Fitzgerald (1990) who replaced concentrates by fodder beet. However, replacing fibrous concentrates by starchy concentrates resulted in reduced fat yields and fat concentrations in a number of experiments (De Visser & De Groot, 1980; Phipps *et al.*, 1987; Sutton *et al.*, 1993; Batajoo & Shaver, 1994; Weiss, 1995). Mansfield *et al.* (1994) found a lower fat concentration when beet pulp was replaced by corn, but fat yield was not affected. Roberts (1987) and Fisher *et al.* (1994) reported increased milk fat yield and concentration when fodder beet was included in the ration. Sabri & Roberts (1988) observed no effect of feeding fodder beet on milk fat yield and milk fat concentration. Meijer *et al.* (1994) found higher fat concentrations, but lower fat yields with fodder beet as a concentrate replacer.

Milk protein yields were not different among treatments when concentrates were replaced by either fodder beet or GMES (Table 3.77, experiment B3-1). However, protein concentration was higher when fodder beet was included in the ration. There was no difference in protein concentration when dietary fibre was replaced by starch in the concentrate portion of the ration. This is in agreement with results of Mayne & Gordon (1984) and De Visser & De Groot (1980).

Several researchers observed higher protein yields and concentrations when dietary fibre was replaced by starch (Phipps *et al.*, 1987; De Visser *et al.*, 1990; Rühle *et al.,* 1992; Gordon *et al.*, 1995; Batajoo & Shaver, 1994; Mansfield *et al.*, 1994). Aston *et al.* (1994) found a lower protein concentration when fibre replaced starch in the concentrate, but protein yield was not affected. Roberts (1987) and Fisher *et al.* (1994) reported a higher milk protein yield and protein concentration when fodder beet was included in the ration, whereas Sabri & Roberts (1988) found a higher protein yield without a change in milk protein concentration. Meijer *et al.* (1994) observed increased protein concentrations when concentrates were replaced by fodder beet, but protein yield was lower. In contrast, Fitzgerald (1990) found no effect on either protein yield or concentration when fodder beet replaced concentrate.

In experiment B3-2, yields of milk, FPCM, milk solids and concentration of milk solids were clearly affected by the level of DVE, but not by the level of forage allowance (Table 3.77). The latter can be explained by the fact that the animals on the restricted forage regimes consumed less than allowed and both TDMI and NE_L intakes were similar for all treatments. Therefore, feeding strategy of the low-forage treatments can be considered *ad libitum.* Modifications of the level of concentrate intake often result in differences in milk yield and milk protein yield (Mayne & Gordon, 1984; Sutton *et al.*, 1987; 1993; Tessmann *et al.*, 1991; Gordon *et al.,* 1995). Such effects on milk performance are probably caused by differences in energy intake. However, Rühle *et al.* (1992) found no effect on milk yield and milk composition of reducing NE_L intake with 10 to 12 per cent by restricting forage allowance to 8.5 kg DM.

The apparent absence of an effect of concentrate level and, consequently, carbohydrate composition on milk composition may seem surprising. However, De Visser (1993) concluded that feeding slowly degradable starch (e.g. GMES) decreases the supply of energy for microbial growth, which results in reduced microbial protein synthesis. In addition, the negative OEB of the low-forage rations (LRLP and LRHP) may also have reduced microbial growth. Likely, replacing grass silage by GMES resulted in a reduced supply of microbial protein to the intestine. However, De Visser (1993) also showed that feeding slowly degradable starch increases the supply of glucose to the small intestine and, thereby, reduces the oxidation of amino acids by the gut wall. Hence, less amino acids will be used for the metabolism of the gut and, consequently, more amino acids will be available for milk protein synthesis. Therefore, when grass silage is replaced by GMES, reduced microbial protein synthesis may be compensated by improved intestinal glucose supply, resulting in similar yields of milk, protein and lactose.

In experiment B3-2, it is likely that insufficient supply of DVE in LRLP and HRLP reduced the availability of amino acids for oxidation and protein synthesis and, consequently, resulted in lower outputs of milk, milk protein and lactose (Table 3.77). The effects of DVE level on milk yield and protein yield are in agreement with results of Hof *et al.* (1994) who also found higher milk and protein yields when the level of DVE in the ration was increased at fixed levels of NE_L intake. However, it was not clear whether the differences in the latter experiment were determined by DVE intake only, since the ratio of DVE available for milk synthesis to NE_L available for milk synthesis was confounded with feeding level.

In experiment B3-2, the reduced concentrations of milk fat and protein in the treatments with a high level of DVE were associated with a higher milk yield, compared to the treatments with a low level of DVE. Sutton (1989) concluded in a review that the effects of reducing the forage to concentrate ratio in the diet on milk fat yield and concentration depend on the carbohydrate composition of the concentrates and the fraction of cell wall constituents in the ration. Maize starch is slowly degradable, and GMES consists of the complete maize cob including some leaves. Therefore, the digestion characteristics of GMES are probably more forage-like than concentrate-like, which may explain the absence of a significant effect of the forage to concentrate ratio on milk fat production in experiment B3-2.

In both experiments, the output of milk urea and urea concentration followed the differences in intake of N and OEB (Tables 3.77 and 3.76). This is in agreement with the findings of Gustafsson & Carlsson (1993) and Gonda & Lindberg (1994). Several reports confirm the positive correlation between the protein balance in the rumen (PBV or OEB) and the concentration of urea in milk (Gustafsson & Carlsson, 1993; Gonda & Lindberg, 1994; Schepers & Meijer, 1998).

When considering the results of the experiments, it is important to bear in mind the stage of lactation. Both experiments were carried out with dairy cows in early lactation (up to 110 days *post partum*). Changes in milk performance during early lactation can affect whole-lactation performance. For example, Broster (1974) calculated that when peak milk yield is increased with 1 kg day^{-1}, total lactation yield will be increased by 200 kg.

Nitrogen utilisation

In both experiments, N utilisation, calculated as (N excretion in milk/N intake) × 100, followed the differences in dietary N intake and OEB (Tables 3.77 and 3.76). Higher intake of N and higher OEB are associated with a lower utilisation of dietary N. A high OEB indicates a surplus of N in the rumen, which is converted into urea. The differences in N intake and OEB in experiment B3-1 were mainly a consequence of the inclusion of larger quantities of a high-N concentrate (SMSE) in the concentrate portion of the rations with fodder beet and GMES in order to obtain equal intakes of DVE. However, in experiment B3-2, replacing grass silage by GMES contributed to a reduced N intake and OEB which resulted in improved N utilisation.

Blood and urine parameters

In both experiments, the levels of urinary nitrogen (UN) and urinary urea nitrogen (UUN) followed the differences in intake of dietary N and OEB (Tables 3.78 and 3.76). This is in agreement with the findings of Gonda & Lindberg (1994) and Ciszuk & Gebregziabher (1994). Differences in both UN and UUN between morning and evening suggest a diurnal variation in N excretion in urine. Diurnal variation in the concentration of UUN in spot samples of urine collected throughout a 24-hours' period was observed by Gonda & Lindberg (1994) and Ciszuk & Gebregziabher (1994). These daily patterns of UUN were affected by the daily pattern of rumen ammonia level as well as by urine flow (Gonda & Lindberg, 1994). Therefore, concentrations of UN or UUN have limited applicability to assess N utilisation.

Plasma ß-HB concentration followed the differences in NE_L coverage. Except for the 4th week after calving in experiment B3-1, there were no indications of insufficient energy supply. In both experiments, the levels of ß-HB were low (<1.2 mmol l^{-1}) according to the reference values for ß-HB of the Public Health Service for Animals (GD, 1994). ß-HB levels higher than 1.2 mmol l^{-1} indicate a lack of energy in the diet.

Conclusions

Replacing purchased concentrates by home-grown concentrates in the grass silage-based diet of dairy cows in early lactation (experiment B3-1), affected the yield of milk and milk composition. These differences in milk performance were associated with variations in the carbohydrate composition of the concentrate portion of the ration. Reduced milk and lactose yields and a higher protein concentration were observed when only fodder beet replaced part of the concentrate. Replacing part of the concentrate by a mixture of fodder beet and GMES had no effect on the yields of milk and milk solids, but increased the protein concentration. Partial replacement of concentrates by GMES only, resulted in higher yields of milk, FPCM and lactose. When purchased concentrates were partially replaced by either fodder beet or GMES, N utilisation in the cow was reduced due to a higher intake of N and OEB from SMSE. The latter had been increased in the concentrate portion to obtain equal intakes of DVE.

Substitution of GMES for grass silage at equal levels of DVE and NE_L intake (experiment B3-2) did not affect milk yield and milk composition. However, replacing grass silage by GMES at equal levels of DVE intake resulted in a reduced N intake and an improved N utilisation. The level of DVE in the ration had a considerable effect on animal performance and N utilisation. Feeding a DVE level of approximately 10 per cent below the requirements according to the DVE/OEB system, resulted in lower yields of milk, FPCM, protein and lactose. Replacing grass silage by GMES at equal levels of DVE intake was most effective to improve N utilisation in dairy cows. However, whole-farm studies must be conducted to assess the potential of home-grown concentrates to reduce the losses of N and energy on dairy farms.

4.9 Sub-task B5

Silage quality in terms of ME content and CP content (Table 3.79) was similar to that reported by Bax & Thomas (1992). Although the CP content was on the higher end of the range for first cut grass/clover silages. The composition of the three supplements was typical (MAFF, 1990). Silage intakes were typical for a silage with a DM content of only 177 g kg^{-1} (Table 3.80). The combinations of silage and supplements resulted in large differences in terms of starch and WSC content of the diets (Table 3.81). However, animal performance was not significantly affected by those differences in diet composition suggesting that the supplements were equally effective in promoting animal performance (Table 3.82). Milk NPN concentrations were lower while milk urea concentrations were higher (Table 3.83) than those reported by Roseler *et al.* (1993). In this experiment the potentially large fluctuations in milk urea content reported by Gustafsson & Palm-Quist (1993) were not detected. This tends to indicate that no excessive amounts of protein were fed in the treatments and that the type of protein consumed did not tend to result in large fluctuations in blood urea. The NPN concentrations of milk were significantly lower on treatment F than on treatment B. However, total NPN output was not significantly different.

Urine-N concentrations were generally lowest in the morning, but diurnal variation was small (Table 3.84). The N concentrations of the urine were very similar to those reported by Betteridge *et al.* (1985) and seem to support the impression from the milk NPN concentration that no excessive amounts of N were consumed. Supplementation with fodder beet significantly decreased N concentration of the urine, possibly because of the rather high K content of fodder beet which enhances urine production.

Due to the fact that barley contained more N than fodder beet (Table 3.79), N content of the overall diet was highest when more barley was consumed (Table 3.81). Animal performance was best when the least barley was consumed (Table 3.82). In this experiment protein supplementation was not adjusted to take account of the increased N content of the barley, therefore the reduced N efficiency for milk production was a consequence of this (Table 3.86). The N efficiencies achieved are similar to those reported by Van Vuuren *et al.* (1993).

Conclusions

This work has shown that home-grown concentrates like fodder beet and the combination of fodder beet and barley can be effective supplements to grass/clover silage. Future dairy production systems could therefore be developed using these home-grown concentrates. The efficiency coefficients observed are very similar to values observed in current dairy production systems. The N and ME efficiency coefficients for the diet with barley (diet B) were slightly lower than for the diets with fodder beet (diet F) or barley and fodder beet (diet BF). This resulted from a slightly higher intake and a lower milk yield on treatment B. No explanation is available for this observation.

4.10 Sub-task B6

Experiment B6-1. Grazing experiment

Average net pasture yield from the swards without applied N was 11.4 t DM ha^{-1} $year^{-1}$ (Table 3.87). This is high and considering the low milk quota of many farms in this region, milk production systems with low input of fertilisers and concentrates should be promoted. White clover may play an important part in such production systems. However, an important disadvantage of white clover and clover-based swards is their poor growth in the cool springs of the maritime climate. Application of N will accelerate pasture growth and this may be an argument to apply N on mixed swards in early spring.

This grazing experiment showed that performance of January-calving cows on good pasture was much better than indoors on a ration of silage supplemented with low rates of concentrates (González *et al.*, 1996). Therefore, in the second and third year of the experiment, cows started grazing immediately after calving from early February onwards. This gave a higher milk yield in spring than in the first year. The grazing experiment confirmed the importance of good grazing management, avoiding old and stemmy herbage. Except in early spring, the stocking rate in the treatment with 240 kg N ha^{-1} $year^{-1}$ was slightly higher than in the treatment with no N applied. This caused small differences in milk yield per ha which, however, were not sufficient to justify the whole rate of N application (González, 1996).

Experiment B6-2. Farm-scale silage production

The seasonality of pasture production in Galicia requires forage conservation for about 4 months per year, 2 in summer and 2 in winter. In addition, good grazing management requires cutting for conservation of a part of the pasture in periods of rapid growth. Probably, this is the most convenient system of producing conserved forage, but it may be difficult to do this on small farms.

Silage was made at farm-scale from grass/clover swards fertilised with 50 kg N ha^{-1} to the first cut and 40 kg N ha^{-1} to the second cut. Dry matter yields of more than 8 t ha^{-1} were achieved in 2 cuts. However, the results of experiment B6-3 showed that the combination of N application in early spring and heavy cuts was rather harmful for the clover content of the sward.

Traditionally, maize is grown in Galicia in rotation with Italian ryegrass, winter cereals or turnips. This work showed that maize can also be grown in rotation with a mixture of vetch and oats. A total of 9 t DM ha^{-1} of maize silage and 4 t DM ha^{-1} of vetch/oats silage was obtained with only 50 kg N ha^{-1}. However, for sustainable forage production, it will be necessary to replace the total amount of plant nutrients withdrawn in the harvested forage and, on the long term, higher rates of N as well as P and K applications will be required. Local scientists expect an increase of the area for maize silage as well as for vetch/oats silage on dairy farms in Galicia. Maize silage is considered a good alternative for grass/clover silage. In addition, the mixture of vetch and oats seems promising to replace Italian ryegrass in the Galician dairy production systems. Therefore, the Spanish Research Institutions finance a PhD thesis on the management of this mixture focusing in particular on the sowing rates of the mixture components as a means of influencing the protein content of the silage. Some results have already been published (Castro & González, 1997).

Experiment B6-3. Grass/clover response to nitrogen and management

Nitrogen applied in February was effective to accelerate herbage growth and to increase the DM yield of the first cut (Table 3.89). The response to N at the highest N rate (120 kg ha^{-1}) was 10, 19 and 25 kg DM per kg N applied, when cutting at about 21 March (T1), 5 April (T2), and 19 April (T3), respectively. The effects of the highest N rate on the annual DM yield were 11, 16 and 14 kg DM per kg N applied for the cutting treatments T1, T2 and T3, respectively. These effects are rather small because of the negative effect of applied N on clover yield: for the highest N rate this was 15, 14 and 21 kg DM per kg N applied, respectively. This is an unexpectedly strong effect and it seems necessary to study long-term implications of N applications for clover persistance. Effects of applied N on total herbage yield as well as on yield of the clover component were linear. This questions the concept of tactical N applications to mixed swards advocated by many scientists.

Conclusions

After three years of experiments conducted under sub-task B6, we conclude that grass/white clover swards have a high potential for milk production in Galicia. Milk production was *ca.* 9000 litres ha^{-1} with only 400 kg concentrate cow^{-1} and without applied N or with low rates of N application. Average milk production in this region is around this figure, but from higher levels of concentrate. Grazing management should aim at a good match of herbage on offer and herbage requirements of the cows, cutting excessive growth for conservation. This is important to maintain good herbage quality and clover performance. A low rate of N application may be recommended to accelerate pasture production and allow early grazing in Spring. Increasing the rate of N application for an early silage cut produced a low annual DM response because of a strong negative effect on the clover component of the sward.

A rotation of maize and a mixture of vetch and oats can be an alternative for the traditional rotation of maize and cereals in the region. The mixture is rather productive and has a higher protein content than the traditional cereals. High-quality silage to supplement dairy cows during the dry summer period can be obtained from an early cut of the mixed sward. Early cutting of a mixed sward slightly reduces its annual DM yield compared to late cutting, but is less harmful to the clover component of the mixture.

4.11 Sub-task C1

The efficiency of use of N from slurry or artificial fertiliser can be expressed in two ways: (1) as the apparent efficiency of N (ANE) which is the increase of harvested DM per kg N applied in slurry or fertiliser, and (2) as the apparent recovery of N (ANR) which is the increase of the amount of N contained in the harvested herbage, expressed as a percentage or a fraction of the amount applied (Van der Meer *et al.*, 1987). The ANE or ANR values are therefore calculated from the respective differences in DM or N yield between the plots that received slurry or fertiliser N and the plots without applied N (PK control). Similarly, the efficiency of use of P from slurry or fertiliser can be expressed as the apparent efficiency of P (APE) or the apparent recovery of P (APR), and the efficiency of K use as AKE or AKR.

