Fractions of Ruminant Feeds:

kinetics of degradation in vitro

Promotor:
Prof. dr. ir. S. Tamminga
Hoogleraar in de Diervoeding, Wageningen Universiteit

Copromotoren:
Dr. ir. A.F.B. van der Poel
Universitair Hoofddocent, leerstoelgroep Diervoeding,
Wageningen Universiteit

Dr. ir. H. Boer
Universitair Docent, leerstoelgroep Diervoeding,
Wageningen Universiteit

Samenstelling van de Promotiecommissie:
Prof. dr. ir. R.M. Boom, Wageningen Universiteit, Nederland

Prof. dr. ir. L.H den Hartog, Wageningen Universiteit, Nederland

Dr. ir. J.O. Goelema, De Heus Voeders, Nederland

Dr. B.A. Williams, The University of Queensland, Australia

Dit onderzoek is uitgevoerd binnen de onderzoekschool WIAS

Fractions of Ruminant Feeds:

kinetics of degradation in vitro

Arash Azarfar

Proefschrift
Ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. M. J. Kropff,
in het openbaar te verdedigen
op Vrijdag 29 Januari 2007
des namiddags te 16:00 uur in de Aula

Fractions of Ruminant Feeds: kinetics of degradation in vitro

Arash Azarfar, 2007.

PhD Thesis, Wageningen Institute of Animal Sciences, Animal Nutrition Group, Wageningen University, the Netherlands

ISBN: 90-8504-557-6

Abstract

A widely adopted procedure to characterise the degradation in the rumen and its dynamics is the *in situ* incubation technique that assumes the washable fraction of feeds (W) to be equal to the soluble (S) fraction and that both are rapidly and completely degraded which may not be the case. Because W fraction is washed out of nylon bags, their behaviour cannot directly be measured. This thesis, therefore, aimed to characterise the degradative behaviour of some important unprocessed and processed concentrate (barley, maize, milo, peas, lupins and faba beans) ingredients and their fractions, using a methodology that fractionates feed samples into its inherent constituents (non-washable, NWF; insoluble washable, ISWF and soluble washable fraction, SWF) in combination with an *in vitro* gas production technique. The results show that the size of the W fraction obtained in nylon bag studies differs from that of the soluble fraction. Except in lupins, ISWF of the concentrate ingredients was very rich in starch. SWF was relatively rich in ash, crude protein, soluble sugars, and a residual fraction (chemically not determined) but contained only a negligible quantity of starch. The degradative behaviour of this difference (ISWF), measured with *in vitro* gas production, is very similar to that of the non-washable fraction. The nature of the VFA profile resulting from the fermentation of the different fractions differs between fractions and changes with time of fermentation. Except in lupins, expander processing decreases the contribution of the truly soluble fraction (SWF) in concentrate ingredients. In maize, milo, peas and faba beans, gelatinised starch serves as a binding agent reducing the size of ISWF. However, the pelleting after expander processing disturbs the binding effect of gelatinised starch to some extent, and increases the size of ISWF. In all cereal grains, faba beans and lupins, thermo-mechanical processing (expander processing and ensuing pelleting) shows a tendency to increase the fractional rate of substrate degradation and rate of gas production at the early stage of fermentation, thereby shifting the pattern of fermentation towards a more glucogenic fermentation. Moreover, it appears that the expander processing and the ensuing pelleting process provides a certain level of protection to dietary protein as represented by a lowered NH_3-N production. Our data show that grinding the samples of technological processing changes the particle size distribution in the samples of processed material. As a consequence, the kinetics of gas production change. When monitoring the effects of feed processing by using an *in vitro* gas production technique, no grinding prior to *in vitro* incubation should be applied.

To my beloved family, Shabnam, Arsalan and Amir

Contents

General Introduction

General introduction

Feeding accounts for up to 60% of the costs of livestock production. An accurate evaluation of feeds is therefore important, because it provides nutritionists with the necessary information to formulate optimal diets from a physiological as well an economical point of view. Since the first ideas in 1725, when ruminant feeds were evaluated as straw unit,[1] laboratory methods to estimate the nutritive value of a feed have been improved. Initially, the techniques were designed mainly to characterise the nutritive value of feedstuffs rather than to predict animal performance. In the evaluation of feedstuffs many different methods are available. Frequently used procedures are proximate analysis or its alternative procedure for fibre [2-4] and modern instrumental techniques like Near Infrared Reflectance Spectroscopy (NIRS). Other developments include buffer solubility tests, a two-stage *in vitro* digestibility technique,[5] enzymatic methods,[6,7] a rumen simulation technique, [8] as well as a gas production technique. [9,10] All these methods are based on the principle of end point measurements and do not include the dynamics of digestion and utilisation in their result. More recent developments do include the dynamics of digestion.

A widely adopted procedure to characterise the degradation in the rumen and its dynamics is the *in situ* incubation technique. This technique is nowadays probably the most widely used method, however, some drawbacks have been pointed out.[11] The method is based on the inclusion of individual feedstuffs into porous bags which are incubated in the rumen of an animal fitted with a rumen fistula. The main objective is to measure the rate of disappearance of dry matter and/or other nutrients. In early experiments [12] silk bags were used to incubate samples. These were later replaced by other types of cloth, e.g. nylon, polyester or dacron. In 1977, Mehrez and Ørskov [13] proposed the use of the *in situ* method as a routine procedure for measuring protein degradation rate. Their approach is based on incubating several bags for different lengths of time in the rumen in order to obtain a kinetic evaluation of the degradation process. Ørskov et al. [14] have later suggested to use fermentation kinetics as a means to obtain data to improve the estimation of the nutritive value of feeds. In the method it is assumed that sample disappearance is synonymous with degradation. The equation which is used to determine the percent of degradation at any time as proposed by Ørskov and McDonald [15] is as follows:

$$P = W + D\,(1 - e^{-ct})$$

where P is the percentage of degradation at time t, W is the washable and D is the insoluble but potentially degradable fraction. The W+D is the potential degradability of the material, and c (or kd) is the degradation rate, expressed in fraction per unit of time, usually hour. In this equation it is assumed that the W (washable fraction) is equal to the soluble fraction (S) and that both are rapidly and completely degraded in the rumen. This assumption has been proven to be incorrect.[16-19] A further question is if the kinetics of degradation of S, W and W-S are the same.

A significant proportion of starches and proteins are present in the W fraction. Starch, a major storage component in the grains of higher plants, is a major source of energy in ruminant feeds. Degradation and digestion behaviour of starch in the rumen depends on many factors such as its origin,[20,21] chemical characteristics,[22] interactions with protein, antinutritional factors (ANF) and the physical form of feeds.[23] Protein is also an important ingredient, notably in legume seeds and its degradation behaviour in the rumen also depends on origin, chemical composition, the presence of ANF (notably tannins) and the physical form of feeds.[24,25]

Because W and S fractions are washed out of nylon bags, their behaviour cannot directly be measured and an alternative method has to be applied. For that purpose a new method of feed fractionation has to be used. This method should mimic the washout procedure with nylon bags, and can subsequently be used to establish the different fermentation characteristics of the non-washable (NWF), total washable (TWF), soluble washable (SWF) and insoluble washable (ISWF) fractions.[26] A suitable method to characterise the dynamic behaviour of feed fractions is an *in vitro* system, which measures gas production continuously.[27] A detailed description of the cumulative gas production system was given by Williams.[28]

Forages are the natural and cheap feed sources for ruminants. Therefore, it is more desirable to maximise the energy and protein intake through them rather than concentrates.[29] However, due to its bulkiness and sometimes a low dry matter (DM) content, to achieve a sufficiently high dry matter intake in high producing dairy cows is very difficult. This is specially the case in the early stage of lactation, resulting in a negative energy balance even when high quality forages are used. This has led to use of concentrate diets in high producing dairy cows. The main purpose of using such diets is to supply sufficient energy via carbohydrates (mainly starch) and proteins. The most important constituents of the concentrates are the cereal grains and legume seeds. Statistics show that in the developed countries cereal grains and grain legumes are mostly used in the feed industries rather than in food sectors (Table 1).

Table 1. Cereal grains and grain legumes used for animal feed and human food (Food balance sheet FAO 2002, http://faostat.fao.org/faostat)

	Barley		Maize		Milo		Pulses		Beans		Peas		Pulses, others	
	Feed (%)	Food (%)	Feed (%)	Food (%)	Feed (%)	Food (%)	Feed (%)	Food (%)	Feed (%)	Food (%)	Feed (%)	Food (%)	Feed (%)	Food (%)
World	66.4	5.3	64.1	17.6	44.2	43.7	19.9	68.1	8.1	80.5	42.8	45	19.2	68.5
Developed countries	72.8	1.7	72.6	5.1	85.0	5.5	53.3	33.3	16.3	76.5	62.3	21.2	66.4	21.5
Developing countries	49	15.2	55.2	30.6	36.5	50.9	11.5	76.9	6.8	81	17.8	75.7	13.1	74.5
Western Europe	77.3	0.9	77	5.4	98.3	0	66.1	26.3	16.7	77.6	74.6	18.3	65.8	25.2
The Netherlands	67.8	2.0	55.2	4	100	0	78.8	20.5	81.6	18.4	83.3	15.7	27.3	72.7
Iran	90.8	1.0	91.6	3.0	93.3	__	__	73.2	__	92.2	__	__	__	57.9

Legume seeds are important sources of proteins and energy in diets of ruminants. Nevertheless, there are some complications when they are included in the ruminants diet:

1) The high protein solubility and degradability in the rumen leading to asynchrony of protein and energy utilisation.[24,30]
2) The presence of anti-nutritional factors in the seed that may cause some problems , particularly in young animals.[31]

These problems can be alleviated either by adding a readily available source of carbohydrate such as cereal grains or applying some technological processes. Cereal grains are normally known as sources of readily available carbohydrates to supply the energy required by ruminants, but the whole grains is largely resistant to ruminal degradation because intact kernels are resistant to microbial attachment.[32] Therefore, prior to their use as an ingredients in ruminant diets, both cereal grains and legume seeds, nowadays undergo non-thermal (grinding, dry rolling) as well as thermal processing (dry: micronising, roasting, popping and wet: cooking, steam pelleting, steam flaking, autoclaving, toasting, expander processing, extruder).[33] These technological processes change the distribution of dry matter (DM), rate and extent of ruminal degradation of protein as well as starch by different ways like disrupting the protein matrix surrounding the starch granules, gelatinizing the starch, protein denaturation and changing the size of particles.

From this background this thesis aimed:

1. To develop a new laboratory method that mimics the result of the washing procedure in the *in situ* method and fractionates the whole grains into a non-washable (NWF), an insoluble washable (ISWF) and a soluble washable (SWF) fraction.

2. To characterise rumen degradation kinetics of these fractions in unprocessed grains (barley, corn, sorghum) and legume seeds (faba beans, peas, lupins).

3. To determine the chemical composition of these fractions by wet chemistry.

4. To determine the fermentation kinetics and the fermentation end products of these fractions with a combination of measuring the cumulative gas production, the organic matter disappearance and the volatile fatty acid (VFA) appearance.

5. To study the effects of expander and pelleting on DM distribution and fermentation characteristics of these concentrate ingredients and their fractions.

Outline of the thesis

Figure 1 gives a schematic overview of the contents of the thesis. The importance of developing a new method of fractionation that fractionates the whole grain into a NWF, ISWF and SWF and factors affecting the contribution of DM over the different fractions (NWF, ISWF and SWF) are described in **Chapter 1**. In **Chapter 2**, we characterised the degradative behaviour of different fractions of some concentrate ingredients using the *in vitro* gas production technique. The aim was to verify if the degradative behaviour of SWF differs distinctly from that of the NWF and ISWF and if the degradative behaviour of ISWF and NWF are similar. Moreover, **Chapter 2** gives an insight in the chemical constituents of the different fractions. Searching through the literature shows that a great deal of information is available about the effects of thermo-mechanical processes (such as expander processing and the ensuing pelleting) on the extent and site of digestion of starch and protein in ruminants. Yet, little is known about the effect of such a thermo-mechanical process on the contribution to DM of the different fractions of ruminant feedstuffs, their chemical composition and degradative behaviour. **Chapters 3** and **4** of this dissertation, therefore, are devoted to the clarification of the effects on degradative behaviour of the different fractions of some processed cereal grains and grains of legume seeds. The results of **Chapters 3** and **4** led us to carry out an experiment, of which the results are presented in **Chapters 5**. The results presented in **Chapters 5** answers the question if the ground technological processed samples are representatives of the original samples. Finally, the **General Discussion**, gives an overall view of the results presented in **Chapters 2** to **5,** and the implications of the results are discussed.

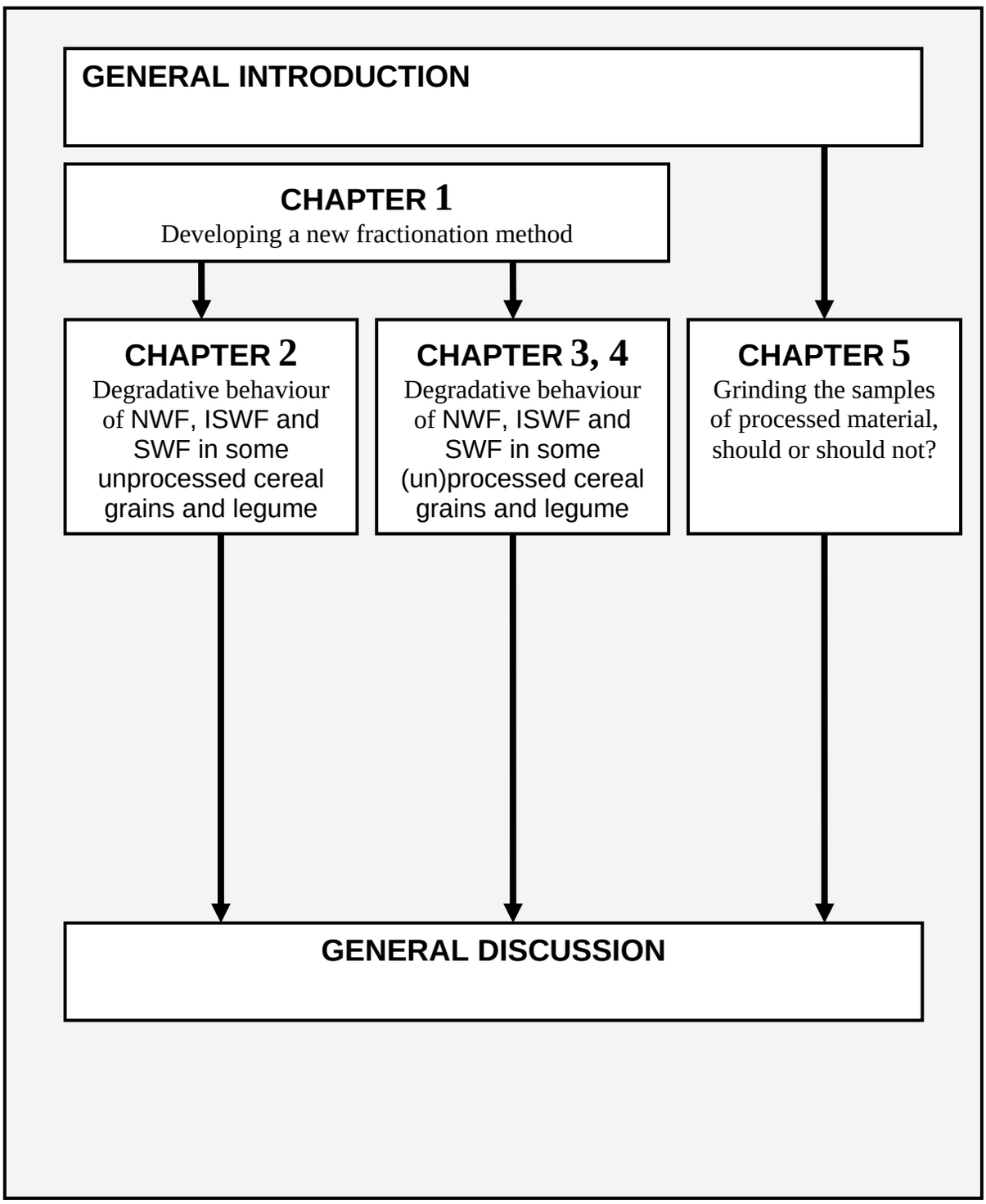


Figure 1. Schematic illustration of thesis outline.

References

1. Blaxtre K, *Energy Metabolism in Animals and Man*, Cambridge University Press, Cambridge (1989).

2. Van Soest PJ, Development of a comprehensive system of feed analysis and its application to forages. *J Anim Sci* **26**:119-128 (1967).

3. Georing HK and Van Soest PJ, *Forage Fibre Analysis (Apparatus, Reagents, Procedures and Some Applications)*, Vol 379, ARS, USDA, Washington, DC (1970).

4. Van Soest PJ, Robertson JB and Lewis BA, Methods for dietary fibre, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**:3583-3597 (1991).

5. Tilley JMA and Terry RA, A two stage technique for the *in vitro* digestion of forage crops. *J Br Grassl Soc* **18**:104-111 (1963).

6. Jones DIH and Hayward MV, The effect of pepsin pretreatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. *J Sci Food Agric* **26**:711-718 (1975).

7. Broderick GA and Cochran RC, *In vitro* and *in situ* techniques for estimating digestibility with reference to protein degradability, in *Feeding Systems and Feed Evaluation Methods*, Ed by Theodorou MK and France J. CABI publishing, Wallingford, pp 53-86 (2000).

8. Czerkawski JW and Breckenridge G, Design and development of a long term rumen simulation technique (Rusitec). *Br J Nutr* **38**:371-384 (1977).

9. Menke KH, Rabb L, Salewski A, Steingass H, Fritz D and Schneider W, The estimation of the digestibility and metabolisable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *J Agric Sci* **93**:374-379 (1979).

10. Menke KH and Steingass H, Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Anim Res Dev* **28**:7-55 (1988).

11. Michalet-Doreau B and Ould-Bah MY, *In vitro* and *in sacco* methods for estimation of dietary nitrogen degradability in the rumen: a review. *Anim Feed Sci and Technol* **40**:57-86 (1992).

12. Quin JI, Van der Walt JG and Myburgh S, Studies on alimentary tract of Merino sheep in South Africa. 4. Description of experimental technique. *J Vet An Ind* **11**:341-360 (1938).

13. Mehrez AZ and Ørskov ER, A study of the artificial bag technique for determining the digestibility of feeds in the rumen. *J Agric Sci* **88**:645-650 (1977).

14. Ørskov ER, Reid GW and Kay M, Prediction of intake by cattle from degradation characteristics of roughages. *An Prod* **46**:29-34 (1988).

15. Ørskov ER and McDonald I, The estimation of protein degradation in the rumen form incubation measurements weighted according to rate of passage. *J Agric Sci* **92**:499-503 (1979).

16. Lopez S, France J and Dhanoa MS, A correction for particular matter loss when applying the polyester bag method. *Br J Nutr* **71**:135-137 (1994).

17. De Boever JL, Vanacker JM, Bogarets DF and Boucque CV, Protein evaluation of cattle compound feeds: Comparison of *in sacco* measurements and tabular values. *Neth J Agric Sci* **43**:297-311 (1995).

18. De Boever JL, Cottyon BG, Vanacker JM and Boucque CV, Potential of solubility, enzymatic methods and NIRS to predict *in situ* rumen escape protein. *Neth J Agric Sci* **45**:291-306 (1997).

19. Madsen J, AAT/PBV protein evaluation system for ruminants. A revision. *Norw J Agri Sci* **19**:1-37 (1995).

20. Tamminga S, van Vuuren AM, van der Koelen CJ, Ketelaar RS and van der Togt PL, Ruminal behaviour of structural carbohydrates, non-structural carbohydrates and crude protein from concentrate ingredients in dairy cows. *Neth J Agric Sci* **38**:513-526 (1990).

21. Nocek JE and Tamminga S, The prediction of nutrient supply to dairy cows from the rate and extent of ruminal degradation of ration components. *J Dairy Sci* **74**:3598-3629 (1991).

22. Rooney LW and Pflugfelder RL, Factors affecting starch digestibility with special emphasis on sorghum and corn. *J Anim Sci* **63**:1607-1623 (1986).

23. Gallant DJ, Bouchet B, Buelon A and Perez S, Physical characteristics of starch granules and susceptibility to enzymatic degradation. *Eur J Clin Nutr* **46**:3-16 (1992).

24. Van Straalen W and Tamminga S, Protein degradability in ruminant diets, in *Feed Evaluation*, Ed by Wiseman J and Cole DJA. Butterworth, London, pp 55-72 (1990).

25. Goelema JO, *Processing of Legume Seeds: Effects on Digestive Behaviour in Dairy Cows*, PhD Thesis, Wageningen University, Wageningen (1999).

26. Yang HJ, Tamminga S, Williams BA, Dijkstra J and Boer H, *In vitro* gas and VFA production profiles of barley and corn and their soluble washout fractions after feed processing. *Anim Feed Sci Technol* **120**:125-140 (2005).

27. Davies ZS, Mason D, Brooks AE, Griffith GW, Merry RJ and Theodorou MK, An automated system for measuring gas production from forages inoculated with rumen fluid and its use in determining the effect of enzymes on grass silage. *Anim Feed Sci Technol* **83**:205-221 (2000).

28. Williams BA, Cumulative gas production techniques for forage evaluation, in *Forage Evaluation in Ruminant Nutrition*, Ed. by Givens DI, Owen E, Axford RFE and Omed HM. CABI Publishing, Bangor, pp. 189-213 (2000).

29. Taweel ZH, *Perennial Ryegrass for Dairy Cows: Grazing Behaviour, Intake, Rumen Function and Performance*, PhD Thesis, Wageningen University, Wageningen, pp 156 (2004).

30. Kozlowaska H, Nutrition, in *Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics*, Ed by Hedley CL. CABI publishing, Wallingford2001).

31. Hill GD, Tamminga S, The Effects of antinutritional factor in legume seeds and rapeseeds on ruminant nutrition, in *Recent Advances of Research in Antinutirtional Factors in Legume Seeds and rapeseed*. EAAP Publication **93**: 157-172 (1998).

32. Beauchemin KA, McAllister TA, Dong Y, Farr BI and Cheng KJ, Effects of mastication on digestion of whole cereal grains by cattle. *J Anim Sci* **72**:236-245 (1994).

33. Tóthi R, *Processed Grains as a Supplement to Lactating Dairy Cows*, PhD Thesis, Wageningen University, Wageningen (2003).

Chapter 1

Effects of washing procedure, particle size and dilution on the distribution between non-washable, insoluble washable, and soluble washable fractions in concentrate ingredients♣

Abstract

The effects of washing procedure, particle size and dilution on the distribution of non-washable (NWF), insoluble washable (ISWF) and soluble washable (SWF) fractions were studied. The effects of three washing procedures (Yang (Y), Melin (M) and *in situ* (IS)) on the size of NWF, ISWF and SWF in six concentrate ingredients (maize, barley, milo, peas, lupins and faba beans), ground at two different particle sizes, were compared. Method M was further developed (method SM) by reducing the dilution ratio; its effect on NWF, ISWF and SWF was compared. A new washing method was developed (method AA); its effect on NWF, ISWF and SWF at different dilutions with water was compared with the IS, M, SM and Y methods. The effects of different dilutions on SWF and soluble true protein (STP) in six concentrate ingredients were studied. The effects of grain, washing method and particle size on the size of NWF and ISWF were significant, with significant interactions between grain and particle size, grain and washing method, particle size and washing method, but no interaction between grain type, washing method and particle size. In method Y the size of NWF was smaller than in the other methods. The results showed that, except in lupins, NWF in grains was significantly higher than in legume seeds. Increasing the particle size significantly increased NWF, whereas ISWF was decreased. The size of SWF in legume seeds was higher than in the grains. Increasing the dilution, increased STP in legume seeds, but not in grains.

♣ Arash Azarfar, Seerp Tamminga and Huug Boer
2006. **Journal of the Science of Food and Agriculture**, Accepted for publication.

Introduction

Nowadays, most available information on the degradative behaviour of feeds in ruminants is based on *in situ* incubation in the rumen. This method divides major feed fractions like starch, proteins and cell walls in a washable (W), a non-washable but degradable (D) and an undegradable (U) fraction.[1] In almost all feed evaluation systems presently in use, the W fraction of protein and starch is assumed to be equal to the soluble fraction (S) and both are assumed to be rapidly and completely degraded. This simplification has been adopted because the *in situ* technique cannot measure the rate at which the W fraction of protein and starch is actually degraded. Therefore, much information is available on the degradative behaviour in the rumen of starches, proteins and cell walls present in the D fraction, but not in the W fraction. It has become apparent that part of the W fraction in fact consists of small insoluble particles and their degradative behaviour is not known,[2–4] but is likely to be more similar to the D fraction than to the S fraction. Some values for the degradation rate for this fraction have been reported and modifications have been made for particle loss,[4,5] but it is still assumed that the washed out particles are degraded at a rate similar to that of the fraction remaining in the bag after washing.[6] The lack of information on the degradative behaviour of the W fraction has encouraged ruminant nutritionists to develop new methods of feed fractionation. Recently, two simple fractionation methods were developed by which we can separate the W fraction into an insoluble washable fraction (ISWF) and a soluble washable fraction (SWF).[7,8] The aims of this study were to:

1. Further develop a new fractionation method.

2. Study the effect of different washing procedures (Method M, filtration according to the procedure of Melin et al.[7]; Method SM, developed M method; Method Y, [8] repeated plunging of nylon bags in a glass beaker; Method IS, washing in a washing machine according to the Dutch protocol [9] for the *in situ* technique; method AA, continuous washing of nylon bags in a centrifuge beaker) on the size of NWF, ISWF and SWF of protein and starch present in some concentrate ingredients (maize, barley, milo, peas, lupins and faba beans).

3. Study the effect of different dilutions (defined as the proportion of consumed water (ml) to sample weight (g); 5, 10, 20, 30 and 40) on the size of SWF, and soluble true protein (STP) in some concentrate ingredients.

Material and Methods

The experiments were carried out in three stages. In the first stage, the effects of two different washing procedures (methods M and Y) on six different concentrate ingredients (maize, barley, peas, lupins, faba beans and milo) and two different sieve opening sizes (1 and 3 mm) on the size of NWF, ISWF and SWF were studied, and the results for NWF compared with those obtained *in situ* (IS). In the second stage, it was attempted to reduce the amount of water used in the washing procedure and method M was modified and a new fractionation method (method AA) was developed and the effects of different dilution ratios on the size of NWF, ISWF and SWF studied. In the third stage, the effects of different dilutions (5, 10, 20, 30 and 40) on the size of SWF, STP and the soluble non-protein fraction (SNPF) were studied.

Sample preparation and chemical analyses

Barley, maize, milo, peas (yellow peas), lupins (a mixture of white and spotted lupins) and round-seeded brown faba beans were supplied from a commercial supplier (Research Diet Services, Wijk bij Duurstede, The Netherlands). The air dry samples were ground in a laboratory grinder (Retsch ZM100, Haan, Germany) to pass through 1 and 3 mm sieves.

The dry matter (DM) content was determined by drying to constant weight at 103 °C for 4 h followed by equilibration in a desiccator (ISO 6496). Ash was determined after incineration for 4 h at 550 °C (ISO 5984), crude fat (CFAT) by extraction with petroleum ether (ISO 6492) and crude protein (CP) by a Kjeldahl method (ISO 5983). Starch content was measured colorimetrically as described by Goelema et al. [10] The sugar content was determined after extracting feed samples with ethanol (400 ml L^{-1}). Carbohydrates in the filtrate were hydrolysed with 0.1 N HCl and after using free sugars as a reducing agent to oxidise copper, the content was measured using a spectrophotometer at 460 nm. [11] Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined with a Fibretec™ (Foss, Denmark) fibre analyser using reagents described by Van Soest et al.[12] Sodium sulphite and amylase were used in the NDF determination.

Experiment 1

This experiment aimed to clarify if the results of previously developed washing procedures (M and Y) in determining the size of NWF resemble the result of the washing

procedure in the IS method and to elucidate if the particle size and grain type had any effect on the size of NWF, ISWF and SWF. In a 3 × 6 × 2 factorial arrangement of treatments with three replicates, the effects of three washing procedures (IS, M and Y) on the size of the NWF and ISWF in six concentrate ingredients (maize, barley, milo, peas, lupins, faba beans) with two particle sizes (1 and 3 mm) were studied.

The size of the NWF and the washable fraction (W) using the IS method was determined according to the Dutch protocol.[9] In brief the principles of the IS methods are as follows. Ground concentrate ingredients were weighed into nylon bags (8 × 15 cm inner size; pore size 40 μm; PA 40/30, Nybolt, Switzerland). To obtain a sample, a surface area ratio of approximately 20 mg cm^{-2}, 5 g of DM per sample, was weighed into each bag. The bags were then washed in a programmable washing machine (AEG Turnamat, Germany) with cold tap water using the gentle 'wool wash' program. The nylon bags and its contents were then freeze-dried to determine the size of the NWF.

In method Y, a 100-g sample of a ground concentrate ingredient (1 and 3 mm) was weighed into a nylon bag (16 × 23 cm inner size; pore size 40 μm; PA 40/30, Nybolt, Switzerland). The bag was placed in a 3-l beaker (height 27 cm) filled with 1 l of de-mineralized water at room temperature. To mimic the standard washing procedure of the *in situ* (IS) technique, the bag was frequently lifted above and submerged into the water for 1 h by an eccentric rod driven by a stirring motor at a speed of 40 rpm. After removal of the bag with its residual contents, to mimic filtration, the liquid mixture in the beaker was centrifuged at 715 × *g* for 20 min in a Beckman 2-21M centrifuge.

After centrifugation, the supernatants were filtered through a fast filter paper (Schleicher & Schuell, Folded Filters, 520 1/2, Ø185 mm, Dassel, Germany), the filtrates pooled together, and dispersed into a pre-weighed aluminium container to be freeze-dried. This yielded the soluble washout fraction (SWF). The pellet inside the centrifuge tubes and the residues on the filter paper were collected in a pre-weighed aluminium container for freeze-drying, which resulted in the insoluble washable fraction (ISWF). The non-washable fraction (NWF), i.e., the residue left inside the nylon bag, was collected and also freeze-dried.

In method M, 3 g of each sample was weighed into a 250-ml beaker and soaked in 25 ml of distilled water during constant shaking at a rate of 150 rpm for 1 h. After soaking, the samples were poured through a funnel with a filter (a piece of nylon bag cloth, 20 × 20 cm; pore size 40 μm; PA 40/30, Nybolt, Switzerland) fixed into the funnel's brim with paper clips. Beakers were washed twice with 10 ml of de-mineralized

water to get the remaining feed onto the filters. The samples in the filter were stirred vigorously with a spatula until water passed through the filters. Then, the samples were rinsed with 10 ml of de-mineralized water. Rinsing was repeated twice. After the final rinse, the samples were left to drip for 15 min to ensure that all rinsing water had passed the filer. Filters and the residue inside the filter were dried at 70 °C for at least 12 h. The residue inside the filter was assumed to be the NWF. The resulting water was centrifuged as in Method Y and the pellet in the centrifuge tubes (ISWF) was collected for freeze-drying into pre-weighed aluminium containers with some water.

Experiment 2

The aim of this experiment was to study the possibility of reducing the dilution in method M and its effect on the size of NWF, ISWF and SWF, and to develop a new fractionation method (method AA) at the laboratory of Animal Nutrition Group of Wageningen University. In a 7 × 6 factorial arrangement of treatments with three replicates the effects of seven washing methods (IS, M, SM, AA10, AA20, AA30 and Y) on the size of NWF, ISWF and SWF in the six concentrate ingredients, ground over a sieve of 3 mm, were studied.

Method SM was almost identical to method M except that the dilution was 25 in method M and 12.5 in method SM. In the AA methods, 5.5 g of feed sample were weighed into a pre-weighed nylon bag. The bags were put into a polypropylene centrifuge tube and distilled water was added to the centrifuge tubes to reach dilutions of 10 g (AA10), 20 g (AA20) and 30 g (AA30) of water per gram of feed. The tubes were then shaken in a shaking bath at 150 rpm for 1 h. The nylon bags were then removed from the centrifuge tubes and their outside rinsed with a small quantity of distilled water. The tubes were then centrifuged at 715 × *g* for 20 min in a Beckman 2-21M centrifuge.

After centrifugation, the supernatant was filtered through a fast filter paper (Schleicher & Schuell, Folded Filters, 520 1/2, Ø185 mm, Dassel, Germany). The resulting water, assumed to be the SWF, was decanted into pre-weighed aluminium containers and freeze-dried to determine the size of the SWF. The pellets in the tubes and the residues on the filter paper were collected, placed into aluminium containers with a small amount of distilled water, and freeze-dried to determine the size of the ISWF.

Experiment 3

The aim of experiment 3 was to elucidate the response of SWF and soluble true protein (STP) with changing the dilution. In a 5 × 6 factorial arrangement of treatments with three replicates the effect of five different dilutions (5, 10, 20, 30 and 40) on the size of the SWF and STP in the six concentrate ingredients, ground over a sieve of 3 mm, was studied. The size of STP was determined according to Melin et al.[7] with some modifications. In brief, 1 g of each sample was weighed accurately into a 50 ml propylene centrifuge tube and distilled water added to each tube to reach a dilution of 5, 10, 20, 30 and 40. The contents of the centrifuge tubes were left to soak while shaking at 150 rpm for 1 h in a shaking bath. The tubes were then centrifuged at 1225 × *g* for 20 min in a Beckman 2-21M centrifuge. The supernatant was filtered through a paper filter (Schleicher & Schuell, 589/ Black Ribbon, Ø185 mm, Dassel, Germany) to separate the solid particles and the resulting liquid was assumed to be the soluble fraction. After filtration, the supernatant was decanted into a 50 ml polypropylene centrifuge tube. To precipitate the STP, an amount of 300 g L^{-1} TCA was added to the centrifuge tube to reach a final concentration of 50 g L^{-1}. The contents of the tubes were left for 2 h at 4 ºC to allow complete precipitation. The tubes were then centrifuged at 1225 × *g* for 20 min. The pellet, assumed to contain all the STP, was freeze-dried and the size of the STP was determined.

Statistical analysis

Data were analysed using the GLM procedure of SAS 9.1 (2002). The models applied were:

$$Y = \mu + G_i + S_j + W_k + (GS)_{ij} + (GW)_{ik} + (SW)_{jk} + (GSW)_{ijk} + \varepsilon_{ijkl} \quad (1)$$

$$Y = \mu + G_i + W_j + (GW)_{ij} + \varepsilon_{ijk} \quad (2)$$

$$Y = \mu + G_i + D_j + (GD)_{ij} + \varepsilon_{ijk} \quad (3)$$

Models 1 and 2 were used to analyse the data of experiments 1 and 2, respectively. To analyse the data of experiments 3 and 4, models 2 and 3 were used. In all experiments, Y is the dependent variable (the size of the NWF and ISWF), μ is the overall mean, G_i is the grain effect (i = 6, maize, barley, peas, lupins, faba beans and milo), S_j is the particle size effect (j=2, 1 and 3 mm), W_k is the effect of the washing procedure (k = 3, 4 and 7 in experiments 1, 2 and 3, respectively) and D_j is the dilution effect (D = 3; 10, 20, 30 in

experiment 3 and D_i = 5; 5, 10, 20, 30 and 40 in experiment 4), and ε_{ijkl} and ε_{ijk} are the error terms.

Results and discussion

Chemical composition

The chemical composition of the concentrate ingredients are shown in Table 1. The values agree with the tabular values[13] and those reported in other studies.[8,110,14] The sugar content was higher for the legume seeds than for the grains (Table 1).

Table 1. Chemical composition of maize, barley, milo, peas, lupins, faba beans

	Maize	Barley	Milo	Peas	Lupins	Faba bean
DM (g kg^{-1})	947.0	934.9	937.6	957.6	955.0	957.4
In dry matter (g kg^{-1})						
OM	933.6	911.8	918.9	926.0	924.0	926.8
Ash	13.4	23.1	18.7	31.6	31.0	30.6
CP	92.0	125.1	111.9	227.4	344.8	325.7
Crude fat	48.0	18.7	39.0	9.7	59.6	11.9
Starch	701.0	548.2	700.3	469.9	9.1	390.4
ADF	26.3	61.0	39.5	72.9	213.4	108.3
NDF	90.5	156.8	70.6	90.5	248.4	116.7
Sugars	19.1	27.9	8.7	54	58.3	37.7
Residue[1]	36.0	100.2	51.2	116.9	248.8	87

[1]Calculated as 1000 – (ash + crude protein + crude fat + starch + NDF + sugars).

This is because legumes contain more oligosaccharides than cereals.[15,16] The results of chemical analysis revealed a residual unknown fraction in all concentrate ingredients. The magnitude of this residual fraction was higher for the legume seeds than for the grains. However, a substantial unknown residual fraction was also found in barley. The values of the residual fraction in barley and maize and the figures given for β-glucan and

soluble non-cellulosic carbohydrates (S-NCP) showed good agreement.[17] The NCPs are the most abundant non-starch carbohydrates (NSP) in both cereal grains and legume seeds.[17] The values of residual fraction in the legume seeds were consistent with figures given for total soluble and insoluble NCPs.[17] It has been reported that water-soluble NSP and water-insoluble pectic substances are lost in the NDF procedure.[18,19] Because legume seeds contain more NSP and pectins than grains,[17] this explains the higher residual fraction in the legume seeds compared to grains. The most prominent aspect of the chemical composition of lupins is a negligible level of starch and high levels of soluble and insoluble NSP.[20]

Experiment 1: effects of different washing procedure, grain type and particle size on the size of NWF and ISWF

The effects of washing procedure, grain type and particle size on the size of the NWF and ISWF are shown in Table 2. The effects of washing method and particle size of grain on the NWF and ISWF were always significant. In agreement with results reported in other studies,[21,22] fine grinding increased the size of the W fraction either by rupturing cell walls and releasing more soluble nutrients or by making particles small enough to be washed out.[23] In this study, decreasing the particle size from 3 to 1 mm increased the size of the SWF (calculated as 1000 – (NWF + ISWF)) as well as that of the ISWF (157.2 vs 148.5 g kg^{-1} DM of whole fractions for 1 and 3 mm particle size, respectively). These results agree with previously published results.[22,24]

The NWF in method Y was smaller than in the other methods (535.5, 580.5 and 604.2 g kg^{-1} DM in Y, M and IS, respectively) indicating that Y was the most intensive of the three methods. Despite an almost equal sample dilution (25 and 30 in methods M and Y, respectively) and a higher ratio of sample weight (mg) to surface area (cm^2) of the filter, in method Y (7.5 and 135 in methods M and Y, respectively), the smaller NWF in method Y than in M indicates that washing duration (2 h vs. 1 h in methods Y and M) can be an important factor affecting not only particle loss, but also the solubility of feed samples. The latter is confirmed by the larger size of the SWF (174 g kg^{-1} DM) in method Y than that in method M (164 g kg^{-1} DM). Regression analysis showed a high correlation between methods M and Y with method IS (Figure 1), although statistical analysis showed the size of the NWF in method Y to be significantly smaller than that in methods IS and M (Table 2).

Table 2. Effects of different washing procedures, grain type and particle size on the size of the non-washable (NWF, g kg^{-1} DM) and insoluble washable fraction (ISWF, g kg^{-1} DM)

Class		NWF	ISWF
Washing method	IS	604.2[a]	–
	M	580.5[b]	256.4[a]
	Y	535.5[c]	291.0[b]
	SEM	1.8	2.8
Grain type	Maize	774.6[a]	154.8[a]
	Barley	680.7[b]	248.2[b]
	Milo	613.2[c]	326.8[c]
	Peas	334.5[d]	409.3[d]
	Lupin	659.4[e]	130.0[e]
	Faba bean	377.7[f]	373.4[f]
	SEM	2.5	4.9
Particle size	1 mm	543.9[a]	298.9[a]
	3 mm	602.9[b]	249.0[b]
	SEM	1.4	2.8
Significance (*P*)	Washing method (W)	<0.001	<0.001
	Grain (G)	<0.001	<0.001
	Particle size (S)	<0.001	<0.001
	G × W	<0.001	<0.001
	G × S	<0.001	<0.001
	S × W	<0.05	<0.05
	G × S × W	NS	NS

NS, non-significant ($P > 0.05$). SEM, standard error of mean. Lower case superscripts in the same column within a class followed by a different letter are significantly different ($P < 0.05$).

Except in lupins, the size of the NWF in grains was significantly larger than in legume seeds (Table 2). The higher NWF in grains can be explained by a lower ISWF and a lower SWF (Tables 2 and 3). Except in lupins, ISWF in legume seeds was higher than in grains. Presumably due to the milling, a larger quantity of fine particles is produced in some legumes than in grains.

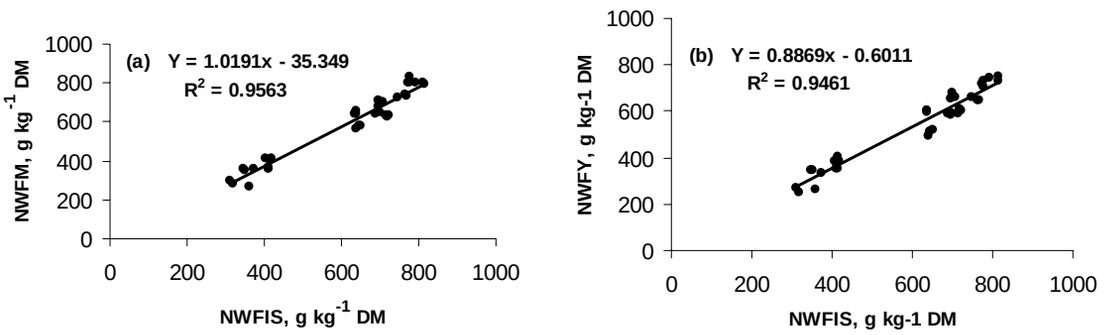


Figure 1. Relationship between the size of the non-washable fraction (NWF) in method IS (NWFIS) with methods M (NWFM) and Y (NWFY) in experiment 1

In grains as well as in legume seeds, the ISWF contributed significantly to the W fraction, indicating that milling through a 1 or 3 mm sieve produces particles small enough to pass through the pores of the nylon bag. The higher ISWF in the legume seeds than in cereal grains can also be explained by the differences between their seeds microstructure. Except in lupins, cells in the legume seeds are loosely packed in the central part of endosperm with large intercellular spaces, whereas in cereal grains cells are tightly packed with small intercellular spaces. [25,26] These differences between the structure of endosperm in legumes and cereal grains cause to produce finer particle during the grinding of legume seeds compared to cereal grains. In a recent study (**Chapter 5**) using the dry sieve analysis, we determined the particle size distribution of samples of barley and peas ground through a 1 and 3 mm sieve. Indeed we demonstrated that the size of particles smaller than 0.071 μm in the samples of peas ground through a 1 and 3 mm sieve were significantly larger than in barley (24.1 vs. 11.7 % in the samples ground through a 1 mm sieve; 22.7 vs. 9.6 % in the samples ground through a 3 mm sieve).

Experiment 2: comparison between methods I, M, SM, Y and AA

The effects on the NWF, ISWF and SWF of grain type, washing method (method IS, AA10, AA20, AA30, M, SM and Y) and the interaction between grain and washing method are shown in Table 3 and all were found to be significant. The largest NWF was observed with method AA10 (Table 3). The higher NWF in method AA10 than in method SM, despite having almost the same dilution (10 vs. 12.5 in method AA10 and SM, respectively) indicates that the washing procedure is an important factor which influences the size of the fractions in the feeds. There was no significant difference in the size of the NWF between methods IS and AA30 (Table 3).

Table 3. Effects of different washing procedure and grain type on the size of non-washable (NWF, g kg^{-1} DM), insoluble washable (ISWF, g kg^{-1} DM) and soluble washable (SWF) fractions

Class		NWF	ISWF	SWF
Method	IS	635.1[a]	–	–
	AA10	675.3[b]	207.6[e]	111.3[f]
	AA20	650.2[c]	212.3[e]	137.6[d]
	AA30	628.5[a]	225.1[d]	146.7[c]
	M	605.9[d]	235.4[c]	158.6[b]
	SM	599.2[d]	278.9[a]	122.12[e]
	Y	567.5[e]	261.7[b]	170.9[a]
	SEM	2.8	3.4	1.8
Grain	Maize	791.2[a]	147.9[d]	65.3[e]
	Barley	722.2[b]	209.9[c]	75.86[d]
	Milo	654.2[c]	284.8[c]	73.9[d]
	Peas	413.4[d]	347.7[a]	237.2[a]
	Lupins	705.2[e]	111.3[e]	183.3[c]
	Faba bean	425.6[f]	345.3[a]	224.5[b]
	SEM	2.4	3.1	1.9
Significance	Grain (G)	<0.001	<0.001	<0.001
	Washing method (W)	<0.001	<0.001	<0.001
	G × W	<0.001	<0.001	<0.001

SEM, standard error of mean. Lower case superscripts in the same column within a class followed by a different letter are significantly different ($P < 0.05$).

Regression analysis revealed that methods AA20 and AA30 were more similar to method IS than the other methods (Figure 2). Therefore, methods AA20 and AA30 can be used as alternative methods to mimic the IS method.

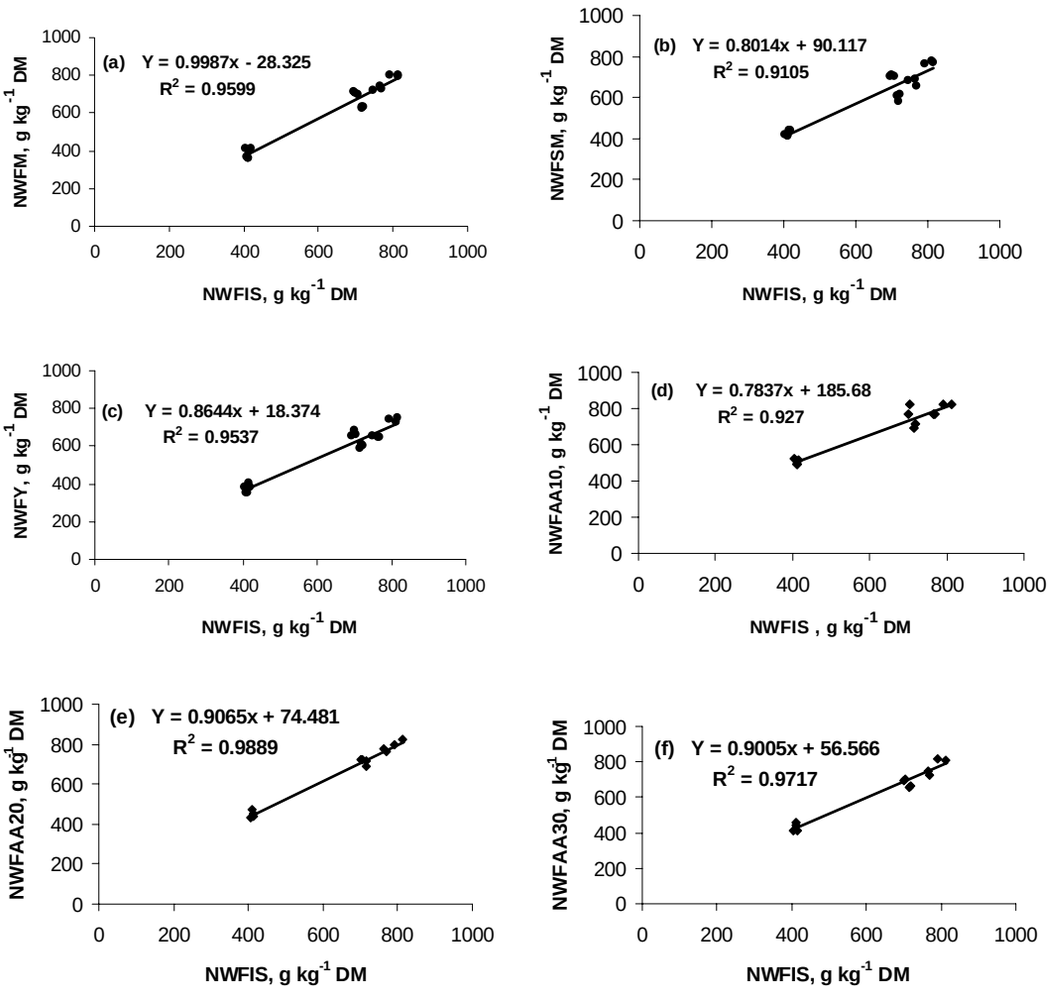


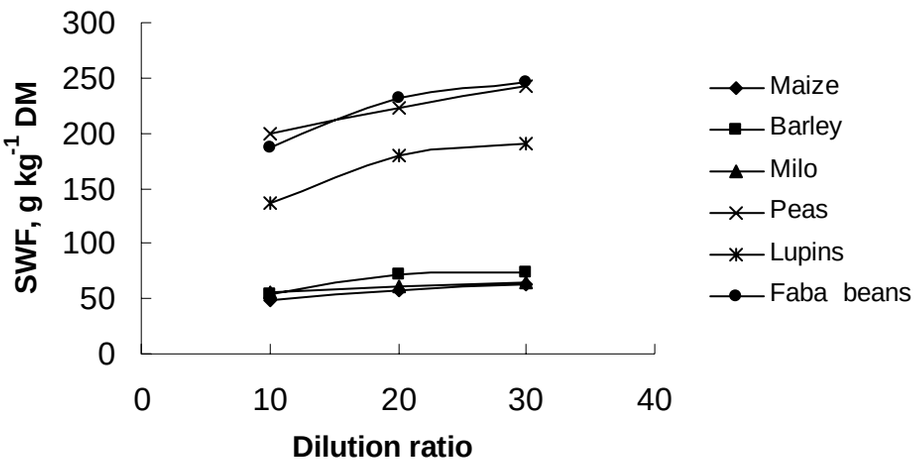
Figure 2. Relationship between the size of the non-washable fraction (NWF) in method IS (NWFIS) with methods M (NWFM), SM (NWSM), Y (NWFY) and AA with dilution ratios of 10 (NWFAA10), 20 (NWFAA20) and 30 (NWFAA30) in experiment 2

The size of the ISWF in method SM was larger than in methods M, AA10, AA20, AA30 and Y, whereas the smallest SWF was found with method SM (Table 3). The largest ISWF and the smallest SWF in method SM indicated that a significant quantity of the W fraction comprises fine particles, small enough to pass through the pores of the nylon bag. As shown in Table 3, the size of the SWF in legume seeds was significantly larger than that in grains, which is due to a higher quantity of soluble proteins in legume seeds than in the grains.[27]

Increasing the dilution ratio in method AA significantly increased the SWF, whereas its effect on the ISWF was less pronounced (Table 3).

Increasing the dilution level in legume seeds increased the SWF, whereas its effect on the SWF in grains was not remarkable (Figure 3). Therefore, it can be concluded that

choosing an appropriate dilution ratio in the concentrate ingredients with a high quantity of SWF (legume seeds) to mimic the results of method IS is of a great importance.



A

Figure 3. Effect of different dilution ratios on the soluble washable fraction (SWF) in different grains in experiment 2

Experiment 3: effect of different dilution ratios on SWF, STP and SNPF

The effects of grain, level of dilution and the interaction of grain and dilution were significant for SWF, STP (expressed as a g kg^{-1} DM of whole grains and as a g kg^{-1} DM of the SWF) and the soluble non-protein fraction SNPF (Table 4). Regression analysis showed a high correlation between the sizes of SWF determined by methods AA in Experiment 2 with those determined in this experiment (R^2 = 0.93, data are not shown), which indicates that the size of SWF in experiment 3 was determined accurate enough.

Not surprisingly, the quantity of STP in legume seeds was significantly higher than in grains, whereas the size of the SNPF in grains was higher than that in legume seeds (Table 4). These results agree with the results of Olaisen *et al*[28], but the SWF in their study was higher than in ours. The higher value for the SWF in the study of Olaisen *et al.* [28] might be due to a smaller particle size of the feeds in their study than in the present study (1.5 vs 3 mm). Although some methods have been developed to determine the solubility and particle loss, none of them used the same particle size, level of dilution or solvent. Therefore, the results are not comparable.[6,23,28,29] In all these methods, particle loss was measured as the difference between W and solubility, which were measured using two different methods, W with the *in situ* method and solubility in a simple filtration method. This may create an extra source of error.

Table 4. Effects of different dilution levels on the soluble washable (SWF), soluble true protein (STP) and soluble non-protein (SNPF) fraction in different grains

Class		SWF[1]	STP[1]	STP[2]	SNPF[2, 3]
Dilution level	5	103.3^{c}	30.0^{c}	196.0^{c}	804.0^{a}
	10	123.9^{b}	50.3^{b}	300.5^{a}	699.5^{c}
	20	121.6^{b}	50.5^{b}	302.2^{a}	697.8^{c}
	30	134.0^{a}	58.3^{a}	281.2^{ab}	718.8^{cb}
	40	137.3^{a}	60.2^{a}	274.0^{b}	72.6^{b}
	SEM	1.8	1.4	6.9	6.9
Grain	Maize	55.9^{d}	1.9^{d}	344.0^{e}	965.6^{a}
	Barley	77.6^{c}	5.8^{d}	76.9^{d}	923.1^{b}
	Milo	57.4^{d}	25.0^{d}	43.2^{e}	956.8^{a}
	Peas	204.0^{a}	118.3^{b}	574.1^{b}	425.9^{d}
	Lupins	144.8^{b}	37.0^{c}	249.7^{c}	750.3^{c}
	Faba bean	204.0^{a}	133.6^{a}	646.2^{a}	353.8^{e}
	SEM	2.0	1.5	7.6	7.6
Significance (*P*)	Grain	<0.001	<0.001	<0.001	<0.001
	Dilution level	<0.001	<0.001	<0.001	<0.001
	Grain × Dilution	<0.001	<0.001	<0.001	<0.001

SEM, standard error of mean. Lower case superscripts in the same column within a class followed by a different letter are significantly different ($P < 0.05$).

[1] As g kg^{-1} DM of whole fractions.

[2]As g kg^{-1} DM of SWF.

[3] Calculated as 1000- (STP (as g kg^{-1} DM of SWF)).

In our study it is shown that the washing method has a significant effect on the size of the NWF, ISWF and SWF. By increasing the dilution, STP increased in legume seeds, but no remarkable response to increasing the dilution ratio was seen in grains (Figure 4).

Higher sensitivity of the legume seeds than the grains in response to change in the dilution ratio can be ascribed to a higher STP in such concentrate ingredients.

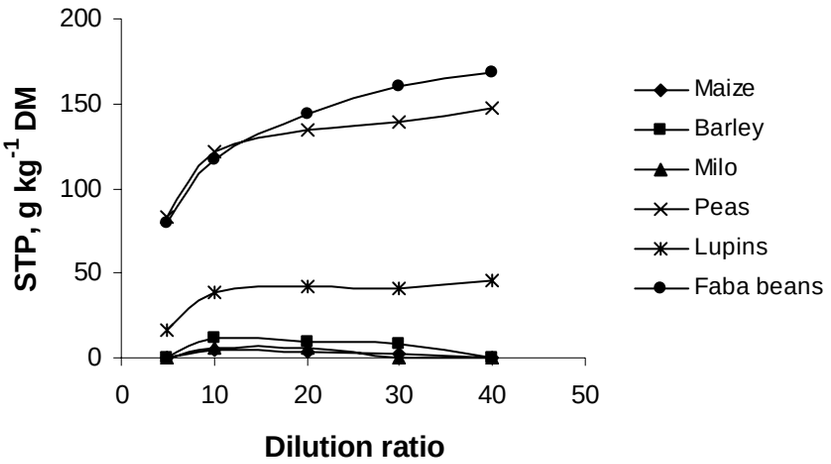


Figure 4. Effect of different dilution ratios on the size of soluble true protein (STP, as percentage of whole fractions) in different grains in experiment 3

A high correlation was observed between the SWF and STP (Figure 5), indicating that the higher SWF in the legume seeds is due to a higher quantity of soluble proteins in legume seeds than in the grains. In legume seeds, 85–100% of the protein is in albumins and globulins, none in prolamins, and 0–15% in glutelins, whereas in grain seeds 10–20% of the protein is in albumins and globulins and the remaining (80–90%) is equally distributed between prolamins and glutelins.[27]

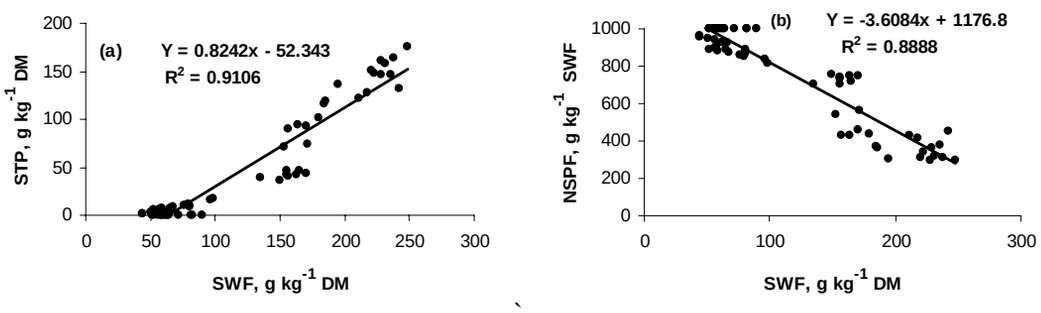


Figure 5. Relationships of soluble true protein (STP, g kg^{-1} DM of whole fractions) and soluble non-protein (SNPF, g^{-1} kg DM of SWF) with the soluble washable (SWF) fraction in experiment 3

The size of the SWF in lupins was lower than that in the other legume seeds. This can be explained by the higher NDF content (Table 1) and the structure of lupin proteins. The storage proteins in lupins are mainly globulins, and this fraction is higher in lupins and soybeans than in most other legumes.[30] The fact that the storage proteins in lupins

are predominantly globulins suggests that they have lower solubility than a legume with higher levels of albumins such as peas and faba beans.[31,32] Of the grains, maize had the highest NWF (Table 3). Almost 60% of protein in maize is in zein, the prolamine fraction of corn protein, which consists of a series of disulfide-linked oligomers that make it less soluble and degradable in comparison with gliadins.[33,34] Zeins have many unique characteristics resulting mainly from their highly hydrophobic nature. They can form water-resistant films which make them less soluble.[35]

In addition to the nature of the proteins, carbohydrates may have a prominent role in determining the size of the SWF in feeds. The results of some studies show that the starch from peas and faba beans is easily washed out from nylon bags.[29,36] However, in a recent study (**Chapter 2**), a negligible quantity of starch was recovered in the SWF indicating a very low solubility of starch granules. Despite a higher NSP[17,37] and sugars (Table 1) reported for lupins than for peas and faba beans, the size of the SWF for lupins was significantly lower than that of peas and faba beans (Tables 2 and 3). Moreover, despite having a higher content of NSP and sugars the size of the SWF in barley was almost similar to that of maize and milo. Therefore, it can be concluded that carbohydrates have only a minor effect on the size of the SWF in feeds.

Conclusions

The results show that methods AA20 and AA30 were the best methods to mimic the washing procedure due to ease of handling and similarity to the conventional washing procedure in the *in situ* technique (method IS). Our data show that the soluble non-protein fraction has a remarkable contribution in the SWF. Therefore, its importance for determining the size of the SWF and its chemical composition has to be elucidated.

Acknowledgements

The first author thanks the Ministry of Science, Research and Technology of Iran for financing the project. The authors wish to express their gratitude to the staff of the laboratory of the Animal Nutrition Group; without their help, this study would not have been possible.

References

1. Lopez S, Dijkstra J and France J, Prediction of energy supply in ruminants, with emphasis on forages, in *Forage Evaluation in Ruminant Nutrition*, Ed by Givens DI, Owen E, Axford RFE and Omed HM. CAB International, Wallingford, pp 63-94 (2000).

2. De Boever JL, Cottyon BG, Vanacker JM and Boucque CV, Protein evaluation of cattle compound feeds: comparison of *in sacco* measurements and tabular values. *Neth J Agric Sci* **43**:297–311 (1997).

3. Lopez S, France J and Dhanoa MS, A correction for particular matter loss when applying the polyester bag method. *Br J Nutr* **71**:135–137 (1994).

4. Madsen J, The AAT/PBV protein evaluation system for ruminants. A revision. *Norw J Agri Sci* **Suppl 19**:1–37 (1995).

5. Weisbjerg MR, Hvelpund T and Bibby BM, Hydrolysis and fermentation rate of glucose, sucrose and lactose in the rumen. *Acta Agric Scand* **48**:12–18 (1998).

6. Hvelpund T and Weisbjerg MR, *In situ* techniques for the estimating of protein degradability and postrumen availability, in *Forage Evaluation in Ruminant Nutrition*, Ed by Givens DI, Owen E, Axford RFE and Omed HM. CAB International, Wallingford, pp 233–258 (2000).

7. Melin M, Gierus M, Meijer GAL and van Vuuren AM, Chemical characteristics and degradability of crude protein in S-fraction of feedstuffs, ID-Leylstad, Leylstad, The Netherlands, Report No. 2079 (2001).

8. Yang HJ, Tamminga S, Williams BA, Dijkstra J and Boer H, *In vitro* gas and volatile fatty acid production profiles of barley and maize and their soluble and washout fractions after feed processing. *Anim Feed Sci Technol* **120**:125–140 (2005).

9. CVB, Centraal Veevoeder Bureau, Voorloping protocol voor *In situ* pensincubatie voor het meten van eiwitbestendigheid, Centraal Veevoeder Bureau, Lelystad, The Netherlands (2003).

10. Goelema JO, Spreeuwenberg MAM, van der Poel AFB and Tamminga S, Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. *Anim Feed Sci Technol* **76**:35–50 (1998).

11. Rijnen MMJA, Verstegen MWA, Heetkamp MJW and Schrama JW, Effects of dietary fermentable carbohydrates on energy metabolism in group-housed sows. J Anim Sci **79**:148-54 (2001).

12. Van Soest PJ, Robertson JB and Lewis BA, Methods for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**:3583–3597 (1991).

13. CVB, Centraal Veevoeder Bureau, Veevoedertabel, gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen. Centraal Veevoeder Bureau, Lelystad, The Netherlands (2004).

14. Tóthi R, Lund P, Weisbjerg MR and Hvelpund T, Effect of expander processing on fractional rate of maize and barley starch degradation in the rumen of dairy cows estimated using rumen evacuation and in situ techniques. *Anim Feed Sci Technol* **104**:71–94 (2003).

15. Kozlowska H, Nutrition, in *Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics*, Ed by Hedley CL. CABI Publishing, Wallingford, pp 322 (2001).