Table 4.1 presents the ANE and ANR values of the experimental treatments that received slurry or fertiliser N. For the calculation of these values the DM and N yields of treatment 5 ($N_0P_2K_2$) have been used as references (Tables 3.90 and 3.91). Because of the large effect of applied N on the botanical composition of the mixture, ANE and ANR values have also been calculated for the mixture components. They are presented in the Annexes C1-5 and C1-6, respectively.

Table 4.1. Apparent N efficiencies (ANE, in kg DM per kg inorganic N applied) and apparent N recoveries (ANR, in % of inorganic N applied) of the experimental treatments with applied N. Experiment C1-1.

Experimental	ANE			ANR		
treatment	1993	1994	1995	1993	1994	1995
Slurry						
1. SA	-6.6	4.5	19.2	-63	9	45
2. SAD	-10.1	15.2	29.0	-91	39	82
3. Inj OS	-0.5	11.3	27.1	-32	33	66
4. Inj	-11.0	10.0	31.6	-53	35	95
Fertiliser N						
7. $N_1P_2K_2$	10.0	19.4	19.5	-8	49	23
8. $N_2P_2K_2$	5.4	14.7	24.2	-21	26	52
9. $N_2P_3K_3$	11.9	16.2	25.1	21	35	57
10. $N_2P_2K_0$	4.9	2.2	12.2	-22	-5	36
11. $N_2P_0K_2$	6.0	7.0	16.5	-11	8	37

Table 4.1 clearly shows the negative effects of slurry on the DM and N yield of the mixture in the first year. Although we only observed damage to the sward, in particular to the white clover, on the plots with surface-applied diluted slurry, the negative ANE and ANR values of the other slurry treatments

also indicate damage to the sward on these plots. Unlike slurry, fertiliser N had only slightly negative ANR values, but positive ANE values. This is in accordance with the results of other studies (Ennik, 1982). The Annexes C1-5 and C1-6 show that the grass in the mixture absorbed (ANR values in Annex C1-6) and utilised (ANE values in Annex C1-5) slurry and fertiliser inorganic N with approximately the same efficiency. Both N sources negatively affected DM and N yields of the clover in the mixture (Annexes C1-5 and C1-6, respectively), but, in 1993 slurry had considerably larger negative effects than fertiliser N. This points to a specific negative effect of slurry on white clover in this first year. From the available information, it can not be concluded whether this effect was caused by N or other substances in the slurry. The Annexes C1-5 and C1-6 show that the specific negative effect of slurry on the clover did not appear in 1994 and 1995. This may indicate a strong susceptibility of white clover in the year of its establishment.

In 1994 and 1995, differences in effectivity between slurry and fertiliser inorganic N were rather small (Table 4.1). Lower than average ANE and ANR values apparently were caused by ammonia volatilisation on the plots with surface-applied undiluted slurry (experimental treatment 1) or by K or P shortage (treatments 10 and 11, respectively). Both ANR and ANE values were higher in 1995 that in the preceding years. This was related to a relatively strong effect of both N sources on grass DM and N yields (Annexes C1-5 and C1-6, respectively) and only a small negative effect on the clover yields, probably in consequence of the low clover content of the sward in that year (Table 3.90). ANE and ANR values in 1995 (Table 4.1) were close to those obtained with pure grass swards in other experiments (Van der Meer *et al.*, 1987; Geurink & Van der Meer, 1995).

Table 4.2 presents the APE and APR values of the experimental treatments that received slurry or fertiliser P. For the calculation of these values, the DM and P yields of treatment 11 ($N_2P_0K_2$) have been used as references. APE and APR values have also been calculated for the mixture components. These values are presented in the Annexes C1-7 and C1-8, respectively.

Table 4.2. Apparent P efficiencies (APE, in kg DM per kg P applied) and apparent P recoveries (APR, in % of P applied) of the experimental treatments with applied P. Experiment C1-1.

Experimental	APE			APR		
treatment	1993	1994	1995	1993	1994	1995
Slurry						
1. SA	-39	-6	12	-6	9	22
2. SAD	-50	38	64	-7	22	40
3. Inj OS	-20	18	38	5	20	31
4. Inj	-49	12	51	-17	12	29
Fertiliser P						
5. $N_0P_2K_2$	-15	-19	-54	-5	-1	-3
6. $N_0P_3K_3$	-11	7	-10	-3	11	9
7. $N_1P_2K_2$	-3	-7	-23	5	11	5
8. $N_2P_2K_2$	-2	20	25	4	13	23
9. $N_2P_3K_3$	10	16	19	8	12	18
10. $N_2P_2K_0$	-3	-13	-14	4	7	13

Table 4.2 shows negative APE values of the treatments that received slurry in 1993, and, in general, of the treatments without applied N (treatments 5 and 6), with the lowest rate of N application (treatment 7), or without applied K (treatment 10). This means that the negative effects of slurry application in 1993 or no N or K application on the DM yield of the mixture were stronger than the positive effects

of P application. Comparison of the APE and APR values in Table 4.2 shows, however, that the negative factors mentioned had stronger effects on DM than on P uptake in the harvested herbage. Or in other words, the positive effect of P application on P uptake did not always lead to an increase of DM yield owing to other factors that hampered growth of the mixture. Comparison of the experimental treatments 5, 7 and 8, or 6 and 9 in Table 4.2 shows the effect of N application on the efficiency of P utilisation (APE and APR), whereas comparison of the treatments 10 and 8 shows the effect of K application.

The rather strong influence on APR and APE of damage caused by slurry or slurry application and of the availability of N and K means that it is very difficult to make a good comparison of the effectivity of slurry P and fertiliser P and to draw proper conclusions from the results presented in Table 4.2. For instance, the high APR and APE values in 1994 and 1995 of slurry applied by low-emission techniques (experimental treatments 2, 3 and 4), compared to those values of fertiliser P (treatment 8) in part appear to be caused by the higher N supply on (some of) the slurry treated plots (Tables 3.91 and 4.1). However, despite this point, the effectivity of P from slurry applied by low-emission techniques at least appears as high as that of fertiliser P.

Table 4.3 presents the AKE and AKR values of the experimental treatments that received slurry or fertiliser K. For the calculation of these values the DM and K yields of treatment 10 ($N_2P_2K_0$) have been used as references. AKE and AKR values have also been calculated for the mixture components. These values are presented in the Annexes C1-9 and C1-10, respectively.

Table 4.3. Apparent K efficiencies (AKE, in kg DM per kg K applied) and apparent K recoveries (AKR, in % of K applied) of the experimental treatments with applied K. Experiment C1-1.

Experimental	AKE			AKR		
treatment	1993	1994	1995	1993	1994	1995
Slurry						
1. SA	-4.9	1.1	4.2	2	41	49
2. SAD	-6.8	6.1	11.2	-19	56	74
3. Inj OS	-2.3	4.1	8.5	10	54	68
4. Inj	-7.2	3.5	11.0	12	59	85
Fertiliser K						
5. $N_0P_2K_2$	-2.3	-0.8	-5.6	16	36	20
6. $N_0P_3K_3$	-1.6	2.1	0.0	8	40	32
7. $N_1P_2K_2$	0.0	2.7	-1.2	29	50	33
8. $N_2P_2K_2$	0.2	4.5	5.6	28	53	60
9. $N_2P_3K_3$	2.2	3.4	4.1	31	41	43
11. $N_2P_0K_2$	0.5	1.7	2.0	36	43	49

Table 4.3 shows negative AKE values of the treatments that received slurry in 1993 and in some cases also of the treatments without applied N or with the lowest rate of N application. These negative values reflect the damage to the sward caused by the slurry or the slurry application technique in the first experimental year, and the effect of N supply on the utilisation of K. Comparison of the experimental treatments 5, 7 and 8, or 6 and 9 in Table 4.3 shows the effect of N application on the efficiency of K utilisation (AKE and AKR), whereas comparison of the treatments 11 and 8 shows the effect of P application. As has been shown for P, the significant effects of damage to the sward caused by slurry application and of the supply of N and P on the efficiency of K utilisation hamper a good comparison of

the effectivity of slurry K and fertiliser K. Nevertheless, in 1994 and 1995 the AKE and AKR values of slurry applied by low-emission techniques (treatments 2, 3 and 4) appear high compared to those of fertiliser K (treatment 8). This indicates a comparable or perhaps even slightly higher effectivity of slurry K.

4.12 Sub-task C2

See paragraph 3.13.

4.13 Sub-task C3

As a consequence of the environmental policy, we expect that the utilisation of animal manure as a source of plant nutrients has to be improved, thus replacing artificial fertilisers, reducing pollution of the environment and saving fossil energy. For a correct and efficient exploitation of slurry as a source of N, it is necessary to know the conditions that induce the maximum availability of slurry N to crops. The comparison of different periods, methods and rates of slurry application on maize was made in experiment C3-1 to obtain information regarding the efficiency of utilisation of slurry N by this crop.

Slurry-fertilised maize yielded, on average, less than maize with N from artificial fertiliser, but there were important differences between slurry treatments. Both spring application and immediate incorporation positively affected maize DM and N yields. Averaged over three years, DM yield on slurry-treated plots was 20% lower than on plots with artificial fertiliser, but considering plots on which slurry was applied in spring and incorporated immediately, the difference was only 11% (Table 3.106).

Nearly the same situation was observed for N yield: N yield on slurry-treated plots was 25% lower than on plots with artificial fertiliser but the difference between artificial fertiliser and ‘spring incorporated’ slurry was only 16% (Table 3.108).

The efficiency of utilisation of N from slurry or mineral fertiliser can be expressed as the apparent recovery of N (ANR). This is the increase of the amount of N contained in the harvested crop, expressed as a percentage of the amount of inorganic or total N applied. Analysis of the N yields in Table 3.109 showed for the main treatments linear relationships between the rate of N application and the N yield in the harvested maize. Consequently, average ANR values can be calculated for each main treatment by linear regression of the N yield in the harvested maize on the rate of inorganic N applied. The calculated values are presented in Table 4.4.

Table 4.4. Apparent recoveries of slurry and fertiliser N (ANR) by silage maize on the main experimental treatments.

Main treatment	ANR (% of inorganic N applied) in:			
	1993	1994	1995	Mean
Winter-applied slurry, incorporated	34.5	8.1	10.2	17.6
Winter-applied slurry, not incorporated	32.7	9.0	6.2	16.0
Spring-applied slurry, incorporated	43.2	10.8	11.5	21.8
Spring-applied slurry, not incorporated	39.6	19.6	0.0	19.7
Spring-applied fertiliser N	12.9	58.4[1)]	22.0	31.1
Slurry + fertiliser N	37.1	59.6	32.9	43.2

[1)] *80.3, if the highest rate of N application, which did not further increase N yield, is excluded.*

Most of the ANR values presented in Table 4.4 are very low, indicating poor utilisation of applied N. On average, the combination of slurry and fertiliser N (95 and 145 kg inorganic N ha^{-1}, respectively) gave the best, and slurry only the worst results. The best results with slurry were obtained in 1993 and with mineral fertiliser in 1994.

The effects of the period and method of slurry application on ANR were very small and not consistent over the years. (Table 4.4). This observation is not in agreement with the significant effects of the period and method of slurry application on N yield, shown in Table 3.108. However, it should be taken into account that the results in Table 3.108 also include the supposed effect of the period of soil tillage on N yield, whereas Table 4.4 presents the pure effect of applied slurry.

The very low ANR values for all the slurry only treatments in 1994 and 1995 indicate large losses of N from these treatments. And the fact that even spring-applied slurry hardly affected the amount of inorganic N in the soil before maize sowing (Table 3.110) indicates that on these plots these losses almost entirely took place in a period of less than 2 weeks. This points to losses by ammonia volatilisation. Measurements of ammonia volatilisation after surface spreading of different types of slurry to grassland in The Netherlands, indeed have shown that 70 to 100% of the amount of inorganic N applied may be lost within 7 to 10 days (Vertregt & Selis, 1990; Selis & Vertregt, 1991). Moreover, 45 to 85% of the total ammonia emission was measured during the first day after application, the actual amount depending on the ambient and soil conditions. Apparently, in these Italian experiments, incorporation of slurry was not effective to reduce ammonia losses, possibly because of a too long period between slurry application and incorporation or insufficient incorporation because of a poor soil structure (heavy soil, high rainfall). In addition, the high pH of the soil also may have contributed to large ammonia losses.

In most cases, the harvested maize contained less than 1.1% N in the DM (compare Tables 3.107 and 3.109). This indicates N deficiency, because with a sufficient N supply silage maize contains about 1.3% N in the DM (Schröder, 1998).

4.14 Sub-task C4

Differences in Italian ryegrass yields among the 3 experimental years were small (Tables 3.113 and 3.115) and did not reflect the differences in the amount of inorganic N in the soil after harvesting the maize (Table 3.118). From the information presented, it is evident that the relatively high amounts of residual inorganic N in the autumn of 1993 were not utilised by the Italian ryegrass in the following winter. Despite some N uptake by the ryegrass and nitrate leaching losses, the amounts of soil inorganic N even increased during the winter. This was not expected and is difficult to explain. The DM and N yields of the Italian ryegrass, in all cases, indicate a low availability of N to the crop. Generally, the harvested herbage contained about 1% N in the DM, whereas a ryegrass crop with an ample N supply may contain more than 2.5% N.

For most of the main treatments, there was a significant positive effect of the rate of N applied to the maize on the N yield of the Italian ryegrass. Table 4.5 presents the apparent recoveries by the ryegrass of N applied to the preceding maize crop. This is calculated by linear regression of the N yield on the rate of inorganic N applied.

Table 4.5. *Apparent recovery (%) by the winter crop Italian ryegrass of slurry and fertiliser inorganic N applied to the preceding maize crop. Experiment C4-1.*

Main treatment to maize	ANR by Italian ryegrass			
	1993	1994	1995	Mean
Winter-applied slurry, incorporated	1.7	1.8	3.1	2.2
Winter-aplied slurry, not incorporated	1.0	2.5	2.3	1.9
Spring-applied slurry, incorporated	5.5	6.0	2.5	4.6
Spring-applied slurry, not incorporated	4.8	5.0	2.7	4.2
Spring-applied fertiliser N	-1.0	5.8	2.0	2.3
Slurry + fertiliser N	3.8	5.2	0.3	3.0

Table 4.5 shows that the residual effect on Italian ryegrass of slurry or fertiliser N application to the preceding maize crop, never exceeded 6% of the rate of inorganic N applied. On average, spring-applied slurry had a slightly higher residual N effect than winter-applied slurry. Slurry incorporation had a negligible effect on the residual N effect measured in Italian ryegrass.

The effects of the period, method and rate of slurry or fertiliser N application to maize on the total N yield of maize and the winter crop Italian ryegrass are presented in Annex C4-1.

5. Evaluation and Integration of Results (Task D)

This project comprised the following tasks:

A. Evaluation of rations and feeding strategies to improve the utilisation of nitrogen in the feed of the dairy herd in order to minimise the production of urinary nitrogen **(Improve the utilisation of dietary nitrogen)**.

B. Comparison of crops, cropping systems and feeding systems to increase the proportion of home-grown feed protein, phosphorus and energy in dairy nutrition, thus reducing the consumption of purchased feeds and the related input of plant nutrients and indirect use of fossil energy **(Increase the proportion of home-grown feeds)**.

C. Quantification of the effectiveness of different slurry utilisation systems on low-input dairy farms and, in particular, on grass/clover swards and maize **(Improve the utilisation of slurry nutrients)**.

D. Evaluation and integration of the results of the experimental work to contribute to the development of dairy farming systems at the project sites with more efficient utilisation of nitrogen, phosphorus and fossil energy **(Evaluation and integration)**.

This Chapter evaluates and integrates the results of the experimental work carried out under the Tasks A (5.1), B (5.2) and C (5.3).

5.1 Improve the utilisation of dietary nitrogen

5.1.1 Minimum content of dietary nitrogen

Better utilisation of dietary N means reducing or avoiding the protein surplus in the diet of the dairy herd. A surplus of dietary protein is common on intensively managed grassland farms. Van der Meer (1991) reported average crude protein (CP) contents of the grazed herbage on the Dutch Nitrogen Pilot Farms in the 1980s of 275 g kg^{-1} DM. These farms applied, on average, between 400 and 450 kg N ha^{-1} year^{-1}. Despite a 10-20% reduction in the rate of N application in the 1990s, average CP content of a large number of samples of pasture grass between 1995 and 1999 was still 250 g kg^{-1} DM, whereas average CP content of grass silage was 205 g kg^{-1} DM (Laboratory for Soil and Crop Testing, Oosterbeek, The Netherlands, unpublished information). Hence, if heavily fertilised grass is the main component of the diet of the dairy herd, retention of dietary N in milk and liveweight gain generally is about 16% and large amounts of N are excreted in faeces and urine. In combination with high N application rates and common practices of manure storage and application, this leads to considerable losses of N by volatilisation of ammonia, denitrification and nitrate leaching. From an environmental point of view, a reduction in the rate of N application on grassland should have priority. This will reduce direct N losses as well as indirect N losses related to the surplus of dietary protein. The mineral accounting system MINAS enforces farmers in The Netherlands to reduce the rate of N application to grassland (Van der Meer, 2001). An additional measure to reduce the protein surplus is supplementation of protein-rich fresh or conserved herbage with high-energy and low-protein forages or concentrates like maize silage, ground maize ear silage (GMES), beet pulp, fodder beet and barley. Both measures were studied in this project.