16. Kent NL, *Technology of Cereals*, 3 edn, Pergamon Press, Oxford, pp 221 (1983).

17. Bach Knudsen KE, Carbohydrate and lignin contents of plants materials used in animal feeding. *Anim Feed Sci Technol* **67**:319–338 (1997).

18. Carre B and Brillote JM, Yeild and composition of cell wall residues isolated from various feedstuffs used for non-ruminants farm animals. *J Sci Food Agric* **37**:341–351 (1986).

19. Reichert RD, Quantitative isolation and estimation of cell wall materials from dehulled pea (*Pisum sativum*) flours and concentrates. *Cereal Chem* **58**:266–270 (1981).

20. Petterson D, Sipas S and Mackintosh JB, *The Chemical Composition and Nutritive Value of Australian Pulses*, 2nd edn. Grains Research and Development Corporation, Canberra (1997).

21. Nocek JE, *In situ* and other methods to estimate ruminal protein and energy digestibility: a review. *J Dairy Sci* **71**:2051–2069 (1988).

22. Michalet-Doreau B and Ould-Bah MY, *In vitro* and *in sacco* methods for the estimation of dietary nitrogen degradability in the rumen: a review. *Anim Feed Sci Technol* **40**:57–86 (1992).

23. De Boever JL, Cottyon BG, Vanacker JM and Boucque CV, Potential of solubility, enzymatic methods and NIRS to predict *in situ* rumen escape protein. *Neth J Agric Sci* **45**:291–306 (1997).

24. Michalet-Doreau B and Cerneau P, Influence of foodstuff particle size on *in situ* degradation of nitrogen in the rumen. *Anim Feed Sci Technol* **35**:69–81 (1991).

25. Otto T, B B-K and Czuchajowska Z, Microstructure of seeds, flours, and starches of legumes. *Cereal Chem* **74**:445-451 (1997).

26. Pomeranz Y and Czuchajowska Z, Structure of coarse and fine fractions of corn samples ground on the Severt hardness tester. *Food Microstructure* **4**:213-219 (1985).

27. Van Straalen W and Tamminga S, Protein degradation of ruminant diets, in *Feedstuff Evaluation*, Ed by Wiesman J and Cole DJ. Butterworths, London, p 55 (1990).

28. Olaisen V, Mejdell T, Volden H and Nesse N, Simplified *in situ* method for estimating ruminal dry matter and protein degradability of concentrates. *J Anim Sci* **81**: 520–528 (2003).

29. Walhain P, Foucart M and Thewis A, Influence of extrusion on ruminal and intestinal disappearance in sacco of pea (*Pisum sativum*) proteins and starch. *Anim Feed Sci Technol* **38**:43–55 (1992).

30. Adsule RN and Kadam SS, Proteins: nutritional chemistry, processing technology and utilization, in *Handbook of World Food Legumes*, Ed by Salunkhe DK and Kadam SS. CRC Press, Boca Raton, FL, pp 75–98 (1989).

31. Gueguen J, Legume seeds protein extraction, processing and end product characteristics. *Plant Foods for Human Nutrition* **32**:267–275 (1983).

32. van Barneveld RJ, Understanding the nutritional chemistry of lupin (*Lupinus* spp.) seed to improve livestock production efficiency. *Nutr Res Rev* **12**:203–230 (1999).

33. Paulis JW, Disulfide structures of zeins proteins from corn endosperm. *Cereal Chem* **58**:542–546 (1981).

34. Sommer A, Chrenokova Z and Peisker M, Effect of pressure conditioning on ruminal protein degradation in selected raw materials. New Aspects of Compound Feed Processing, Proceedings of the 3rd International Kahl Symposium, Hamburg, Germany, November 1994, 13 pp (1994).

35. Batterman-Azcona SJ, Lawton JW and Hamker BR, Effect of specific mechanical energy on protein bodies and a-zeins in corn flour extrudates. *Cereal Chem* **76**:316–320 (1999).

36. Nocek JE and Tamminga S, Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. *J Dairy Sci* **74**:3598–3629 (1991).

37. Czukor B, Processing, in *Carbohydrates in Grain Legume Seed: Improving Nutritional Quality and Agronomic Characteristics*, Ed by Hedley CL. CABI, Wallingford, pp 322 (2001).

Chapter 2

In vitro gas production profile and the formation of end products from non-washable, insoluble washable and soluble washable fractions in some concentrate ingredients[♣]

Abstract

A procedure that mimics washing in the in situ incubation technique, combined with an in vitro gas and volatile fatty acids (VFA) production technique, was used to verify the assumption that rumen degradation behaviour of material washed out of nylon bags is instantaneous and complete. In a 6×4 factorial arrangement of treatments with three replicates, fractions of maize, barley, milo, yellow peas, lupins (a mixture of white and spotted lupins) and round-seeded brown faba beans were subjected to an in vitro incubation technique. Fractions were whole (WHO), non-washable (NWF), insoluble washable (ISWF) and soluble washable (SWF)) fraction. In a manually operated in vitro fermentation system, another 24 samples of the same substrates were fermented for VFA and ammonia analysis. Except in lupins, ISWF in the concentrate ingredients was very rich in starch. SWF was relatively rich in ash, crude protein, soluble sugars, and a residual unknown fraction but contained only a negligible quantity of starch. Thus, the fermentation characteristics of ISWF were more like WHO and NWF than SWF. Total gas production of SWF was considerably lower than the other fractions. A very rapidly degradable fraction was seen in the first phase of degradation of SWF. The pattern of fermentation end-product formation for SWF differed from that of the other fractions.

[♣] Azarfar, A., Williams, B. A., Boer, H. and Tamminga, S. 2006. **Journal of the Science of Food and Agriculture**, Accepted for publication.

Introduction

High producing dairy cows have a high requirement for energy supplied by nutrients that differ in nature. Nowadays, it has become common practice to divide energy for dairy cows into ketogenic, glucogenic and aminogenic energy. The ratio in which such nutrients are supplied to organs and tissues is highly influenced by microbial fermentation in the rumen. Although dynamic models to describe rumen fermentation and to predict the quantity and quality of the nutrients resulting from it have been developed,[1,2] as yet, insufficient information is available on the conversions and type of nutrients supplied by different feed fractions that differ in degradative behaviour in the rumen. The available information is mainly based on in situ incubations in the rumen, which measure the disappearance of feed fractions rather than the appearance of nutrients. This method divides major feed fractions like starch, proteins and cell walls into washable (W), non-washable but degradable (D) and undegradable (U) fractions. Much information is available on the degradative behaviour in the rumen of starches, proteins and cell walls present in the D fraction, but this is not the case for these components in the W fraction. It has become apparent that part of the W fraction may in fact consist of small insoluble particles and their degradative behaviour is likely to be more similar to the D fraction than to the W fraction.[3,4] The combination of feed fractionation that separates the W fraction into an insoluble washable (ISWF) and a soluble washable fraction (SWF), and *in vitro* gas and VFA production techniques is considered a promising method to characterise the degradative behaviour of these fractions in feedstuffs.[5–7] Therefore, the current study aimed to characterise the degradative behaviour and the end products of the non-washable fraction (NWF), ISWF and SWF in some concentrate ingredients (maize, barley, milo, yellow peas, lupins (a mixture of white and spotted lupins) and round-seeded brown faba beans.

Materials and Methods

The experiment was carried out in two stages. The first stage was a preliminary study, carried out to establish the possible effects of different sample weights, volume of medium and volume of rumen fluid on the pattern of production of volatile fatty acids (VFA) through the incubation of the sample in a manually operated in vitro fermentation system. In the second stage, the degradative behaviour of different concentrate

ingredients (maize, barley, milo, peas, lupins and faba beans) and their fractions (whole (WHO), NWF, ISWF and SWF) were studied *in vitro*.

Experiment 1

The aim of this study was to investigate the possibility of reducing the quantity of sample and medium used in an in vitro gas production study and the effects on the resulting end-products. An insufficient yield of SWF from the fractionation led us to do preliminary experiments. In a completely randomised design with three replicates, the effects of five treatments on the pH and VFA production throughout a 48-h incubation in a temperature-controlled incubator were studied in whole maize. The treatments included a combination of different sample weights, volume of medium and volume of rumen inoculum (control, 0.5 g sample + 82 ml medium B[8] + 5 ml rumen inoculum; A, 0.1 g sample + 20 ml medium B + 1.25 ml rumen inoculum; B, 0.05 g sample + 20 ml medium B + 1.25 ml rumen inoculum; C, 0.05 g sample + 10 ml medium B + 0.6 ml rumen inoculum; D, 0.05 g sample + 8 ml medium B + 0.6 ml rumen inoculum). In each treatment, the production VFA was corrected for a blank.

Experiment 2

In a 6 × 4 factorial arrangement of treatments with three replicates in two runs, samples of six concentrate ingredients (maize, barley, milo, peas, lupins and faba beans) and four fractions (WHO, NWF, ISWF and NWF) were subjected to an in vitro incubation technique, which measures gas production continuously in an automated system (APES-IGER, Aberystwyth, Wales) for 72 h. In a manually operated in vitro fermentation system, the production of VFA and ammonia (NH_3-N) were simultaneously measured at 0, 3, 6, 12, 24, and 48 h. The whole concentrate ingredients were included in each *in vitro* incubation run as a control throughout the runs to establish possible effects of the runs on gas production.

Fractionation of concentrate ingredients in different fractions

Fractionation of concentrate ingredients into NWF, ISWF and SWF has been described in detail by Azarfar et al.[5] To fractionate the whole grain, 5.5 g of a feed sample was weighed into a pre-weighed nylon bag. The bag was put into a polypropylene centrifuge tube and distilled water was added to reach a dilution of 20 ml of water per gram of feed. The tube was then shaken in a shaking bath at 150 rpm for 1 h. Then, the

nylon bag was removed from the centrifuge tube and rinsed on the outside with a small quantity of distilled water. The tube was then centrifuged at 715×*g* for 20 min in a Beckman 2-21M centrifuge. After centrifugation, the supernatant was filtered through a fast filter paper (Schleicher & Schuell, Folded Filters, 520 1/2, diameter 185 mm, Dassel, Germany). The resulting water, assumed to be the SWF, was decanted into a pre-weighed aluminium container and freeze-dried to determine the size of the SWF. The pellet in the tube and the residue on the filter paper were collected, put into an aluminium container with a small amount of distilled water, and freeze-dried to determine the size of the ISWF.

In vitro fermentation and chemical analysis

The cumulative gas production technique of Theodorou,[9] as modified by Williams et al.[10], was used to determine the fermentation characteristics of the concentrate ingredients and their different fractions (WHO, NWF, ISWF and SWF). The whole experiment was conducted in two runs; an automated pressure system (APES)[11] and a manually operated in vitro fermentation system were applied simultaneously. Three replicates of each substrate were fermented in specific 100-ml APES bottles containing 0.5 g dry matter (DM) of each substrate, 82 ml of medium B and 5 ml of rumen fluid inoculum in the automated pressure system for 72 h. Meanwhile, in the manually operated *in vitro* fermentation system, another 24 samples of the same substrates were fermented up to a maximum of 48 h. These samples, except for the SWF, containing 0.5 g DM of each substrate, 82 ml of medium B and 5 ml of the same rumen fluid inoculum, were also fermented in 100-ml serum bottles and kept in a temperature-controlled incubator. Because of an insufficient amount of SWF, only 0.05 g of SWF samples were incubated with 10 ml of medium B and 0.6 ml of the same rumen inoculum. The inoculum was a mixture of rumen fluid obtained from three non-lactating cows, fed once daily on a diet of moderate quality ryegrass hay (crude protein 100 g kg^{-1}; organic matter: 920 g kg^{-1} DM) ad libitum and 1 kg of a commercial concentrate. The sample of rumen fluid for the inoculum was taken at 08.00 h, prior to feeding. In order to stop the fermentation at 0, 3, 6, 12, 24 and 48 h, at each time point, bottles with each substrate were taken out and immediately autoclaved at 130 °C and 2.0 kg cm^{-2} steam pressure for 30 min. After cooling, a sub-sample of 0.5 ml for measuring VFA was withdrawn and added to a 1 ml Eppendorf tube containing 25 µl of phosphoric acid (850 g L^{-1}). A second sub-sample of 1 ml, to which 1 ml of trichloroacetic acid (TCA; 100 g L^{-1}) was added, was taken for measurement of NH_3-N. The remainder of the fermentation mixture was used to

determine pH. After incubation in the APES, the amount of fermented organic matter (FOM) after 72 h incubation was calculated as the difference between incubated and residual organic matter (OM). The latter was determined by filtering the contents of the APES bottles through sintered glass crucibles (Schott Duran, porosity # 2, Mainz, Germany), and drying, followed by ashing at 530 °C.

Chemical analysis

Feeds and fractions were analysed for dry matter (DM), ash, crude fat (CFAT) crude protein (CP), neutral detergent fibre (NDF), starch and reducing sugars.

The dry matter (DM) content was determined by drying to constant weight at 103 °C for 4 h followed by equilibration in a desiccator (ISO 6496). Ash was determined after incineration for 4 h at 550 °C (ISO 5984), crude fat (CFAT) by extraction with petroleum ether (ISO 6492) and crude protein (CP) by a Kjeldahl method (ISO 5983). Starch content was measured colorimetrically as described by Goelema et al. [12] The sugar content was determined after extracting feed samples with ethanol (400 ml L^{-1}). Carbohydrates in the filtrate were hydrolysed with 0.1 N HCl and after using free sugars as a reducing agent to oxidise copper, the content was measured using a spectrophotometer at 460 nm. [13] Neutral detergent fibre (NDF) was determined with a Fibretec™ (Foss, Denmark) fibre analyser using reagents described by Van Soest et al.[14] Sodium sulphite and amylase were used in the NDF determination. Dietary fibre (DF, g kg^{-1} DM) in WHO calculated as 1000 – (ash + CP+ CFAT + starch + sugars).

VFA were measured using gas liquid chromatography (Packard 419 glass column, CE instruments, Milan, Italy) filled with Chromosorb 101, carrier gas N_2 saturated with methanoic acid, at 190 °C, with iso-caproic acid as an internal standard. Acetic (HAc), propionic (HPr), *iso*-butyric (i-HBu), butyric (HBu), *iso*-valeric (i-HVal) and valeric (HVal) acid concentrations were expressed in mmol g^{-1} OM incubated. The NH_3-N concentration was expressed in mg g^{-1} OM incubated. The ratio of non-glucogenic to glucogenic acids (NGR) was calculated using the following equation:

$$NGR = \frac{HAc + 2 \times HBu + Hval}{HPr + Hval}$$

which was adapted from Ørskov.[15] Additionally, *iso*-butyric and *iso*-valeric acid were considered to originate primarily from the degradation of protein and were expressed as a proportion of the total VFA, called the branched chain ratio (BCR).

Kinetic model analysis

Gas production profiles, obtained with the automated system, were fitted by iteration for individual incubation flasks to the multi-phasic model as described by Groot et al.[16] and shown in the following equation:

$$\mathrm{OMCV} = \sum_{i=1}^{n} \frac{A_i}{1 + (C_i / t)^{B_i}}$$

where i is the number of phases, OMCV is the cumulative gas production at time t (ml g^{-1} OM incubated), A_i is the estimated asymptotic gas production in phase i (ml g^{-1} OM incubated) at time t, B_i represents the sharpness of the switching characteristic for the profile and C_i is the time (h) of incubation at which half of the asymptotic gas production has been formed. The maximum fractional rate of substrate degradation was calculated according the following equation:

$$R_{\max} S = \frac{(B-1)^{(B-1)/B}}{C}$$

where $R_{max}S$ is the maximum fractional rate of substrate degradation, B is the switching characteristic, and C the time (h) at which half of the asymptote (A) is reached.

Statistical anlaysis

The distribution of the mean square error (MSE) of the three models (mono-phasic, MSE1; di-phasic, MSE2; tri-phasic, MSE3) are presented using box-and-whisker plots.[17]

In this study, the VFA, NH_3-N and OM degradation data at 72 h were obtained from the 72-h automated in vitro gas production. Data from the first experiment were analysed using the GLM procedure of SAS 8.2. The model applied was:

$$Y = \mu + T_i + \varepsilon_{ij}$$

where Y is the dependent variable under examination (pH and VFA production), μ is the overall mean, T_i is the treatment effect (i=5), and ε_{ij} is the error term.

Analysis of variance for cumulative gas production, parameter fittings of the results of cumulative gas production, VFA production, NH_3-N production, NGR, BCR and disappearance of OM in the second experiment, were done using the GLM procedure of SAS 9.1 (2002). The models applied were:

$$Y = \mu + G_i + F_j + (GF)_{ij} + \varepsilon_{ijk} \quad (1)$$

$$Y = \mu + G_i + F_j + T_k + (GF)_{ij} + (GT)_{ik} + (FT)_{jk} + (GFT)_{ijk} + \varepsilon_{ijkl} \quad (2)$$

where Y is the dependent variable under examination, μ is the overall mean, G_i is the grain effect (i = 6; maize, barley, milo, peas, lupins and faba beans), F_j is the fraction type effect (j = 4; WHO, NWF, ISWF, SWF), T_k is the time type effect (k = 5; 0, 3, 6, 24 and 72 h), ε_{ijk} and ε_{ijkl} are the error term. Differences between individual substrates were analysed by a multiple comparison test (Duncan).

Results

The contribution of the fractions (WHO, NWF, ISWF and SWF) in the concentrate ingredients and the chemical composition of the concentrate ingredients and their fractions are shown in Table 1. The size of NWF in grains (barley, maize, milo) and lupins was much larger than in the two legume seeds (peas, faba beans). Except in lupins, the size of the ISWF was considerably larger than that of SWF. SWF was small (4–5%) in grains, but much larger (16–22%) in legume seeds. Striking differences between fractions were observed for chemical composition. NDF was almost entirely recovered in the NWF. The SWF contained virtually no starch, a considerable quantity of reducing sugars and a large residual fraction (12–45%) of unknown origin especially in the grains. Except in lupins, the ISWF contained a high quantity of starch. Compared to the original material (WHO), the SWF contained higher levels of ash specifically in the grains. In lupins, a large residual fraction with an unknown origin was observed, not only in SWF, but also in the other fractions.

Experiment 1

The results of the first experiment are shown in Table 2. There was no significant difference between the control treatment and treatment D. In the other cases, the total VFA was significantly different among the treatments. Surprisingly, the total VFA

production in treatment C was higher than that in the other treatments. Treatment had no significant effect on pH.

Table 1. Chemical composition (g kg^{-1} DM) of the concentrate ingredients and their fractions

		% of total	OM	Ash	Starch	CP	CFAT	NDF	Sugar	DF[1]	Residue[2]
Maize	WHO	100	933.6	13.4	701.0	92.0	48.0	90.5	19.1	126.5	36.0
	NWF	82.5	953.1	5.2	759.6	91.8	ND	103.1	1.1	NC	39.2
	ISWF	13.1	964.1	5.1	765.2	60.9	ND	19.5	4.5	NC	144.8
	SWF	4.4	805.9	184.8	5.2	134.9	ND	ND	334.2	NC	340.9
Barley	WHO	100	911.8	23.1	548.2	125.1	18.7	156.8	27.9	257	100.2
	NWF	76.7	939.9	18.6	563.2	128.9	ND	191.1	9.2	NC	89.0
	ISWF	18.0	966.8	6.3	817.1	81.3	ND	16.9	2.2	NC	76.2
	SWF	5.3	834.9	152.6	6.1	156.0	ND	ND	328.1	NC	357.2
Milo	WHO	100	918.9	18.7	700.3	111.9	39.0	70.6	8.7	121.4	50.8
	NWF	70.2	941.9	9.7	729.6	135.4	ND	90.1	0.5	NC	34.7
	ISWF	24.3	961.8	6.6	896.5	38.7	ND	19.0	0.7	NC	38.5
	SWF	5.5	788.0	196.7	18.9	187.3	ND	ND	134.7	NC	462.4
Peas	WHO	100	926.0	31.6	469.9	227.4	9.7	90.5	54	207.4	116.9
	NWF	41.3	903.3	20.3	480.6	147.7	ND	231.4	16.1	NC	103.9
	ISWF	37.0	906.1	11.2	769.5	139.4	ND	22.3	10.4	NC	47.2
	SWF	21.7	886.9	89.9	1.6	491.8	ND	ND	253.7	NC	163
Lupins	WHO	100	924.0	31.0	9.1	344.8	59.6	248.4	58.3	497.2	248.8
	NWF	70.4	919.5	19.9	5.9	279.0	ND	344.8	22.5	NC	327.9
	ISWF	13.6	928.8	18.7	5.2	380.9	ND	280.5	16.6	NC	298.1
	SWF	16.0	897.5	86.3	0.9	266.0	ND	ND	284.2	NC	362.6
Faba beans	WHO	100	926.8	30.6	390.4	325.7	11.9	116.7	37.7	203.7	87
	NWF	43.2	904.2	19.2	368.9	218.2	ND	285.5	11.7	NC	96.5
	ISWF	37.9	906.8	9.5	461.6	251.8	ND	189.7	7.9	NC	79.5
	SWF	18.9	878.5	93.4	0.7	589.5	ND	ND	169.4	NC	147

ND, not determined; NC, not calculated; [1] Dietary fiber (DF) calculated as 1000 - (ash + CFAT + CP + starch + sugar). [2]Calculated as 1000 – (ash + CP + CFAT + starch + NDF + sugars).

Table 2. Effects of different volumes of medium and inoculum, and sample weight on the concentration of VFA (mmol g^{-1} DM) and pH

Treatment	HAc	HPr	iHBu	HBu	iVal	Hval	Total	pH
Control	4.3^{e}	2.9^{a}	0.073^{cd}	1.35^{b}	0.094^{cd}	0.166^{cd}	8.9^{d}	7.00^{a}
A	5.6^{b}	2.8^{a}	0.123^{b}	1.31^{b}	0.161^{b}	0.256^{b}	10.3^{b}	6.8^{a}
B	7.9^{a}	3.0^{a}	0.163^{a}	1.78^{a}	0.303^{a}	0.357^{a}	13.4^{a}	7.03^{a}
C	5.2^{c}	2.6^{b}	0.094^{c}	1.36^{b}	0.130^{bc}	0.2^{bc}	9.6^{c}	6.9^{a}
D	4.9^{d}	2.6^{b}	0.053^{d}	1.37^{b}	0.066^{d}	0.113^{d}	9.1^{d}	6.7^{a}

Means in the same column followed by a different letter are significantly different (P<0.05).

Experiment 2

Additivity of treatments and fractionation

Gas production was measured and calculated for WHO on the basis of the contributions of NWF, ISWF and SWF. No significant differences between measured and calculated amounts were found (data are not shown).

Gas production results

Figure 1 shows the gas production profiles for the concentrate ingredients and their fractions. In all concentrate ingredients, the SWF tended to be degraded more rapidly than the other fractions. Total gas production of SWF was considerably lower than that of WHO, NWF and ISWF. Except in lupins, the pattern of gas production of the ISWF was more similar to WHO and NWF than to SWF.

In Figure 2, the distribution of the MSE of the three models (mono-phasic, MSE1; di-phasic, MSE2; tri-phasic, MSE3) are presented using box-and-whisker plots.[17] The distribution of MSE1 is skewed towards larger values. The large outliers of MSE1 were due to ill-fitting of SWF gas production profiles. The results presented in Figure 2 suggest that the di- and tri-phasic models are superior. However, Figure 2 shows that the di-phasic model describes the profiles of gas production quite accurately. Although in all substrates a third phase could be discriminated mathematically, whether this phase is biologically relevant has to be investigated further.

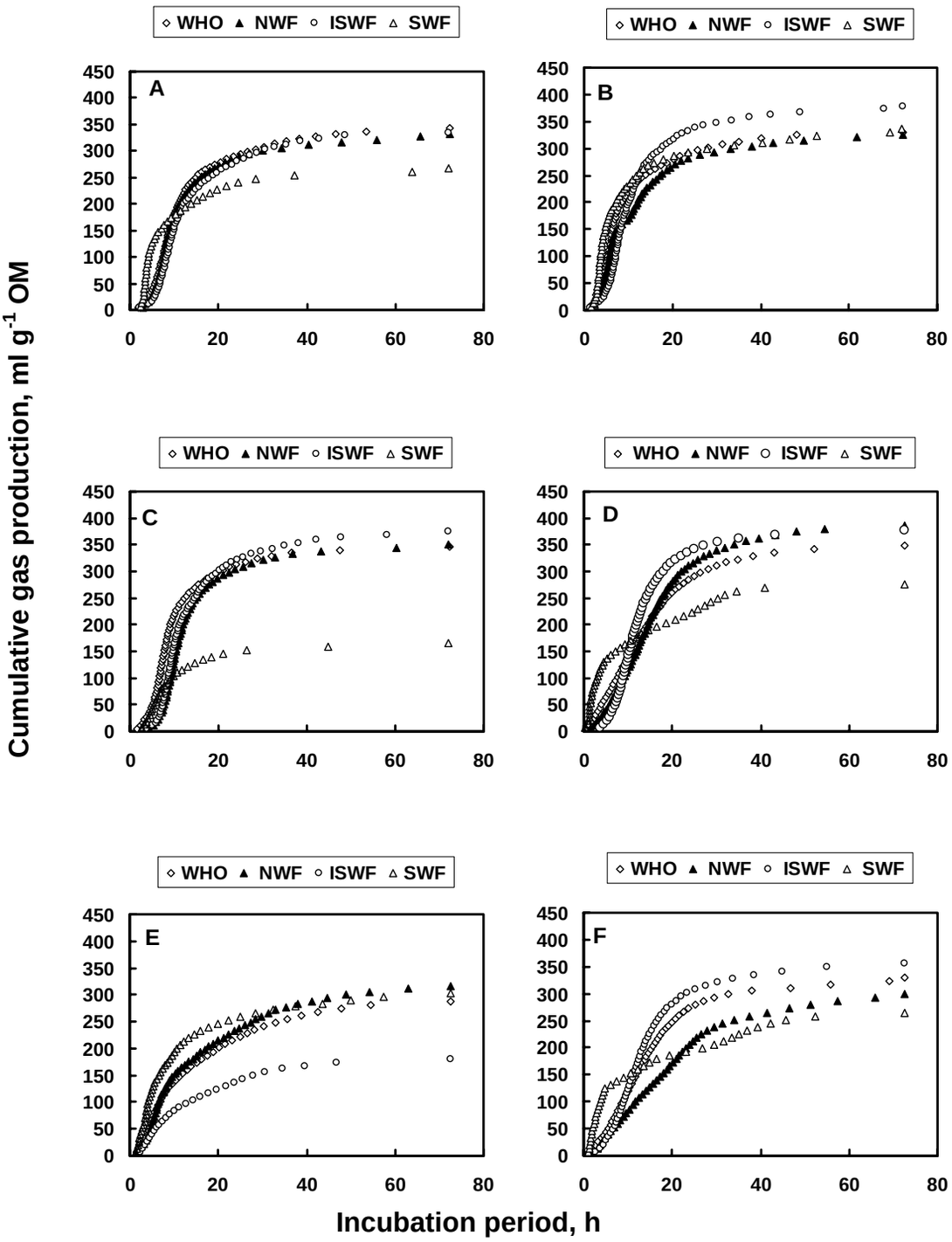


Figure1. Cumulative gas production in maize (A), barley (B), milo (C), peas (D), lupins (E) and faba beans (F) and their fractions.

A multi-phasic gas production profile was observed with the incubation of the SWF of peas and faba beans (Figure 1). From the significant improvement in fit when adding a third phase, gas production of SWF in peas is considered to be tri-phasic (data are not shown). In the other cases, a di-phasic model seems appropriate to describe the gas production profiles of the feeds and their fractions.

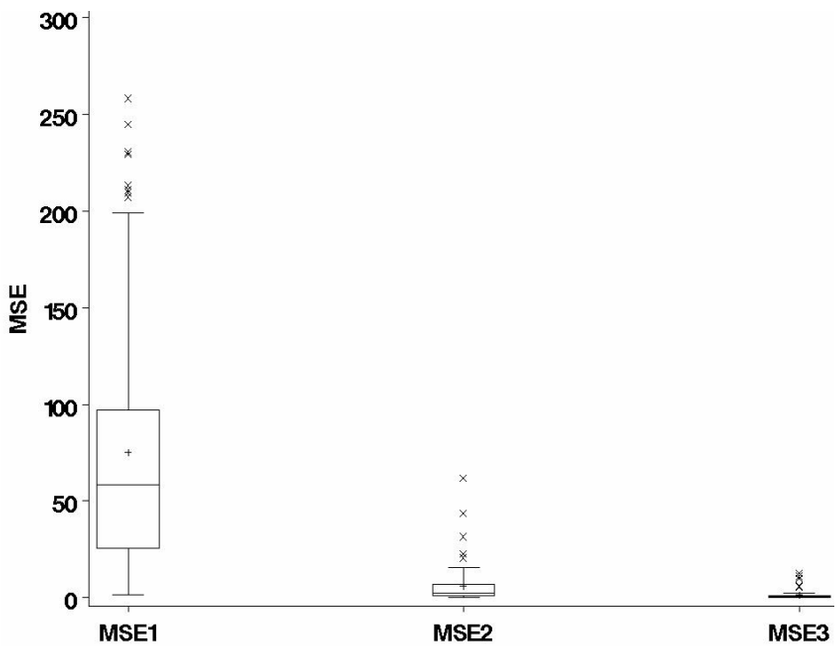


Figure 2. Box-and-whisker plots[19] of the distribution of error mean square (MSE) of mono-phasic (MSE1), di-phasic (MSE2) and tri-phasic Groot models. Means of each group indicated are labelled as '+', outliers indicated as '×' and the median of the distribution splits the box into two parts.

The effects on the gas production parameters (A, C, $R_{max}S$) of grain, fraction and the interaction between grain and fraction for both mono and di-phasic models are shown in Table 3 and all were found to be significant. Comparing the mono-phasic C values and $R_{max}S$ of the fractions revealed that the fermentation characteristics of the ISWF were more like those of NWF and WHO than SWF (Table 3).

The gas production profile of the SWF was characterised by two distinct phases. The first phase represented a very rapidly degraded fraction; the half-time was reached much earlier than that of the first phase of WHO, NWF and ISWF. In the second phase; however, $R_{max}S$ of the SWF was more or less similar to the other fractions. Gas production in the second phase of fermentation of SWF accounted for 57 percent of the total gas production with a C value at 16.5 h.