Under Tasks A and B, several feeding experiments were carried out. For these experiments, grass, grass/clover mixtures and forage legumes were grown with moderate rates of N application or no N application at all. Table 5.1 summarises the CP contents of the forages produced. In general, there was a remarkable variation in CP content of the different forages produced, and in several cases, CP content was unexpectedly low, particularly in 1994.

Table 5.1. Rates of N application and CP contents of grass, grass/clover and forage legumes used in the feeding experiments of this project.

Crop/product	Year/month	N rate [1] (kg ha^{-1})	CP content (g kg^{-1} DM)	Sub-task and Country
Grass, fresh	1993	80, 60, 40, 40, 40	183	A3, The Netherlands
Grass, fresh	1994	80, 60, 40, 40, 40	135	A3, The Netherlands
Grass, silage	May 1993	80	189	B3, The Netherlands
Grass, silage	May 1994	80	140	B3, The Netherlands
Grass/clover, fresh	May 1993	-	153	A4, Scotland
	June 1993	-	181	A4, Scotland
	July 1993	-	197	A4, Scotland
Grass/clover, silage	May 1993	slurry, 50 t/ha	145	B5, Scotland
Grass/clover, fresh	May 1994	-	126	A5, Scotland
	July 1994	-	155	A5, Scotland
Grass/clover, silage	May 1994	slurry, 50 t/ha	160	A6-1, Scotland
	July 1994	-	144	A6-1, Scotland
Grass/clover silage	1993	50, 40	135	A6-2, Spain
Vetch/oats, silage	1994	-	97	A6-2, Spain
Grass/clover, silage	1993	slurry, 25 t/ha	130	A1, Denmark
Grass/clover, silage	1994	slurry, 24 t/ha	133	A1, Denmark
Pea, silage	1993	-	156	A1, Denmark
Pea, silage	1994	-	176	A1, Denmark

1) *N rates to the first, second and following cuts, respectively*

From the results of the feeding experiments in Denmark (sub-task A1), the scientists involved concluded that with balanced diets, containing the recommended minimum levels of both AAT and PBV, maximum milk yield may be obtained with about 160 g CP kg^{-1} DM. However, Figure 3.1 and the related Tables 3.4, 3.5, 3.6 and 3.7 rather seem to support the conclusion that a minimum CP content of about 170 g kg^{-1} DM is required for productive dairy cows in early lactation. For dairy cows in late lactation, the minimum CP content for maximum production of milk protein was *ca.* 130-140 g kg^{-1} DM (Tables 3.8 and 3.9). In addition, these experiments showed a rather strong negative effect of a sub-optimum protein supply on milk protein yield, *viz.* on average, a reduction in milk N yield of 22 g per 100 g reduction in N intake below the optimum (Figure 3.1). This means that it is important to supply sufficient protein as well as to avoid a protein surplus. However, this is difficult under farming conditions, because both CP content and protein degradability of the forages are variable and no simple methods are available to determine these on the farm. Therefore, CP contents of the diets under farming conditions should have a slight margin of safety and should be slightly higher than the minimum CP contents observed in feeding experiments. As a consequence, the Danish scientists concluded that there are no real possibilities to reduce the CP content of winter diets of Danish dairy cows below the present level of 170 to 180 g kg^{-1} DM without risk of a significant reduction in milk and milk protein yield. A considerable part of the CP contents presented in Table 5.1 is lower than 170 g kg^{-1} DM and, consequently, these forages had a protein shortage rather than a protein surplus for productive dairy cows in early lactation. In fact, we only expected too low CP contents in the first growth of mixed swards in regions with a cool spring like Scotland (sub-tasks A4 and A5). The results presented in Table 5.1 indicate that it is difficult to provide sharply balanced protein levels to cows fed fresh grass or grass/clover.

In conclusion, the variability of the protein value of fresh grass and grass/clover and the need to supply the dairy herd with well balanced diets, require a quick and simple method to determine protein content and degradability of available herbage.

5.1.2 Urea content of the milk as an indicator of the protein supply of dairy cows

The results obtained in this project confirm that the urea content of the milk may be used as an indicator of the protein supply of the dairy herd. According to Dutch experience, the optimum urea content of milk is 20-30 mg per 100 ml (Bruins & Beltman, 1997; Teenstra & Boxem, 1998; Van Duinkerken *et al.*, 2000). Concentrations <15-20 mg per 100 ml indicate a shortage of protein in the rumen and concentrations >30 mg per 100 ml a protein surplus. These figures are averages for the whole dairy herd. Table 3.16 shows a strong effect of the protein content of the diet on the concentration of urea in the milk, both for cows in early and in late lactation. Comparison of Table 3.16 and Figure 3.1 shows that for productive cows in early lactation urea contents under *ca.* 20-25 mg per 100 ml indicate a too low protein supply to the cow. Comparison of the Tables 3.16, 3.8 and 3.9 suggests that the critical milk urea content for cows in late lactation is lower, possibly *ca.* 10-15 mg per 100 ml. These results support the threshold value at herd level of 15-20 mg urea per 100 ml milk, used in Dutch advisory work.

Application of the critical milk urea contents mentioned above indicates that the following experiments and experimental treatments possibly had a sub-optimal protein supply to the cows:

- Experiment A3-2 (sub-task A3), in particular the treatments M25, M50 and M75 (Table 3.20). Inadequate protein supply possibly caused the relatively low milk protein contents and yields in this experiment. Differences in milk urea content were associated with differences in OEB intake, but not with milk protein yield (Tables 3.18 and 3.20), suggesting other effects than dietary protein content on milk urea content and/or milk protein yield.
- Experiment B3-2 (sub-task B3), in particular the treatments HRLP and LRLP, but also HRHP and LRHP if a critical milk urea content of 20-25 mg per 100 ml is considered for productive cows in early lactation (Table 3.77). In this experiment, differences in milk urea content were associated with differences in milk protein yield.
- Possibly also experiment A5-1 (sub-task A5) (Tables 3.38 and 3.41). In this experiment supplementation of low-protein herbage with a urea containing concentrate (concentrate B) did not affect milk protein yield nor milk urea content. This questions the effectiveness of urea as a supplement to low-protein herbage.
- In experiment A6-1.1 (sub-task A6-1), a concentrate with soybean protein (concentrate B) increased both milk urea content and milk protein yield of cows on a diet of grass/clover silage, barley and fodder beet, whereas a urea based concentrate (concentrate C) increased milk urea content but did not affect milk protein yield (Tables 3.58 and 3.61). This again points to ineffectivity of supplementation with urea.
- In experiment A5-2 (sub-task A5), supplementation of herbage with a moderate CP content with a concentrate with a moderate CP content strongly reduced the milk urea content and increased milk protein yield (Tables 3.47 and 3.50). This suggests a too low energy supply to the cows on the control diet (diet A) and possibly a too low protein supply to the cows in the group with the highest level of supplementation (diet C).

The too low urea concentrations in the milk, observed in this project, were all obtained when cows were fed diets based on forages with lower than expected CP contents (Table 5.1). The only experiment in this project with (slightly) too high levels of milk urea was experiment B3-1, conducted under subtask B3 (Table 3.77).

5.1.3 Utilisation of dietary nitrogen and nitrogen excretion in faeces and urine

Excreta of dairy cows contain large amounts of N, because only a relatively small part of dietary N is retained in the products (milk, calf and liveweight gain). On many commercial farms, N efficiency of the lactating cows (N in products/N intake) varies between 20 and 25%. This means that for a cow of 600 kg live weight and an annual milk yield of 7000 kg and a corresponding feed intake of approximately 5800 kg DM cow^{-1} year^{-1} and an N retention in products of 39 kg cow^{-1} year^{-1} (Ketelaars & Van der Meer, 1999a and b), N intake varies between 195 and 156 kg cow^{-1} year^{-1} and N excretion in faeces and urine between 156 and 117 kg cow^{-1} year^{-1}, respectively. The corresponding average N contents of the diets are 33.6 and 26.9 g kg^{-1} DM (210 and 168 g CP kg^{-1} DM), respectively.

In the feeding experiments conducted in this project, N efficiency of the cows ranged between 24 and 38%. Values of more than 30%, however, were often associated with milk urea contents of less than 15-20 mg per 100 g, indicating protein deficiency in the rumen and, probably, a reduced yield of milk protein. This is clearly shown in the Danish feeding experiments with productive cows in early lactation (sub-task A1). The results of these experiments also indicate that the use of N efficiency values may be misleading, because the experimental group of cows with the highest output of milk N had the lowest N efficiency, *viz.* 29.3%, whereas all other groups had higher N efficiency values but lower yields of milk N (Figure 3.1 and Tables 3.10 and 3.11). In addition, differences in N efficiency were relatively small, because reductions in N intake were associated with reductions in N yield, *viz.*, on average, 22 g N yield per 100 g N intake, a value not much smaller than the N efficiency of the most productive group.

Several publications state that excretion of N in the faeces of both cattle and sheep is approximately 8 g kg^{-1} DM consumed (Barrow & Lambourne, 1962; Lantinga *et al.*, 1987; Whitehead, 1995). Although some studies showed a slight increase of excretion of faecal N with increasing N concentration in the diet (Blaxter *et al.*, 1971; Kemp *et al.*, 1979), it is generally assumed that excretion of faecal N per unit of DM consumed is fairly constant, and that changes in dietary N concentration are mainly reflected in the amount of N excreted in urine (Whitehead, 1995; Monteny & Erisman, 1998; Sommer & Hutchings, 2001). From the results obtained under sub-task A1 of this project, it can be calculated that N excretion in faeces averaged 9.6 g kg^{-1} DM consumed (Tables 3.10 and 3.11). This value was rather variable (range: 8.2-10.9) and tended to be lower for cows on diets with a low N content. This was also observed in N balance experiments with lactating dairy cows in The Netherlands (J.J.M.H. Ketelaars, Plant Research International, unpublished results). In these studies, diets containing between 20 and 36 g N kg^{-1} DM were fed and slurry was collected and sampled weekly. The low-N diets were based on maize silage, the high-N diets on grass silage and an intermediate group on both maize and grass silage. This reflects the situation on commercial farms where fresh and conserved grass often are partly replaced by maize silage to reduce the N content of the diet. The slurry collected contained between 7.4 and 11.4 g organic N kg^{-1} DM consumed, and there was a significant positive effect of dietary N content on this figure. As a consequence, N content of the diet had a surprisingly small effect on the proportion of inorganic N in the slurry. This averaged 45% of total slurry N for the diets based on maize silage (containing on average 22 g N kg^{-1} DM) and 52% for the diets based on grass silage (containing on average 35 g N kg^{-1} DM). These results show that the effect of dietary N content on excretion of N in urine and, hence, on N losses might be smaller than often is assumed.

Published information on the proportion of excreted N in urine varies from about 45% for N-poor diets to 80% for diets with approximately 40 g N kg^{-1} DM (Kemp *et al.*, 1979; Van der Meer, 1982; Van Vuuren & Meijs, 1987; Whitehead, 1970 and 1995). Most of the figures in these publications are from studies with grass as the main diet component, fertilised with varying rates of N to obtain different N contents. In the feeding experiments conducted under sub-task A1 of this project, the proportion of excreted N in urine varied from 50% in the experimental diet with the highest CP content (98 g AAT FU^{-1} in experiment A1-4), to about 25% in the treatments with the lowest CP contents (- 660 g PBV cow^{-1} day^{-1} in experiment A1-5) (Tables 3.10 and 3.11). The milk urea contents observed in these experiments (Table 3.16), however, indicate that a proportion of excreted N in urine of less than 40-45% was associated with protein deficiency in the rumen and a reduced yield of milk protein. These experiments showed clearly that the possibilities to improve N efficiency of dairy cows and to reduce excretion of urinary N without negative effects on animal performance, are limited. Attempts to further reduce excretion of urinary N should only be considered if this reduction leads to a considerable reduction of N losses.

5.1.4 Composition of faeces and urine of dairy cows

Relatively little information has been published on the composition of faeces of dairy cows fed different diets. Kolenbrander & De la Lande Cremer (1967) reported a DM content of the faeces of housed dairy cows of 145 g kg^{-1} fresh faeces and an N content of 27.5 g kg^{-1} DM. For grazing dairy cows these figures were 105 g kg^{-1} and 33.3 g kg^{-1} DM. According to Whitehead (1995), the DM content of the faeces of dairy cows varies between 80 and 160 g kg^{-1} and the N content between 15 and 40 g kg^{-1} DM. This author did not provide information on the causes of these differences. In the present project, faecal N contents of 36.4-38.4 g kg^{-1} DM were reported (Table 3.85). According to Whitehead (1995), about 3% of faecal N is inorganic N; other figures reported are 1-4% (Oenema *et al.*, 2000), and 7.5% (Van der Meer, 1991). Possibly triggered by biological farming, there is increasing interest in the composition of faeces and its effects on soil organisms, and, as a consequence, on soil quality and nutrient cycling and losses. Systematic research on this topic is required.

The N content of urine may vary widely. It depends on the excretion of urinary N and the volume of urine produced. Excretion of urinary N is related to the surplus of degradable protein in the diet (Van Vuuren *et al.*, 1993), whereas urine volume depends on the intake of N, K and Na (Van Vuuren & Smits, 1997). Groenwold & Keuning (1988) extensively studied N content of dairy cow urine and found between 2.3 and 17.3 g N $litre^{-1}$ (mean: 9.7 g l^{-1}) in the urine of cows grazing heavily fertilised grass on the experimental farm 'De Olde Weije'. In their study they observed large differences in urinary N content between cows, between days and between periods of the day (Lantinga *et al.*, 1987). In another study, the rate of N application (0, 150, 600 and 1000 kg ha^{-1} $year^{-1}$) and the corresponding N contents of the herbage (27.9, 28.0, 48.6 and 47.0 g kg^{-1} DM, respectively) did not have a clear effect on the mean N content of the urine of grazing dairy cows. These N contents were 7.8, 6.5, 9.0 and 7.2 g $litre^{-1}$, respectively (Groenwold & Heringa, 1981; Van der Meer, 1991). Apparently, the cows with higher N intakes produced more urine and/or, possibly, more faecal N. According to Whitehead (1995), the N concentration in cattle urine varies usually between 2 and 20 g $litre^{-1}$, and averages 8-10 g $litre^{-1}$.

Urea is the main nitrogenous compound of urine. Groenwold & Keuning (1988) found a good linear relation between the contents of urea-N and total N in urine ($r = 0.97$); on average, urea-N accounted for 65% of total N. In the study of Groenwold & Heringa (1981), urea-N accounted for 55-93% of the total urinary N content; the highest values were obtained on the fields with the highest N contents in the herbage (Van der Meer, 1991). According to Whitehead (1995), the proportion of urea-N in total urinary N generally varies between 60 and 90%, but much lower values were reported for sheep fed diets with extremely low protein contents. Allantoin, hippuric acid and creatinine are other important urinary N compounds (Whitehead, 1995).

In this project, urine has been sampled for determination of total N and urea-N in the experiments conducted under the sub-tasks A3, A4, A5, A6-1, B3 and B5. The results are presented in the Tables 3.21, 3.33, 3.42, 3.51, 3.60, 3.78 and 3.84. Mean total N content of the urine of the experimental groups generally was between 4.5 and 8.0 g $litre^{-1}$, *i.e.* considerably lower than the mean values reported by Groenwold & Heringa (1981), Groenwold & Keuning (1988) and Whitehead (1995). Diets with very low N contents, particularly in experiment A3-2 (sub-task A3), experiment A5-1 (sub-task A5), and the low-protein diets in experiment B3-2 (sub-task B3), generally gave mean N contents of the urine of less than 6.0 g litre^{-1} (Tables 3.21, 3.42 and 3.78, respectively). In addition, the proportion of urea-N in total urinary N in these treatments was lower than 60% (range: 41-60%), whereas in the experimental treatments with diets with higher N contents, all values were higher than 60% (range: 60-87%). These results suggest that the proportion of urea-N in total urinary N also might be used as an indicator of the protein supply of dairy cows.

The Dutch feeding experiments conducted in this project, showed that substitution of ground maize ear silage (GMES) for beet pulp in a diet with fresh grass (experiment A3-1) or for beet pulp and/or fodder beet in a diet with grass silage (experiment B3-1), significantly increased the N content of the urine (Tables 3.21 and 3.78). On the other hand, partly replacement of N-poor grass or grass silage by GMES (experiments A3-2 and B3-2, respectively), only slightly increased the N content of the urine (Tables 3.21 and 3.78). The effect of GMES on the proportion of urea-N in total urinary N reflected its effect on the OEB (degradable protein balance in the rumen) content of the diet. In general, this effect was rather small for positive OEB values (experiments A3-1 and B3-1; Tables 3.18, 3.21, 3.75 and 3.78) and significant for negative OEB values (experiments A3-2 and B3-2; Tables 3.18, 3.21, 3.75 and 3.78). Monteny & Smits (1997) reported that cows on a maize silage-based diet urinated on average *ca.* 8 times per day, and cows on a grass silage-based diet with the same OEB content *ca.* 12 times. The OEB level of both types of diet (range: *ca.* 20-680 g OEB cow^{-1} day^{-1} in the maize silage group and *ca.* 200-960 g OEB cow^{-1} day^{-1} in the grass silage group) hardly affected urination frequency. As a consequence, urinary urea-N content was positively correlated with OEB level and varied between 5.5 and 8.0 g litre^{-1} for the cows on the maize silage-based diet and between 3.5 and 6.0 g litre^{-1} for the cows on the grass silage-based diet. Hence, diet components as well as level of OEB intake affected the urea-N concentration in the urine. In several publications, urea-N concentration in urine has been mentioned to have a strong effect on ammonia volatilisation from buildings and urine patches (see next paragraph).

In the Scottish feeding experiment B5-1, conducted under sub-task B5, replacement of barley as a supplement of grass/clover silage by fodder beet decreased urinary N content and urinary urea-N content, but did not affect the proportion of urea-N in total urinary N (Table 3.84). The relatively high K content of fodder beet apparently increased urine volume.

5.1.5 Nitrogen losses from faeces and urine of dairy cows

The main processes causing N losses from dairy farms are ammonia (NH_3) volatilisation, denitrification and nitrate (NO_3^-) leaching. An important part of these N losses originates from animal excreta, *viz.* from excreta voided on the floor of the buildings and the yard, from stored slurry, from field applied slurry, and from dung pats and urine patches in grazed grassland. Ammonia volatilisation and denitrification losses generally are expressed as a percentage of N excreted or N in slurry applied. Nitrate leaching losses generally are expressed in kg N ha^{-1} year^{-1}.

Ammonia volatilisation

Ammonia volatilisation has generally been reported to depend strongly on the production of urinary N and, particularly, on the production and concentration of urinary urea-N (Whitehead *et al.*, 1989; Smits *et al.*, 1995; Whitehead, 1995; Smits *et al.*, 1997; Elzing & Monteny, 1997). Very soon after urine production, urea is hydrolysed to ammonium (NH_4^+) salts (Monteny & Erisman, 1998). This process leads to a strong increase of the pH and, as a consequence, to volatilisation of NH_3. Urea hydrolysis is catalised by the enzyme urease, produced by micro-organisms in faeces, in a layer of precipitated minerals on the fouled floor of the barn (Ketelaars & Rap, 1994) and in grassland soils.

Elzing & Monteny (1997) found a linear relationship between the concentration of urinary urea and peak NH_3 emission in experiments conducted in a scale model of a dairy cow house. In this scale model a fouled slatted floor was sprinkled with urine from groups of lactating cows fed different diets. The urine contained between 3.2 and 7.3 g urea-N kg^{-1}; this was 62-83% of Kjeldahl-N. In these experiments, NH_3 emission was measured during 24 hours following urine application. It reached the highest rate *ca.* 2 hours after sprinkling the urine and decreased rather quickly afterwards. Although the regression equation shows a strong effect of the urea content on peak NH_3 emission, it doesn't exclude a contribution of other urinary and faecal constituents. In addition, the relationship between peak emission and total emission during 20 hours was not strong ($r = 0.721$). This indicates that even under the controlled conditions of this study, other factors than urinary urea concentration played a role in total emission. These possibly are the amount of urine and urea remaining on the floor, the contribution of other constituents of urine in total emission, and the contribution of emission from excreta under the slatted floor in total emission. On commercial farms, factors like (1) the fouled floor area per cow, (2) the amount and residence time of urine and faeces (and, hence, of N compounds contributing to emission) on the floor, (3) the NH_3 emission from the slurry under the floor, and (4) the environmental conditions (e.g. temperature, humidity, ventilation) will affect total NH_3 emission from the building. Given these factors, it is not sure that a reduction in the production and concentration of urinary urea will always lead to a reduction in NH_3 volatilisation from the buildings. More research is required on these aspects.

From a large number of measurements of ammonia losses from buildings and closed slurry silos on dairy farms in The Netherlands, average ammonia losses were estimated at 12.7% of excreted N (11.7% from the buildings and 1% from the slurry storage). In addition, other gaseous losses related to nitrification and denitrification were estimated at 1.8% of excreted N (Oenema *et al.*, 2000). These figures relate to farms with free-stall barns with a slatted floor, and a covered slurry silo outside the buildings. Gaseous N losses from systems with farmyard manure may amount to 30% of excreted N.

The preceding means that about 85% of N excreted in the barn is available for field application. Large NH_3 volatilisation losses occur after surface application of slurry in the field. Huijsmans (1999) reported average losses of 68% of inorganic N from surface-applied cattle or pig slurry to grassland (range: 27-98%; $n = 47$), as well as to arable land (range: 20-100%; $n = 29$). In another series of experiments with surface-applied cattle slurry on grassland (Vertregt & Selis, 1990; Selis & Vertregt, 1991), average losses by NH_3 volatilisation amounted to 63% of inorganic N applied (range: 28-101%; $n = 39$). The Dutch figures are slightly higher than figures from other countries, possibly because of the higher DM content of the Dutch slurries. Sommer *et al.* (1991) reported average losses from surface-applied cattle slurry of 56% of inorganic N applied ($n = 12$). Thompson *et al.* (1990a and 1990b) conducted 5 experiments and found average NH_3 losses of 44% of inorganic N applied. So far, it has not been possible to satisfactorily explain the large differences in NH_3 volatilisation from surface-applied animal slurries. Weather, soil and crop factors play a part, but it has been observed that conditions causing low initial losses often lead to a prolonged period of emission (Van der Meer, 1991). The preceding means that it is not well possible to select proper conditions for surface spreading of slurry. Low-emission techniques have to be employed to ensure low NH_3 losses and high N utilisation by the crop (Van der Meer *et al.*, 1987; Van der Meer, 1994; Sommer & Hutchings, 2001). From a large number of experiments on grassland and using statistical analyses, Huijsmans *et al.* (2001) estimated mean NH_3 volatilisation at 77 (range: 27-98), 20 (range: 8.5-50) and 6% (range: 1.5-25) of the total ammoniacal nitrogen (TAN) applied by surface spreading, narrow-band application and shallow injection, respectively. Sommer & Hutchings (2001) as well as Huijsmans *et al.* (2001) indicate that the TAN content of slurry has an important effect on the rate of NH_3 volatilisation.

Whitehead *et al.* (1989) studied the dynamics of NH_3 volatilisation from five nitrogenous constituents of urine applied to soil and reported the highest losses from urea, followed by allantoin and creatinine, whereas losses from creatine and hippuric acid were negligible. However, hippuric acid, when mixed with urea in solution, substantially increased NH_3 losses from urea, particularly during the first 2 days after application. In addition, Whitehead *et al.* (1989) studied NH_3 volatilisation from artificial urine. This consisted of solutions containing urea + hippuric acid, with the concentration of urea-N varying from 1 to 15 g N litre^{-1}, and the hippuric acid-N equivalent to 2.5% of urea-N. The proportion of N volatilised was similar for the urines with 5 and 10 g urea-N litre^{-1} (*viz.* slightly over 25%), and decreased at lower as well as at higher concentrations. Vertregt & Rutgers (1988) studied the fate of N in artificial urine, containing 12 g N litre^{-1}, in small-plot experiments in grassland on a sandy soil. On average, NH_3 volatilisation amounted to 13% of applied N (60 g N m^{-2}), whereas other losses in the first 10 days, probably related to nitrification (Van der Meer & Whitehead, 1990) averaged 28% of applied N. The remaining part was absorbed by the sward or present as inorganic N in the soil profile at the end of the growing season (Van der Meer, 1996). In a limited number of experiments with artificial urine containing 6 g N litre^{-1}, NH_3 losses averaged 8.5% of applied N (Van der Meer, 1991).

Information on NH_3 losses from fresh faeces is conflicting. In a study in The Netherlands, NH_3 losses from fresh (artificial) dung pats amounted to 10% of total N during the first 12 days after production, and to about 3% in the next 16 days (Vertregt & Rutgers, 1988; Van der Meer & Whitehead, 1990). This study was carried out with faeces of grazing dairy cows containing 3.92 g total N kg^{-1} (28.4 g kg^{-1} DM; a rather low N content compared to the figures reported in paragraph 5.1.4). Sugimoto & Ball (1989) measured NH_3 losses of 3-8% of total N in the faeces. The faeces applied in their experiments contained approximately 3.25 g total N kg^{-1}. Some older reports mention NH_3 losses from dung of 1-5% of total N (Floate & Torrance, 1970; MacDiarmid & Watkin, 1972). Petersen *et al.* (1998) reported insignificant NH_3 losses from faeces of grazing dairy cows containing 3.4-5.0 g total N kg^{-1}. However, their experimental design, with 1 dung pat of 0.05 m^2 in a strongly ventilated tunnel of 3 m^2, was not sufficiently accurate to estimate NH_3 losses, because these losses were too small compared to the air flow in the tunnel.

From the different studies, it can be calculated that the peak rate of NH_3 volatilisation differs largely among the different sources. Ammonia volatilisation from an artificial urine patch, containing 60 g N m^{-2}, reached its maximum rate (0.15-0.3 g N m^{-2} hour^{-1}) already during the first day after production, and decreased rapidly after the second day (Vertregt & Rutgers, 1988; Vertregt & Rutgers, 1990). Ammonia losses from an artificial dung pat, containing about 125 g N m^{-2}, were low during the first two days after production, but increased on the third day to 0.1 g N m^{-2} hour^{-1} and maintained or exceeded this rate until the seventh day (Vertregt & Rutgers, 1988). The low rate of NH_3 volatilisation during the first two days is remarkable because the faeces applied contained 10 g inorganic N m^{-2} (almost 8% of total N). Ammonia volatilisation from the floor of the barn is much higher than from faeces and urine voided in the pasture. With a typical emission of 40 g N cow^{-1} day^{-1} from 3.5 m^2 of floor, the average emission rate is 0.48 g N m^{-2} hour^{-1}. In the experiments of Elzing & Monteny (1997) with applications of urine on the floor of a scale model of a dairy cow house, the highest peak NH_3 emission observed was 1.02 g N m^{-2} hour^{-1}. Huijsmans *et al.* (2001) reported a typical peak NH_3 emission of 1.2 g N m^{-2} hour^{-1} (12 kg N ha^{-1} hour^{-1}) immediately after surface spreading of slurry to grassland. In their experiments, the average rate of slurry application was 14 m^3 ha^{-1} and average composition of cattle slurry 77 g DM and 2.15 g TAN kg^{-1}. These results show the dominant role of the slurry application technique in (reducing) total NH_3 losses from a dairy farm. Effects of emission reduction techniques in the barn and slurry storage may be lost in a very short time after field application of the slurry.

Nitrate leaching

There is a general opinion that N from animal manure strongly contributes to NO_3^- leaching. Possibly, this dates from the 1970s and 1980s when most livestock farmers disposed of slurry as cheaply as possible, avoiding damage to grassland and crops. As a consequence of the limited storage capacity for slurry and the problems involved in surface spreading on grassland during the growing season, a large proportion of animal slurries were applied throughout the winter to maize land and to the driest grassland fields. Typical apparent recoveries of slurry total N in the year of application were lower than 25% and, particularly from winter-applied slurry as low as 10% (Van der Meer, 1994). Very high NO_3^-

leaching losses were measured after high rates of slurry application to maize land (Schröder & Dilz, 1987). In addition, high NO_3^- leaching losses were observed in grazed grasslands fertilised with high rates of N, as recommended in those years (Garwood & Ryden, 1986; Van der Meer & Meeuwissen, 1989; Macduff *et al.*, 1990; Fraters *et al.*, 1998). Current regulations on manure management in some European countries cause a better distribution of animal slurries over the total area of farmland as well as applications just before or during the growing season with better equipment. As a result, apparent recoveries of slurry N improved, reaching values between 35 and 60%, depending on the method of slurry application and the crop grown (Van der Meer, 1994). Geurink & Van der Meer (1995) studied N utilisation from different types of animal slurries applied in spring or early summer to grassland by different techniques. They concluded that for injection techniques apparent recoveries of total slurry N in the year of application were equal or slightly higher than the proportion of inorganic N in total slurry N. The N not recovered in the harvested herbage appears to be rather stable organic N.

Recently, it has been shown that NO_3^- leaching from grassland depends on the total rate of N application rather than on the rate of application of manure N (Ten Berge *et al.*, 2002). This has been concluded from a comparison of the effects of N from injected or surface-spread cattle slurry and fertiliser N on grass N yield under cutting management and on NO_3^- leaching (Van der Meer *et al.*, 1987; Jarvis *et al.*, 1987; Van der Meer, 2001). This comparison showed that in each of the 5 experimental years, effective slurry N had the same effect on NO_3^- leaching as fertiliser N (effective slurry N = total slurry N multiplied by the efficiency index, *i.e.* the ratio of the apparent recoveries of total slurry N and fertiliser N). Hence, if slurry N is correctly included in the fertilisation plan of grassland, it has no specific effect originating from the N not recovered by the crop, on NO_3^- leaching. The critical rate of N application in this study for the maximum admissible NO_3^- concentration and a drainage volume of 300 mm per year, was approximately 330 kg ha^{-1} $year^{-1}$. This is common for grass swards on sandy soils and cutting management in The Netherlands (Van der Meer & Van der Putten, 1995).

It is not sure whether the preceding conclusion for grassland, *viz.* that the total rate of N application (fertiliser N + effective slurry N) and not the source of N determines NO_3^- leaching, also applies to silage maize and other annual crops. These crops have a lower N uptake capacity than grass and, consequently, critical rates of N application are smaller. In addition, the period of active N uptake is smaller and some of the easily mineralisable organic N from the slurry may become available after this period and be lost by denitrification or leaching. Probably, this is of limited importance, but should be studied.

Nitrate leaching from grazed grassland is higher, and critical rates of N application lower, than from cut swards because of the contribution of urinary N to leaching. Model calculations for exclusively grazed swards on sandy soil in The Netherlands indicate a rapidly increasing effect of urinary N on NO_3^- leaching at annual N application rates of >150 kg ha^{-1} (Van der Meer & Meeuwissen, 1989; Van der Meer & van der Putten, 1995). According to these calculations, the critical N application rate to grazed swards for drinking water quality is 170-180 kg ha^{-1} $year^{-1}$; for a combination of cutting and grazing, *i.e.* normal grassland management practice on many dairy farms, it is 200-250 kg ha^{-1} $year^{-1}$. Macduff *et al.* (1990) concluded that with average excess winter rainfall in the UK and The Netherlands, leaching losses from grazed pastures fertilised with <200-250 kg N ha^{-1} $year^{-1}$, are acceptable with respect to maintaining mean concentrations of NO_3^- in drainage below the EC limit for drinking water. If these critical rates of N application to grassland are observed, it does not seem necessary to set specific limits for manure N (applied slurry N + excreted N of grazing livestock); at higher N rates, such a limit does not make sense.

5.1.6 Effects of dietary N on N losses

Ammonia volatilisation

Published information on the effects of the N content of the diet on NH_3 volatilisation from dairy farms is scarce. It is generally assumed that a reduction in the protein surplus of the diet causes a reduction in excretion of urinary N and, possibly, also in the excretion and concentration of urea in urine (e.g. Whitehead, 1995). Based on this assumption and on the reported linear relationship between the concentration of urea-N in urine and NH_3 volatilisation (Muck & Steenhuis, 1982; Elzing & Kroodsma, 1993), many scientists advocate a reduction in the N content of the diet as an effective measure to reduce NH_3 volatilisation from dairy farms. However, it has been shown that a reduction in dietary N content may also cause a reduction in N excretion in faeces and, as a consequence, a smaller than expected reduction in excretion of urinary N (sub-task A1 in this report; J.J.M.H. Ketelaars, Plant Research International, unpublished results). In addition, the concentration of urea in urine also depends on the volume of urine produced and this is largely affected by the intake of K and Na (Van Vuuren & Smits, 1997). Often, a reduction in N intake is associated with a reduction in K and Na intake and, consequently, the effect on the concentration of urinary urea-N also will be smaller than expected. Finally, NH_3 volatilisation from a dairy farm depends on a series of complicated processes and it appears not sufficiently established that the amount of excreted N always is the most limiting factor for total NH_3 losses. More experimental work is required on this point.