Table 3. Gas production characteristics of the concentrate ingredients and their fractions fitted with a mono- and a di-phasic model

		Mono-phasic				Di-phasic					
Class		OMCV	A	C	$R_{max}S$	A_1	C_1	$R_{max}S_1$	A_2	C_2	$R_{max}S_2$
Fraction	WHO	326.5^{b}	333.3^{a}	10.9^{c}	0.118^{c}	140.5^{a}	7.8^{c}	0.379^{b}	194.9^{ab}	14.9^{b}	0.153^{a}
	NWF	339.3^{a}	333.9^{a}	12.8^{a}	0.111^{c}	154.9^{a}	8.5^{b}	0.465^{b}	191.1^{ab}	16.9^{a}	0.071^{b}
	ISWF	335.1^{a}	325.6^{ab}	11.6^{b}	0.144^{b}	131.8^{a}	9.3^{a}	0.511^{b}	207.6^{a}	15.8^{ab}	0.028^{b}
	SWF	268.2^{c}	254.8^{c}	5.6^{d}	0.200^{a}	122.2^{a}	3.3^{d}	1.099^{a}	164.8^{b}	16.5^{ab}	0.022^{b}
	SEM	3.0	3.1	0.3	0.005	11.9	0.2	0.063	12.8	0.6	0.026
Grain	Maize	326.6^{b}	310.1^{b}	9.2^{c}	0.165^{a}	112.0^{b}	7.5^{b}	0.847^{a}	226.0^{a}	14.0^{c}	0.014^{c}
	Barley	327.5^{b}	314.6^{b}	7.3^{d}	0.176^{a}	126.9^{b}	5.7^{c}	0.866^{a}	203.6^{ab}	10.0^{d}	0.187^{a}
	Milo	308.4^{c}	294.8^{c}	9.3^{c}	0.177^{a}	104.3^{b}	8.0^{b}	0.658^{ab}	215.6^{ab}	13.0^{c}	0.018^{c}
	Peas	351.1^{a}	370.8^{a}	12.5^{a}	0.106^{b}	180.6^{a}	8.0^{b}	0.450^{bc}	179.9^{bc}	18.1^{b}	0.137^{ab}
	Lupins	281.4^{d}	292.4^{c}	11.2^{b}	0.082^{c}	135.5^{b}	5.7^{c}	0.346^{cd}	156.6^{c}	21.3^{a}	0.060^{bc}
	Faba beans	314.7^{c}	319.3^{b}	12.5^{a}	0.102^{b}	175.2^{a}	9.0^{a}	0.176^{d}	151.3^{c}	19.6^{ab}	0.113^{ab}
	SEM	4.1	4.2	0.3	0.005	16.4	0.3	0.086	16.9	0.8	0.036
Significance (P)	Grain (G)	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	NS	<0.001	NS
	Fraction (F)	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001	NS	<0.05	<0.001
	G × F	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05

OMCV, cumulative gas production (ml g^{-1} OM); A, asymptotic gas production (ml g^{-1} OM); C, half-time of gas production (h); and $R_{max}S$, maximum fractional rate of substrate degradation (h^{-1}). Means with different superscripts within class and column differ significantly (P<0.05).

Comparing the mono-phasic C values and $R_{max}S$ of grains showed that the cereal grains (maize, barley and milo) fermented faster than the legume seeds. The half time of gas production was reached earlier and the $R_{max}S$ was higher in the cereal grains than in the legume seeds, which is further confirmed by a significantly higher $R_{max}S_I$ of cereal grains compared to legume seeds (Table 3). Comparing the mono and di-phasic models, C and $R_{max}S$ revealed that the degradative behaviour of milo is more similar to that of maize than to barley. In the legume seeds, the degradative characteristics of lupins with regard to mono-phasic C values and $R_{max}S$ were different from those in peas and faba beans (Table 3).

Correlation between chemical composition and gas production characteristics

The correlation coefficients between the chemical composition of feeds and feed fractions and gas production characteristics are given in Table 4. The results show that cumulative gas production was negatively correlated with protein and sugars (P<0.001), whereas a strong and significant positive correlation was observed between starch and gas production. The half-time of gas production (C) was negatively correlated with the sugar content, whereas a positive correlation was observed between NDF and C. $R_{max}S$ was positively correlated with sugars and negatively with NDF.

Table 4. Correlation between chemical composition (g kg^{-1} DM) and gas production characteristics fitted with a mono-phasic model

	OMCV	C	$R_{max}S$	$R_{max}G$
Starch	0.65***	0.24*	0.12	0.08
Protein	–0.39***	0.01	–0.30**	–0.04
NDF	–0.02	0.59***	–0.71***	–0.63***
Sugar	.–0.37***	–0.64***	0.45***	0.41***

*P<0.05, **P<0.01 and ***P<0.001.

Fermentation results for individual time points

The effects on the VFAs production and NH_3-N of grain, fraction, time and interaction between these parameters are shown in Table 5 and all were significant.

Table 5. VFA production profiles (mmol g^{-1} OM), total VFA production (TVFA, mmol g^{-1} OM), NGR, BCR and NH_3-N (mg g^{-1} OM)

Class		HAc	HPr	HBu	TVFA	NGR	BCR	NH_3-N
Fraction	WHO	3.01^{b}	1.56^{a}	0.66^{b}	5.55^{b}	3.59^{b}	0.030^{c}	43.5^{b}
	NWF	2.94^{b}	1.55^{a}	0.59^{c}	5.36^{c}	3.43^{c}	0.028^{c}	36.5^{d}
	ISWF	2.62^{c}	1.55^{a}	0.69^{a}	5.17^{d}	3.32^{d}	0.034^{b}	39.8^{c}
	SWF	3.34^{a}	1.51^{a}	0.52^{d}	5.75^{a}	4.01^{a}	0.038^{a}	55.2^{a}
	SEM	0.03	0.02	0.01	0.06	0.03	0.0008	0.6
Grain	Maize	2.76^{c}	1.73^{b}	0.65^{b}	5.39^{b}	3.39^{cd}	0.028^{c}	33.1^{c}
	Barley	3.02^{b}	1.81^{a}	0.60^{c}	5.71^{a}	3.45^{c}	0.026^{c}	35.6^{d}
	Milo	2.71^{c}	1.73^{b}	0.61^{c}	5.27^{b}	3.31^{d}	0.027^{c}	33.2^{e}
	Peas	3.11^{b}	1.45^{c}	0.69^{c}	5.59^{a}	3.73^{b}	0.035^{b}	49.1^{c}
	Lupins	3.27^{a}	1.36^{d}	0.54^{d}	5.60^{a}	3.77^{b}	0.039^{a}	58.5^{a}
	Faba beans	3.05^{b}	1.22^{e}	0.66^{ab}	5.31^{b}	3.91^{a}	0.037^{ab}	52.4^{b}
	SEM	0.04	0.03	0.01	0.08	0.04	0.001	0.8
Time (h)	0	1.06^{e}	0.24^{e}	0.12^{e}	1.48^{e}	4.99^{a}	0.025^{c}	27.9^{d}
	3	1.31^{d}	0.35^{d}	0.16^{d}	1.92^{d}	4.43^{b}	0.037^{b}	31.9^{c}
	6	1.54^{c}	0.46^{c}	0.22^{c}	2.29^{c}	4.20^{c}	0.023^{d}	31.4^{c}
	24	4.13^{b}	2.62^{b}	1.11^{b}	8.17^{b}	2.48^{d}	0.025^{cd}	48.4^{b}
	72	5.55^{a}	3.21^{a}	1.21^{a}	10.77^{a}	2.43^{d}	0.044^{a}	66.8^{a}
	SEM	0.04	0.03	0.01	0.07	0.03	0.0009	0.7
Significance (*P*)	Grain (G)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Fraction (F)	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001
	Time (T)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	G × F	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	G × T	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	F × T	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001
	G × F × T	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means with different superscripts within fermentation time, grain and column differ significantly (P<0.05).

There were significant differences between fractions with regard to HAc, HBu, total VFA production (TVA), NGR, BCR and NH_3-N. VFA production profile, as represented by NGR, of the SWF was different from that of the ISWF. In legume seeds a significantly higher NGR, BCR and NH_3-N were observed compared to grains (Table 5). Throughout the fermentation, the VFA production profiles shifted from the production of non-glucogenic to glucogenic fatty acids as indicated by the decreasing NGR. The NH_3-N production; however, increasd as fermentation progressed (Table5). Unlike NH_3-N, no consistant pattern was observed with regard to changes in BCR throughout the fermentation. However, it is evidient that the BCR increased up to 3 h and thereafter backed to its value at 0 h, remained constant for a period of 18 h, and deramatically increased thereafter (Table 5).

In Figure 3, the differences between the profiles of VFA, as represented by NGR, in different fractions of concentrate ingredients are shown.

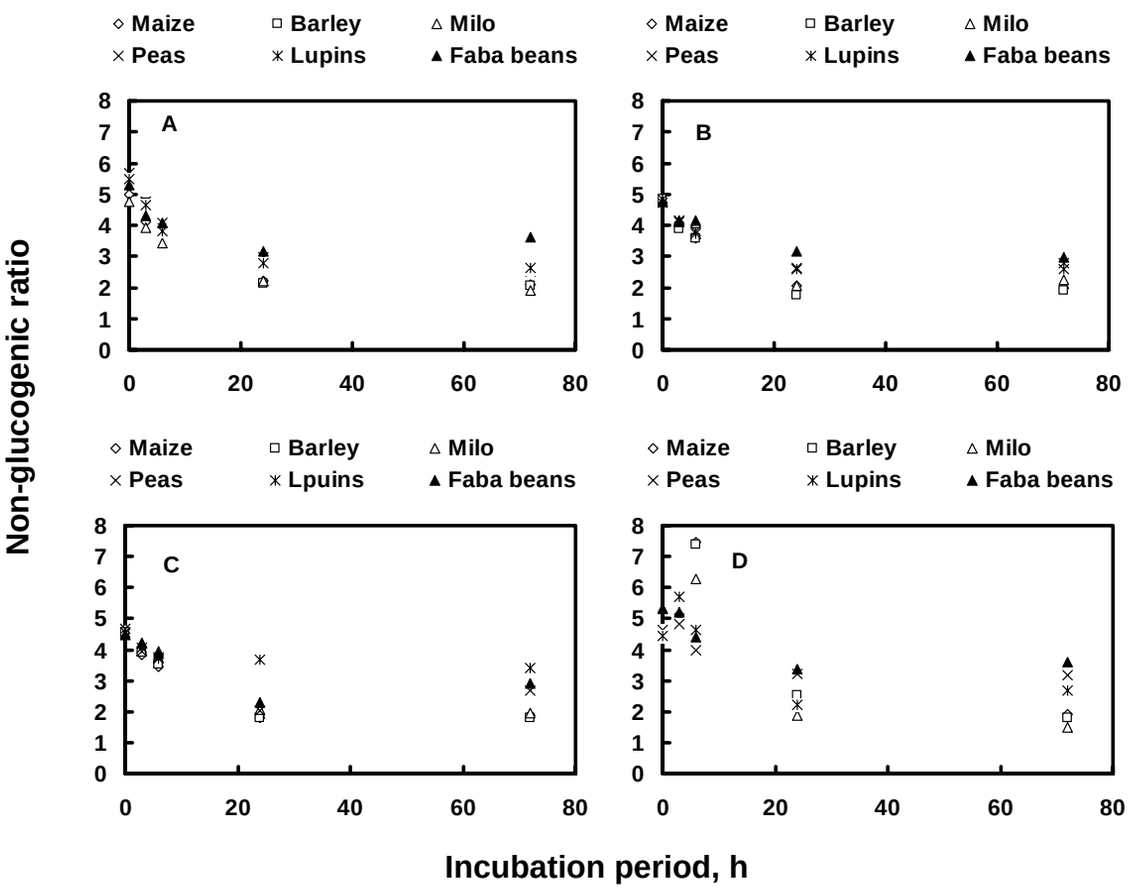


Figure 3. Non-glucogenic ratio in whole (A), non-washable (B), insoulbe washable (C) and soluble washable fraction (D).

The results show that up to 6 h, the pattern of VFA production in WHO, NWF and ISWF were almost identical between the concentrate ingredients; however, after 24 h of incubation the differences became more apparent. In SWF; however, the differences between the patterns of VFA production became already apparent after 6 h (Fig 3).

Gas production related to VFA production

The relationship between gas production and VFA production is shown in Table 6. For each concentrate ingredient and its fraction a high correlation (R^2 = 0.81–0.98) was observed between gas production and total VFA production. R^2 was always highest for ISWF and lowest for SWF.

Relationship between NH_3 production BCR and gas production

Ammonia production after 72 h of incubation was significantly correlated to BCR (R=0.9). A negative correlation was observed not only between cumulative gas production and NH_3-N production but also between gas production and BCR (R=–0.59 and –0.62, respectively; data are not shown).

Discussion

Chemical composition of concentrate ingredients and their fractions

The results in Table 1 show that, except in lupins, the ISWF in the concentrate ingredients is very rich in starch. Indeed, a significant proportion of the starch, for instance up to 60% in peas, was washed out and recovered in the ISWF. The average diameter of the starch granules in grains and legume seeds is smaller than 20 μm and the pore size of the bags (40 μm) would most likely allow a portion of the starch granules to be washed out.[18] SWF is relatively rich in ash, crude protein, soluble sugars and a residual unknown fraction. A large proportion, between 35 and 62%, of the ash appeared to be soluble and was recovered in the SWF. This was also the case for protein in legume seeds, but not in grains. As expected, a large proportion of the sugars (between 62 and 100%) was also found in the SWF. The fraction of unknown origin is most likely composed of water soluble non-starch polysaccharides containing β-glucans, pectins or galactans, at least in barley and legume seeds.[19]

Table 6. Parameters for the linear regression of total VFA production (mmol g^{-1} OM) against cumulative gas production (ml g^{-1} OM)

Grain	Fraction	Intercept	Coefficient	R^2
Maize	WHO	0.99	0.03	0.96
	NWF	1.23	0.03	0.98
	ISWF	1.17	0.02	0.99
	SWF	0.95	0.03	0.81
Barley	WHO	1.16	0.03	0.96
	NWF	0.58	0.03	0.97
	ISWF	1.03	0.03	0.99
	SWF	0.67	0.04	0.87
Milo	WHO	1.16	0.03	0.95
	NWF	1.43	0.02	0.98
	ISWF	1.19	0.02	0.99
	SWF	1.54	0.04	0.94
Peas	WHO	1.38	0.02	0.97
	NWF	1.22	0.02	0.97
	ISWF	1.09	0.03	0.98
	SWF	0.72	0.04	0.92
Lupins	WHO	1.78	0.03	0.97
	NWF	0.90	0.03	0.96
	ISWF	0.96	0.04	0.97
	SWF	0.65	0.04	0.90
Faba beans	WHO	1.45	0.02	0.98
	NWF	2.08	0.02	0.93
	ISWF	1.25	0.02	0.98
	SWF	–0.05	0.04	0.81

The negligible quantity of starch found in the SWF demonstrates that, contrary to the results suggested in other studies,[20,21] even in legume seeds, the starch in the granules has very low solubility. The results show that the quantity of SWF in grains is very small (4.4–5.5% of whole grain), whereas in legume seeds the SWF contributes substantially (16–21.7%).

Gas production profiles

As shown in Figure 1, the gas production pattern of the SWF differed from the other fractions. The total gas production of the SWF was considerably lower than that of WHO, NWF and ISWF. The relatively high crude protein content is kept responsible for this, because it is known that gas yield of the degradation of protein is lower than that of carbohydrates such as starch and NDF. Cone and van Gelder[22] showed that the fermentation of a protein-rich substrate causes less gas production than carbohydrate-rich fractions, partly due to binding of H^+ ions with ammonia, limiting the indirect gas production by preventing the release of CO_2 from the inoculum. A negative correlation between protein content and gas production was also found in our study (Table 4).

The higher gas yield of the ISWF than the other fractions in barley, milo and faba beans is explained by the higher starch content of this fraction in these feeds. In our study, a modest correlation (R^2=0.65) was found between the starch content of the substrate and gas yield (Table 4). Chai et al.[23] also observed a modest relationship between gas production and starch degradation.

Dividing the gas production profile of the SWF into two phases showed that the first phase represents a very rapidly degraded fraction; the half-time was reached much earlier than the first phase of WHO, NWF and ISWF. Because of the positive correlation between sugars and $R_{max}S$ (Table 4), the higher sugar content of the SWF is kept responsible for this. Comparing the $R_{max}S$ of NWF and SWF in the mono-phasic model showed that degradation of the SWF in grains was 1.2–1.3 times faster than of the NWF, but in legume seeds that difference was even 2–4 times faster (data not shown). Taking into account that the rumen outflow of the SWF is with the fluid and the NWF with the solids, the rate of outflow of the SWF would be faster than that of the NWF and a significant proportion of the SWF can also escape the rumen before being degraded.

Statistical analysis revealed that the degradative behaviour of the ISWF was more like the NWF than with the SWF, but some exceptions (Figure 1) suggest that the assumption that the degradative behaviour of the ISWF is similar to that of the NWF may

not always be true.[24] It must however be concluded that, with concentrate ingredients, the nylon bag method gives inaccurate results, at least for protein and starch, and should be corrected with a fractionation method as suggested by various authors.[4,5,25]

Fermentation pattern of concentrate ingredients and their fractions

As expected, the total production of VFA was initially higher in the SWF than in the other fractions. This can be explained by the highly degradable nature of this fraction (data not shown). Surprisingly, the NGR of the SWF in grains tended to increase up to 6 h, indicating the production of predominantly HAc. This may either result from the characteristics of the inoculum used, which was obtained from predominantly hay-fed cows, or, more likely, by the fermentation of the residual fraction consisting of soluble non-starch polysaccharides (NSP). It has been demonstrated [26,27] that the fermentation of soluble NSP can produce a high HAc/HPr ratio. The fermentation of an extract from cell walls with a high glucose content gave a high proportion of acetic acid.[28] Moreover, the high glucose content would be expected to lead to an acetogenic fermentation.[29,30]An initial increase in NGR was not observed for the SWF of legume seeds, probably because the protein of legume seeds in the SWF in our study was less degradable in the early stages of fermentation than that of grains. Evidence for this was found by a lower BCR in the SWF of legume seeds than in the SWF of grains up to 3 h. In the other fractions, however, a decrease in NGR up to 24 h for grains and up to 12 h for legume seeds indicates fermentation of cell content, predominantly starch, producing substantial amounts of HPr and valerate.[30]

Since production of VFA and in vitro gas production are stoichiometrically related,[31–33] the amount of substrate converted to the VFA-gas complex can be determined by multiplying the in vitro gas production by a stoichiometric factor.[34] This parameter can be used to determine the efficiency of microbial production (EMP) in a substrate with a known true substrate degradability value.[33] The efficiency of microbial production is normally determined at a fixed time point.[35] However, the results in Figure 3 show that using a fixed time point, for example 24 h, to determine in vitro EMP might be misleading. Figure 3 shows that the differences in the NGR of WHO, NWF and ISWF in the concentrate ingredients were apparent after 24 h. In the SWF, however, this difference was already apparent after 6 h. These results indicate that to improve the estimation of EMP, measurements of end-product formation along with the determination of substrate degradability has to be conducted at substrate-specific incubation times.

A significantly higher BCR of the SWF than the other fractions could be explained by a higher level of soluble true protein (measured by precipitation of true protein by TCA; **Chapter 1**) in this fraction. The difference in the production of NH_3-N between the SWF and the other fractions indicates a different fermentation pattern of nitrogenous compounds in these fractions. This could have resulted not only from a higher protein content, but also due to a higher non-protein nitrogen content of this fraction (**Chapter 1**).

Gas production related to end-product formation

According to Cone and van Gelder [22] gas production is an indication of quantitative VFA production. Fermentation gases are produced mainly when feedstuffs are fermented to acetate and butyrate, with propionate yielding gas only indirectly due to the buffering of the acid. Thus, feeds that produce high amounts of propionate yield lower gas volumes.[32] A close relationship between gas production and total VFA formation has been reported by many researchers.[31,36,37] Our data confirm the close relationship between gas production and VFA formation. However, the relationship between gas production and VFA formation in SWF was less than the other fractions. This can be explained by the higher protein content of SWF which interferes with indirect gas production. Recently, Getachew[38] also reported a strong relationship (R^2=0.76) between gas production and total VFA after 24 h; however, this relationship was weaker than that found in our study and that reported by Beuvink and Spoelstra[32] It has been reported that gas production can be calculated from the total VFA production.[22] However, other factors, notably the protein content of the substrate, may influence gas production. A negative effect of protein on gas production has been reported[22,38] and our study also showed a negative correlation (R=–0.4) between protein content and gas production which may explain the lower gas production of the SWF than the other fractions.

Ammonia production could also have a negative effect on indirect gas production in an in vitro system using bicarbonate as a buffer.[22,32] Our study also showed a significant and negative correlation (R=–0.58) between NH_3-N and cumulative gas produced after 72 h incubation. The gas is produced from gaseous fermentation end-products (CO_2, CH_4, direct gas production) and CO_2 released from the buffer (bicarbonate) by the VFAs produced.[32] The latter can be diminished by ammonia production as a result of neutralising of H^+ ions by ammonia (indirectly). However, Cone et al.[22] showed that diminishing gas production due to the fermentation of proteinaceous feeds is not only caused indirectly but could also be related to an altered stoichiometry due to the

fermentation of protein. This explains the modest correlation between NH_3-N and cumulative gas production found in our study. The mechanism by which protein fermentation reduces the direct gas production is not yet fully understood, but a correction on the gas production may be needed when proteinaceous substrates (like SWF) are fermented.

Conclusions

Rumen degradative behaviour differs between concentrate ingredients because of differences in the distribution of the chemical components among non-washable, insoluble washable and soluble washable fractions. The size of the washable fraction obtained in nylon bag studies differs from that of the soluble fraction. The degradative behaviour of this difference (ISWF), measured with *in vitro* gas production, is very similar to that of the non-washable fraction. The nature of the VFA profile resulting from the fermentation of the different fractions differs between fractions and changes with time of fermentation.

Acknowledgement

A.A. thanks the Ministry of Science, Research and Technology of Iran for financing the project. The authors wish to express their gratitude to the staff of the Laboratory of the Animal Nutrition Group; without their help this study would not have been possible.

References

1. Baldwin RL, Thronley JHM and Beever DE, Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. *J Dairy Res* **54**:107-131 (1987).

2. Dijkstra J, Neal HDSC, Beever DE and France J, Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *J Nutr* **122**:2239–2256 (1992).

3. De Boever JL, Cottyn BG, Vanacker JM and Boucque CV, Potential of solubility, enzymatic methods and NIRS to predict *in situ* rumen escape protein. *Neth J Agric Sci* **45**:291–306 (1997).

4. Lopez S, France J and Dhanoa MS, A correction for particular matter loss when applying the polyester bag method. *Br J Nutr* **71**:135–137 (1994).

5. Azarfar A, Tamminga S and Boer H, Effects of washing procedure, grain type, and particle size on the size of non-washout and insoluble washout fractions in concentrate ingredients, in *EAAP – 55th Annual Meeting*. p 96 (2004).

6. Melin M, Gierus M, Meijer GAL and van Vuuren AM, Chemical Characteristics and Degradability of Crude Protein in s-Fraction. ID-Leylstad, Leylstad, The Netherlands, Report No. 2079 (2001).

7. Yang HJ, Tamminga S, Williams BA, Dijkstra J and Boer H, *In vitro* gas and VFA production profiles of barley and corn and their soluble washout fractions after feed processing. *Anim Feed Sci Technol* **120**:125–140 (2005).

8. Lowe SE, Theodorou MK, Trinci APJ and Hespel RB, Growth of anaerobic rumen fungi on defined and semi-defined media lacking rumen fluid. *J Gen Microb* **131**:2225–2229 (1985).

9. Theodorou MK, Williams BA, Dhanoa MS, McAllan AB and France J, A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim Feed Sci Technol* **48**:185–197 (1994).

10. Williams BA, van der Poel AFB, Boer H and Tamminga S, The use of cumulative gas production to determine the effect of steam explosion on fermentability of two substrates with different cell wall quality. *J Sci Food Agric* **69**:33–39 (1995).

11. Davies ZS, Mason D, Brooks AE, Griffith GW, Merry RJ and Theodorou MK, An automated system for measuring gas production from forages inoculated with rumen fluid and its use in determining the effect of enzymes on grass silage. *Anim Feed Sci Technol* **83**:205–221 (2000).

12. Goelema JO, Spreeuwenberg MAM, van der Poel AFB and Tamminga S, Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. *Anim Feed Sci Technol* **76**:35–50 (1998).

13. Rijnen MMJA, Verstegen MWA, Heetkamp MJW and Schrama JW, Effects of dietary fermentable carbohydrates on energy metabolism in group-housed sows. J Anim Sci **79**:148-54 (2001).

14. Van Soest PJ, Robertson JB and Lewis BA, Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**:3583–3597 (1991).

15. Ørskov ER, Manipulation of rumen fermentation for maximum food utilization. *World Rev Nutr Diet* **22**:152–182 (1975).

16. Groot JCJ, Cone JW, Williams BA, Debersaques FMA and Lantinga EA, Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. *Anim Feed Sci Technol* **64**:77–89 (1996).

17. Tukey JW, *Expalnatory Data Analysis*. Addison-Wesley, Reading (1977).

18. Petterson D, Sipas S and Mackintosh JB, *The Chemical Composition and Nutritive Value of Australian Pulses*, 2nd edn. Grains Research and Development Corporation, Canberra, p. 125 (1997).

19. Chesson A, Feed characterization, in *Feeding Systems and Feed Evaluation Models*, Ed by Theodorou MK and France J. CAB International, Wallingford, p. 11 (2000).

20. Tamminga S, van Vuuren AM, van der Koelen CJ, Ketelaar RS and van der Togt PL, Ruminal behaviour of structural carbohydrates, non-structural carbohydrates and crude protein from concentrate ingredient in dairy cows. *Neth J Agric Sci* **38**:513–526 (1990).

21. Walhin PM, Foucant M and Thewis A, Influence of extrusion on ruminal and intestinal disappearance *in sacco* of pea (*Pisum sativum*) proteins and starch. *Anim Feed Sci Technol* **38**:43–55 (1992).

22. Cone JW and Van Gelder AH, Influence of protein fermentation on gas production profiles. *Anim Feed Sci Technol* **76**:251–264 (1999).

23. Chai WZ, Van Gelder AH and Cone JW, Relationship between gas production and starch degradation in feed samples. *Anim Feed Sci Technol* **114**:195–204 (2004).

24. Dhanoa MS, France J, Lopez S, Dijkstra J, Lister SJ, Davis DR and Bannink A, Correcting the calculation of extent of degradation to account for particulate matter loss at zero time when applying the polyester bag method. *J Anim Sci* **77**:3385–3391 (1999).

25. De Boever JL, Vanacker JM, Bogarets DF and Boucque CV, Protein evaluation of cattle compound feeds: comparison of *in sacco* measurements and tabular values. *Neth J Agric Sci* **43**:297–311 (1995).

26. Marounek M, Bartos S and Brezina P, Factors influencing the production of volatile fatty acids from hemicellulose, pectin and starch by mixed culture of rumen microorganisms. *Z Tierphsiol Tierenah* **53**:50–58 (1985).

27. Sunvold GD, Hussien HS, Fahey JR GC, Merchen NR and Reinhart GA, *In vitro* fermentation of cellulose, beet pulp, and citrus pectin using faecal inoculum from cats, dog, horses, humans, and pigs ruminal fluid from cattle. *J Anim Sci* **73**:3639–3648 (1995).

28. van Laar H, Tamminga S, Williams BA and Verstegen MWA, Fermentation of the endosperm cell walls of monocotyledon and dicotyledon plants species faecal microbes from pigs: the relationship between cell wall characteristics and fermentability. *Anim Feed Sci Technol* **88**:13–30 (2000).

29. Hungate RE, *The Rumen and its Microbes*. Academic Press, New York, 533 pp. (1966).

30. van Houtert MFJ, The production and metabolism of fatty acids by ruminants fed roughages: a review. *Anim Feed Sci Technol* **88**:13–30 (1993).

31. Wolin MJ, A theoretical rumen fermentation balance. *J Dairy Sci* **43**:1452–1459 (1960).

32. Beuvink JMW and Spoelstra SF, Interaction between substrate, fermentation end-products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms *in vitro*. *Appl Microbiol Biotechnol* **37**:505–509 (1992).

33. Blümmel M and Lebzien P, Predicting ruminal microbial efficiencies of dairy ration by in vitro techniques. *Livest Prod Sci* **68**:107–117 (2001).

34. Grings EE, Blummel M and Sudekum KH, Methodological considerations in using gas production techniques for estimating ruminal microbial efficiencies for silage-based diets. *Animal Feed Sci Technol* **123–124**:527 (2005).

35. Blümmel M, Schroder A, Sudekum K-H and Becker K, Estimating ruminal microbial efficiencies of dairy ration by in silage-fed cattle: comparison of an in vitro method with a combination of in situ and in vivo measurements. *J Anim Physiol Anim Nutr* **81**:157–167 (1999).

36. McBurney MI, Cuff DJ and Thompson LU, Rates of fermentation and short chain fatty acid and gas production of six starches by human faecal microbiota. *J Sci Food Agric* **50**:79–88 (1990).

37. Naga MA and Harmeyer JH, Gas and volatile fatty acid production at different rates of rumen microbial protein synthesis *in vitro*. *J Anim Sci* **40**:374–379 (1975).

38. Getachew G, Robinson PH, Depeters EJ and Taylor SJ, Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. *Anim Feed Sci Technol* **111**:57–71 (2004).

Chapter 3

In vitro gas production profiles and formation of fermentation end-product in processed barley, maize and milo ♣

Abstract

The effects of using a pre-compacting device (expander) on the degradative behavior of barley, maize and milo and their different fractions were studied using an *in vitro* gas production. The aim was to establish whether the processing changes the contribution of dry matter (DM), chemical constituents and degradative behaviour of these concentrate ingredients over the different fractions (non-washable, NWF; insoluble washable, ISWF and soluble washable fraction, SWF). The samples were fractionated into SWF, ISWF, and NWF fractions. In three consecutive gas production runs, samples of different fractions (NWF, ISWF and SWF) of the entire concentrate ingredients (WHO) were subjected to three processes (R, Retsch mill ground samples; E, expander treated samples; EP, expander-pelleted samples) and their fermentation characteristics were evaluated using an *in vitro* incubation technique (Automated Pressure Evaluation System, APES) for 72h. In a manually operated *in vitro* fermentation system, the same substrates were fermented for 6, 12, 24 and 48 h in order to collect samples for volatile fatty acids (VFA) and ammonia (NH_3-N) analysis. In maize and milo, the E process significantly ($P < 0.05$) increased the size of NWF compared to process R, whilst by the ensuing pelleting it was decreased. Fraction SWF in the processed samples E was significantly ($P < 0.05$) lower than in the R samples. The ISWF was very rich in starch. Fraction SWF was relatively rich in ash, crude protein, soluble sugars, and a residual (chemically not determined; 24-42 %), which was elevated by expander-pelleting. Compared to the R samples, the EP samples gave a faster fermentation, as presented by a significantly (in barley numerically) higher maximum fractional rate of substrate degradation ($P < 0.05$) and a significantly (in maize numerically) higher maximum rate of gas production ($P < 0.05$) in the first phase of fermentation. In barley and maize, E and EP samples shifted the pattern of fermentation towards a more glucogenic fermentation as represented by a lower non-glucogenic to glucogenic ratio (NGR). In all cereal grains, the NH_3-N and branched chain ratio (BCR) for E and EP samples were significantly ($P < 0.05$) lower than in R samples.

♣ Azarfar, A., Namgay, K., Pellikaan, W. F., Tamminga, S. and van der Poel, A. F. B. **Submitted**.

Introduction

Processing of cereal grains is a well known concept to ruminant nutritionists and the feed industry. Various feed processing methods that are deemed beneficial to animals are widely used in feed industries around the world. Expander processing is one such technique in feed manufacturing, which involves heat, pressure and shear. The major parameters characterizing the expander process are temperature (80-130 ^{o}C), moisture content of the product (18-30%), residence time (5-15 s) and shear force.[1]

Expander processing may improve the nutritive value of feeds for ruminants by increasing the availability of energy and by synchronizing the nutrient supply in animals as well.[2-4] Subjecting cereal grains to such a hydrothermal process increases the degree of starch gelatinization and makes the starch granule more accessible for both bacterial attachment and ruminal fermentation.[5] Moreover, expander treatment and the ensuing pelleting process involve shear forces that may affect the size of particles. Therefore, processing of feeds may change degradability of feeds in ruminants either by changing the degradation rate or by changing the distribution of feed's dry matter (DM) over the different fractions (washable, potentially degradable and undegradable fractions) in whole feeds.[3,6]

The *in situ* technique is a very common procedure that is often used to characterize ruminal degradation of unprocessed as well as processed feeds. This technique fractionates feed material into a washable fraction (W), a potentially degradable fraction (D) and an undegradable fraction (U), and gives information on the fractional degradation rate of the D-fraction. The *in situ* technique cannot measure the rate at which the W fraction of protein and starch is actually degraded. Therefore, assumptions are made regarding the part of it that escape from rumen fermentation.[7] This means that a great deal of information is available on the degradative behaviour in the rumen of starches, proteins and cell walls present in the D fraction, but not in the W fraction. Since the W fraction quantitatively constitutes an important part of the feedstuffs' dry matter (DM) (up to 50 % of DM),[8] characterising its degradative behaviour is of great importance. The combination of feed fractionation that further fractionates the W fraction into an insoluble washable (ISWF) and a soluble washable fraction (SWF) in combination with an *in vitro* gas production techniques is considered to be a promising method to characterise the degradative behaviour of these fractions in feedstuffs.[9-11] Since the development of the new feed fractionation methods, no study has been done on the possible effects of using a pre-compacting device (expander) on the contribution and the degradative

behaviour of the feed fractions. This study, therefore, aimed to elaborate on the effects of expander treatment and the ensuing pelleting process on the *in vitro* measured degradative behaviour and formation of fermentation end-product in some cereal grains (barley, maize and milo) and their fractions (NWF, ISWF and SWF).

Materials and Methods

Experimental setup

The schematic set up of experiment is presented in Figure 1.

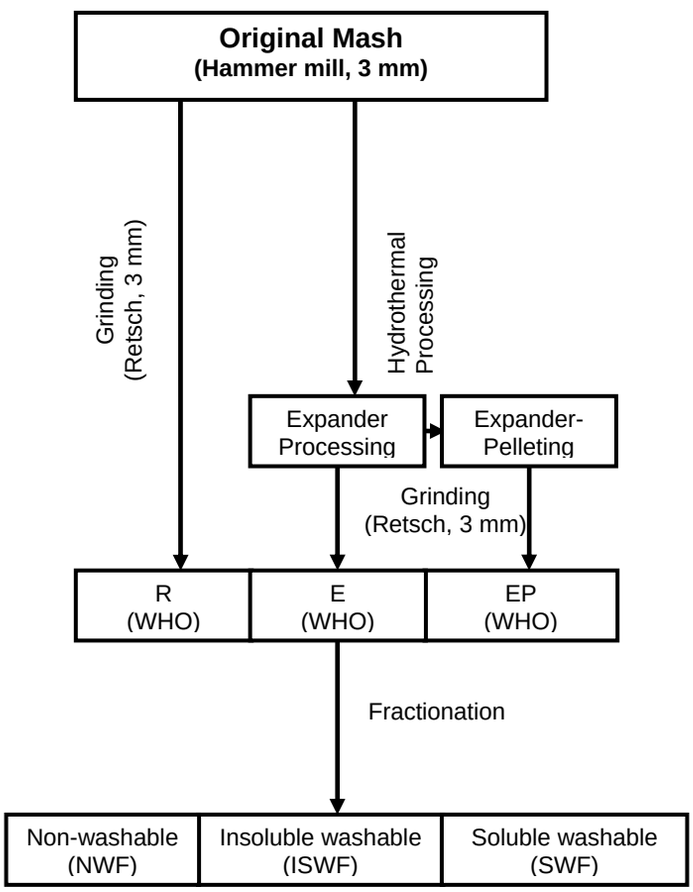


Figure 1. Schematic presentation of the experimental set up.

The effects of using a pre-compacting device (expander) alone and in combination with pelleting on the degradative characteristics of three single concentrate ingredients (barley, maize and milo) were studied. The kinetics of gas production in samples of three

entire (WHO) concentrate ingredients processed differently (Retsch milled, R; expander processed, E; expander-pelleted, EP) and their fractions (non-washable fraction, NWF; insoluble washable fraction, ISWF; soluble washable fraction, SWF) were evaluated using an *in vitro* incubation technique, which measures gas production continuously in an automated system (APES-IGER, Aberystwyth, Wales) for 72 h. In a manually operated *in vitro* fermentation system, the production of VFA and ammonia (NH_3-N) were simultaneously measured at 6, 12, 24, and 48 h, respectively. The entire concentrate ingredients (WHO) were included in each *in vitro* incubation run to test for possible run effects.

Sample processing

All concentrate ingredients were supplied by a commercial supplier (Research Diet Services, Wijk bij Duurstede, The Netherlands). As is common in practice, all ingredients were already ground (hammer mill; 3 mm sieve) prior to further agglomeration. Further processing was carried out at the Wageningen Feed Processing Centre (WFPC). An Almex expander (150 Φ, Almex BV Zutphen, The Netherlands), fitted with a 22 kW engine was used for the expander treatment. Steam at a pressure of 60 kPa was added to the mash materials in the conditioner. Processing temperature, determined using thermocouples in the mixing bolts of the expander, were 99 ºC, 110 ºC and 96 ºC for barley, maize and milo, respectively.

Pelleting was carried out with a 5 × 45 mm die (bore × hole), using a V2-30 pelletizer (Robinson Milling System B.V., Boxtel, The Netherlands). After pelleting, the pellets were cooled in a two-deck counter flow bunker cooler for 20 min. The feed samples were then dried in a forced oven for 16 h at 35°C.

Subsequently, identical samples of original mashes (hammer mill; 3 mm sieve) and all the processed materials (E and EP) were ground through a 3 mm sieve (Retsch ZM1 centrifugal mill). The samples were then subjected to a fractionation method (**Chapter 2**) to quantify the size of the NWF, ISWF and SWF (g kg^{-1} DM of WHO).

Sample fractionation into NWF, SWF and ISWF

The R, E and EP samples of three concentrate ingredients (barley, maize and milo) were fractionated using the method developed by Azarfar et al. (**Chapter 2**). To fractionate the whole grain, 5.5 g of each sample were weighed into pre-weighed nylon bags (8 × 16.5 cm inner size; pore size 40 μm; PA 40/30, Nybolt, Switzerland). The bags

were put into polypropylene centrifuge tubes and de-mineralized water at room temperature was added to reach a dilution factor of 20 (20 ml of de-mineralized water per g of sample). The tubes were then shaken in a shaking bath at 150 rpm for 1h. The nylon bags were then removed from the centrifuge tubes and their outside rinsed with a small quantity of distilled water and were left to drip for 15 min over a funnel to ensure that all rinsing water had passed the nylon bags. The tubes were then centrifuged at 715 × *g* for 30 min in a Beckman 2-21 M centrifuges.

After centrifugation the supernatant was filtered through a fast filter paper (S&S, Folded paper, 520 ½, Ø185 mm). The resulting liquid, assumed to be SWF, was decanted into pre-weighed aluminium containers and freeze-dried (FTS, Dura-Dry programmable tray freeze drier) to quantify the size of SWF. The residue left in the container together with left over on filter papers were brought quantitatively into pre-weighed aluminium container and freeze-dried to quantify the size of ISWF. The residual material in the nylon bag, which did not get washed out of the bag during 1h shaking was freeze dried as well to determine NWF.

In vitro fermentation

The cumulative gas production technique of Theodorou et al.[12] as modified by Williams et al.[13] was used to characterize the fermentation behaviour of feed samples. In three consecutive gas production runs, the WHO (R, E and EP) samples of 3 concentrate ingredients (maize, barley, and milo) and their fractions (NWF, ISWF and SWF) were subjected to an *in vitro* incubation technique, which continuously measured gas production in an automated pressure evaluation system (APES)[14] for 72 h. Simultaneously, in a manually operated *in vitro* fermentation system, the concentration of VFA and ammonia (NH_3-N) was measured after 6, 12, 24, and 48h.

The rumen inoculum was taken weekly at 0800 h for three consecutive weeks from three rumen fistulated Holstein-Friesian non lactating dairy cows, fed a basic diet of grass silage and hay *ad libitum*. About 500 ml of rumen fluid was collected from each cow in pre-warmed thermos flasks that were filled with CO_2. The rumen fluid samples were proportionally pooled, blended for 60 s and strained through two layers of cheese cloth. All handlings were done under CO_2 to ensure anaerobic conditions.

Representative samples of each substrate fractions were incubated in rumen fluid with medium B (modified from Lowe et al.[15] by Williams et al.[16]) at 38°C under anaerobic conditions to mimic the rumen environment for microbes to ferment the substrates.

Medium B, a complex semi-defined medium, in principal is expected to provide most of the rumen micro-organisms with all the necessary nutrients except for energy, which is provided by the test substrate.

The experiment was conducted in three runs in which an automated pressure evaluation system (APES), and a manually operated *in vitro* fermentation system were applied simultaneously. Three replicate samples of each substrate were fermented for 72 h in 100 ml specific APES bottles (Schott Duran, Mainz, Germany) containing approximately 0.5 g of each substrate, 82 ml medium B and 5 ml of rumen fluid inoculum. Meanwhile, in the manually operated *in vitro* fermentation system, triplicate samples of the same substrates were fermented at four different time points (6, 12, 24 and 48 h, respectively). From the WHO, NWF and ISWF 0.5 g DM was fermented in 100-ml serum bottles in 82 ml medium B and 5 ml rumen inoculum kept in a temperature-controlled incubator. Because of an insufficient quantity of the SWF, only 0.05 g of SWF samples were incubated with 10 ml of medium B and 0.6 ml of rumen inoculum. For measuring the VFA, a sub-sample of 0.5 ml was withdrawn and added to a 1 ml Eppendorf tube containing 25 µl of phosphoric acid (850 g L^{-1}). A second sub-sample of 0.75 ml, to which 0.75 ml of trichloroacetic acid (TCA; 100 g L^{-1}) was added, was taken for measurement of NH_3-N by the indophenol method as described by Searle.[17] The remainder of the fermentation fluid was used to determine pH.

Chemical analysis

The WHO samples and their different fractions (NWF, ISWF and SWF) were analyzed for dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF), starch, reducing sugars and the degree of starch gelatinisation.

The dry matter (DM) content was determined by drying to constant weight at 103 °C for 4 h followed by equilibration in a desiccator (ISO 6496). Ash was determined after incineration for 4 h at 550 °C (ISO 5984) and CP by a Kjeldahl method (ISO 5983). Starch content was measured colorimetrically by a modification of the procedure described by Goelema et al.[18] The sugar content was determined after extracting feed samples with ethanol (400 ml L^{-1}). Carbohydrates in the filtrate were hydrolysed with 0.1 N HCl and after using free sugars as a reducing agent to oxidise copper, the content was measured using a spectrophotometer at 460 nm.[19] The degree of starch gelatinization (SGD, as percentage of total starch) was determined as described by Goelema et al.[18] NDF was determined with a Fibretec™ (Foss, Denmark) fibre analyser using reagents

described by Van Soest et al.[20] Sodium sulphite and amylase were used in the NDF determination.

The VFAs were measured by a GC (Fisons Instruments, HRGC mega 2, Milan, Italy) with a split/splitless injector operated in split mode (split ratio 1:10), fitted to a flame ionization detector (FID), using a capillary column (Alltech Capillary Column, EC™ 1000, length 30 meters, inside diameter 0.53 mm, film thickness 1 micron). Helium was used as the carrier gas at a flow rate of 30 ml min^{-1} with an initial temperature of 110°C which was held for 2 min after which the temperature was increased at 18°C per minute to 200°C and then kept at 200°C for another 1 min, with *iso*-caproic acid as an internal standard.

Acetic acid (HAc), propionic acid (HPr), *iso*-butyric (*i*-HBu), butyric acid (HBu), *iso*-valeric (*i*-HVal) and valeric (HVal) acid concentrations were expressed in mmol g^{-1} incubated OM. The non-glucogenic to glucogenic acids ratio (NGR) was calculated using the equation given below (Ørskov, 1975):[21]

$$\mathrm{NGR} = \frac{\mathrm{HAc} + 2 \times \mathrm{HBu} + \mathrm{Hval}}{\mathrm{HPr} + \mathrm{Hval}}$$

Iso-butyric and *iso*-valeric acid were assumed to originate from the degradation of protein and were expressed as proportion of the total VFA and named the branched chain ratio (BCR).

Kinetic model analysis

Gas production profiles, obtained with the automated system, were fitted by iteration for individual incubation flasks to the di-phasic model as described by Groot et al.[22] and shown in the following equation:

$$OMCV = \sum_{i=1}^{2} \frac{Ai}{1 + \left(Ci / ti\right)^{Bi}}$$

where i is the number of phases, OMCV is the cumulative gas production (ml g^{-1} incubated OM), A_i is the estimated asymptotic gas production (ml g^{-1} incubated OM), B_i represents a constant that determines the sharpness of the switching characteristic of the profile, and C_i is the time (h) after incubation at which half of the asymptotic gas production has been reached. The *in vitro* cumulative gas production profiles were further

characterised by estimating the maximum rate of gas production ($R_{max}G$, ml h^{-1}) and the time at which this maximum rate was reached ($TR_{max}G$, h):[11]

$$R_{\max}G = AC^{B}B\frac{TR_{\max}G^{(-B-1)}}{\left[1+C^{B}\times TR_{\max}G^{-B}\right]^{2}}$$

Maximum fractional rate of substrate degradation was estimated according to Yang et al.[11] as below:

$$R_{\max}S = \frac{(B-1)^{(B-1)/B}}{C}$$

where $R_{max}S$ (h^{-1}) is the maximum fractional rate of substrate degradation.

Statistical analysis

Additivity of fractions NWF, ISWF and SWF in WHO was tested by a two-tailed *t*-test. Analysis of variance for the distribution of DM of WHO samples over the different fractions, gas production kinetics' parameters and fermentation end-product were done using the GLM procedure of SAS 9.1 (SAS/STAT(R) package, 2003). The models used were:

$$Y = \mu + P_i + \varepsilon_{ij} \quad (1)$$

$$Y = \mu + P_i + F_j + (PF)_{ij} + \varepsilon_{ijk} \quad (2)$$

$$Y = \mu + T_k + R(T)_l + P_i + F_j + (TP)_{ik} + (TF)_{jk} + (PF)_{ij} + (TPF)_{ijk} + \varepsilon_{ijklm} \quad (3)$$

Model 1 was used to analyse the effects of processing on the distribution of DM. We used the second and third model to analyse the effects of processing on gas production kinetics parameters and end-product formation, respectively. In all models Y is the dependent variable under examination, μ is the overall mean, P_i is the processing effect (i = 4 in model (1), i = 3 in models (2) and (3); R, E and EP), F_j is the fraction effect (j = 3; NWF, ISWF and SWF), T_k is the time effect (k = 5; 6, 12, 24, 48 and 72 h), ε_{ij}, ε_{ijk} and ε_{ijklm} are the error term. Because in the third model the results were analysed as a split-plot design, T_k and $R(T)_l$ (the effect of replicates nested in time) are regarded as the main plot and the error term of the main plot, respectively.

In all models, differences between the main effects were analysed by a multiple comparison test (Tukey/Kramer), and the least square means listed in results tables using the LSMEANS statement of SAS 9.1 (SAS/STAT(R) package, 2003).

Results

Consistency of runs

The control samples in three runs showed significantly different results with regard to cumulative gas production, VFA and NH_3-N concentrations at 72 h of incubation. Therefore, data of each run were analysed separately.

Additivity of fractionation

The gas and VFA production were calculated for the whole fraction (WHO) on the basis of the contributions of NWF, ISWF and SWF. No significant differences were found between observed and calculated values (data are not shown). Therefore, WHO was discarded from the statistical analysis.

Effect of processing on the contribution of DM and chemical composition of the concentrate ingredients

The distribution of DM over the different fractions (NWF, ISWF and SWF) in the R, E and EP cereal grains and their chemical composition are shown in Tables 1 and 2.

In barley, processing had a significant effect on the size of NWF (EP 58, 40 and 140 g kg^{-1} DM lower than E, R and O respectively; $P < 0.05$), ISWF (EP 59, 58 and 151 g kg^{-1} DM higher than E, R and O respectively; $P < 0.05$) and SWF (E and EP 17 and 10 g kg^{-1} DM higher than R and O, respectively; $P < 0.05$). Unlike barley, expander treatment in maize and milo significantly increased the size of the NWF compared with that of EP, R and O whereas the size of the ISWF and SWF were decreased (Table 1). However, like in barley, a further reduction in the size of NWF was observed after pelleting the expander processed maize (Table 1). Although the size of the SWF in maize and milo was decreased after expander processing, pelleting after expander treatment rather increased it (Table 1).

Striking differences were observed between the cereal grains and their fractions with regard to their chemical composition (Table 2). The results show that NDF was almost completely recovered in the NWF. The ISWF is characterised by a high starch (up to 892 g kg^{-1}) content and a negligible quantity of reducing sugars (< 10 g kg^{-1} DM). The SWF contained a considerable quantity of ash, CP, reducing sugars and a large residual (24-42 %) chemically not determined fraction.

Table 1. Effect of processing (O, original; R, Retsch; E, expander processed; EP, expander-pelleted) on the contribution of dry matter (g kg^{-1} DM) in barley, maize and milo

Feed ingredient	Treatment	Fraction		
		NWF	ISWF	SWF
Barley	O	768[a]	168[c]	64[b]
	R	668[b]	261[b]	71[a]
	E	686[b]	260[b]	54[c]
	EP	628[c]	319[a]	53[c]
	SEM	13	13	2
Maize	O	831[b]	122[C]	47[c]
	R	772[d]	176[a]	52[b]
	E	900[a]	55[d]	45[d]
	EP	797[c]	137[b]	66[a]
	SEM	8	6	2
Milo	O	840[a]	124[b]	36[b]
	R	750[d]	197[a]	53[a]
	E	893[a]	71[c]	36[b]
	EP	806[c]	140[b]	54[a]
	SEM	12	10	2

Means in the same column within a feed followed by different superscript differ ($P < 0.05$); SEM, standard error of mean.

The expander processing and the ensuing pelleting resulted in only minor changes in CP content of WHO. In the SWF, however, both the E and EP samples had a lower CP content compared to the R samples. The results show that the expander treatment and the followed pelleting had a more profound effect in barley and decreased the NDF content of WHO and NWF compared to those of R samples. Compared to the R samples, the E and EP samples of WHO contained marginally lower reducing sugars. In the SWF, no consistent effect of processing on reducing sugar content was observed. However, it is evident that in barley and milo, the reducing sugars content of the SWF was higher in the expander treated samples than in the R samples.

Table 2. Chemical composition (g kg^{-1} DM) of cereal grains and their fractions

Feed	Process	Fraction	Ash	CP	NDF	Starch	Sugar	Residue[1]
Barley	Retsch	WHO	26.1	130.3	188.0	522.7	28.9	104.0
		NWF	22.0	130.0	290.7	498.5	7.7	51.1
		ISWF	11.7	101.3	-6.2 [2]	815.9	7.9	63.2
		SWF	150.1	192.2	ND	-23.2 [2]	342.0	315.7
Barley	E	WHO	22.4	128.2	155.5	576.9	25.6	91.4
		NWF	20.7	148.4	208.6	512.4	11.2	98.7
		ISWF	8.7	51.4	12.4 [2]	870.2	1.4	55.9
		SWF	177.4	106.0	ND	-0.9 [2]	385.2	331.4
Barley	EP	WHO	22.3	127.2	151.5	592.8	24.1	82.1
		NWF	20.2	142.5	190.8	520.4	18.3	107.8
		ISWF	7.6	51.5	31.7 [2]	862.6	0.9	45.7
		SWF	183.0	108.4	ND	-9.2 [2]	343.6	365.0
Maize	Retsch	WHO	15.7	107.2	98.5	687.8	23.5	67.3
		NWF	6.8	107.3	124.5	717.2	ND	44.2
		ISWF	6.6	75.9	2.4 [2]	824.6	1.5	89.0
		SWF	177.7	157.7	ND	-11.0 [2]	420.2	244.4
Maize	E	WHO	15.4	104.0	86.3	709.1	19.8	65.4
		NWF	7.3	105.6	97.8	731.2	0.9	57.2
		ISWF	5.6	96.7	-1.7 [2]	797.7	1.7	98.3
		SWF	179.5	102.2	ND	7.1 [2]	418.4	292.8
Maize	EP	WHO	15.8	103.3	80.3	745.8	20.7	34.1
		NWF	7.2	112.9	100.1	715.7	3.3	60.8
		ISWF	7.7	68.6	0.5 [2]	866.2	ND	57.0
		SWF	152.1	90.9	ND	56.7 [2]	319.6	380.7
Milo	Retsch	WHO	16.4	116.2	87.6	711.9	14.5	53.4
		NWF	6.8	125.8	104.2	732.7	2.0	28.5
		ISWF	5.2	59.3	9.5 [2]	883.9	3.0	39.2
		SWF	200.8	185.8	ND	-11.8 [2]	260.3	353.1
Milo	E	WHO	16.2	120.7	79.1	702.1	11.3	70.6
		NWF	7.9	118.6	87.9	762.1	2.0	21.5
		ISWF	12.6	118.3	0.6 [2]	795.3	8.4	64.8
		SWF	231.3	109.2	ND	-34.9 [2]	336.6	322.9
Milo	EP	WHO	16.2	122.9	72.3	719.9	12.9	55.8
		NWF	7.1	129.1	87.3	724.7	1.2	50.6
		ISWF	9.4	65.7	1.9 [2]	891.6	0.9	30.5
		SWF	200.2	98.3	ND	10.9 [2]	271.0	419.5

ND, not determined. [1] Calculated as 1000 – (ash + CP + starch + NDF + sugars).[2] Calculated as component in WHO– (component in the other fractions × their contribution to the DM of WHO).

The results show that compared to the R samples, the expander treatment increased SGD which was further alleviated by the ensuing pelleting process (Table 3). The increase in SGD was higher in E and EP treated maize and milo than in E and EP treated barley.

Table 3. The effect of processing on total starch, degree of starch gelatinisation (SGD) and gelatinised starch (GS)

Feed	Process	Fraction	Starch (g kg^{-1} DM)	GS (g kg^{-1} DM)	SGD %
Barley	Retsch	WHO	522.7	48.1	9.2
	E	WHO	576.9	158.6	27.5
	EP	WHO	592.8	258.6	43.6
Maize	Retsch	WHO	687.8	41.3	6.0
	E	WHO	709.1	352.4	49.7
	EP	WHO	745.8	469.9	63.0
Milo	Retsch	WHO	711.9	24.9	3.5
	E	WHO	702.1	275.9	39.3
	EP	WHO	719.9	365.7	50.8

Effect of processing on in vitro cumulative gas production kinetics

Barley

The effects on the gas production parameters (cumulative gas production, OMCV; A, *C*, $R_{max}S$ *and* $R_{max}G$) of processing, fraction and the interaction between processing and fraction are shown in Table 4.

A significant effect of processing was observed with regard to all gas production parameters except OMCV and $R_{max}S_1$. Gas production in the first phase of fermentation of samples accounted for 32-48 percent of the total gas production. The half time of gas production occurred earlier; $R_{max}S_1$ (numerically) and $R_{max}G_1$ was higher in the E and EP samples than in the R sample. In the second phase, no consistent effects of processing were observed with regard to the gas production parameters.

Except for C_2 and $R_{max}S_2$, the effects of fraction on the gas production parameters were significant (Table 4). As can be seen from Table 4 in all fractions most of the gas was produced in the second phase with a half time ranging from 13 (NWF) to 15.5 (ISWF) h. The fermentation of SWF in the first phase differed distinctly from those of NWF and ISWF and showed a higher $R_{max}S$ and $R_{max}G$ and occurred sooner (5.4 h).

A significant interaction between process and fraction was observed with regard to all kinetics of gas production profiles except A_1 and A_2. In NWF, expander processing and the ensuing pelleting decreased $R_{max}S_1$, $R_{max}S_2$ and $R_{max}G_2$ whereas $R_{max}G_1$ was

increased (data not shown). In SWF, the processes of E and EP compared to R, significantly increased $R_{max}S_1$, $R_{max}S_2$ and $R_{max}G_1$ (data not shown).

Maize

The kinetics of gas production in the samples of maize are shown in Table 5. A significant effect of processing was observed with regard to C_1 (E 0.6 h shorter than R, $P < 0.05$), $RmaxS_1$ (EP 0.3 and 0.4 h^{-1} higher than E and R, respectively; $P < 0.05$) and $R_{max}G_1$ (EP 14.1 ml h^{-1} higher than E; $P < 0.05$).

Within the model, fraction contributed significantly to all the kinetic profile characteristics except for C_2 (Table 5). The results show no significant differences (except for C_1) between the kinetics of gas production of ISWF and NWF. Regarding the first phase, C_1 was reached earlier and the $R_{max}S_1$ and $R_{max}G_1$ were higher in the SWF than in the NWF and ISWF.

The results show that the interaction between process and fraction was only significant for the $R_{max}S_1$. In NWF, processes E and EP significantly increased $R_{max}S_1$ (EP and E were 0.5 and 0.45 h^{-1} higher than R, respectively; data not shown). Surprisingly, in the SWF the highest $R_{max}S_1$ was observed for expander-pelleted samples (2.0, 1.5 and 1.3 h^{-1} in EP, R and E, respectively; data not shown).

Milo

The results (Table 6) show that the interaction between process and fraction was only significant for the OMCV ($P < 0.05$) and $R_{max}S_1$ ($P < 0.1$). In NWF, processes E and EP significantly increased $R_{max}S_1$ (EP and E were 0.5 and 0.45 h^{-1} higher than R, respectively; data not shown). Surprisingly, in the SWF the highest $R_{max}S_1$ was observed for expander-pelleted samples (2.0, 1.5 and 1.3 h^{-1} in EP, R and E, respectively; data not shown). Like in maize, the gas production profile of SWF in milo showed two distinct phases. The first phase showed a very rapidly degraded fraction. The C_1 was reached much earlier, and the $R_{max}S_1$ and A_1 were higher than those of the first phase of NWF and ISWF. In the second phase, however, $R_{max}S$ of the SWF was more or less similar to the other fractions.

Table 4. Gas production characteristics in samples of barley and its fractions fitted to a di-phasic Groot model

Class			OMCV	A_1	C_1	$R_{max}S_1$	$R_{max}G_1$	A_2	C_2	$R_{max}S_2$	$R_{max}G_2$
Process	R		281.9^{a}	0.326^{a}	8.6^{a}	0.583^{a}	18.8^{a}	0.674^{a}	14.2^{ab}	0.041^{ab}	12.2^{a}
	E		291.0^{a}	0.365^{ab}	7.8^{b}	0.788^{a}	30.6^{b}	0.635^{ab}	12.8^{b}	0.042^{ab}	11.5^{a}
	EP		276.4^{a}	0.458^{b}	7.6^{b}	0.765^{a}	36.6^{b}	0.542^{b}	16.0^{a}	0.036^{a}	8.1^{b}
Fraction	NWF		254.7^{a}	0.481^{a}	9.1^{a}	0.397^{a}	20.4^{a}	0.519^{a}	13.2^{b}	0.043^{a}	6.9^{a}
	ISWF		306.2^{b}	0.248^{b}	9.6^{a}	0.591^{a}	16.0^{a}	0.752^{b}	15.5^{a}	0.037^{b}	14.9^{b}
	SWF		288.4^{c}	0.419^{a}	5.4^{b}	1.148^{b}	49.7^{b}	0.581^{a}	14.3^{ab}	0.039^{b}	10.1^{c}
	SEM		5.2	0.040	0.2	0.0880	3.4	0.035	0.65	0.002	0.7
Significance (P)	Process	(P)	0.1942	0.0464	0.0150	0.2412	0.0069	0.0463	0.0137	0.0304	0.0024
	Fraction	(F)	0.0002	0.0015	<0.0001	<0.0001	<0.0001	0.0015	0.0910	0.0527	<0.0001
	P × F		0.0010	0.1284	0.0775	0.0050	0.0210	0.1279	0.0155	0.0252	0.0386

OMCV, cumulative gas production (ml g^{-1} OM); A_1 and A_2, proportion of gas produced at the first and second phase relative to the total produced gas; C_1 and C_2, half-time of gas production (h); $R_{max}S$, maximum fractional rate of substrate degradation (h^{-1}); $R_{max}G$, maximum rate of gas production (ml h^{-1}); and SEM, standard error of least square mean. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Table 5. Gas production characteristics in samples of maize and its fractions fitted to a di-phasic Groot model

Class			OMCV	A_1	C_1	$R_{max}S_1$	$R_{max}G_1$	A_2	C_2	$R_{max}S_2$	$R_{max}G_2$
Process	R		243.2^{a}	0.330^{a}	8.2^{a}	0.876^{b}	31.1^{ab}	0.670^{a}	17.0^{a}	0.106^{b}	8.3^{a}
	E		218.5^{a}	0.384^{a}	7.6^{b}	1.009^{b}	27.5^{b}	0.616^{a}	18.0^{a}	0.141^{a}	9.2^{a}
	EP		236.1^{a}	0.340^{a}	8.3^{a}	1.309^{a}	42.6^{a}	0.659^{a}	16.2^{a}	0.125^{ab}	9.3^{a}
Fraction	NWF		254.8^{a}	0.304^{b}	9.1^{a}	0.796^{b}	22.7^{b}	0.696^{a}	15.5^{a}	0.145^{a}	11.3^{a}
	ISWF		232.1^{a}	0.260^{b}	9.5^{b}	0.803^{b}	17.5^{b}	0.740^{a}	17.9^{a}	0.129^{a}	10.5^{a}
	SWF		210.9^{b}	0.490^{a}	5.5^{c}	1.596^{a}	61.1^{a}	0.510^{b}	17.8^{a}	0.097^{b}	5.1^{b}
	SEM		8.5	0.029	0.1	0.071	4.0	0.030	0.7	0.009	0.4
Significance (*P*)	Process	(P)	0.1252	0.4026	0.0048	0.0027	0.0382	0.4054	0.2150	0.0677	0.2296
	Fraction	(F)	0.0074	0.0002	<0.0001	<0.0001	<0.0001	0.0003	0.0705	0.0082	<0.0001
	P × F		0.4869	0.5054	0.5066	0.0412	0.1286	0.5066	0.3473	0.4644	0.4595

Gas production parameters explained in Table 4. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Table 6.Gas production characteristics in samples of milo and its fractions fitted a di-phasic model Groot model

Class			OMCV	A_1	C_1	$R_{max}S_1$	$R_{max}G_1$	A_2	C_2	$R_{max}S_2$	$R_{max}G_2$
Process	R		202.9[a]	0.421[a]	10.4[a]	0.685[a]	17.0[a]	0.579[a]	19.6[a]	0.128[a]	7.8[a]
	E		235.8[b]	0.370[a]	8.8[b]	0.987[b]	27.8[b]	0.630[a]	18.9[a]	0.109[a]	7.9[a]
	EP		238.2[b]	0.402[a]	8.2[c]	1.297[b]	36.5[c]	0.598[a]	17.7[a]	0.131[a]	9.5[a]
Fraction	NWF		238.4[a]	0.436[a]	10.4[a]	0.716[b]	24.1[b]	0.564[b]	17.4[b]	0.129[a]	9.9[a]
	ISWF		270.3[b]	0.288[b]	10.0[a]	0.897[b]	21.7[b]	0.712[a]	17.8[ab]	0.109[a]	11.4[a]
	SWF		168.3[c]	0.469[a]	6.4[b]	1.357[a]	35.4[a]	0.531[b]	20.9[a]	0.131[a]	3.9[b]
	SEM		5.2	0.043	0.2	0.099	2.7	0.043	1.0	0.013	0.8
Significance (P)	Process	(P)	0.0008	0.6992	0.0017	0.0042	0.0012	0.6999	0.4235	0.4489	0.2860
	Fraction	(F)	<0.0001	0.0239	<0.0001	0.0029	0.0089	0.0238	0.0527	0.0980	<0.0001
	P × F		0.0139	0.4836	0.4984	0.0962	0.1087	0.4845	0.3339	0.5363	0.3216

Gas production parameters explained in Table 4. Means with different superscripts within class and column differ significantly ($P < 0.05$).