Smits *et al.* (1995) studied the effect of dietary N content on NH_3 emission in a commercial cubicle house with 34 high-yielding dairy cows. In this experiment, a low-N (146 g CP kg^{-1} DM) and a high-N (198 g CP kg^{-1} DM) diet, resulting in intakes of 40 and 1060 g OEB cow^{-1} day^{-1}, respectively, were fed alternately during six 3-week periods. Salt was added to the low-N diet to obtain similar urine productions in both experimental groups. The mean concentration of urea-N in the urine of the cows on the low-N diet was 42% lower than that of the cows on the high-N diet. Absolute values were 4.8 and 8.4 g urea-N $litre^{-1}$, respectively. The low-N diet caused a 39% reduction in NH_3 emission from the building, *i.e.* from about 41 to 25 g N cow^{-1} day^{-1} in the first part of the experiment. This study confirmed the linear relationship between the concentration of urea-N in urine and NH_3 volatilisation, reported by Elzing & Kroodsma (1993). Unfortunately, it does not elucidate the separate effects of the low N content of the diet and the additional salt in the low-N diet. As a consequence, the results possibly over-estimate the effect of a reduction in dietary N content on NH_3 volatilisation in the building.

In another experiment in the same building but with a different floor, salt was added to the high-N diet to increase urine production of the cows on this diet (Smits *et al.*, 1997). This led to similar urea-N concentrations in the urine of both experimental groups, *viz.* 4.9 and 4.7 g $litre^{-1}$ in the high-N and low-N group, respectively. In this experiment, the emission reduction by the low-N diet was 20%. Addition of salt to the low-N diet caused a 30% reduction in the concentration of urinary urea-N and only a 5% reduction in NH_3 volatilisation. Hence, in this experiment emission reduction was caused by the lower total N excretion in (faeces and) urine and there was no relationship between urinary urea-N concentration and NH_3 volatilisation. Unfortunately, this inconsistency between the results of both experiments was not discussed in this paper.

The N balance experiments with lactating dairy cows in The Netherlands (J.J.M.H. Ketelaars, Plant Research International, unpublished results) did not show a clear effect of dietary N content on N losses from the barn. These losses were determined as ‘N not accounted for’ in feeding experiments with 6 cows during 1 week; they included gaseous N losses from the barn unit, N losses in faeces and urine voided during the short period in which the cows were outside the unit for milking, and changes in N content of the cows during the experimental week. Average N contents of the low-N (maize silage + concentrates), medium-N (maize and grass silage + concentrates) and high-N (grass silage + concentrates) diets were 21.7, 27.4 and 32.3 g kg^{-1} DM and mean N losses 37, 43 and 40 g cow^{-1} day^{-1}, respectively. These values are averages of 18, 18 and 12 experiments, respectively. Temperature rather than dietary N content affected N losses. Mean temperature of the barn floor during these feeding experiments was 9.1, 11.0 and 9.8 °C for the low-N, medium-N and high-N diets, respectively. Mean N losses in experiments in periods with higher temperatures (mean: 16.8 and 15.1 °C) were 53 and 62 g cow^{-1} day^{-1} for low-N and high-N diets, respectively. These values are averages of 16 and 8 experiments, and mean N contents of the diets were 23.2 and 34.5 g kg^{-1} DM, respectively. As shown in paragraph 5.1.3, a reduction in the N content of the diet by replacing grass silage by maize silage, increased the DM content of the slurry and decreased the excretion of N in faeces. As a consequence, the cows on the low-N and medium-N diets produced more urinary N than expected, which may explain the absence of a clear effect of dietary N content on N losses from the barn. The higher DM content of excreta in the low-N and medium-N groups (than in the high-N group) may have caused a slower removal of urinary N from the floor of the barn to the (closed) slurry storage. This may also partly explain the poor effect of the reduced dietary N content on N losses.

Paul *et al.* (1998) studied the effects of protein content in dairy cattle diets on NH_3 volatilisation from slurry in laboratory experiments. Diets contained 123-164 g CP kg^{-1} DM for the slurry used in a first series of experiments and 153-183 g kg^{-1} for the slurry in a second series. No information is given how the differences in CP content were obtained. A reduction in dietary N content reduced both total-N and NH_4^+-N content of the slurry. Average total-N content, estimated from the graphs presented, varied from about 4.7 to 6.3 g kg^{-1} fresh slurry in the first series of experiments and from about 6.2 to 7.6 g kg^{-1} in the second series. The average NH_4^+-N content was between 2.2 and 3.9 g kg^{-1} fresh slurry (47-62% of total-N) in the first series and between 2.6 and 3.8 g kg^{-1} (42-50% of total-N) in the second series. Total-N and NH_4^+-N contents were high compared to European conditions. Ammonia volatilisation was measured by drawing air over a 50 g sample of slurry in a 1 litre sidearm flask. Average NH_3 losses during the first 24 hours were 38 and 23% of total slurry N from the high-N and low-N diets in the first series of experiments and 22 and 15% of total slurry N from the high-N and low-N diets in the second series of experiments. NH_3 losses after 48 hours amounted to about 90% of the NH_4^+-N content of all the types of slurry, except for the slurry from the low-N diet in the first series of experiments where these losses were about 70% of the NH_4^+-N content. This study showed a significant effect of the CP content of the diet on NH_3 volatilisation from the slurry. However, NH_3 losses in these laboratory experiments with a stationary thin layer of slurry were much higher than in the barn of commercial dairy farms and it is doubtful whether these results are representative for on-farm conditions.

Paul *et al.* (1998) compared 2 of the slurries from the diets, discussed above, in a pot experiment with maize and reported that the reduction in dietary CP content had a significant negative effect on the availability of slurry N in the year of slurry application. On the contrary, Misselbrook *et al.* (1998) reported a positive effect of a reduction in dietary protein for finishing pigs on herbage N uptake from the slurry produced. They compared N losses and herbage N uptake from slurries produced by pigs fed a high-N commercial (205 g CP kg^{-1}) or a low-N experimental diet (140 g CP kg^{-1}). The reduction in dietary protein resulted in a lower slurry pH (7.2 *versus* 7.8) and in lower total-N and NH_4^+-N content (4.46 *versus* 5.56 g kg^{-1} and 2.28 *versus* 3.78 g kg^{-1}, respectively). Both slurries were surface-applied to a grass/clover sward with an estimated clover content of less than 5%. Unfortunately, no objective assessments of clover contents were made and given the experimental set up, an interaction between clover and slurry type, favouring herbage N uptake on the low-N slurry plots, cannot be ruled out. Ammonia losses from the low-N and high-N slurries were 38 and 58%, denitrification losses 5.3 and 12%, and herbage N uptake 58 and 47%, respectively, of NH_4^+-N applied. According to the authors, the observed difference in slurry pH can give a substantial effect on NH_3 volatilisation. More and particularly long-term experiments are necessary to study the effect of a reduction in dietary N content on the utilisation of slurry N by grass and forage crops.

Annual NH_3 losses from grazed swards increase strongly with the rate of N application (Jarvis *et al.*, 1989; Bussink, 1996). This can be explained to a large extent by the higher herbage yield and N content and, consequently, the higher N intake and excretion in faeces and urine. The effects of the rate of N application to grazed swards on the partition of excreted N over faeces and urine, on the composition of faeces and urine, and on the emission of NH_3 from faeces and urine are not clear (see paragraphs 5.1.3, 5.1.4 and 5.1.5). According to Bussink (1994), annual NH_3 volatilisation increases progressively with annual N excretion on the sward. However, the results presented do not exclude a linear relationship between N excretion and NH_3 emission, and it appears that the exponential relationship drawn is mainly based on the assumption of zero NH_3 emission at zero N excretion. Similarly, the results presented for the relationship between the N concentration of the diet (predominantly fresh grass) and the fraction of excreted N lost as NH_3 does not exclude a linear relationship. Again, the exponential relationship presented is rather based on an assumption than on the values observed. It should be taken into account that the sward may play an important role in these relationships because of the direct exchange of NH_3 between the sward and the atmosphere. Direct NH_3 emission or absorption by the sward depends on its N status, on soil water content and on the NH_3 content of the atmosphere (Whitehead & Lockyer, 1987; Harper *et al.*, 1996; Bussink *et al.*, 1996). As a consequence, it is possible that at medium or low rates of N application absorption of NH_3 by the sward (and the soil) approaches or equals NH_3 volatilisation from excreta of the grazing animals.

Nitrate leaching

The rate and period of N application are the dominant factors determining NO_3^- leaching from grassland and maize land (Garwood & Ryden, 1986; Van der Meer & Meeuwissen, 1989; Macduff *et al.*, 1990; Van der Meer, 1994; Schröder, 1998). The rate of N *(i.e.* fertiliser N + effective slurry N) has a direct effect on NO_3^- leaching that does not depend on the N source (paragraph 5.1.5). The rate of N application also has an indirect effect on NO_3^- leaching, because it increases the N content of the herbage and the production of faecal and urinary N, and, particularly in grazed grassland, it decreases the capacity of the sward to re-utilise this N (Van der Meer, 1982). Hence, it is evident that a reduction in the rate of N application is the most effective method to reduce NO_3^- leaching from dairy farms. Besides, replacement of a part of the N-rich herbage by high-energy and low-protein feeds is often advocated as a measure to reduce NO_3^- leaching. However, the effects of this measure on NO_3^- leaching may be positive or negative, depending on the management of the forage crop involved and the place where the excreta are produced. If maize silage or GMES is the home-grown, high-energy and low-protein supplement, it should be taken into account that NO_3^- leaching from maize land often is higher than from grassland. This has even been observed on the experimental farm 'De Marke', where special care is taken to reduce NO_3^- leaching (Aarts *et al.*, 2000; Conijn, 2000). But it is a particular problem on many commercial farms, where traditional practices of slurry disposal on maize land have not yet been replaced by proper nutrient management practices like a low rate of N application and growing of a winter crop. If a part of the grassland is replaced by a high-energy and low-protein fodder crop, grazing intensity and production of faeces and urine on the remaining grassland generally will increase. This may cause a small increase of NO_3^- leaching. On the other hand, if the dairy herd is kept inside, a reduction in dietary N content will reduce the content of total N and, possibly, of effective N in slurry. As a consequence, the farmer will increase the rate of chemical fertiliser N to apply the same total N rate to the crops as before. This will not affect NO_3^- leaching (Ten Berge *et al.*, 2002).

5.2 Increase the proportion of home-grown feeds

The objective of Task B was to conduct a 'comparison of crops, cropping systems and feeding systems to increase the proportion of home-grown feed protein, phosphorus and energy in dairy nutrition, thus reducing the consumption of purchased feeds and the related input of plant nutrients and indirect use of fossil energy'. Dairy farms with a high animal density and/or producing forages with low protein and P contents need large N and P inputs in purchased feeds and generally have a surplus of N and P in animal manure and related environmental problems. These problems can be reduced by (1) increasing the on-farm production of feed protein and P, e.g. by the use of forages with higher N and P contents or by better grassland and forage crop management and utilisation, (2) better animal feeding practices, avoiding surpluses of protein and P in the diets, and (3) manure export to other farms. Economic factors determine the suitability of these measures for a given farm. However, good quality fresh or conserved herbage from well managed grass or grass/clover swards may constitute a large part of the diet of productive dairy cows as was shown in different feeding experiments carried out in this project (e.g. in sub-tasks A3, A5, B3 and B6).

Increasing the on-farm production of dietary protein and P has the advantage that it also increases the capacity to re-utilise manure N and P on the farm. Nitrogen-fertilised grasses, grass/clover mixtures and leguminous and cruciferous forage crops generally have a medium or high N and P content, in particular when these leafy forages are harvested in a relatively young growing stage. If these forages are the main component of the diet and if they are properly supplemented, *i.e.* with high-energy/low-protein and low-P forages and concentrates, the related production of manure N and P can generally be utilised on the farm without major environmental problems. On the contrary, forage crops with a storage organ for energy, like maize, cereals and fodder beet have low N and P contents and require supplements with high N and P contents. If these crops occupy a large part of the farm area, the production of manure N and P easily exceeds their N and P requirements. Farm N and P balances can be used to analyse potential pollution from animal feeding practices. In fact, the imports of N and P in roughage and concentrates from outside the farm should not exceed the exports of N and P in animal products + the ecologically acceptable N and P losses. This point of view has been developed in the course of the project (Van der Meer *et al.*, 1997b). Unfortunately, definition of ecologically acceptable losses may be difficult, in particular for N because of the different pathways of losses, *viz.* volatilisation of ammonia, losses related to nitrification, denitrification and nitrate leaching. For P, however, it can be argued that ecologically acceptable losses are less than 0.45 kg P ha^{-1} year^{-1}. Recent model calculations show that on average Dutch dairy farms (mainly grassland with up to 30% silage maize and N inputs limited by the nutrient accounting system MINAS), P input in purchased feeds equals P output in animal products at an animal density of about 1.5 cows + additional young stock per ha (Van der Meer, 2002). This means that at higher cattle densities P will accumulate in the soils unless manure is exported or higher than average grassland and forage crop yields are obtained.

Too low N and P contents of home-grown feeds will be common on dairy farms with a large proportion of silage maize. In this project, very low protein contents in the home-grown feeds were observed in the crop rotations studied under sub-task B1 in Denmark. The objective of the 'protein rotation' was to maximise yield of milk protein per hectare with minimum use of fertiliser N in the fields and a minimum of protein supplements in the diets (paragraph 2.7). The objective of the 'energy rotation' was to maximise yield of metabolisable energy per ha. The protein rotation was not successful, because DM and ME yields of the crops in this rotation were low (Table 3.69) and despite the objective of this rotation, the mean CP content of the feeds produced was only 122 g kg^{-1} DM. This means that even with this rotation relatively large amounts of protein supplements have to be purchased to obtain a diet with 160-170 g CP kg^{-1} DM, required for productive dairy cows, as concluded from the results of the feeding experiments conducted under sub-task A1. Although average DM and ME yields in the energy rotation were considerably higher than in the protein rotation, average CP content was only 98 g kg^{-1} DM (Table 3.69). Hence, large amounts of N (and P) will have to be imported in supplements to make good diets and this certainly will cause too high N and, on the long term, also P losses, unless manure is exported. Good nutrient management strategies for dairy farms should aim to produce a higher proportion of dietary N and P on the farm than in these rotations.

On dairy farms with a surplus of roughage or where the feed production capacity is not fully utilised, it may be interesting to grow concentrate replacers, like ground maize ear silage (GMES), barley and fodder beet. The use of GMES in dairy cow rations based on fresh grass (sub-task A3) or grass silage (sub-task B3) was studied in The Netherlands. Effects of GMES on cow performance were good. Given the rather high nitrate leaching losses, often observed on maize land, and the relative increase of urinary N and urinary urea-N contents resulting from inclusion of maize silage or GMES in the diet of dairy cows (paragraph 5.1.4), the effects of maize silage or GMES on N losses may be less favourable than assumed and should be studied in detail at farm level. A feeding experiment in Scotland (sub-task B5) showed that a mixture of barley and fodder beet (*i.e.* of starch and sugar containing feeds) was a good supplement for a dairy cow ration based on grass/clover silage. Results with fodder beet in the Dutch feeding experiments (sub-task B3) were less positive, and despite the very high yields of this crop, its use decreased in recent years.

5.3 Improve the utilisation of slurry nutrients

This project (sub-tasks C1 and C2) showed that dairy cow slurry, applied by low-emission techniques, is an effective source of P and K and, possibly, also of trace elements for a grass/clover sward. Inorganic N, total P and total K from slurry gave approximately the same effects on DM and NPK yields than N, P and K from chemical fertilisers. In all cases, application of slurry or fertiliser N to the mixed sward increased DM and N yield of the grass component and decreased DM and N yield of the clover component. In the first year after sward establishment, the positive effect of slurry N on grass yield tended to be smaller than the negative effect on clover yield. In the following years, the positive effect on grass yield exceeded the negative effect on clover yield, particularly in the third year when clover content on all the plots had decreased owing to unfavourable weather conditions. Overall, these experiments showed a small effect of slurry N (and fertiliser N) on the DM yield of a clover-rich sward and, as a consequence, biological (dairy) farmers may consider alternatives for slurry use. Interesting opportunities are (1) application to non-leguminous forage and food crops, (2) application to mixed swards with a low clover content, (3) application to mixed swards in early spring to accelerate early growth, and (4) application to clover-rich swards to avoid a too high clover content. If a considerable part of the slurry is used on other crops, attention should be given to P and, particularly, K supply to the mixed sward.