VFA production and formation of fermentation end- product

Barley

VFA production profiles and formation of fermentation end-product in the samples of barley are presented in Table 7. Processing (P), fraction (F) and sampling time (T) had significant effects, and also many interactions were significant. Compared to the R samples, TVFA significantly increased whereas the NGR, BCR and NH_3-N were decreased by E and EP treatment.

Striking differences were observed between the fractions with regards to profile of VFA and formation of fermentation end-products (Table 7). The fermentation of the SWF was quite distinct from that of in NWF and ISWF with a significantly higher TVFA, NGR and NH_3-N. A significant interaction of processing and incubation time was found for NH_3-N. The changes in NH_3-N throughout the incubation period in the samples of barley are presented in Figure 2A. Both E and EP reduced the NH_3-N after 72 h of incubation compared to R. Compared to the R sample, the BCR was marginally lower in E and EP samples over the course of incubation (data not shown). In all fractions, NGR decreased dramatically up to 24 h and remained almost constant thereafter (data not shown). At the beginning of fermentation, processes E and EP caused a lower NGR in the SWF of barley (data not shown). However, the differences in the NGR in the other fractions of barley caused by the E and EP became more apparent at the later stages of fermentation where processes E and EP showed a lower NGR (data not shown).

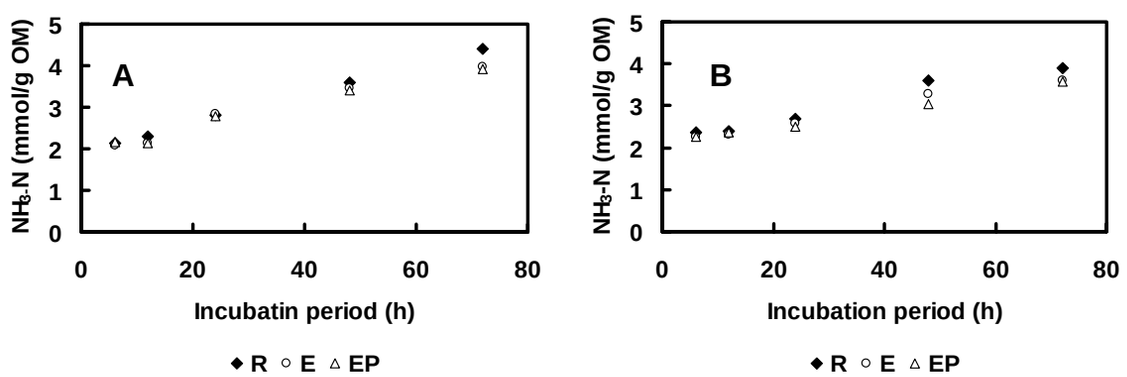


Figure 2. NH_3-N production in Retsch milled (R), expander processed (E) and expander-pelleted (EP) barley (A) and maize (B).

Table 7. VFA production profiles (mmol g^{-1} OM), total VFA production (TVFA, mmol g^{-1} OM), non-glucogenic ratio (NGR), branched chain ratio (BCR) and NH_3-N (mmol g^{-1} OM) for the barley samples and its fractions

Class		HAc	HPr	HBu	TVFA	NGR	BCR	NH_3-N
Process	R	3.2[a]	2.6[a]	0.7[a]	6.9[a]	2.1[a]	0.026[a]	3.0[a]
	E	3.5[b]	2.9[b]	0.7[a]	7.5[b]	2.0[b]	0.022[b]	2.9[b]
	EP	3.5[b]	3.0[b]	0.7[a]	7.6[b]	1.9[b]	0.021[b]	2.9[b]
Fraction	NWF	3.0[a]	2.5[a]	0.7[a]	6.5[a]	1.9[a]	0.024[a]	2.7[a]
	ISWF	3.0[a]	2.7[b]	0.7[a]	6.7[a]	1.9[a]	0.022[b]	2.4[b]
	SWF	4.3[b]	3.3[c]	0.9[b]	6.9[b]	2.1[b]	0.023[ab]	3.7[c]
	SEM	0.04	0.07	0.03	0.12	0.04	0.0005	0.03
Time (h)	6	1.5[a]	0.6[a]	0.2[a]	2.4[a]	3.2[a]	0.022[a]	2.1[a]
	12	2.4[b]	1.9[b]	0.6[b]	5.0[b]	1.9[b]	0.016[b]	2.2[a]
	24	3.9[c]	3.7[c]	1.0[c]	9.0[c]	1.6[c]	0.020[a]	2.8[b]
	48	4.5[d]	3.8[c]	1.0[c]	9.8[d]	1.6[c]	0.028[c]	3.5[c]
	72	4.5[d]	4.2[d]	0.9[c]	10.4[d]	1.6[c]	0.029[c]	4.1[d]
	SEM	0.07	0.05	0.05	0.15	0.06	0.0007	0.05
Significance	Process (P)	<0.0001	<0.0001	0.8128	0.0003	0.0002	<0.0001	0.0002
	Fraction (F)	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	0.0106	<0.0001
	Time (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P × F	0.0036	0.8062	0.3377	0.3244	0.8125	0.1354	0.2095
	P × T	0.2110	0.0534	0.5181	0.1543	0.9428	0.0515	0.0077
	F × T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	F × P × T	0.1430	0.1356	0.7590	0.3160	0.0089	0.0002	<0.0001

SEM, standard error of least square mean. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Maize

Significant differences were observed in VFA production profiles, NGR, BCR and NH_3-N with regard to process, fraction, time and interactions between these factors (Table 8). Regardless of fractions and incubation time, the NGR, BCR, NH_3-N were lower for samples of E and EP compared to those in the R maize sample. Significant differences between fractions were observed with respect to VFA production profiles (a higher HAc, HPr and a lower HBu produced for SWF compared to the NWF and ISWF), TVFA (SWF was 1.3 and 1.6 unit higher than ISWF and NWF, respectively), NGR (SWF

was 0.2 lower than NWF), BCR (SWF was relatively 11 and 5% higher than ISWF and NWF) and NH_3-N (SWF was 1.8 and 1.7 unit higher than ISWF and NWF).

Table 8. VFA production profiles (mmol g^{-1} OM), total VFA production (TVFA, mmol g^{-1} OM), NGR, BCR and NH_3-N (mmol g^{-1} OM) for the maize samples and its fractions

Class		HAc	HPr	HBu	TVFA	NGR	BCR	NH_3-N
Process	R	3.1^{a}	3.0^{b}	0.71^{a}	7.1^{b}	2.0^{a}	0.021^{a}	3.0^{a}
	E	3.1^{a}	3.1^{b}	0.65^{b}	7.1^{b}	1.9^{b}	0.019^{b}	2.8^{b}
	EP	3.3^{a}	3.3^{a}	0.66^{ab}	7.5^{a}	1.8^{c}	0.019^{b}	2.7^{b}
Fraction	NWF	2.8^{b}	2.8^{c}	0.7^{b}	6.6^{b}	2.0^{a}	0.019^{b}	2.3^{a}
	ISWF	2.8^{b}	3.1^{b}	0.8^{b}	6.9^{b}	1.9^{b}	0.018^{c}	2.2^{a}
	SWF	3.9^{a}	3.4^{a}	0.5^{a}	8.2^{a}	1.8^{b}	0.020^{a}	4.0^{b}
	SEM	0.05	0.04	0.02	0.1	0.02	0.0002	0.02
Time (h)	6	1.2^{a}	0.4^{a}	0.2^{a}	1.9^{a}	3.6^{a}	0.021^{b}	2.3^{a}
	12	2.3^{b}	1.8^{b}	0.5^{b}	4.7^{b}	2.0^{b}	0.013^{d}	2.4^{a}
	24	3.6^{c}	4.0^{c}	0.9^{c}	8.7^{c}	1.4^{cd}	0.022^{c}	2.6^{b}
	48	4.2^{d}	4.4^{d}	1.1^{d}	10.1^{d}	1.5^{cd}	0.021^{b}	3.3^{c}
	72	4.5^{d}	4.9^{e}	0.7^{e}	10.6^{d}	1.2^{d}	0.024^{a}	3.7^{d}
	SEM	0.09	0.07	0.03	0.16	0.06	0.0004	0.02
Significance	Process (P)	0.058	<0.0001	0.0395	0.0111	<0.0001	<0.0001	<0.0001
	Fraction (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Time (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P × F	0.9342	0.9348	0.1313	0.9931	<0.0001	<0.0001	<0.0001
	P × T	0.4267	0.2482	0.0119	0.2937	0.2186	<0.0001	0.0004
	F × T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	F × P × T	0.7559	0.5507	0.7454	0.7562	<0.0001	<0.0001	<0.0001

SEM, standard error of least square mean. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Significant interactions of processing (P) and incubation time (T) were found for BCR and NH_3-N (Table 8). Figure 2.B shows that both E and EP treatment reduced the NH_3-N at 48 and 72 h after incubation. In the fractions of maize, most differences in the pattern of VFA production occurred up to 12h of incubation, where the ISWF of E and EP processed maize showed a lower NGR than that of R of maize (data not shown). Processes E and EP did not change the pattern of NH_3-N production in the NWF and ISWF of maize (data are not shown). However, with regard to the SWF a lower NH_3-N

was observed for the E and EP than for the R after 48 and 72 h of incubation (data not shown).

Milo

Like barley and maize, not only processing, fraction and time of sampling had significant effects, but also there were many significant interactions (Table 9). Compared to the R samples, both E and EP treatment significantly increased HAc, HPr and TVFA whereas BCR and NH_3-N were decreased. Like barley and maize striking differences were observed between the fractions with respect to fermentation end-product.

Figure 3 shows that profiles of VFA, represented as NGR, and BCR both were affected by expander processing and the ensuing pelleting. Compared to the R sample, in both E and EP samples a lower NGR (marginally) and BCR was observed throughout the incubation.

The results show that the processes E and EP had a more profound effect on NGR in NWF than they had in ISWF and SWF. In the NWF these processes significantly decreased the NGR ($P < 0.05$; data not shown).

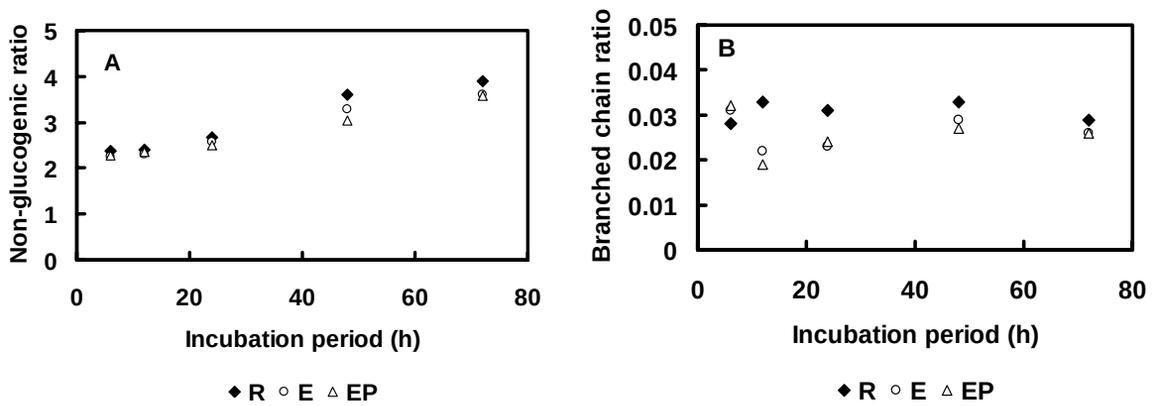


Figure 3. Non-glucogenic ratio (A) and branched chain ratio (B) in Retsch milled (R), expander processed (E) and expander-pelleted (EP) milo.

Table 9. VFA production profiles (mmol g^{-1} OM), total VFA production (TVFA, mmol g^{-1} OM), NGR, BCR and NH_3-N (mmol g^{-1} OM) for the milo samples and its fractions

Class		HAc	HPr	HBu	TVFA	NGR	BCR	NH_3-N
Process	R	3.0[a]	2.6[a]	0.59[a]	6.4[a]	2.2[a]	0.031[a]	2.9[a]
	E	3.4[b]	2.9[b]	0.70[b]	7.3[b]	2.2[a]	0.026[b]	2.8[b]
	EP	3.5[b]	2.9[b]	0.71[b]	7.4[b]	2.2[a]	0.026[b]	2.7[b]
Fraction	NWF	2.8[b]	2.3[c]	0.7[b]	6.2[c]	2.5[a]	0.027[b]	2.2[a]
	ISWF	3.0[b]	2.9[b]	0.8[a]	7.1[b]	2.1[b]	0.029[a]	2.3[a]
	SWF	4.0[a]	3.2[a]	0.4[c]	7.9[a]	2..0[c]	0.026[b]	4.0[b]
	SEM	0.05	0.06	0.02	0.1	0.03	0.0005	0.02
Time (h)	6	1.2[a]	0.4[e]	0.2[d]	1.9[d]	4.1[a]	0.031[a]	2.4[a]
	12	2.4[b]	1.7[d]	0.5[c]	4.8[c]	2.3[b]	0.025[c]	2.5[b]
	24	3.8[c]	3.6[c]	0.9[a]	8.7[b]	1.5[c]	0.026[b]	2.7[c]
	48	4.5[d]	3.8[b]	1.0[a]	9.8[a]	1.7[c]	0.030[a]	3.3[d]
	72	4.4[d]	4.4[a]	0.8[b]	10.1[a]	1.3[d]	0.027[b]	3.3[d]
	SEM	0.05	0.05	0.02	0.1	0.04	0.0005	0.02
Significance	Process (P)	<0.0001	<0.0001	<0.0001	<0.0001	0.1984	<0.0001	<0.0001
	Fraction (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Time (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P × F	0.0005	0.4359	0.4695	0.1141	<0.0001	0.2560	<0.0001
	P × T	0.0290	0.0416	0.2490	0.0515	<0.0001	<0.0001	0.0192
	F × T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	F × P × T	0.0996	0.7854	0.8899	0.3904	<0.0001	<0.0001	0.0317

SEM: standard error of least square mean. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Discussion

Effect of expander processing and expander-pelleting on the contribution of fractions and chemical components in the cereal grains

Processing may alter the physico-chemical characteristics of a feed in two ways. Because processing often affects the particle size distribution, it may interfere with analytical procedures that involve filtration, such as NDF analysis. Changes in the contribution of DM to different fractions are also possible if processing either alters the solubility, thereby changing the size of true soluble fractions (SWF), or reduces the size of particles, thereby increasing the ISWF. Indeed changes in the distribution of DM over the

different fractions were observed, most likely due to changes in particle size distribution, gelatinization of starch and protein denaturation. Compared to the original mash material that was pre-ground with a hammer mill through a 3-mm sieve, process R increased the total washable fraction (SWF+ISWF). The subsequent grinding of feed samples in the Retsch mill not only reduced the particle size such that more particles were small enough to escape the nylon bag pores, it also increased the size of the SWF. However, the extent of increase in the size of ISWF was much larger than that of the SWF.

From the results in Tables 1 and 2 the shifts in the distribution of the different chemical components over the three fractions NWF, ISWF and SWF caused by the different treatments can be calculated. The NDF contents of WHO and NWF were lower in thermo-mechanical processed samples (E and EP) than in R samples, mostly with a concomitant increase of the starch content. The shift between starch and NDF is likely the result of the amylase activity in the NDF analysis. Processing apparently made starch more susceptible for enzymatic degradation. Expander processing and the ensuing pelleting process drastically increased the content of gelatinised starch (Table 3), therefore made the starch more available for enzymatic breakdown. It has been well documented that both mechanical and thermal energy transferred to starch break the main and secondary valance bonds between neighbouring starch polymers in the starch structure, which increase the susceptibility of starch to enzyme action.[23] The reduced NDF content of WHO and NWF of thermo-mechanical processed samples could also result from a reduced particle size, causing the loss of more fine particles during the filtration procedure of the NDF analysis or by partly solubilising the NDF due to processing. Indeed, the reduced NDF content of E and EP samples was concomitant with an elevated chemically not determined fraction that is thought to be soluble NSP.[11] In accordance with findings of Yang et al.,[11] the SWF of expander processed samples of barley and milo had a higher sugar content compared to the R samples (Table 2). Starch dextrinisation and depolymerisation that occur due to shear forces in expander processing,[24] may explain such higher sugar contents of SWF of samples compared to the R samples.

It further appears that E and EP shift CP from SWF and ISWF to NWF, whereas starch is shifted from ISWF to NWF in maize and milo, but not in barley. The shift in CP could be explained by the occurrence of protein denaturation and therefore decreasing the size of soluble proteins. A decrease in the size of NWF after pelleting the expander processed barley can also be explained by the reduction of particles size during the pelleting process. It has further been shown that gelatinised starch has potential to act as a

binder.[25] In the current study though, the content of gelatinised starch was drastically higher in E compared to R treated barley (Table 3), yet the size of ISWF (fine particles smaller than 40 μ) was not different from that of R treated barley. Probably the extent of starch gelatinisation in the current study was not high enough to allow starch to act as a binder in barley.

The decrease in the size of ISWF of maize and milo due to EP treatment observed in this experiment was inconsistent with the results of Tothi et al.,[26] where the particle loss was reported to have increased with EP treatment in both maize and barley. The discrepancy between the results of the current study with findings of Tothi et al.[26] can be explained by the different methods used to determine the size of particle loss (ISWF).Yang et al.,[11] in agreement with the present study reported similar decreases in the size of the washable fraction with both barley and maize upon pelleting, toasting and the combination of both. Interestingly, in the samples of maize and milo used in the present study a decreased ISWF due to E treatment was increased by the ensuing pelleting. It implies that although in expander treated samples of maize and milo gelatinised starch serves as a binding agent, its binding role could be disturbed by physical forces imposed upon it by the additional pelleting. The extent of decrease in water solubility (SWF) due to expander processing in our research was higher in barley than in maize and milo. There are two possible explanations. Firstly, agglomerated fine particles due to the expander processing may restrict the contact of feed particles with the solvent (water) upon the fractionation procedure. Secondly, chemical reactions (like protein denaturation) can be kept responsible for the observed decreased SWF due to expander processing. Indeed in an earlier experiment we observed a positive correlation between the magnitude of SWF and the enthalpy of protein denaturation (data not shown), indicating that changes in protein solubility affect the size of SWF. To bear out the effect of feed particles on the size of SWF, it is worthwhile mentioning that the decreased SWF in maize and milo upon expander processing, increased again with consecutive pelleting. These increases in the size of SWF in maize and milo corresponded with an increased ISWF (particle loss during the washing procedure). The reduced protein content of SWF after the E and EP in the current study was concomitant with a reduced transition enthalpy of the protein denaturation peak measured using differential scanning calorimetry (data not shown). Yang et al.[11] also found a lower CP content of SWF for samples of toasted and toasted-pelleted barley and maize compared to the unprocessed samples.

Effect of processing on in vitro cumulative gas production kinetics of processed cereal grains

In barley differences in the distribution of gas production between the two phases were observed between treatments (Table 4). The higher A_1 with processes E and EP compared to R, is an indication that E and EP processed barley was more easily accessible for microbes and R material initially offered some resistance to microbial degradation. This is further confirmed by a higher $R_{max}S_1$ and $R_{max}G_1$ in both E and EP treated barley compared to the R barley. The results of the current study were in fair agreement with some *in vivo* trails where it was found that expander treatment increased rumen degradation of starch in grains like barley and oats.[27] When the gas production profiles were separately analysed by fractions, the lower $R_{max}S_1$ observed for NWF in E and EP processed barley (data not shown) is consistent with the results of Ljøkjel et al.,[28] Tothi et al.[26] and Prestløkken[29] where a decreased fractional degradation rate of barley was observed with expander treatment. These inconsistent results of expander treatment on ruminal degradation of feeds like barley, may not only be explained by different treatment conditions but also by the different fractions used (WHO vs. NWF).[2,27] While in the *in situ* trials rate of degradation is calculated for NWF, in the *in vivo* trial rate of ruminal degradation are normally given for WHO. The lowered $R_{max}S_1$ of NWF in E and EP barley was in contrast with the higher gelatinised starch found for the E and EP samples. This indicates that in a complex matrix of chemical components like the NWF, other components rather than starch might have been affected by the processing. It has been well documented that thermal processing physically protects the starch granules from being fermented by denaturing the protein matrix surrounding the starch granules.[30] Moreover, it is shown that when barley and oats were subjected to heat and pressure during the expander processing, starch and protein were transformed into a dough, in which the total surface area was decreased. This may limit the extent of microbial attachment and thereby ruminal degradation.[29] Although in NWF of barley, expander and expander pelleting decreased the $R_{max}S_1$ (data not shown), an opposite effect was observed for SWF in that these processes significantly increased $R_{max}S_1$. This might be due to the effect of E and EP processing on soluble non-starch polysaccharides (NSP) content of this fraction. It is thought that E and EP processing might have broken down the soluble NSP to its monomer constituents thereby increasing the $R_{max}S_1$. Besides, the increased $R_{max}S_1$ of SWF in barley was accompanied with an increased chemically not determined fraction (Table 2) that is thought to constitute soluble carbohydrates.[11] It has been

suggested that soluble fibres increase after pelleting and extrusion.[31-33] However, the authors believe that a further experiment has to be carried out to bear out this theory.

In maize and milo overall, it is noted that expander processing and expander-pelleting did show a tendency to increase the maximum fractional rate of substrate degradation ($R_{max}S_1$) (Tables 5 and 6). This was in line with the increase in effective degradability reported by Tóthi et al.[26] in maize using an expander treatment. Arieli et al.,[34] however, found no effect of expander processing on effective ruminal degradability of maize DM. The discrepancy between the effects of these processes in barley, maize and milo can be explained by the nature of their starch and the protein matrix surrounding the starch granules. In maize, the protein matrix surrounding the starch granules is extremely resistant to microbial degradation whereas in barley this structure is quite susceptible to microbial degradation.[35] It is thought that thermal processing such as expander treatment and subsequent pelleting might disrupt the surrounding protein matrix of starch granules in maize and make them more susceptible to microbial degradation. In barley, however, such thermal processing may lead to the formation a protein-starch or protein-protein (*iso*-peptide bond) complex[29] thereby making the barley starch less degradable. In fact in barley with a higher protein and lysine content compared to maize and milo,[36] the chance of occurring of such complexes is higher than in maize and in milo.

Some theories have been put forward by authors such as Dijkstra et al.;[37] Cone and van Gelder;[38] Groot et al.[22] that the first phase of gas production is presumably from the fermentation of highly soluble and easily fermentable fractions while the second phase is due to the fermentation of slowly fermentable insoluble fractions. Cone et al.[38] stated that gas production in the first 3 h of incubation can be regarded as fast initial fermentation of the water-soluble components and gas production between 3 and 20 h as moderately fast fermentation of the non-soluble components. However, the current study showed that, when fitting a di-phasic model to the gas production profiles of the soluble fraction, 51 to 58 % of the gas was produced in the second phase of fermentation with a C_2 value ranging from 14.3 to 20.9 h. This would suggest that the SWF consists of a complex of solubilised monomers and polymers of different degree of polymerisation.

Effect of processing on formation of fermentation end-product in the cereal grains

Heat treatment normally results in gelatinisation of starch[39] and denaturation of protein,[6] rendering the starch into a more accessible form (gelatinised starch) and protein

into a less available form (denaturated protein) for microbial degradation. In all concentrate ingredients used in the present study, the general tendency of increased TVFA and reduced NGR (except in milo), BCR and NH_3-N indicate that the expander processing and expander-pelleting increased starch degradation *in vitro* whereas the degradation of protein was decreased. These effects were in agreement with the findings of Prestløkken and Harstad[2] who found in a barley-based concentrate, that expander processing increased TVFA whereas NH_3-N was decreased. Crocker et al.[40] reported a significant increase in the molar proportion of propionate and a decrease in the molar proportion of acetate, and NGR when maize was heat treated. In another study,[41] it was found that steam treatment of maize increased the proportion of propionate whilst the concentration of acetate and *iso*-valerate decreased. In our research processing caused maize to degrade at a faster rate allowing propionate producing bacteria to thrive. It has been demonstrated that a rapid fermentation of starch by amylolytic bacteria tends to produce more propionate.[26]

It appears that the processes E and EP shifted the pattern of fermentation of carbohydrates towards a glucogenic fermentation, which is indicated by a higher HPr and a lower NGR compared to the R samples. A lower NGR, BCR and NH_3-N due to the processes E and EP compared to that of R indicates that the processing could have a positive effect by synchronising the fermentation of proteins and readily fermentable carbohydrates (gelatinised starch). Prestløkken and Harstad[2] demonstrated that the milk production and milk constituents were increased in cows fed with expander processed barley based concentrate, indicating that the expander treatment increased the supply of nutrients for milk synthesis. Indeed we found that the efficiency of fermented feed carbohydrates incorporated into microbial biomass was elevated when barley, maize and milo were subjected to expander and expander-pelleting processes (Azarfar et al., unpublished data).

As is evident from the results in Tables 7 to 9 the fermentation of SWF with respect to fermentation end-product was distinctly different from that of NWF and ISWF. It can therefore be expected that expander processing and the followed pelleting process change the formation of end-product by changing the distribution of DM over these fractions (NWF, ISWF and SWF). More importantly, processing can change the chemical compositions of these fractions thereby changing the pattern of fermentation. For instance, it is clear from Table 2 that expander and expander-pelleting reduced the CP content of SWF which explains why in the present study a lower BCR and NH_3-N were

observed with the processed samples. These results are in agreement with the findings of Yang et al. (2005).[11]

Conclusion

Expander processing decreases the contribution of the truly soluble fraction (SWF) in cereal grains. Pelleting ensuing expander treatment also decreases the soluble fraction (SWF) in barley but increases it in maize and milo. In maize and milo, gelatinised starch serves as a binder agent reducing the size of ISWF. However, the ensuing pelleting disturbs the binding effect of gelatinised starch, and increases the size of ISWF.

Processing shows a tendency to increase the fractional rate of substrate degradation and rate of gas production at the early stage of fermentation. The general tendency of reduced NGR and BCR due to the expander processing and the followed pelleting compared with all three concentrate ingredients indicates that the processing synchronised the fermentation of proteins and carbohydrate, as both expander and expander-pelleting increased the gelatinised starch content whilst the water soluble protein was decreased. Moreover, it appears that the expander processing and the ensuing pelleting process shifted the pattern of fermentation of carbohydrates towards a glucogenic fermentation besides providing certain level of protection to dietary protein as represented by a lowered NH_3-N production.

Acknowledgement

First author thanks the Ministry of Science, Research and Technology of Iran for financing the project. The authors wish to express their gratitude to Saskia van Laar, Dick Bongers, Jane-Martine Muijlaert, Truus Post and Meijke Booij of the Laboratory of the Animal Nutrition Group; without their help this study would not have been possible. Tamme Zandstra is thanked for his co-operation in processing the feed samples.

References

1. Tóthi R, Babinszky L and Tamminga S, Effect of hydrothermal processing on the feed quality, the ruminal degradation of grains and the milk compositions in high producing dairy cows. *Krimva* **44**:203-210 (2002).

2. Prestløkken E and Harstad OM, Effects of expander-treating a barley-based concentrate on ruminal fermentation, bacterial N synthesis, escape of dietary N, and performance of dairy cows. *Anim Feed Sci Technol* **90**:227- 246 (2001).

3. Tóthi R, *Processed Grains as a Supplement to Lactating Dairy Cows*, PhD Thesis, Wageningen University, Wageningen (2003).

4. Nielsen I, Effect of expander treatment on protein quality in different raw materials and mixture, Intercoop Feedstuffs congress, Lofoten, Norway.

5. Huntington JA, Starch utilization by ruminants: from basic to the bunk. *J Anim Sci* **75**:852-867 (1997).

6. Goelema JO, *Processing of Legume Seeds: Effects on Digestive Behaviour in Dairy cows*, PhD Thesis, Wageningen University, Wageningen (1999).

7. Tamminga S, van Straalen WM, Subnel APJ, Meijer RMG, Steg A, Wever CJG and Blok MC, The Dutch protein evaluation system: DVE/OEB system. *Livest Prod Sci* **40**:139-155 (1994).

8. Michalet-Doreau B and Ould-Bah MY, *In vitro* and *in sacco* methods for estimation of dietary nitrogen degradability in the rumen: a review. *Anim Feed Sci and Technol* **40**:57-86 (1992).

9. Melin M, Gierus M, Meijer GAL and van Vuuren AM, Chemical characteristics and degradability of crude protein in S-fraction. ID-Lelystad, Lelystad, The Netherlands, Report No. 2079 (2001).

10. Azarfar A, Tamminga S and Boer H, Effects of washing procedure, grain type, and particle size on the size of non-washout and insoluble washout fractions in concentrate ingredients, in *EAAP – 55th Annual Meeting*. p 96 (2004).

11. Yang HJ, Tamminga S, Williams BA, Dijkstra J and Boer H, *In vitro* gas and VFA production profiles of barley and corn and their soluble washout fractions after feed processing. *Anim Feed Sci Technol* **120**:125-140 (2005).

12. Theodorou MK, Williams BA, Dhanoa MS, McAllan AB and France J, A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim Feed Sci Technol* **48**:185-197 (1994).

13. Williams BA, van der Poel AFB, Boer H and Tamminga S, The use of cumulative gas production to determine the effect of steam explosion on fermentability of two substrates with different cell wall quality. *J Sci Fd Agric* **69**:33-39 (1995).

14. Davies ZS, Mason D, Brooks AE, Griffith GW, Merry RJ and Theodorou MK, An automated system for measuring gas production from forages inoculated with rumen fluid and its use in determining the effect of enzymes on grass silage. *Anim Feed Sci Technol* **83**:205-221 (2000).

15. Lowe SE, Theodorou MK, Trinci APJ and Hespel RB, Growth of anaerobic rumen fungi on defined and semi-defined media lacking rumen fluid. *J Gen Microb* **131**:2225-2229 (1985).

16. Williams BA, Bosch MW, Boer H, Verstegen MWA and Tamminga S, An *in vitro* batch culture method to assess potential fermentability of feed ingredients for monogastric diets. *Anim Feed Sci Technol* **123-124**:445-462 (2005).

17. Searle PL, The Berthelot or indophenol reaction and its use in the analytical chemistry. a review. *Analyst* **109**:549-568 (1984).

18. Goelema JO, Spreeuwenberg MAM, Hof G, van der Poel AFB and Tamminga S, Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. *Anim Feed Sci Technol* **76**:35-50 (1998).

19. Rijnen MMJA, Verstegen MWA, Heetkamp MJW and Schrama JW, Effects of dietary fermentable carbohydrates on energy metabolism in group-housed sows. *J Anim Sci* **79**:148-154 (2001).

20. Van Soest PJ, Robertson JB and Lewis BA, Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**:3583-3597 (1991).

21. Ørskov ER, Manipulation of rumen fermentation for maximum food utilization. *World Rev Nutr Dietet* **22**:152-182 (1975).

22. Groot JCJ, Cone JW, Williams BA, Debersaques FMA and Lantinga EA, Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. *Anim Feed Sci Technol* **64**:77-89 (1996).

23. Klinger RW, Meuser F and Niediek E, Depolymerization of starch by high pressure extrusion. *Starch / Stärke* **38**:40-44 (1986).

24. Gomez MH and Aguilera JM, Changes in the starch fraction during extrusion-cooking of corn. *J Food Sci* **48**:378-381 (1983).

25. Svihus B, Uhlen AK and Harstad OM, Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Anim Feed Sci Technol* **122**:303-320 (2005).

26. Tóthi R, Lund P, Weisbjerg MR and Hevlpund T, Effect of expander processing on fractional rate of maize and barley starch degradation in the rumen of dairy cows estimated using rumen evacuation and *in situ* techniques. *Anim Feed Sci Technol* **104**:71-93 (2003).

27. Harstad OM, Prestløkken E, Nordheim H, Ruminal fermentation and digestion of carbohydrate fractions in lactating cows fed pelleted or expanded barley and oats based concentrates, in *EAAP- 47th Annual Meeting*, p 82 (1996).

28. Ljøkjel K, Skrede A and Harstad OM, Effects of pelleting and expanding on vegetable feeds on in situ protein and starch digestion in dairy cows. *J Anim Feed Sci* **12**:435-449 (2003).

29. Prestløkken E, In situ ruminal degradation and intestinal digestibility of dry matter and protein in expanded feedstuffs. *Anim Feed Sci Technol* **77**:1-23 (1999).

30. Kotarski SF, Waniska RD and Thurn KK, Starch hydrolysis by rumen micro flora. *J Nutr* **122**:178-190 (1992).

31. Graham H, Fadel JG, Newman CK and Newman RK, Effect of pelleting and β-glucanase supplementation on the ileal and faecal digestibility of a barley-based diet in the pig. *J Anim Sci* **67**:1293-1298 (1989).

32. Shinnick FL, Longacre MJ, Ink SL and Marlett JA, Oat fibre: composition versus physiological function in rats. *J Nutr* **144-151** (1988).

33. Vranjes MV and Wenk C, The influence of extruded vs. untreated barley in the feed, with and without dietary enzyme supplement on broiler performance. *Anim Feed Sci Technol* **54**:21-32 (1995).

34. Arieli A, Bruckental I, Kedar O and Sklan D, Insacco disappearance of starch, nitrogen and fat in processed grains. *Anim Feed Sci Technol* **51**:287-295 (1995).

35. Rooney LW and Pflugfelder RL, Factors affecting starch digestibility with special emphasis on sorghum and corn. *J Anim Sci* **63**:1607-1623 (1986).

36. CVB, Centraal Veevoeder Bureau, Veevoedertabel, gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen. Centraal Veevoeder Bureau, Lelystad, The Netherlands (2004).

37. Dijkstra J, Kebreab E, Bannink A, France J and Lopez S, Application of gas production technique to feed evaluation systems for ruminants. *Anim Feed Sci Technol* **123-124**:561-578 (2005).

38. Cone JW and Van Gelder AH, Influence of protein fermentation on gas production profiles. *Anim Feed Sci Technol* **76**:251-264 (1999).

39. van Soest PJ, *Nutritional Ecology of the Ruminant*, Comstock, Cornell University Press, Ithaca, NY, pp 476 (1994).

40. Crocker LM, Depeters EJ, Fadel JG, Perez-Monti H, Taylor SJ, Wyckoff JA and Zinn RA, Influence of processed corn grain in diets of dairy cows on digestion of nutrients and milk composition. *J Dairy Sci* **81**:2394-2407 (1998).

41. Joy MT, Depeters EJ, Fadel JG and Zinn RA, Effects of corn processing on the site and extent of digestion on lactating cows. *J Dairy Sci* **80**:2087-2097 (1997).

Chapter 4

In vitro gas production profiles and formation of fermentation end-product in processed peas, lupins and faba beans[♣]

Abstract

The effects of using a pre-compacting device (expander) on the degradative behaviour of peas, lupins and faba beans and their different fractions were studied using an *in vitro* gas production technique. The aim was to establish whether the thermo-mechanical processing changes the contribution of dry matter (DM), chemical constituents and degradative behaviour of these concentrate ingredients over the different fractions (non-washable, NWF; insoluble washable, ISWF and soluble washable fraction, SWF). The entire samples (WHO) were fractionated into SWF, ISWF, and NWF fractions. In three consecutive gas production runs, samples of the entire concentrate ingredients (WHO) and their different fractions (NWF, ISWF and SWF) were subjected to three processes (R, Retsch mill ground samples; E, expander treated samples; EP, expander-pelleted samples) and their fermentation characteristics were evaluated using an *in vitro* incubation technique (Automated Pressure Evaluation System, APES) for 72h. In a manually operated *in vitro* fermentation system, the same substrates were fermented for 6, 12, 24 and 48 h in order to collect samples for volatile fatty acids (VFA) and ammonia (NH_3-N) analysis. In peas and faba beans, both the E and EP process significantly ($P < 0.05$) increased the size of NWF compared to process R. The SWF in the processed samples E and EP was lower than in the R samples. The ISWF of peas and faba beans were very rich in starch. Fraction SWF was relatively rich in ash, crude protein, soluble sugars, and a residual chemically undefined fraction (3.7-37.0 % of DM), which was elevated by expander processing and the ensuing pelleting process. In the legume seeds compared to the R samples; both the E and EP samples had a significantly higher maximum fractional rate of gas production in the first phase of fermentation ($P < 0.05$). In lupins and faba beans, E and EP shifted the pattern of fermentation towards a more glucogenic fermentation as represented by a lower non-glucogenic to glucogenic ratio (NGR). The NH_3-N for E and EP samples were significantly ($P < 0.05$) lower than in R samples.

[♣] Azarfar, A., Tamminga, S. Pellikaan, W. F. and van der Poel, A. F. B. 2006. **Submitted**.

Introduction

Since inclusion of proteins of animal origin in ruminant concentrates is not allowed in EU countries, supplies of rumen undegradable protein from plant proteins are of special importance for high producing dairy cows. Because of the high protein and in some cases the high starch or oil content, the nutritional use of legume seeds is globally applied in animal feeds.[1] Apart from soybean and lupin species, legume seeds have a high protein (20-30%) and high carbohydrate (50-65%) content. In the high carbohydrate legume seeds, 35-45% is made up of starch, the major energy source for (most) farm animals. However, legume seeds contain a considerable quantity of protein that is highly soluble and rapidly degradable (up to 75%) in the rumen,[2,3] which are generally associated with nitrogen loss as ammonia and makes them less suitable as a protein source. To avoid this problem dairy diets are usually supplemented either by a source of highly fermentable carbohydrates or with a source of rumen undegradable protein.[4] The latter can be included in the animal's diet via an ingredient source of undegradable protein, either by nature, or resulting from technological processing.[4] In the feed industry, many forms of technological processing are applied to manipulate the site and extent of digestion in ruminants. Expander treatment is such a process which involves heat, pressure and shear. Such processing manipulates the site of digestion in ruminants by reducing the size of feed particles,[5] protecting the proteins from being degraded in the rumen,[6,7] and making the starch granules more accessible for microbial digestion.[7,8] The effects of expander processing and/or pelleting on *in situ* rumen starch[8,9] and protein[6,7,10] degradation of feedstuffs have intensively been studied. In most of these researches the effects of the expander processing and ensuing pelleting on the degradative behaviour of feed sample was evaluated using the *in situ* technique. This technique fractionates feed material into a washable fraction (W), a potentially degradable fraction (D) and an undegradable fraction (U). However, the *in situ* technique cannot measure the rate at which protein and starch in the W fraction are actually degraded. Since the W fraction quantitatively constitutes an important part of feedstuffs' dry matter (DM) (up to 50 % of DM),[11] characterising its degradative behaviour in the rumen is of great importance. Using an appropriate fractionation method to fractionate the W fraction into an insoluble (ISWF) and a soluble washable fraction (SWF) along with an *in vitro* gas production technique is considered an appropriate method to characterise the degradative behaviour of the W fraction (**Chapters 2 and 3**). The current study, therefore, aimed to established the effects of expander processing alone or in combination with pelleting on the chemical composition,

dry matter distribution, *in vitro* degradative behaviour and formation of fermentation end-product in some legume seeds (peas, lupins and faba beans) and their fractions (NWF, non-washable fraction; ISWF and SWF).

Materials and Methods

Experimental setup

The effects of using a pre-compacting device (expander) alone and in combination with pelleting on the degradative characteristics of three legume seeds considered suitable to be included in dairy concentrates (peas, lupins and faba beans) were studied. Feed samples were taken before and after expander processing and after pelleting. The kinetics of gas production in samples of three entire (WHO) concentrate ingredients processed differently (Retsch milled, R; expander processed, E; expander-pelleted, EP) and their fractions (non-washable fraction, NWF; insoluble washable fraction, ISWF; soluble washable fraction, SWF) were evaluated using an *in vitro* incubation technique, which measures gas production continuously in an automated system (APES-IGER, Aberystwyth, Wales) for 72 h. In a manually operated *in vitro* fermentation system, the production of VFA and ammonia (NH_3-N) were simultaneously measured at 6, 12, 24, and 48 h, respectively. The entire concentrate ingredients (WHO) were included in each *in vitro* incubation run to test for possible run effects.

Sample processing

The schematic set up of the experiment is presented in **Chapter 3**. All concentrate ingredients were supplied by a commercial supplier (Research Diet Services, Wijk bij Duurstede, The Netherlands). The ingredients were already ground through a 3 mm sieve using a hammer mill. Further processing was carried out at the Wageningen Feed Processing Centre (WFPC). An Almex expander (150 Φ, Almex BV Zutphen, The Netherlands), fitted with a 22 kW engine was used for the expander treatment. Steam at a pressure of 60 kPa was added to the mash materials in the conditioner. Product temperature determined using thermocouples in the last mixing bolts before the exit of expander, were 105 ºC, 110 ºC and 106 ºC for peas, lupins and faba beans, respectively.

Pelleting was carried out with a 5 × 45 mm die (bore × hole), using a V2-30 pelletizer (Robinson Milling System B.V., Boxtel, The Netherlands). After pelleting, the

pellets were cooled in a two-deck counter flow bunker cooler for 20 min. The feed samples were then dried in a forced oven for 16 h at 35°C.

Subsequently, identical samples of original mashes (hammer mill; 3 mm sieve) and all the processed materials (E and EP) were ground through a 3 mm sieve (Retsch ZM1 centrifugal mill). The samples were then subjected to a fractionation method (**Chapter 2**) to quantify the size of the NWF, ISWF and SWF (g kg^{-1} DM of WHO).

In vitro fermentation

The cumulative gas production technique of Theodorou et al.[12] as modified by Williams et al.[13] was used to characterize the fermentation behavior of feed samples. In three consecutive gas production runs, the WHO (R, E and EP) samples of 3 concentrate ingredients (peas, lupins and faba beans) and their fractions (NWF, ISWF and SWF) were subjected to an *in vitro* incubation technique, which continuously measured gas production in an automated pressure evaluation system (APES)[14] for 72 h. Simultaneously, in a manually operated *in vitro* fermentation system, the concentration of VFA and ammonia (NH_3-N) was measured after 6, 12, 24, and 48h.

The rumen inoculum was taken weekly at 0800 h for three consecutive weeks from three rumen fistulated Holstein- Friesian non lactating dairy cows, fed a basic diet of grass silage and hay *ad libitum*. About 500 ml of rumen fluid was collected from each cow in pre-warmed thermos flasks that were filled with CO_2. The rumen fluid samples were proportionally pooled, blended for 60 s and strained through two layers of cheese cloth. All handlings were done under CO_2 flushing to ensure anaerobic conditions.

Representative samples of each substrate fractions were incubated in rumen fluid with medium B (modified from Lowe et al.[15] by Williams et al. [16]) at 38°C under anaerobic conditions to mimic the rumen environment suitable for microbes to ferment the substrates. The experiment was conducted in three runs in which an automated pressure evaluation system (APES), and a manually operated *in vitro* fermentation system were applied simultaneously. Three replicate samples of each substrate were fermented in 100 ml specific APES bottles (Schott Duran, Mainz, Germany) containing approximately 0.5 g of each substrate, 82 ml medium B and 5 ml of rumen fluid inoculum for 72 h. Meanwhile, in the manually operated *in vitro* fermentation system, triplicate samples of the same substrates were fermented at four different time points (6, 12, 24 and 48 h, respectively). From the WHO, NWF and ISWF 0.5 g DM was fermented in 100-ml serum bottles in 82 ml medium B and 5 ml inoculum kept in a temperature-controlled incubator. Because of an insufficient quantity of the SWF, only 0.05 g of SWF samples

were incubated with 10 ml of medium B and 0.6 ml of rumen inoculum. For measuring the VFA, a sub-sample of 0.5 ml was withdrawn and added to a 1 ml Eppendorf tube containing 25 µl of phosphoric acid (850 g L^{-1}). A second sub-sample of 0.75 ml, to which 0.75 ml of trichloroacetic acid (TCA; 100 g L^{-1}) was added, was taken for measurement of NH_3-N by the indophenol method as described by Searle.[17] The remainder of the fermentation fluid was used to determine pH.

Chemical analysis

The feed samples and their different fractions (NWF, ISWF and SWF) were analyzed for dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF), starch, reducing sugars and degree of starch gelatinisation.

The DM, Ash, CP, NDF, starch and degree of starch gelatinisation (SGD) were determined as described by Goelema et al.[18] The reducing sugar content was determined according to Rijnen et al. (2001).[19]

The VFAs were measured as described in **Chapter 3**. Acetic acid (HAc), propionic acid (HPr), *iso*-butyric (*i*-HBu), butyric acid (HBu), *iso*-valeric (*i*-HVal) and valeric (HVal) acid concentrations were expressed in mmol g^{-1} incubated OM. The non-glucogenic to glucogenic acids ratio (NGR) was calculated using the equation given in **Chapter 3**. *Iso*-butyric and *iso*-valeric acid were assumed to originate from the degradation of protein and were expressed as proportion of the total VFA and named the branched chain ratio (BCR).

Kinetic model analysis

Gas production profiles, obtained with the automated system, were fitted by iteration for individual incubation flasks to the di-phasic model as described by Groot et al.[20] The gas production profiles were characterised by the cumulative gas production (OMCV, ml g^{-1} incubated OM); the estimated asymptotic gas production (A_i, ml g^{-1} incubated OM); a constant that determines the sharpness of the switching characteristic of the profile (B_i); the time (h) after incubation at which half of the asymptotic gas production has been reached (C_i). The maximum rate of gas production ($R_{max}G$, ml h^{-1}) and the maximum fractional rate of substrate degradation ($R_{max}S$, h^{-1}) were calculated according to Yang et al.[21]

Statistical analysis

Additivity of fractions NWF, ISWF and SWF in WHO was tested by a two-tailed *t*-test. Analysis of variance for the distribution of DM of processed samples over the different fractions, gas production kinetics' parameters and fermentation end-product were done using the GLM procedure of SAS 9.1 (SAS/STAT(R) package, 2003). The models used were:

$$Y = \mu + P_i + \varepsilon_{ij} \quad (1)$$

$$Y = \mu + P_i + F_j + (PF)_{ij} + \varepsilon_{ijk} \quad (2)$$

$$Y = \mu + T_k + R(T)_l + P_i + F_j + (TP)_{ik} + (TF)_{jk} + (PF)_{ij} + (TPF)_{ijk} + \varepsilon_{ijklm} \quad (3)$$

Model 1 was used to analyse the effects of processing on distribution of DM. We used the second and third model to analyse the effects of processing on gas production kinetics parameters and end-product formation, respectively. In all models Y is the dependent variable under examination, μ is the overall mean, P_i is the processing effect (i = 4 in model (1), i = 3 in models (2) and (3); R, E and EP), F_j is the fraction effect (j = 3; NWF, ISWF and SWF), T_k is the time effect (k = 5; 6, 12, 24, 48 and 72 h), ε_{ij}, ε_{ijk} and ε_{ijklm} are the error term. Because in the third model the results were analysed as a split-plot design, T_k and $R(T)_l$ (the effect of replicates nested in time) are regarded as the main plot and error term of the main plot, respectively.

In all models, differences between the main effects were analysed by a multiple comparison test (Tukey/Kramer), and the least square means listed in results tables using the LSMEANS statement of SAS 9.1 (SAS/STAT(R) package, 2003).

Results

Consistency of runs

The control samples in the three runs showed significantly different results with regard to cumulative gas production, VFA and NH_3-N concentrations at 72 h of incubation. Therefore, data of each run were analysed separately.

Additivity of fractionation

The gas and VFA production were calculated for the WHO on the basis of the contributions of NWF, ISWF and SWF. No significant differences were found between observed and calculated values (data not shown). Therefore, WHO was discarded from the statistical analysis.

Effect of processing on contribution of DM and chemical composition of the concentrate ingredients

The distribution of DM over the different fractions (NWF, ISWF and SWF) in the R, E and EP legume seeds and their chemical composition are shown in Tables 1 and 2.

Significant differences were observed between grinding procedures (O vs. R) with regard to NWF (O 124 g kg^{-1} DM higher than R, $P < 0.0001$), ISWF (O 80.7 g kg^{-1} DM lower than R, $P < 0.0001$) and SWF (R 43.3 g kg^{-1} DM higher than O, $P < 0.05$). Overall, statistical analysis revealed significant differences between E and EP regarding the size of NWF (E 42.4 g kg^{-1} DM higher than EP, $P < 0.0001$), ISWF (E 36.6 g kg^{-1} DM lower than EP, $P < 0.0001$) and SWF (E 5.1 g kg^{-1} DM lower than EP, $P < 0.05$).

In peas, processing had a significant effect on the size of NWF (E 155.0 and 74.0 g kg^{-1} DM higher than R and EP, respectively; $P < 0.05$), ISWF (R 100.3, 131.8 and 77.0 g kg^{-1} DM higher than O, E and EP, respectively; $P < 0.05$) and SWF (R 47.7 and 23.2 g kg^{-1} DM higher than O and E, respectively; $P < 0.05$). Like in peas, expander treatment and the ensuing pelleting process in faba beans significantly increased the size of the NWF compared with that of R, whereas the size of the ISWF and SWF were decreased (Table 1). Like in peas, a further reduction in the size of NWF was observed after pelleting the expander processed faba beans (Table 1). In lupins, the thermo-mechanical processing (E and EP) significantly decreased the size of NWF and increased the size of SWF and ISWF when the results were compared to the original sample. Comparing the size of the different fractions with the samples ground with the Retsch mill, however, revealed that the processing did not have any significant effect on the contribution of DM in lupins (Table 1).

Table 1.Effect of processing (O, original; R, Retsch; E, expander processed; EP, expander-pelleted) on the contribution of dry matter (g kg^{-1} DM) in peas, lupins and faba beans

	Peas				Lupins				Faba beans					Significance (*P*)	
	O	R	E	EP	O	R	E	EP	O	R	E	EP	SEM	O vs. R	E vs. EP
NWF	610.0^{c}	462.0^{e}	617.0^{c}	543.0^{d}	777.0^{a}	710.0^{b}	706.5^{b}	692.3^{b}	627.3^{c}	470.0^{e}	764.0^{a}	725.0^{abc}	10.1	< 0.0001	< 0.0001
ISWF	237.0^{c}	337.3^{a}	205.5^{d}	260.3^{b}	84.0^{g}	113.7^{ef}	123.5^{ef}	131.7^{e}	211.7^{d}	324.0^{a}	55.7^{h}	102.3^{fg}	7.3	< 0.0001	< 0.0001
SWF	153.3^{de}	200.7^{a}	177.5^{bc}	196.3^{a}	138.7^{e}	176.3^{bc}	170.0^{bc}	176.0^{bc}	161.0^{cd}	206.3^{a}	180.7^{b}	171.3^{d}	5.0	0.0462	0.0157

NWF; nonwashable fraction; ISWF, insoluble washable fraction; SWF, soluble washable fraction; O, original; R, Retsch; E; expander processed; EP; expander-pelleted; SEM: standard error of mean. Means in the same row followed by different superscript differ ($P < 0.05$).

Striking differences were observed between the legume seeds and their fractions with regard to their chemical composition (Table 2). The results show that in lupins and faba beans a considerable quantity of NDF was recovered in the ISWF. The ISWF was further characterised by a high starch (up to 795.0 g kg^{-1}) content (except in lupins) and a negligible quantity of reducing sugars (less than 6 g kg^{-1} DM). The SWF contained a considerable quantity of ash, CP, reducing sugars and a residual (37.2-369.3 g kg^{-1} DM) chemically not determined fraction.

Table 2. Chemical composition (g kg^{-1} DM) of legume seeds and their fractions

Feed	Process	Fraction	Ash	CP	NDF	Starch	Sugar	Residue[1]
Peas	Retsch	WHO	32.6	229.7	96.1	510.7	47.9	83.0
		NWF	23.2	157.5	193.7	465.5	24.5	135.6
		ISWF	8.1	167.2	6.6 [2]	766.9	ND	51.2
		SWF	115.5	519.4	ND	37.2 [2]	263.2	64.7
Peas	E	WHO	32.4	226.9	99.4	510.6	52.9	77.8
		NWF	15.8	188.3	151.8	519.6	7.6	116.9
		ISWF	8.8	132.3	68.1 [2]	766.9	4.6	19.3
		SWF	123.2	444.3	ND	32.0 [2]	288.0	112.5
Peas	EP	WHO	32.7	227.1	90.6	479.1	51.6	118.9
		NWF	17.1	196.1	174.0	476.5	1.5	134.8
		ISWF	6.8	114.8	45.4 [2]	795.0	3.5	34.5
		SWF	119.4	459.6	ND	13.7 [2]	273.7	133.6
Lupins	Retsch	WHO	31.3	333.9	236.1	7.1	64.5	327.1
		NWF	18.8	257.2	372.0	6.3	10.1	335.6
		ISWF	18.6	702.1	193.7 [2]	ND	5.4	80.2
		SWF	96.4	296.2	ND	2.6 [2]	295.2	309.6
Lupins	E	WHO	30.5	332.2	244.4	14.0	62.5	316.4
		NWF	18.4	313.0	327.9	10.6	13.3	316.8
		ISWF	18.0	578.1	203.7 [2]	ND	2.9	197.3
		SWF	95.6	231.5	ND	6.5 [2]	297.1	369.3
Lupins	EP	WHO	30.6	336.0	230.3	16.8	65.1	321.2
		NWF	19.7	322.5	297.8	12.7	17.6	329.7
		ISWF	17.0	602.7	191.0 [2]	ND	0.4	188.9
		SWF	96.3	231.6	ND	8.0	298.3	365.8
Faba beans	Retsch	WHO	35.5	283.6	144.3	407.5	39.4	89.7
		NWF	24.1	203.7	245.1	337.4	19.1	170.6
		ISWF	7.4	177.6	64.9 [2]	743.7	2.4	4.0
		SWF	116.7	652.8	ND	8.0 [2]	185.3	37.2
Faba beans	E	WHO	35.3	290.0	129.7	421.1	38.6	85.3
		NWF	16.5	222.2	160.4	482.6	9.7	108.6
		ISWF	19.6	322.1	120.7 [2]	518.6	ND	19.0
		SWF	134.1	554.3	ND	23.4 [2]	215.0	73.2
Faba beans	EP	WHO	35.4	287.7	128.0	418.0	33.1	97.8
		NWF	17.3	221.7	177.1	456.6	10.4	116.9
		ISWF	7.8	167.0	97.4 [2]	749.1	5.4	-27.1
		SWF	117.5	581.6	ND	ND	197.2	103.7

ND: not determined. [1] Calculated as 1000 – (ash + CP + starch + NDF + sugars). [2] Calculated as component in WHO– (component in the other fractions × their contribution to the DM of WHO).

The expander processing and ensuing pelleting resulted in only minor changes in CP content of entire R samples. In the SWF, however, both expander treated and expander pelleted samples had a lower CP content compared to the R samples. The results show that expander treatment followed by pelleting decreased the NDF content of NWF compared to those of R samples, with the most profound effect in faba beans. In the WHO, no consistent effect of processing on reducing sugar content was observed. Compared to the R samples, the E and EP samples of SWF contained higher reducing sugars.

The results show that compared to the R samples, the expander treatment increased SGD which was further alleviated by the ensuing pelleting (Table 3). The increase in SGD was higher in treated faba beans than in treated peas.

Table 3.The effect of processing on starch, gelatinised starch (GS) and degree of starch gelatinisation (SGD)

Feed	Process	Fraction	Starch (g kg^{-1} DM)	GS (g kg^{-1} DM)	SGD %
Peas	Retsch	WHO	510.7	35.2	6.9
	E	WHO	510.6	57.7	11.3
	EP	WHO	479.1	69.0	14.4
Faba beans	Retsch	WHO	407.5	0.0	0.0
	E	WHO	421.1	104.0	24.7
	EP	WHO	418.0	118.7	28.4

Effect of processing on in vitro cumulative gas production kinetics

Peas

The effects of processing, fraction and the interaction between processing and fraction on the gas production parameters (cumulative gas production, OMCV; A, C, $R_{max}S$ *and* $R_{max}G$) in peas are shown in Table 4. A significant effect of processing was observed on $R_{max}G_1$. Compared to the R, the E and EP treatments increased the $R_{max}G_1$ by 33 and 55 %, respectively. The $R_{max}S_1$ and $R_{max}S_2$ were numerically higher in E and EP than in R sample. All the kinetic profile characteristics differed between fractions (Table 4). In the ISWF and SWF most of the gas (A_2) was produced in the second phase of

incubation with a half time of 19.3 and 26.2 h, respectively. The fermentation of SWF in the first phase was quite distinct from those of NWF and ISWF and showed a lower $R_{max}S$ and $R_{max}G$ and occurred sooner (C=8.9 h). A significant interaction between process and fraction was observed for $R_{max}G_1$. In NWF and ISWF, expander processing (E) and ensuing pelleting (EP) increased $R_{max}G_1$ (data not shown) as compared to R.

Lupins

The kinetics of gas production in the lupins are shown in Table 5. A significant effect of processing was observed for OMCV (E 37.5 ml g^{-1} OM higher than R, $P < 0.01$), $RmaxS_1$ (E and EP 0.5 and 0.6 h^{-1} higher than R, respectively; $P < 0.05$) and $R_{max}G_1$ (E and EP 10.5 and 10.2 ml h^{-1} higher than R, respectively; $P < 0.0001$). Significant differences were observed between fractions for A_1, $RmaxS_1$, $R_{max}G_1$, A_2 and $R_{max}S_2$. In the SWF more than 50 % of the total gas was produced in the first phase of the fermentation whereas in the NWF and ISWF gas produced in the first phase only accounted for 32 % of the total produced gas. Surprisingly however, despite a significantly higher *RmaxG1*, the *RmaxS1* was lower in SWF than in the NWF and ISWF.

The results show that the interaction between process and fraction was only significant for the $R_{max}S_1$ and $R_{max}S_2$. In NWF, process EP significantly increased $R_{max}S_1$ (EP was 0.3 h^{-1} higher than R, $P < 0.05$; data not shown) and $R_{max}S_2$ (EP was 0.03 h^{-1} higher than R, $P < 0.05$; data not shown). Like in NWF, in the SWF the highest $R_{max}S_1$ was observed for the EP samples (0.496, 0.422 and 0.255 h^{-1} in EP, E and R, respectively; data not shown). Contrary to that, in the ISWF the highest $R_{max}S_1$ was observed for the Retsch milled samples (0.675, 0.422 and 0.483 h^{-1} in R, E and EP, respectively; data not shown).

Faba beans

Parameters of the gas profiles of faba beans and its fractions were fitted to the di-phasic model and are displayed in Table 6. As is evident from the results, processing had only significant effects on the kinetics of gas production for C_1 and $R_{max}G_1$. In the first phase of fermentation, $R_{max}G_1$ was significantly higher and C_1 occurred significantly sooner in E and EP samples compared to the R sample. Compared to the R sample, the E and EP samples had a numerically higher $R_{max}S$ in the first phase of fermentation.