The rate of slurry application to the mixed sward in experiment C1-1 intended to replace P yield in the harvested herbage. Actual rates of slurry P application were slightly lower than P yields, whereas the rates of fertiliser P slightly exceeded P yields (Table 3.92 and Annex C1-1). Different rates and sources of P application hardly affected the P status of the soil (Table 3.94 and Annex C1-1). This indicates that the P-AL values are well buffered by P reserves of lower availability. The rates of slurry applied in the experiments C1-1 and C2-1 contained between *ca.* 200 and 300 kg total N ha^{-1} year^{-1} (calculated from Annex C1-1 and Table 2.19 for experiment C1-1 and from Annex C2-1 and Table 2.21 for experiment C2-1) and between *ca.* 230 and 315 kg K ha^{-1} year^{-1} (Annex C1-1). These rates of slurry N and the equivalent rates of fertiliser N did not significantly increase the amount of soil inorganic N in Autumn compared to the treatment without applied N (Table 3.105). A comparison of the amounts of soil inorganic N in Autumn under the mixed sward and the pure grass sward shows no effect of white clover on this NO_3^- leaching indicator. The highest amounts of soil inorganic N observed varied between 20 and 30 kg ha^{-1} (Table 3.105). This is well below the critical levels for drinking water quality. The rate of slurry application aiming at P replacement did not contain sufficient K for K replacement. Particularly in the first experimental year, K yields in the harvested herbage were significantly higher than K application rates (Table 3.93 and Annex C1-1). Experiment C1-1 showed a strong effect of K supply on K uptake and DM yield of white clover (Tables 3.90 and 3.93) and on the K status of the soil (Table 3.94). Therefore, K fertilisation of grassland should receive much attention in white clover-based production systems, particularly on light soils.

Experiments in Italy (sub-tasks C3 and C4) showed poor utilisation of slurry N (and urea-N) by silage maize (Table 4.4), even when this crop was followed by Italian ryegrass as a winter crop (Table 4.5). The effects of the period and method of slurry application on N utilisation were unexpectedly small. A possible explanation is that incorporation of the surface-spread slurry was too late or not effective on the heavy soil of the experimental field. As a consequence, most inorganic N was probably lost by NH_3 volatilisation. This assumption is supported by the observation that applied N had a relatively small effect on the amount of soil inorganic N (Tables 3.110 and 3.118) and on NO_3^- leaching (Tables 3.112 and 3.119). This work stresses the need for careful slurry management.

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Annex B1

Annex B1-1. Yields of protein crops at Silstrup Research Station in 1993. Experiment B1-1.

Crops	Plant part	Area1) (ha)	DM (kg ha^{-1})	Protein (kg ha^{-1})	ME (1000 MJ ha^{1})
Barley + grass/clover	Straw		1349	99	8.4
Barley + grass/clover	Grain	8.1	3635	451	46.8
Grass/clover, 1st prod. year	Herbage	5.5	6634	831	72.3
Grass/clover, 2nd prod. year	Herbage	5.9	4012	499	45.1
Whole barley + Ital. ryegrass	Herbage	3.9	11475	1237	117.3
Whole pea + Ital. ryegrass	Herbage	5.4	7914	1137	90.8
Average		**28.8**	**6528**	**796**	**71.5**

1) Bold value is the total area of protein crops; DM = dry matter; ME = metabolisable energy

Annex B1-2. Yields of protein crops at Silstrup Research Station in 1994. Experiment B1-1.

Crops	Plant part	Area1) (ha)	DM (kg ha^{-1})	Protein (kg ha^{-1})	ME (1000 MJ ha^{-1})
Barley + Grass/clover	Straw		1333	65	8.0
Barley + Grass/clover	Grain	10.6	4065	400	51.4
Grass/clover, 1st prod. year	Herbage	6.1	5826	754	68.4
Grass/clover, 2nd prod. year	Herbage	3.9	4986	666	62.4
Whole barley + Ital. ryegrass	Herbage	2.5	6994	558	78.8
Whole pea + Ital. ryegrass	Herbage	5.5	10099	1365	124.4
Average		**28.5**	**6475**	**735**	**75.9**

1) Bold value is the total area of protein crops; DM = dry matter; ME = metabolisable energy

Annex B1-3. Yields of protein crops at Silstrup Research Station in 1995. Experiment B1-1.

Crops	Plant part	Area1) (ha)	DM (kg ha^{-1})	Protein (kg ha^{-1})	ME (1000 MJ ha^{-1})
Barley + Grass/clover	Straw		1936	69	12.2
Barley + Grass/clover	Grain	5.8	5732	588	71.9
Grass/clover, 1st prod. year	Herbage	3.7	5316	791	55.9
Grass/clover, 2nd prod. year	Herbage	6.1	4565	645	47.5
Whole barley + Ital. ryegrass	Herbage	5.5	7676	666	63.9
Whole pea + Ital. ryegrass	Herbage	5.9	7758	1181	76.9
Average		**27.0**	**6666**	**789**	**66.3**

1) Bold value is the total area of protein crops; DM = dry matter; ME = metabolisable energy

Annex B1-4. Yields of energy crops at Silstrup Research Station in 1993. Experiment B1-1.

Crops	Plant part	Area[1)] (ha)	DM (kg ha^{-1})	Protein (kg ha^{-1})	ME (1000 MJ ha^{-1})
Barley + per. ryegrass	Straw		2194	211	13.7
Barley + per. ryegrass	Grain	5.4	3929	436	50.6
Per. ryegrass, 1st prod. year	Herbage	5.4	12224	1699	117.6
Oat	Straw		1594	94	9.8
Oat	Grain	5.8	5246	660	61.1
Whole wheat	Herbage	5.3	13857	1193	138.6
Fodder beet	Root	5.3	15234	1103	181.4
Fodder beet	Top		3612	627	39.5
Average		**27.2**	**11473**	**1196**	**121.3**

1) *Bold value is the total area of energy crops; DM = dry matter; ME = metabolisable energy.*

Annex B1-5. Yields of energy crops at Silstrup Research Station in 1994. Experiment B1-1.

Crops	Plant part	Area[1)] (ha)	DM (kg ha^{-1})	Protein (kg ha^{-1})	ME (1000 MJ ha^{-1})
Barley + per. ryegrass	Straw		2134	81	9.8
Barley + per. ryegrass	Grain	5.2	4034	382	51.1
Per. ryegrass, 1st prod. year	Herbage	4.7	7839	916	92.0
Oat	Straw		1998	63	10.6
Oat	Grain	5.4	3739	345	43.2
Whole wheat	Herbage	5.2	9674	723	109.2
Fodder beet	Root	3.4	10486	637	124.2
Fodder beet	Top		2107	370	29.4
Average		**23.9**	**8088**	**675**	**89.3**

1) *Bold value is the total area of energy crops; DM = dry matter; ME = metabolisable energy.*

Annex B1-6. Yields of energy crops at Silstrup Research Station in 1995. Experiment B6-1.

Crops	Plant part	Area[1)] (ha)	DM (kg ha^{-1})	Protein (kg ha^{-1})	ME (1000 MJ ha^{-1})
Barley + per. ryegrass	Straw		2131	83	13.4
Barley + per. ryegrass	Grain	5.3	4424	454	55.5
Per. ryegrass, 1st prod. year	Herbage	5.3	9302	852	87.2
Oat	Straw		1733	69	10.7
Oat	Grain	5.4	3196	366	35.6
Whole wheat	Herbage	4.1	13017	849	105.5
Fodder beet	Root	4.0	12461	741	144.1
Fodder beet	Top		1788	360	18.3
Average		**24.1**	**9171**	**730**	**89.6**

1) *Bold value is the total area of energy crops; DM = dry matter; ME = metabolisable energy.*

Annex B1-7. Application of cattle slurry and fertiliser to crops in the protein cropping system at Silstrup Research Station in 1993. Concentration of total N in slurry was 3.2 kg ton^{-1}. Experiment B6-1.

Protein crops, 1993	Area[1)]	Slurry	Fertiliser[2)]	Total
	(ha)	(kg total N ha^{-1})		
Barley + Grass/clover	8.1	74	3	77
Grass/clover, 1st production year	5.5	80	0	80
Grass/clover, 2nd production year	5.9	86	3	89
Whole barley + Ital. ryegrass	3.9	124	6	130
Whole pea + Ital. ryegrass	5.4	14	9	23
Average	**28.8**	**73**	**4**	**77**

[1)] *Bold value is the total area of protein crops.*

[2)] *One field received fertiliser by mistake. When averaging application over several fields, values are low.*

Annex B1-8. Application of cattle slurry and fertiliser to crops in the protein cropping system at Silstrup Research Station in 1994. Concentration of total N in slurry was 4.8 kg ton^{-1}. Experiment B6-1.

Protein crops, 1994	Area[1)]	Slurry	Fertiliser	Total
	(ha)	(kg total N ha^{-1})		
Barley + Grass/clover	10.6	194	29	223
Grass/clover, 1st prod. year	6.1	93	0	93
Grass/clover, 2nd prod. year	3.9	135	0	135
Whole barley + Ital. ryegrass	2.5	146	0	146
Whole pea + Ital. ryegrass	5.5	0	0	0
Average	**28.6**	**123**	**11**	**134**

[1)] *Bold value is the total area of protein crops.*

Annex B1-9. Application of cattle slurry and fertiliser to crops in the protein cropping system at Silstrup Research Station in 1995. Concentration of total N in slurry was 4.8 kg ton^{-1}. Experiment B6-1.

Protein crops, 1995	Area[1)]	Slurry	Fertiliser	Total
	(ha)	(kg total N ha^{-1})		
Barley + Grass/clover	5.8	106	126	233
Grass/clover, 1st prod. year	3.7	112	0	112
Grass/clover, 2nd prod. year	6.1	191	0	191
Whole barley + Ital. ryegrass	5.5	286	0	286
Whole pea + Ital. ryegrass	5.9	112	0	112
Average	**27.0**	**164**	**27**	**191**

[1)] *Bold value is the total area of protein crops.*

Annex B1-10. Application of cattle slurry and fertiliser to crops in the energy cropping system at Silstrup Research Station in 1993. Concentration of total N in slurry was 3.2 kg ton^{-1}. Experiment B6-1.

Energy crops, 1993	Area[1]	Slurry	Fertiliser	Total
	(ha)	(kg total N ha^{-1})		
Barley + per. ryegrass	5.4	21	95	116
Per. ryegrass, 1st prod. year	5.4	54	234	288
Oat	5.8	71	34	105
Whole wheat + Ital. ryegrass	5.3	68	167	235
Fodder beet	5.3	94	96	190
Average	**27.2**	**62**	**124**	**186**

[1] *Bold value is the total area of energy crops.*

Annex B1-11. Application of cattle slurry and fertiliser to crops in the energy cropping system at Silstrup Research Station in 1994. Concentration of total N in slurry was 4.8 kg ton^{-1}. Experiment B6-1.

Energy crops, 1994	Area[1]	Slurry	Fertiliser	Total
	(ha)	(kg total N ha^{-1})		
Barley + per. ryegrass	5.2	157	70	227
Per. ryegrass, 1st prod. year	4.7	161	109	270
Oat	5.4	192	0	192
Whole wheat + Ital. ryegrass	5.2	144	92	236
Fodder beet	3.4	260	62	322
Average	**23.9**	**178**	**66**	**243**

[1] *Bold value is the total area of energy crops.*

Annex B1-12. Application of cattle slurry and fertiliser to crops in the energy cropping system at Silstrup Research Station in 1995. Concentration of total N in slurry was 4.8 kg ton^{-1}. Experiment B6-1.

Energy crops, 1995	Area[1]	Slurry	Fertiliser	Total
	(ha)	(kg total N ha^{-1})		
Barley + per. ryegrass	5.3	161	120	281
Per. ryegrass, 1st prod. year	5.3	187	192	379
Oat	5.4	242	0	242
Whole wheat + Ital. ryegrass	4.1	353	91	444
Fodder beet	4.0	258	57	315
Average	**24.1**	**234**	**94**	**327**

[1] *Bold value is the total area of energy crops.*

Annex B1-13. Nitrogen balances of crops in the protein cropping system at Silstrup Research Station. Concentration of total N in slurry was 3.2 kg ton⁻¹ in 1993, and 4.8 kg ton⁻¹ in 1994 and 1995. Experiment B6-1.

Protein crops	Area	Applied	Harvested	Balance[1]	Corrected balance[2]
	(ha)	(kg total N ha⁻¹)			
1993					
Barley + Grass/clover	8.1	77	88	-11	-11
Grass/clover, 1st prod. year	5.5	80	133	-53	-53
Grass/clover, 2nd prod. year	5.9	89	80	9	9
Whole barley + Ital. ryegrass	3.9	130	198	-68	-68
Whole pea + Ital. ryegrass	5.4	23	182	-159	-159
Average	**(28.8)**	**77**	**127**	**-50**	**-50**
1994					
Barley + Grass/clover	10.6	223	74	149	84
Grass/clover, 1st prod. year	6.1	93	121	-28	-59
Grass/clover, 2nd prod. year	3.9	135	107	28	-17
Whole barley + Ital. ryegrass	2.5	146	89	57	8
Whole pea + Ital. ryegrass	5.5	0	218	-218	-218
Average	**(28.6)**	**134**	**118**	**16**	**-25**
1995					
Barley + Grass/clover	5.8	233	105	128	92
Grass/clover, 1st prod. year	3.7	112	127	-15	-52
Grass/clover, 2nd prod. year	6.1	191	103	88	24
Whole barley + Ital. ryegrass	5.5	286	107	179	84
Whole pea + Ital. ryegrass	5.9	112	189	-77	-117
Average	**(27.0)**	**191**	**126**	**65**	**10**

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1) *Calculated as the difference between N applied and N removed in harvested crops.*

2) *Amount of slurry N applied is recalculated on the basis of 3.2 kg total N ton⁻¹ instead of 4.8 kg ton⁻¹.*

Annex B1-14. Nitrogen balances of crops in the energy cropping system at Silstrup Research Station. Concentration of total N in slurry was 3.2 kg ton^{-1} in 1993, and 4.8 kg ton^{-1} in 1994 and 1995. Experiment B6-1.

Energy crops	Area	Applied	Harvested	Balance[1)]	Corrected balance[2)]
	(ha)	(kg total N ha^{-1})			
1993					
Barley + per. ryegrass	5.4	116	104	12	12
Per. ryegrass, 1st prod. year	5.4	288	272	16	16
Oat	5.8	105	121	-16	-16
Whole wheat + Ital. ryegrass	5.3	235	191	44	44
Fodder beet	5.3	190	277	-87	-87
Average	**(27.2)**	**186**	**192**	**-6**	**-6**
1994					
Barley + per. ryegrass	5.2	227	74	153	101
Per. ryegrass, 1st prod. year	4.7	270	147	123	69
Oat	5.4	192	65	127	63
Whole wheat + Ital. ryegrass	5.2	236	116	120	72
Fodder beet	3.4	322	161	161	74
Average	**(23.9)**	**243**	**108**	**135**	**76**
1995					
Barley + per. ryegrass	5.3	281	86	195	141
Per. ryegrass, 1st prod. year	5.3	379	136	243	181
Oat	5.4	242	70	172	91
Whole wheat + Ital. ryegrass	4.1	444	136	308	190
Fodder beet	4.0	315	176	139	53
Average	**(24.1)**	**327**	**117**	**210**	**132**

[1)] *Calculated as the difference between N applied and N removed in harvested crops.*

[2)] *Amount of slurry N applied is recalculated on the basis of 3.2 kg total N ton^{-1} instead of 4.8 kg ton^{-1}.*

Annex B1-15. Chemical analyses of 'high' dry matter slurry sampled at Silstrup Research Station in 1994. Identical samples were sent to three different laboratories in Denmark. Amounts of each nutrient relate to a slurry application of 40 tons ha^{-1}. Experiment B6-1.

Laboratory	Dry matter	Total N	NH_4^+-N	P	K
	(g kg^{-1})			(kg ha^{-1})	
1	53	96	50	24	84
2	52	76	56	24	84
3	49	108	60	22	84
Average	**51**	**93**	**55**	**23**	**84**

Annex B1-16. Chemical analyses of 'low' dry matter slurry sampled at Silstrup Research Station in 1994. Identical samples were sent to three different laboratories in Denmark. Amounts of each nutrient relate to a slurry application of 40 tons ha^{-1}. Experiment B6-1.

Laboratory	Dry matter	Total N	NH_4^+-N	P	K
		(g kg$^{-1)}$		(kg ha^{-1})	
1	17	48	30	10	60
2	17	36	32	8	56
3	18	60	28	10	60
Average	**17**	**48**	**30**	**9**	**59**

Annex B5

Annex B5-1: Renal and mammary purine derivative excretion in Holstein/Friesian dairy cows

The significance and contribution of rumen-synthesised microbial protein (MCP) in the context of current metabolisable protein (MP) systems and variations in the energetic efficiency of MCP synthesis, reported in the literature, are reviewed. The estimation of MCP supply from urinary purine derivative (PD) excretion is discussed and reviewed in detail, with the conclusion that it is a reliable non-invasive technique. HPLC methodologies were developed to determine PD, pseudo-uridine and creatinine in bovine urine and allantoin in bovine milk. A series of experiments were conducted to evaluate the potential of the PD technique, using spot urine samples or measurements of allantoin in milk as on-farm diagnostics of MCP supply. Prediction of daily mean urinary molar ratios of PDs to creatinine (PD/c) from spot urine samples was poor due to diurnal variations, the extent of which was influenced by feeding regime. Furthermore, prediction of urinary PD excretion from daily mean PD/c ratios was poor due to between-cow variations in urinary creatinine excretion. On this basis the spot urine sampling technique was considered unreliable and a total urine collection proved necessary. Variability of urinary creatinine excretion precludes its use as a urinary output marker for individual cows. Urinary pseudo-uridine excretion was independent of nutrient supply but appeared to be influenced by metabolic changes occurring during lactation. In two experiments, dietary fermentable metabolisable energy (FME) supply was manipulated during early and late lactation. For both experiments, individual cow urinary PD excretion was poorly predicted from calculated FME intake or MCP supply. Based on mean treatment values, urinary PD excretion was accurately predicted from calculated MCP. Individual cow milk allantoin excretion or concentration were poorly correlated with urinary PD excretion, calculated FME intake or MCP. Relationships derived using mean treatment values indicated that milk allantoin excretion or concentration were strongly correlated with urinary PD excretion or calculated MCP. Variability precludes the use of milk allantoin as an index of MCP supply for individual cows, but it appears as reliable as urinary PD excretion when used on a herd or group feeding basis.

Annex B6

Annex B6-1. Pasture production parameters of the grazed grass/clover swards in 1993. Experiment B6-1.

	Spring		Autumn		Total	
N applied (kg ha^{-1})	20	120	0	80	20	200
Days of growth	157	150	90	90	247	240
Grazing rotations	4	4	2	2	6	6
Net pasture yield						
Total (t DM ha^{-1})	9.68	9.85	2.90	3.42	12.6	13.3
Clover (t DM ha^{-1})	4.33	3.05	1.50	1.28	5.8	4.3
(%)	45	31	52	37	46	32
Grazing pressure (cows ha^{-1})	4.00	4.53	2.20	2.26	3.34	3.68
Intake (t DM ha^{-1})	8.37	8.88	2.54	3.13	10.9	12.0
(kg DM cow^{-1} day^{-1})	13.3	13.1	12.8	15.4	13.2	13.6
Net pasture Utilisation (%)	86	90	87	91	86	90

Annex B6-2. Production parameters of the grazed grass/clover swards in 1994. Experiment B6-1.

	Spring		Autumn		Total	
Days of growth	211		67		278	
N applied (kg ha^{-1})	0	160	0	80	0	240
Pasture offered (t DM ha^{-1})	10.70	11.37	3.05	3.30	13.75	14.67
Net pasture yield (t ha^{-1})						
Total (t DM ha^{-1})	8.30	8.91	2.90	3.10	11.20	12.00
Clover (t DM ha^{-1})	6.03	5.52	1.35	1.17	7.38	6.69
(%)	73	62	44	35	66	53
Grazing pressure (cows ha^{-1})	3.28	3.38	2.82	3.07	3.16	3.30
Intake (t DM ha^{-1})	7.80	8.37	2.77	3.11	10.57	11.48
(kg DM cow^{-1} day^{-1})	11.3	11.7	14.7	15.1	12.00	12.14

Annex B6-3. Production parameters of the grazed grass/clover swards in 1995. Experiment B6-1.

		Spring		Autumn		Total	
Days of growth		195		85		280	
N applied (kg ha^{-1})		0	160	0	80	0	240
Pasture offered (t DM ha^{-1})		8.65	9.85	2.80	3.20	11.45	13.05
Net pasture yield							
Total	(t DM ha^{-1})	7.91	9.04	2.54	2.94	10.45	12.00
Clover	(t DM ha^{-1})	2.78	2.35	0.84	0.84	3.62	3.19
	(%)	32	24	30	26	32	24
Grazing pressure (cows ha^{-1})		2.26	2.34	2.19	2.36	2.24	2.35
Intake	(t DM ha^{-1})	7.55	8.65	2.06	2.37	9.61	11.02
	(kg DM cow^{-1} day^{-1})	17.1	19.6	11.1	11.8	15.3	16.7

Annex B6-4. DM yield of conservation cuts from grass/clover swards with low N fertiliser. Experiment B6-2.

	Cutting date	DM yield (kg ha^{-1})
Early silage	27/4/93	3 000
Normal silage	10/5/93	4 500
	6/5/94	3 700
	19/5/95	3 500
	11/5/96	4 800
Mean		**4 100**
Late silage	24/5/94	5 500
(after early grazing)	30/5/95	2 400
	20/5/96	5 100
Mean		**5 300**
Hay	14/7/93	4 100
(second cut)	21/6/94	4 600
	20/6/95	5 000
	22/6/96	4 900
Mean		**4 650**

Annex B6-5. DM yields of a rotation of a vetch/oats mixture and silage maize. Experiment B6-2.

Vetch/oats			Silage maize		
Sowing	Cutting	DM (kg ha^{-1}) (% vetch)	Sowing	Cutting	DM (kg ha^{-1}) (%)
19/11/92	7/5/93	5 220 (32%)	30/5	1/10	8 900 (25%)
25/10/93	25/4/94	4 640 (33%)	26/5	4/10	9 030 (30%)
25/10/94	26/4/95	4 010 (42%)	15/5	12/9	6 000 (28%)
9/10/95	24/4/96	4 000 (50%)			

Annex B6-6. DM yield of a mixed sward (t ha^{-1}) as affected by the rate of N application to the first cut and the date of the first cut. Experiment B6-3.

	First cut			Second cut			Total annual		
N applied (kg ha^{-1})	1993	1994	1995	1993	1994	1995	1993	1994	1995
First cut mid March									
0	1.12	1.34	1.14	0.60	1.84	1.99	9.38	7.78	9.96
40	1.17	1.65	2.64	1.15	2.54	1.84	10.02	8.03	10.89
80	1.30	1.79	3.01	1.53	3.04	1.96	9.83	8.99	10.98
120	1.38	2.23	3.76	1.92	3.44	2.44	10.76	9.73	10.92
First cut end of March									
0	1.39	1.80	2.06	0.61	2.10	3.39	7.81	8.32	10.70
40	1.56	2.61	3.93	0.93	2.39	2.44	8.84	9.18	11.90
80	2.10	3.54	5.15	0.99	2.60	1.78	9.24	10.08	11.51
120	2.36	4.19	5.69	1.13	3.03	1.85	9.81	11.39	11.63
First cut mid April									
0	2.21	2.89	3.24	0.95	3.44	3.99	9.15	9.60	11.79
40	2.51	4.13	5.49	1.06	2.61	3.12	9.34	9.80	13.03
80	3.19	4.73	6.68	1.15	2.96	2.32	10.42	10.73	13.35
120	3.75	5.58	8.17	1.29	2.07	1.75	10.80	10.68	13.89

Annex C1

Annex C1-1. Rates of inorganic N, total P and total K (kg ha^{-1}) applied in 1993, 1994 and 1995. Experiment C1-1.

Experimental	Inorganic N			Total P			Total K		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SA	104	123	137	33	34	41	242	282	326
2. SAD	140	141	165	41	37	41	284	315	361
3. Inj OS	104	119	137	33	33	41	243	273	326
4. Inj	106	115	135	36	35	42	232	266	320
5. $N_0P_2K_2$	0	0	0	40	40	40	218	292	355
6. $N_0P_3K_3$	0	0	0	59	59	58	326	437	480
7. $N_1P_2K_2$	51	53	64	40	40	40	218	292	355
8. $N_2P_2K_2$	102	105	130	40	40	40	218	292	355
9. $N_2P_3K_3$	102	105	130	59	59	58	326	437	480
10. $N_2P_2K_0$	102	105	130	40	40	40	0	0	75
11. $N_2P_0K_2$	102	105	130	0	0	0	218	292	355

Annex C1-2. Mean N contents (g kg^{-1} DM) of the mixture and the mixture components in 1993, 1994 and 1995. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SA	35.0	29.9	23.4	29.4	26.7	24.7	43.8	42.1	37.6
2. SAD	30.3	29.6	25.5	26.0	28.0	26.0	43.8	42.3	40.5
3. Inj OS	36.1	30.4	23.9	31.4	29.7	26.6	44.5	42.2	37.3
4. Inj	38.2	31.0	26.0	32.9	32.5	29.1	45.8	44.1	39.3
5. $N_0P_2K_2$	39.4	30.5	23.5	30.2	26.3	23.7	44.3	41.0	35.4
6. $N_0P_3K_3$	38.7	33.2	25.3	28.1	29.3	25.8	44.7	41.7	37.6
7. $N_1P_2K_2$	37.0	30.0	21.8	29.8	27.5	22.8	44.7	41.4	34.7
8. $N_2P_2K_2$	35.1	28.6	22.8	30.0	26.8	25.1	43.7	40.8	36.8
9. $N_2P_3K_3$	36.9	29.1	23.3	33.1	27.8	25.6	43.4	41.3	36.4
10. $N_2P_2K_0$	35.2	29.2	24.7	29.3	28.4	26.5	44.0	43.9	38.5
11. $N_2P_0K_2$	35.9	29.0	23.3	31.3	26.7	24.6	44.0	40.8	36.0
Grand mean	36.2	30.0	24.0	30.2	28.3	25.6	44.3	41.9	37.2

Annex C1-3. Mean P contents (g kg^{-1} *DM) of the mixture and the mixture components in 1993, 1994 and 1995. Experiment C1-1.*

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SA	4.3	3.9	4.1	4.9	4.4	4.5	3.5	3.2	2.8
2. SAD	4.6	3.8	4.0	4.7	4.6	4.3	3.6	3.5	3.1
3. Inj OS	4.4	4.0	4.0	4.9	4.5	4.3	3.6	3.4	2.7
4. Inj	4.0	3.8	3.8	4.6	4.0	4.2	3.5	3.6	3.2
5. $N_0P_2K_2$	4.0	3.8	4.2	4.8	4.4	4.6	3.6	3.4	3.0
6. $N_0P_3K_3$	4.0	4.0	4.2	5.1	4.8	4.6	3.5	3.6	3.1
7. $N_1P_2K_2$	4.2	3.8	3.9	4.7	4.5	4.2	3.7	3.6	2.9
8. $N_2P_2K_2$	4.1	3.8	3.9	4.4	4.2	4.1	3.7	3.5	2.9
9. $N_2P_3K_3$	4.1	3.9	4.0	4.2	4.4	4.3	3.9	3.6	3.1
10. $N_2P_2K_0$	4.1	4.0	4.1	4.2	4.4	4.3	3.6	3.9	3.1
11. $N_2P_0K_2$	3.9	3.5	3.3	3.8	3.8	3.5	3.5	3.2	2.6
Grand mean	4.2	3.8	4.0	4.5	4.3	4.2	3.6	3.5	2.9

Annex C1-4. Mean K contents (g kg^{-1} *DM) of the mixture and the mixture components in 1993, 1994 and 1995. Experiment C1-1.*

Experimental	Mixture			Grass			White clover		
Treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SA	42.1	32.2	27.9	42.7	34.2	30.5	41.1	32.5	28.7
2. SAD	38.5	33.0	30.3	38.3	35.9	31.8	40.0	34.0	30.5
3. Inj OS	41.3	32.7	29.5	42.1	36.6	33.0	38.9	29.2	27.5
4. Inj	40.5	34.4	31.4	43.3	38.5	35.6	39.9	31.3	28.6
5. $N_0P_2K_2$	42.1	32.9	28.5	43.5	34.0	31.0	40.5	32.0	31.5
6. $N_0P_3K_3$	41.2	36.1	31.8	43.8	38.5	32.6	39.6	36.4	35.2
7. $N_1P_2K_2$	42.8	33.6	28.5	44.3	37.1	31.3	40.9	32.2	30.5
8. $N_2P_2K_2$	42.5	32.8	30.9	42.6	36.5	34.2	40.8	31.5	30.9
9. $N_2P_3K_3$	43.7	34.9	31.3	45.2	37.4	35.7	42.3	34.9	33.6
10. $N_2P_2K_0$	36.5	20.7	16.7	39.0	25.7	18.1	31.3	16.9	7.7
11. $N_2P_0K_2$	43.9	32.5	30.8	44.1	36.3	32.2	41.3	34.0	32.0
Grand mean	41.5	32.4	29.1	42.3	35.6	31.7	40.2	31.9	31.6

Annex C1-5. Apparent N efficiencies (ANE, in kg DM kg^1 inorganic N applied) of the treatments with applied N. The ANE values have been calculated for the mixture and the mixture components, using the DM yields of treatment 5 ($N_0P_2K_2$) as references. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
Slurry									
1. SA	-6.6	4.5	19.2	12.5	10.7	17.2	-19.2	-6.3	2.0
2. SAD	-10.1	15.2	29.0	22.9	13.6	28.9	-33.2	1.6	0.1
3. Inj OS	-0.5	11.3	27.1	25.9	18.1	32.8	-26.4	-6.7	-5.8
4. Inj	-11.0	10.0	31.6	16.7	23.6	39.2	-27.7	-13.5	-7.6
Fertiliser N									
7. $N_1P_2K_2$	10.0	19.4	19.5	29.0	21.5	25.2	-19.2	-2.1	-5.5
8. $N_2P_2K_2$	5.4	14.7	24.2	24.7	21.0	28.9	-19.5	-6.3	-4.8
9. $N_2P_3K_3$	11.9	16.2	25.1	22.6	18.1	26.6	-10.9	-1.9	-1.5
10. $N_2P_2K_0$	4.9	2.2	12.2	29.3	17.1	21.2	-24.5	-15.0	-9.0
11. $N_2P_0K_2$	6.0	7.0	16.5	29.0	11.5	22.8	-23.1	-4.5	-6.2

Annex C1-6. Apparent N recoveries (ANR, in % of inorganic N applied) of the treatments with applied N. The ANR values have been calculated for the mixture and the mixture components, using the N yields of treatment 5 ($N_0P_2K_2$) as references. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
Slurry									
1. SA	-63	9	45	35	30	47	-88	-23	10
2. SAD	-91	39	82	51	44	83	-148	11	4
3. Inj OS	-32	33	66	85	67	99	-116	-24	-20
4. Inj	-53	35	95	62	103	136	-118	-49	-26
Fertiliser N									
7. $N_1P_2K_2$	-8	49	23	84	70	50	-80	-6	-20
8. $N_2P_2K_2$	-21	26	52	74	58	79	-89	-27	-16
9. $N_2P_3K_3$	21	35	57	83	57	76	-53	-7	-5
10. $N_2P_2K_0$	-22	-5	36	83	58	69	-110	-54	-32
11. $N_2P_0K_2$	-11	8	37	94	32	60	-104	-19	-22

Annex C1-7. Apparent P efficiencies (APE, in kg DM kg^{-1} P applied) of the experimental treatments with applied P. The APE values have been calculated for the mixture and the mixture components, using the DM yields of treatment 11 ($N_2P_0K_2$) as references. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
Slurry									
1. SA	-39	-6	12	-50	3	-15	11	-9	26
2. SAD	-50	38	64	6	19	44	-56	19	20
3. Inj OS	-20	18	38	-8	29	38	-12	-10	0
4. Inj	-49	12	51	-33	43	56	-16	-31	-5
Fertiliser P									
5. $N_0P_2K_2$	-15	-19	-54	-74	-30	-74	59	12	20
6. $N_0P_3K_3$	-11	7	-10	-45	-23	-38	35	30	28
7. $N_1P_2K_2$	-3	-7	-23	-37	-2	-34	35	9	11
8. $N_2P_2K_2$	-2	20	25	-11	25	20	9	-5	5
9. $N_2P_3K_3$	10	16	19	-11	12	9	21	5	10
10. $N_2P_2K_0$	-3	-13	-14	1	15	-5	-4	-28	-9

Annex C1-8. Apparent P recoveries (APR, in % of P applied) of the experimental treatments with applied P. The APR values have been calculated for the mixture and the mixture components, using the P yields of treatment 11 ($N_2P_0K_2$) as references. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
Slurry									
1. SA	-6	9	22	-6	13	13	4	-1	8
2. SAD	-7	22	40	15	21	34	-19	10	7
3. Inj OS	5	20	31	16	25	31	-3	-1	0
4. Inj	-17	12	29	-2	21	36	-6	-7	-1
Fertiliser P									
5. $N_0P_2K_2$	-5	-1	-3	-21	-5	-12	23	6	7
6. $N_0P_3K_3$	-3	11	9	-11	-1	-2	12	13	9
7. $N_1P_2K_2$	5	11	5	-5	10	0	14	7	4
8. $N_2P_2K_2$	4	13	23	3	16	21	5	1	2
9. $N_2P_3K_3$	8	12	18	-1	11	15	11	4	4
10. $N_2P_2K_0$	4	7	13	6	15	14	0	-4	-2

Annex C1-9. Apparent K efficiencies (AKE, in kg DM kg^{-1} K applied) of the experimental treatments with applied K. The AKE values have been calculated for the mixture and the mixture components, using the DM yields of treatment 10 ($N_2P_2K_0$) as references. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
Slurry									
1. SA	-4.9	1.1	4.2	-7.0	-1.7	-1.6	2.1	2.8	5.7
2. SAD	-6.8	6.1	11.2	0.8	0.4	7.1	-7.6	5.7	4.1
3. Inj OS	-2.3	4.1	8.5	-1.2	1.3	7.0	-1.0	2.8	1.5
4. Inj	-7.2	3.5	11.0	-5.3	3.4	10.4	-1.9	0.0	0.6
Fertiliser K									
5. $N_0P_2K_2$	-2.3	-0.8	-5.6	-13.7	-6.2	-9.8	11.5	5.4	4.2
6. $N_0P_3K_3$	-1.6	2.1	0.0	-8.3	-4.5	-4.9	6.7	6.5	4.9
7. $N_1P_2K_2$	0.0	2.7	-1.2	-6.9	-2.3	-4.1	7.0	5.0	2.9
8. $N_2P_2K_2$	0.2	4.5	5.6	-2.2	1.4	3.6	2.3	3.1	2.0
9. $N_2P_3K_3$	2.2	3.4	4.1	-2.1	0.2	1.8	4.3	3.1	2.4
11. $N_2P_0K_2$	0.5	1.7	2.0	-0.1	-2.0	0.8	0.6	3.8	1.3

Annex C1-10. Apparent K recoveries (AKR, in % of K applied) of the experimental treatments with applied K. The AKR values have been calculated for the mixture and the mixture components, using the K yields of treatment 10 ($N_2P_2K_0$) as references. Experiment C1-1.

Experimental	Mixture			Grass			White clover		
treatment	1993	1994	1995	1993	1994	1995	1993	1994	1995
Slurry									
1. SA	2	41	49	-21	14	36	25	23	18
2. SAD	-19	56	74	1	23	62	-18	33	14
3. Inj OS	10	54	68	2	31	73	9	19	5
4. Inj	12	59	85	-12	45	96	7	14	3
Fertiliser K									
5. $N_0P_2K_2$	16	36	20	-48	-2	8	63	31	14
6. $N_0P_3K_3$	8	40	32	-28	-2	14	37	35	18
7. $N_1P_2K_2$	29	50	33	-17	18	26	46	30	10
8. $N_2P_2K_2$	28	53	60	1	30	60	27	23	7
9. $N_2P_3K_3$	31	41	43	2	19	42	32	22	9
11. $N_2P_0K_2$	36	43	49	13	17	44	21	28	5

Annex C2

Annex C2-1. Rates of inorganic N, total P and total K (kg ha^{-1}) actually applied in 1993, 1994 and 1995. Experiment C2-1.

Experimental	Inorganic N			Total P			Total K		
treatment [1]	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SAD 1,2	140	140	162	65	59	59	396	458	418
2. SAD 3,4	137	150	178	59	55	60	412	438	448
3. SAD 1,3	139	140	166	60	56	61	390	451	420
4. Inj OS 1,2	107	113	136	56	54	61	348	408	394
5. Inj OS 3,4	114	126	150	56	53	61	377	402	419
6. Inj OS 1,3	107	117	134	53	53	59	357	418	375
7. Inj 1	106	113	149	57	54	65	340	410	400
8. Inj 3	52	121	138	35	51	61	237	430	408
9. Inj OS NPK 1,2	103	104	138	58	59	61	330	442	432
10. Inj OS NPK 3,4	102	104	115	59	59	59	326	437	410
11. Inj OS NPK 1,3	102	105	130	59	59	59	326	437	410
12. NPK 1,2	103	104	138	58	59	61	330	442	432
13. NPK 3,4	102	104	115	59	59	59	326	437	410
14. NPK 1,3	102	105	130	59	59	59	326	437	410
15. PK 1,2	0	0	0	58	59	61	330	442	432
16. PK 3,4	0	0	0	59	59	59	326	437	410
17. PK 1,3	0	0	0	59	59	59	326	437	410

[1] *see Table 2.20 for the meaning of the codes*

Annex C2-2. Apparent efficiencies (ANE, in kg DM kg^{-1} inorganic N applied) and apparent recoveries (ANR, in % of inorganic N applied) of slurry and fertiliser N applied to the grass in monoculture. The ANE and ANR values have been calculated using the average DM and N yields of the PK treatments (15, 16 and 17) as references. Experiment C2-1.

Experimental	ANE (kg DM/kg N)				ANR (%)			
treatment [1]	1993	1994	1995	Mean	1993	1994	1995	Mean
1. SAD 1,2	24.8	31.9	30.9	29.3	55	59	70	62
2. SAD 3,4	17.4	15.4	16.9	16.6	47	43	43	45
3. SAD 1,3	21.2	23.5	23.9	22.9	49	44	49	48
4. Inj OS 1,2	25.5	34.9	34.0	31.8	72	74	84	78
5. Inj OS 3,4	17.3	22.5	25.4	22.1	56	69	61	63
6. Inj OS 1,3	21.2	32.2	36.2	30.4	56	75	85	74
7. Inj 1	21.3	40.7	32.1	31.7	75	92	88	86
8. Inj 3	9.2	27.0	30.3	25.6	29	86	90	79
9. Inj OS NPK 1,2	35.2	33.7	33.5	34.1	95	53	77	76
10. Inj OS NPK 3,4	28.1	20.9	28.4	25.9	80	75	71	76
11. Inj OS NPK 1,3	32.4	25.2	25.4	27.5	83	51	51	62
12. NPK 1,2	43.3	36.6	33.1	37.2	107	59	77	81
13. NPK 3,4	35.0	27.0	32.4	31.5	96	86	78	87
14. NPK 1,3	36.7	28.4	23.7	29.2	90	55	47	64

[1] *see Table 2.20 for the meaning of the codes*

Annex C2-3. Apparent efficiencies (ANE, in kg DM kg^{-1} inorganic N applied) of slurry and fertiliser N, as affected by method and period of application. The ANE values have been calculated for the mixture and the mixture components, using the average DM yields of the PK treatments (15, 16 and 17) as references. Experiment C2-1.

Experimental	Mixture			Grass			White clover		
treatment 1)	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SAD 1,2	3.7	10.8	22.3	19.3	13.6	24.1	-15.6	-2.8	-1.8
2. SAD 3,4	6.3	8.5	12.6	12.2	5.0	16.8	-5.8	3.5	-4.2
3. SAD 1,3	-5.8	11.3	14.0	15.0	15.2	19.1	-20.8	-4.0	-5.2
4. Inj OS 1,2	3.8	10.9	23.3	22.3	22.9	34.0	-18.5	-12.0	-10.7
5. Inj OS 3,4	7.3	9.0	11.6	10.1	13.7	21.1	-2.8	-4.6	-9.5
6. Inj OS 1,3	3.1	12.0	24.9	20.2	19.9	33.1	-17.1	-8.0	-8.2
7. Inj 1	-10.7	5.8	14.0	13.9	30.2	27.7	-24.6	-24.4	-13.7
8. Inj 3	9.8	-1.6	20.4	1.7	4.6	24.5	8.1	-6.1	-4.1
9. Inj OS NPK 1,2	0.9	9.3	17.0	28.1	20.6	25.1	-27.2	-11.4	-8.2
10. Inj OS NPK 3,4	9.4	-2.1	15.6	22.5	11.1	23.3	-13.1	-13.2	-7.8
11. Inj OS NPK 1,3	6.4	5.4	21.8	26.5	17.7	28.9	-20.1	-12.3	-7.2
12. NPK 1,2	12.5	14.0	19.4	29.9	20.2	27.3	-17.4	-6.1	-7.9
13. NPK 3,4	11.0	0.9	8.8	21.0	11.7	20.5	-10.0	-10.8	-11.7
14. NPK 1,3	11.4	7.8	15.3	27.0	15.1	24.8	-15.6	-7.3	-9.5

1) *see Table 2.20 for the meaning of the codes*

Annex C2-4. Apparent recoveries (ANR, in % of the rate of inorganic N applied) of slurry and fertiliser N, as affected by method and period of application. The ANR values haven been calculated for the mixture and the mixture components, using the average N yields of the PK treatments (15, 16 and 17) as references. Experiment C2-1.

Experimental	Mixture			Grass			White clover		
treatment 1)	1993	1994	1995	1993	1994	1995	1993	1994	1995
1. SAD 1,2	-26	31	58	48	46	70	-68	-10	-6
2. SAD 3,4	12	27	31	39	24	52	-34	17	-18
3. SAD 1,3	-64	24	24	40	46	50	-94	-16	-19
4. Inj OS 1,2	-6	23	65	68	71	99	-79	-46	-39
5. Inj OS 3,4	27	24	27	38	58	60	-17	-17	-38
6. Inj OS 1,3	-22	38	62	64	70	101	-78	-28	-30
7. Inj 1	-61	-1	40	48	117	95	-105	-96	-52
8. Inj 3	12	15	74	-4	35	90	27	-9	-14
9. Inj OS NPK 1,2	-36	10	42	91	61	78	-123	-48	-28
10. Inj OS NPK 3,4	17	-15	29	68	41	70	-69	-52	-33
11. Inj OS NPK 1,3	-15	4	50	77	62	82	-95	-56	-27
12. NPK 1,2	5	17	49	83	63	86	-80	-25	-28
13. NPK 3,4	24	3	11	66	49	54	-49	-47	-49
14. NPK 1,3	-3	-3	32	82	48	72	-76	-35	-38

1) *see Table 2.20 for the meaning of the codes*

Annex C2-5. Contribution of white clover to the DM and N yield of the mixture. This is expressed as extra DM yield (in metric tons ha^{-1}) or extra N yield (apparent N fixation, ANF, in kg ha^{-1}) and calculated as the difference between the DM and N yields of the mixture and the grass in monoculture. Experiment C2-1.

Experimental	Extra DM yield			ANF		
treatment [1]	1993	1994	1995	1993	1994	1995
1. SAD 1,2	2.73	4.13	2.80	173	234	122
2. SAD 3,4	4.17	6.05	3.43	238	249	120
3. SAD 1,3	1.94	5.38	2.54	129	244	99
4. Inj OS 1,2	3.36	4.37	2.73	203	215	114
5. Inj OS 3,4	4.54	5.39	2.12	253	216	91
6. Inj OS 1,3	3.74	4.72	2.68	202	229	109
7. Inj 1	2.29	3.14	1.50	142	168	69
8. Inj 3	5.71	3.62	2.82	277	187	118
9. Inj OS NPK 1,2	2.15	4.55	1.90	151	228	93
10. Inj OS NPK 3,4	3.77	4.69	2.71	221	179	91
11. Inj OS NPK 1,3	3.04	5.01	3.71	186	223	139
12. NPK 1,2	2.51	4.74	2.30	181	230	104
13. NPK 3,4	3.23	4.36	1.47	212	187	64
14. NPK 1,3	3.11	4.92	3.09	191	212	121
15. PK 1,2	5.78	6.78	3.22	293	266	111
16. PK 3,4	5.78	7.40	4.53	285	282	154
17. PK 1,3	5.54	7.07	4.81	286	271	157
Grand mean	3.73	5.08	2.84	213	225	110

(1) *see Table 2.20 for the meaning of the codes*

Annex C2-6. N fixation efficiencies (NFE, in kg N t^{1} clover DM harvested) as affected by the main experimental treatments and period of N application. Experiment C2-1.

Expermental treatment[1]	NFE (kg N per ton clover DM) 1993	1994	1995	Mean
1. SAD 1,2	43.9	43.9	41.9	43.4
2. SAD 3,4	44.7	39.8	48.6	43.2
3. SAD 1,3	39.8	47.3	42.4	43.9
4. Inj OS 1,2	48.9	49.2	65.1	51.8
5. Inj OS 3,4	43.5	42.0	51.0	44.0
6. Inj OS 1,3	47.0	47.8	51.8	48.2
7. Inj 1	40.3	56.6	59.4	49.5
8. Inj 3	42.3	37.6	44.6	41.1
9. Inj OS NPK 1,2	45.3	50.2	44.8	47.4
10. Inj OS NPK 3,4	46.1	41.1	39.5	42.9
11. Inj OS NPK 1,3	45.6	50.3	61.3	50.8
12. NPK 1,2	41.7	45.2	49.0	44.6
13. NPK 3,4	41.5	40.7	34.7	40.0
14. NPK 1,3	42.1	42.7	61.4	45.7
15. PK 1,2	47.2	49.3	46.1	47.8
16. PK 3,4	46.5	45.4	43.2	45.3
17. PK 1,3	47.3	48.7	43.0	46.8
Mean 1-14	43.8	44.7	48.9	45.2
Mean 15-17	47.0	47.7	44.0	46.6
Grand mean	44.5	45.4	48.7	45.4

[1] *see Table 2.20 for the meaning of the codes*

Annex C4

The total N yields of maize and Italian ryegrass, as affected by the period, rate and method of slurry application, are presented in the Annexes C4-1 and C4-2.

Annex C4-1. Effects of the period and method of slurry or fertiliser N application to maize on the total N yield of maize and Italian ryegrass (kg ha^{-1}). The figures presented are averages of the 4 N levels. Experiments C3-1 and C4-1.

Period and method of application	1993-94		1994-95		1995-96		Average	
Winter	171	b	117	b	95	b	128	b
Spring	186	a	149	a	115	a	150	a
Incorporated	183	n.s.	140	a	110	a	144	a
Not incorporated	186	n.s.	126	b	101	b	138	b
Winter, incorporated	177	ab	127	c	101	c	135	c
Winter, not incorporated	164	bc	107	d	90	d	120	d
Spring, incorporated	189	a	153	b	118	b	153	b
Spring, not incorporated	184	ab	144	b	112	b	147	b
Spring, artificial fertiliser	153	c	219	a	146	a	173	a

Averaged over the 3 years, total N yield of maize and Italian ryegrass was significantly higher on the plots with spring-applied slurry than on the plots with winter-applied slurry. Also incorporation of slurry significantly increased total N yield of the two crops (Annex C4-1).

Annex C4-2. Effects of the rate, period and method of slurry or fertiliser N application on the total N yield of maize and Italian ryegrass (kg ha^{-1}). Experiments C3-1 and C4-1.

Rate of	Slurry												Fertiliser		
inorganic	winter application						spring application						spring		
N	incorp.			not incorp.			incorp.			not incorp.			application		
1993-94															
0	C	120	a	B	136	a	D	125	a	C	124	a	B	138	a
80	B	182	a	B	153	ab	C	179	a	B	181	a	B	143	b
160	B	179	a	AB	161	a	B	200	a	B	192	a	A	165	a
240	A	228	ab	A	206	b	A	248	a	A	239	a	A	164	c
Slurry + fertiliser N: control (0 N): 146; combination (240 N): 243															
1994-95															
0	B	111	bc	B	98	c	B	129	ab	A	114	abc	C	133	a
80	A	132	bc	B	99	c	AB	161	ab	A	138	b	B	194	a
160	AB	125	bc	B	104	c	B	145	B	A	149	b	A	270	a
240	A	139	bc	A	127	c	A	179	B	A	176	b	A	280	a
Slurry + fertiliser N: control (0 N): 113; combination (240 N): 268															
1995-96															
0	C	85	bc	A	79	c	A	97	abc	A	107	ab	A	123	a
80	BC	97	bc	A	85	c	A	116	ab	A	113	ab	A	131	a
160	B	102	b	A	96	b	A	132	ab	A	116	ab	A	150	a
240	A	119	bc	A	98	c	A	129	b	A	113	bc	A	180	a
Slurry + fertiliser N: control (0 N): 105; combination (240 N): 185															
3-year average															
0	C	108	b	C	106	b	C	117	ab	C	112	ab	C	125	a
80	B	139	ab	B	115	b	B	153	a	B	139	ab	B	153	a
160	B	139	bc	B	123	c	B	157	b	B	149	b	AB	190	a
240	A	166	bc	A	146	c	A	185	b	A	175	b	A	202	a
Slurry + fertiliser N: control (0 N): 121; combination (240 N): 232															

Capital letters show significance ($p \leq 0.05$) in vertical.
Small letters show significance ($p \leq 0.05$) in horizontal.

Annex C4-2 shows significant effects of the rate of slurry on total N yield of maize and Italian ryegrass in the first year and much smaller, often not significant effects in the second and third year. The rate of fertiliser N had a significant effect on total N yield in the second year and small effects in the first and third year. Both in the second and third year, there were significant differences in N yield among the plots that did not receive N, indicating possible effects of soil cultivation on N availability.