Table 4. Gas production characteristics in samples of peas and its fractions fitted to a di-phasic Groot model

Class		OMCV	A_1	C_1	$R_{max}S_1$	$R_{max}G_1$	A_2	C_2	$R_{max}S_2$	$R_{max}G_2$
Process	R	227.7[a]	0.426[a]	9.7[a]	0.439[a]	13.9[c]	0.574[a]	23.9[a]	0.073[a]	5.6[a]
	E	222.1[a]	0.374[a]	9.4[a]	0.493[a]	18.5[b]	0.629[a]	23.3[a]	0.083[a]	5.5[a]
	EP	240.7[a]	0.429[a]	9.6[a]	0.502[a]	21.5[a]	0.571[a]	24.7[a]	0.093[a]	6.1[a]
Fraction	NWF	271.0[a]	0.616[a]	9.4[b]	0.455[ab]	31.1[a]	0.384[b]	26.3[a]	0.061[b]	3.3[b]
	ISWF	259.0[a]	0.355[b]	10.6[a]	0.592[a]	16.4[b]	0.645[a]	19.3[b]	0.100[a]	9.0[a]
	SWF	160.4[b]	0.258[b]	8.9[b]	0.387[b]	6.4[c]	0.742[a]	26.2[a]	0.087[a]	4.8[b]
	SEM	5.2	0.046	0.3	0.045	0.7	0.036	1.6	0.006	0.5
Significance (P)	Process (P)	0.0817	0.6605	0.7436	0.5456	<0.0001	0.6592	0.8402	0.0809	0.6900
	Fraction (F)	<0.0001	0.0002	0.0017	0.0226	<0.0001	0.0002	0.0134	0.0020	<0.0001
	P × F	0.6379	0.3882	0.0871	0.0859	<0.0001	0.3885	0.6389	0.6787	0.1541

OMCV, cumulative gas production (ml g^{-1} OM); A_1 and A_2, proportion of gas produced at the first and second phase relative to the total produced gas; C_1 and C_2, half-time of gas production (h); $R_{max}S$, maximum fractional rate of substrate degradation (h^{-1}); $R_{max}G$, maximum rate of gas production (ml h^{-1}); and SEM, standard error of least square mean. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Table 5. Gas production characteristics in samples of lupins and its fractions fitted to a di-phasic Groot model

Class		OMCV	A_1	C_1	$R_{max}S_1$	$R_{max}G_1$	A_2	C_2	$R_{max}S_2$	$R_{max}G_2$
Process	R	223.0^{b}	0.353^{a}	8.4^{a}	0.045^{b}	13.1^{b}	0.647^{a}	24.4^{a}	0.092^{a}	5.9^{a}
	E	260.5^{a}	0.400^{a}	7.8^{a}	0.527^{a}	23.6^{a}	0.600^{a}	21.9^{a}	0.088^{a}	6.3^{a}
	EP	241.1^{ab}	0.399^{a}	7.6^{a}	0.628^{a}	23.3^{a}	0.601^{a}	21.3^{a}	0.078^{a}	6.1^{a}
Fraction	NWF	275.1^{a}	0.321^{b}	7.9^{a}	0.633^{a}	20.9^{c}	0.679^{a}	23.8^{a}	0.061^{b}	6.6^{a}
	ISWF	189.2^{b}	0.325^{b}	8.1^{a}	0.521^{ab}	12.5^{b}	0.675^{a}	22.7^{a}	0.103^{a}	5.9^{a}
	SWF	260.3^{a}	0.507^{a}	7.8^{a}	0.453^{b}	26.4^{a}	0.493^{b}	21.2^{a}	0.095^{a}	5.8^{a}
	SEM	6.7	0.035	0.2	0.044	1.3	0.035	1.0	0.005	0.4
Significance (P)	Process (P)	0.0055	0.5775	0.0546	0.0392	<0.0001	0.5792	0.0845	0.1039	0.8673
	Fraction (F)	<0.0001	0.0019	0.5732	0.0351	<0.0001	0.0019	0.1948	<0.0001	0.4693
	P × F	0.1710	0.4494	0.0847	0.0081	0.1546	0.4503	0.1089	0.0128	0.1395

Gas production parameters explained in Table 4. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Table 6. Gas production characteristics in samples of faba beans and its fractions fitted to a di-phasic Groot model

Class		OMCV	A_1	C_1	$R_{max}S_1$	$R_{max}G_1$	A_2	C_2	$R_{max}S_2$	$R_{max}G_2$
Process	R	194.4[a]	0.452[a]	11.4[a]	0.361[a]	12.0[b]	0.548[a]	27.1[a]	0.137[a]	5.4[a]
	E	225.1[a]	0.569[a]	8.5[b]	0.474[a]	20.9[a]	0.431[a]	23.2[a]	0.077[a]	4.5[a]
	EP	221.9[a]	0.412[a]	8.5[b]	0.578[a]	21.0[a]	0.588[a]	23.0[a]	0.076[a]	6.0[a]
Fraction	NWF	224.2[ab]	0.662[a]	10.7[a]	0.386[a]	26.3[a]	0.338[b]	30.9[b]	0.043[b]	1.6[b]
	ISWF	236.3[a]	0.512[a]	9.9[a]	0.569[a]	18.4[b]	0.488[ab]	17.9[a]	0.160[a]	8.6[a]
	SWF	180.8[b]	0.259[b]	7.7[b]	0.457[a]	9.2[c]	0.741[a]	24.6[ab]	0.087[ab]	5.7[ab]
	SEM	11.9	0.048	0.4	0.067	1.0	0.067	3.4	0.022	0.9
Significance (P)	Process (P)	0.1920	0.2734	0.0003	0.1117	0.0001	0.2736	0.6435	0.1352	0.4764
	Fraction (F)	0.0220	0.0049	0.0004	0.2000	<0.0001	0.0049	0.0660	0.0109	0.0005
	P × F	0.6854	0.5916	0.0057	0.5914	0.0001	0.5912	0.7597	0.3771	0.2729

Gas production parameters explained in Table 4. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Like in peas, the gas production profile of SWF was quite distinct from those of NWF and ISWF. In the first phase the half-time was reached earlier; however, the maximum fractional rate of the substrate degradation and gas production were lower than those of the first phase of NWF and ISWF. In the second phase, the kinetics of gas production of SWF in faba beans were very similar to those of peas (Tables 4 and 6). A significant interaction of processing and fraction was found for C_1 and $R_{max}G_1$. In NWF, the E and EP samples had a significantly higher $R_{max}G_1$ (40.1, 29.7 and 8.9 ml h^{-1} in EP, E and R, respectively; $P < 0.05$) and C_1 occurred much sooner than in the R sample (8.6, 9.1 and 17.0 h in EP, E and R, respectively; $P < 0.05$).

VFA production and formation of fermentation end- product

Peas

VFA production profiles and formation of fermentation end-product in the samples of peas are presented in Table 7. The results show that not only processing, fraction and sampling time had significant effects, but also that there were many significant interactions. Compared to the R samples, TVFA and NGR increased whereas NH_3-N was decreased by E and EP. Striking differences were observed between the fractions with regards to profile of VFA and formation of fermentation end-products. The fermentation of the SWF was quite distinct from that of in NWF and ISWF with a significantly higher TVFA, BCR and NH_3-N. A significant interaction of processing and incubation time was found for NGR.

The changes in TVFA, NGR and NH_3-N throughout the incubation period in samples of peas are presented in Figure 1. Expander treatment increased the NGR after 12 h of incubation. Compared to the R sample, the TVFA was marginally higher whereas NH_3-N was marginally lower in E and EP samples over the course of incubation. In all fractions, NGR decreased dramatically up to 24 h and remained almost constant thereafter (data not shown). Results show that up to 24 h of fermentation, processes E and EP caused a higher NGR in the NWF of peas (data not shown). However, the differences in the NGR in the SWF of peas caused by the E and EP became more apparent at the earlier stages of fermentation where processes E and EP showed a lower NGR (data not shown). No differences between the ISWF of E, EP and R were observed for NGR (data not shown).

.

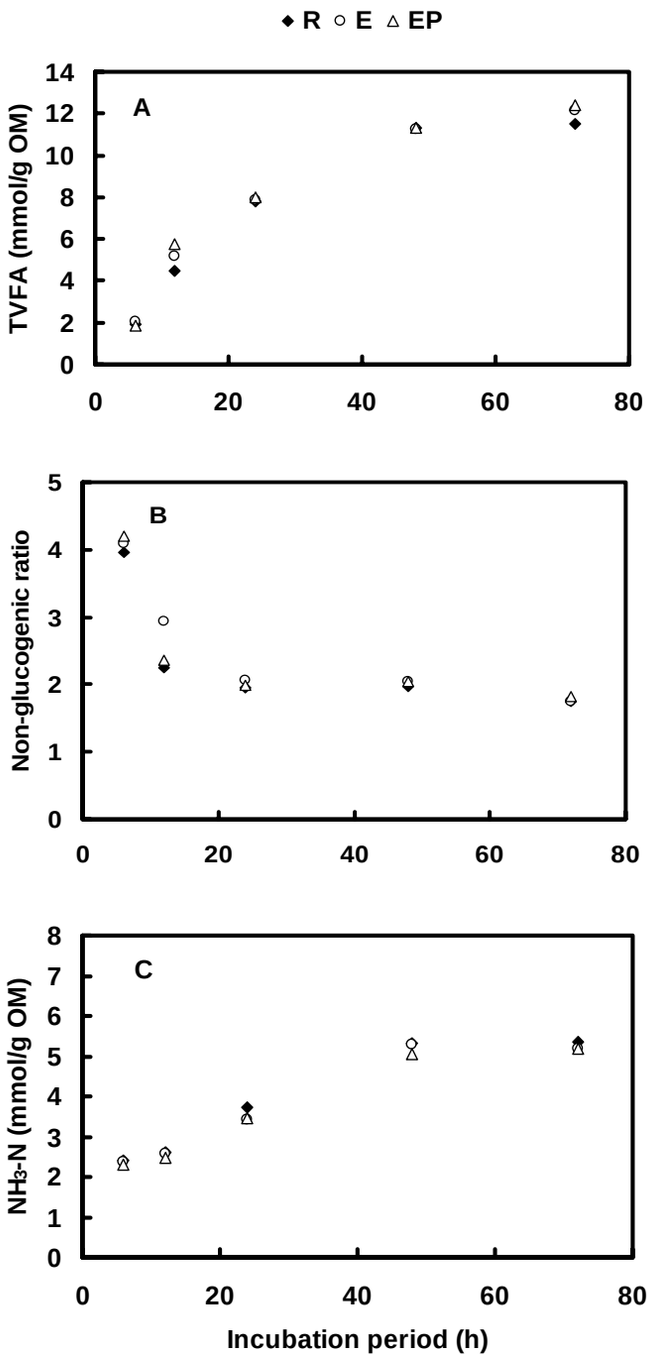


Figure 1. Total volatile fatty acids production (TVFA), non-glucogenic to glucogenic ratio (Non-glucogenic ratio) and ammonia production (NH_3-N) in Retsch milled (R), expander processed (E) and expander-pelleted (EP) peas.

Table 7 .VFA production profiles (mmol g^{-1} OM), total VFA production (TVFA, mmol g^{-1} OM), non-glucogenic ratio (NGR), branched chain ratio (BCR) and NH_3-N (mmol g^{-1} OM) for the peas samples and its fractions

Class		HAc	HPr	HBu	TVFA	NGR	BCR	NH_3-N
Process	R	3.7^{b}	2.6^{a}	0.7^{b}	7.4^{b}	2.4^{b}	0.035^{a}	3.9^{a}
	E	3.8^{ab}	2.6^{a}	0.8^{a}	7.7^{ab}	2.6^{a}	0.035^{a}	3.8^{b}
	EP	3.9^{a}	2.7^{a}	0.8^{a}	7.9^{a}	2.5^{ab}	0.033^{a}	3.8^{b}
Fraction	NWF	3.8^{b}	2.4^{b}	1.0^{a}	7.6^{b}	2.7^{a}	0.031^{b}	3.1^{b}
	ISWF	3.1^{c}	2.8^{a}	0.8^{b}	7.0^{c}	2.2^{a}	0.029^{b}	2.6^{c}
	SWF	4.5^{a}	2.7^{a}	0.6^{c}	8.4^{a}	2.5^{c}	0.043^{a}	5.7^{a}
	SEM	0.05	0.05	0.02	0.1	0.05	0.0015	0.03
Time (h)	6	1.3^{e}	0.4^{e}	0.2^{d}	1.9^{d}	4.1^{a}	0.024^{b}	2.4^{d}
	12	2.7^{d}	1.7^{d}	0.6^{c}	5.1^{c}	2.5^{b}	0.029^{b}	2.6^{c}
	24	3.7^{c}	2.9^{c}	1.0^{b}	7.9^{b}	2.0^{c}	0.032^{b}	3.5^{b}
	48	5.4^{b}	3.9^{b}	1.2^{a}	11.3^{a}	2.0^{c}	0.044^{a}	5.2^{a}
	72	5.8^{a}	4.4^{a}	1.0^{a}	12.0^{a}	1.8^{c}	0.043^{a}	5.2^{a}
	SEM	0.08	0.06	0.03	0.2	0.05	0.002	0.03
Significance	Process (P)	0.010	0.1203	<0.0001	0.0053	<0.0001	0.6807	<0.0001
	Fraction (F)	<0.0001	<0.0001	<0.0001	<0.0001	0.0099	<0.0001	<0.0001
	Time (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
	P × F	0.3679	0.0002	<0.0001	0.3106	<0.0001	0.0541	<0.0001
	P × T	0.1351	0.2284	0.0189	0.0746	0.0127	0.0879	0.1866
	F × T	<0.0001	<0.0001	<0.0001	0.0011	<0.0001	<0.0001	<0.0001
	F × P × T	0.1697	0.0852	0.0530	0.0971	0.3162	0.2718	0.0343

SEM: standard error of least square mean. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Lupins

Significant differences were observed in VFA production profiles, NGR, BCR and NH_3-N for process, fraction, time and interactions between these factors (Table 8). Regardless of fractions and incubation time, the NGR, BCR and NH_3-N were lower for E and EP samples compared to those in the R sample (Table 8). Significant differences between fractions were observed for VFA production profiles (a higher HAc and HPr produced for SWF compared to the NWF and ISWF; $P < 0.05$), TVFA (SWF was 2.5 and 2.7 unit higher than NWF and ISWF, respectively; P ,< 0.05), NGR (SWF was 0.5 and 0.9

lower than NWF and ISWF, respectively; $P < 0.05$), BCR (SWF was 1 and 3.3 percent lower than NWF and ISWF; $P < 0.05$) and NH_3-N (SWF was 1.0 unit higher and 1.1 unit lower than NWF and ISWF; $P < 0.05$). Significant interactions of processing and incubation time were found for NGR and NH_3-N.

Table 8. VFA production profiles (mmol g^{-1} OM), total VFA production (TVFA, mmol g^{-1} OM), non-glucogenic ratio (NGR), branched chain ratio (BCR) and NH_3-N (mmol g^{-1} OM) for the lupins samples and its fractions

Class		HAc	HPr	HBu	TVFA	NGR	BCR	NH_3-N
Process	R	3.8^{a}	2.0^{b}	0.65^{b}	7.1^{a}	2.7^{a}	0.046^{a}	5.5^{b}
	E	3.7^{a}	2.2^{a}	0.66^{ab}	7.2^{a}	2.4^{b}	0.039^{b}	5.0^{a}
	EP	3.8^{a}	2.3^{a}	0.68^{a}	7.3^{a}	2.4^{b}	0.039^{b}	5.1^{a}
Fraction	NWF	3.4^{b}	1.9^{b}	0.67^{b}	6.4^{b}	2.5^{b}	0.037^{b}	4.2^{c}
	ISWF	3.3^{b}	1.5^{c}	0.71^{a}	6.2^{b}	2.9^{a}	0.060^{a}	6.3^{a}
	SWF	4.7^{a}	3.1^{a}	0.62^{c}	8.9^{a}	2.0^{c}	0.027^{c}	5.2^{b}
	SEM	0.04	0.03	0.009	0.08	0.04	0.0006	0.02
Time (h)	6	1.6^{d}	0.6^{e}	0.2^{d}	2.5^{d}	3.3^{a}	0.021^{c}	2.7^{e}
	12	3.1^{c}	2.0^{a}	0.6^{c}	5.6^{c}	2.3^{b}	0.021^{c}	3.5^{a}
	24	4.0^{b}	2.4^{c}	0.8^{b}	7.8^{b}	2.4^{b}	0.050^{b}	5.5^{c}
	48	5.1^{a}	2.9^{b}	0.9^{a}	9.8^{a}	2.4^{b}	0.059^{a}	7.1^{b}
	72	5.0^{a}	3.1^{a}	0.9^{a}	10.0^{a}	2.1^{c}	0.057^{a}	7.4^{a}
	SEM	0.04	0.02	0.008	0.07	0.04	0.0007	0.02
Significance	Process (P)	0.1871	<0.0001	0.0093	0.1210	<0.0001	<0.0001	<0.0001
	Fraction (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Time (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P × F	0.4751	<0.0001	0.0138	0.1464	<0.0001	<0.0001	<0.0001
	P × T	0.1084	0.2415	0.1760	0.2056	0.0386	0.1208	<0.0001
	F × T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	F × P × T	0.3328	0.0169	0.2749	0.1642	0.0889	0.0020	<0.0001

SEM: standard error of least square mean. Means with different superscripts within class and column differ significantly ($P < 0.05$).

Figure 2 shows that both E and EP reduced the NGR and NH_3-N throughout the incubation. Except in the ISWF, the pattern of VFA production, as represented by NGR, did not change due to the E and EP process (data not shown). In the ISWF, the processes E and EP caused a lower NGR (3.5, 2.7 and 2.8 in R, E and EP, respectively; *P*

< 0.05). Processes E and EP did not change the BCR and the pattern of NH_3-N production in the NWF (data not shown). In contrast to that, in the ISWF and SWF a significantly lower BCR and NH_3-N was observed for the ISWF and SWF of E and EP samples than for the R (data not shown, $P < 0.05$).

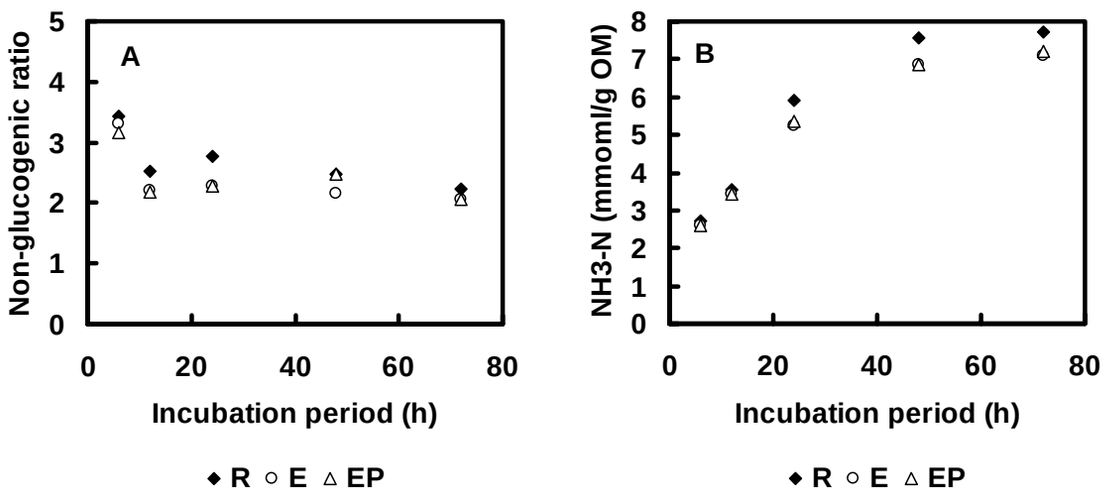


Figure 2. Non-glucogenic to glucogenic ratio (Non-glucogenic ratio) and ammonia production (NH_3-N) in Retsch milled (R), expander processed (E) and expander-pelleted (EP) lupins.

Faba beans

Like with peas and lupins, not only processing, fraction and time of sampling had significant effects, but there were also many significant interactions. As presented in Table 9 compared to the R samples, both E and EP significantly increased HPr and TVFA whereas NGR, BCR and NH_3-N were decreased. Like in peas and lupins, striking differences were observed between the fractions with regard to fermentation end-products.

Figure 3 shows that profiles of VFA, represented as NGR, and BCR both were affected by E and EP. Compared to the R sample, in both E and EP samples a lower NGR was observed throughout the incubation. As is evident from the figure, both E and EP lowered the BCR compared to R samples up to 24 h of incubation. The results show that the processes E and EP had a profound effect on NGR in all fractions. In all fractions (NWF, ISWF and SWF) these processes significantly decreased the NGR ($P <$ 0.05; data not shown). Both NWF and SWF of E and EP samples had a lower BCR and NH_3-N compared to the R sample (data not shown). Surprisingly, however, a significantly

lower BCR and NH_3-N was observed for the ISWF of expander treated sample than for the EP and R (data not shown, $P < 0.05$).

Table 9. VFA production profiles (mmol g^{-1} OM), total VFA production (TVFA, mmol g^{-1} OM), non-glucogenic ratio (NGR), branched chain ratio (BCR) and NH_3-N (mmol g^{-1} OM) for the faba beans samples and its fractions

Class		HAc	HPr	HBu	TVFA	NGR	BCR	NH_3-N
Process	R	3.5^{a}	2.0^{b}	0.76^{a}	6.8^{b}	2.9^{a}	0.045^{a}	4.2^{a}
	E	3.5^{a}	2.3^{a}	0.75^{a}	7.0^{a}	2.4^{b}	0.040^{b}	4.0^{b}
	EP	3.5^{a}	2.3^{a}	0.78^{a}	7.1^{a}	2.4^{b}	0.039^{b}	4.0^{b}
Fraction	NWF	3.2^{b}	1.9^{c}	0.79^{a}	6.3^{c}	2.8^{a}	0.036^{c}	2.9^{b}
	ISWF	3.1^{b}	2.6^{a}	0.79^{a}	6.9^{b}	2.2^{b}	0.033^{b}	2.8^{b}
	SWF	4.1^{a}	2.1^{b}	0.70^{b}	7.6^{a}	2.7^{a}	0.056^{a}	6.5^{a}
	SEM	0.04	0.03	0.01	0.06	0.03	0.0006	0.03
Time (h)	6	1.4^{e}	0.4^{e}	0.22^{d}	2.1^{e}	3.9^{a}	0.032^{c}	2.5^{d}
	12	2.3^{d}	1.3^{d}	0.58^{c}	4.4^{d}	2.7^{b}	0.029^{c}	2.7^{c}
	24	3.6^{c}	2.7^{c}	0.92^{b}	7.7^{c}	2.0^{c}	0.042^{b}	3.9^{b}
	48	4.8^{b}	3.0^{b}	1.05^{a}	9.8^{b}	2.2^{c}	0.054^{a}	5.7^{a}
	72	5.4^{a}	3.5^{a}	1.03^{a}	10.8^{a}	2.1^{c}	0.051^{a}	5.7^{a}
	SEM	0.04	0.03	0.01	0.07	0.06	0.0007	0.03
Significance	Process (P)	0.8170	<0.0001	0.1453	0.0007	<0.0001	<0.0001	<0.0001
	Fraction (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Time (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P × F	0.0025	<0.0001	<0.0001	0.0113	<0.0001	<0.0001	<0.0001
	P × T	0.0422	0.0008	0.0084	0.0356	0.0010	0.0019	<0.0001
	F × T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	F × P × T	0.4437	<0.0001	<0.0001	0.1048	<0.0001	<0.0001	<0.0001

SEM: standard error of least square mean. Means with different superscripts within class and column differ significantly ($P < 0.05$).

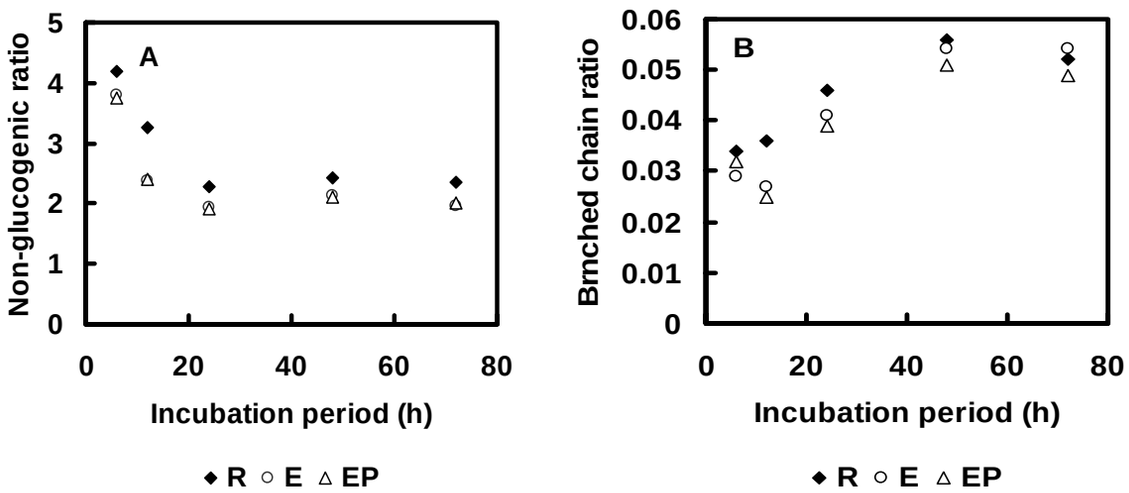


Figure 3. Non-glucogenic ratio and branched chain ratio in unprocessed (R), expander processed (E) and expander-pelleted (EP) faba beans

Discussion

Effect of expander processing and expander-pelleting on the contribution of fractions and chemical components

In feed manufacturing, technological processing is used to manipulate the extent of degradation in the rumen and the site of digestion of feed components.[4] In processing of ruminant feeds, both changes in particle size distribution and changes in physico-chemical characteristics of feeds (e.g. gelatinisation of starch and denaturation of proteins) are the means to manipulate the site and extent of nutrient digestion.[22] Processing may alter the physico-chemical characteristics of a feed in two ways. Firstly, because processing often affects the particle size distribution,[5,23] it may interfere with analytical procedures that involve filtration, such as NDF analysis, and those that involve enzymatic determination, such as starch analysis. Secondly, changes in the contribution of DM to different fractions (NWF, ISWF and SWF) are also possible if processing either alters the solubility, thereby changing the size of true soluble fractions (SWF), or reduces the size of particles, thereby increasing the ISWF. Indeed changes in the distribution of DM were observed, most likely due to particle size reduction, gelatinization of starch and denaturation of protein. Compared to the original mash material that was pre-ground with a hammer mill through a 3mm sieve, process R increased the total washable fraction (SWF+ISWF, Table 1). The repeated grinding of feed samples not only reduced the particle size in such a way that more particles were small enough to escape through the nylon bag pores; it also increased

the size of the SWF. However, the extent of increase in the size of ISWF was much larger than that of the SWF (45 and 29 percent in ISWF and SWF). These results were in a good agreement with findings for cereal grains (**Chapter 3**).We found the same tendency in reducing the size of the washable fraction when the samples of pre-ground cereal grains (with a hammer mill) were ground again with a laboratory mill (through a 3 mm sieve). The results, however, show that the extent of increase in the size of ISWF fraction due to grinding the pre-ground samples was considerably higher in peas and faba beans than in lupins (42, 35 and 53 percent in peas, lupins and faba beans, respectively). The higher ISWF in peas and faba beans than in lupins can be explained by the differences between their seeds microstructure. Except in lupins, cells in the legume seeds are loosely packed in the central part of the endosperm with large intercellular spaces.[24] These differences between the structure of endosperm in legume seeds produce finer particles during the grinding of peas and faba beans compared to lupins.

From the results in Tables 1 and 2 the shifts in the distribution of the different chemical components over the three fractions NWF, ISWF and SWF caused by the different treatments can be calculated. The NDF content of NWF was lower in the E and EP samples than in the R samples, mostly with a concomitant increase of the size of the chemically not determined fraction in the SWF. This shift between the chemically not determined fraction of SWF and NDF might be the result of partly solubilising of hemicellulose due to processing.[25] It has been found that expander and extrusion processing increase the ratio of soluble to insoluble dietary fibre.[26] Expander processing and the ensuing pelleting drastically increased the content of gelatinised starch in peas and faba beans (Table 3). In agreement with our results, Goelema et al.[27] also showed that in a mixture of broken peas, lupins and faba beans, SGD was higher in E and EP samples than in the unprocessed sample. It has been well documented that both mechanical and thermal energy transferred to starch break the main and secondary valence bonds between neighbouring starch polymers in the starch structure which increases the susceptibility of starch to enzyme action.[28] In accordance with the findings of Yang et al.[21] in barley and maize, the SWF of E and EP samples had a higher sugar content compared to the R sample in peas and faba beans (Table 2). In lupins, however, such a tendency of increasing the soluble sugars due to processing was not observed, most likely due to a lack of starch in lupins. The higher sugars observed in SWF of E and EP peas and faba beans compared to the R samples could be explained by starch dextrinisation and depolymerisation that occur due to shear forces in expander processing.[29]

It further appears that both E and EP shift CP from SWF and ISWF to NWF. The shift in CP could be explained by the occurrence of protein denaturation and therefore decreasing the size of soluble proteins. Goelema et al.[27] found a positive correlation between the magnitude of the washable fraction and the enthalpy of protein denaturation. In an additional experiment using differential scanning calorimetry (DSC) we found a decreased enthalpy of protein denaturation in E and EP samples of peas, lupins and faba beans compared to the R sample (Azarfar et al., unpublished data).

An increase in the size of NWF after expander processing in the samples of peas and faba beans can be explained by the role of gelatinised starch as binding agent. It has been shown that gelatinised starch has potential to act as a binder.[30] In the current study though, the content of gelatinised starch in peas and faba beans was higher in EP compared to E treated samples (Table 3), yet the size of ISWF (fine particles smaller than 40 μ) was higher in E samples than in EP. It implies that although in expander treated samples of peas and faba bean gelatinised starch serves as a binding agent, its binding role could be disturbed by physical forces imposed upon it by the pelleting. Moreover, the increased ISWF in both E and EP samples of lupins indicates that in concentrate ingredients that lack starch, technological processes that involve shear forces (like expander and expander-pelleting) increase the pool of fine particles. The increase in the size of ISWF of peas, lupins and faba beans due to the EP treatment observed in this experiment was consistent with the results of Tóthi et al.,[31] where the particle loss was reported to have increased with EP in both maize and barley. The declined water solubility reported for peas, lupins and faba beans upon the pressure toasting[18] was in agreement with our study (Table 1). There are two possible explanations. Firstly, agglomerated fine particles due to the expander processing may restrict the contact of feed particles with the solvent (water) upon the fractionation procedure. Secondly, chemical reactions (like protein denaturation) can be kept responsible for such decreased SWF due to expander processing. Indeed in an experiment we found a positive correlation between the magnitude of SWF and the enthalpy of protein denaturation, indicating that changes in protein solubility affect the size of SWF (Azarfar et al., unpublished data). To bear out the effect of feed particles on the size of SWF, it is worthwhile mentioning that the decreased SWF in peas and faba beans after expander processing corresponds with a decreased ISWF (particle loss during the washing procedure). Moreover, in all concentrate ingredients used in the current study a higher SWF were observed for O samples than for R (Table 1). The reduced protein content of SWF after the E and EP in the current study was concomitant with a reduced transition enthalpy of the protein denaturation peak measured with DSC (Azarfar et al, unpublished

data). Yang et al.[21] also found a lower CP content of SWF for samples of toasted and toasted-pelleted barley and maize compared to the unprocessed samples.

Effect of processing on in vitro cumulative gas production kinetics

In peas and faba beans differences in $R_{max}G_1$ were observed between treatments. The significantly higher $R_{max}G_1$ with processes E and EP which was concomitant with an earlier occurring C_1 in faba beans, is an indication that E and EP processed peas and faba beans were made easily accessible for microbes and that R material initially offered some resistance to microbial degradation. The results of the current study were in fair agreement with some *in situ* trails where it was found that expander treatment increased rumen degradation of starch in legume seeds like peas.[7] Heat treatment that is often associated with an increased level of gelatinised starch,[32,33] is generally thought to increase both the rate and the extent of starch degradation *in vitro* and *in situ*. Indeed in the current study the increased $R_{max}G_1$ in E and EP treated peas and faba beans were concomitant with increased gelatinised starch (Table 3). It has recently been shown that expander processing in peas increased the starch gelatinisation that was accompanied with a greater gas production during the first 8 hours of fermentation.[25] Technological processes that involve shear forces like expander processing not only can alter the degradative behaviour of feedstuffs by changing the chemical compositions of feedstuffs,[4] they can also change the degradative behaviour of feeds by changing the distribution of fractions (NWF, ISWF and SWF) in feed's DM.[34] In fact in both peas and faba beans, the higher $R_{max}G_1$ of NWF in E and EP compared to R implies that E and EP have changed the degradative behaviour of feeds by changing the distribution of fractions (in this case NWF) in feed's DM.

In lupins, $R_{max}S_1$ and $R_{max}G_1$ tended to increase after the E and EP processing. These increased rates are consistent with findings of Goelema et al.,[3] where EP increased the *in situ* fractional rate of degradation of CP in a mixture of broken legume seeds. However, other authors reported a decrease in the size of the soluble fraction and the rate of degradation after expander treatment,[8,35,36] which is in contrast with our findings. The discrepancy between our results and the findings of these authors might have arisen from the fact that these authors used a higher range of temperatures (120-135°c) than used in our study (110°c). Denaturation temperature of proteins determined by DSC in some legume seeds ranged between 88 and 105°C.[37] This indicates that probably the processing temperature along with the short residence time (5-20 s in expander processing)[4] in our study was not high enough to denature all the protein of lupins thereby decreasing the

quantity of SWF and fractional rate of substrate degradation. Indeed transition enthalpy of the protein denaturation peak measured with DSC in lupins was not affected by treatments E and EP (Azarfar et al., unpublished data). This was in line with the findings of Goelema et al.[27] who found no effect on denaturation enthalpies of pressure toasted lupins heated up to 100°C at various residence times ranging from 7-30 s. It is well documented that reducing the size of particles increases ruminal degradation of feeds by increasing the surface area for microbial attachment.[38] The higher $R_{max}S_1$ and $R_{max}G_1$ with processes E and EP were concomitant with a higher ISWF. Nevertheless, the ISWF of E and EP sample had a lower $R_{max}S_1$ and $R_{max}G_1$ compared to R. Therefore, such elevated rates due to E and EP can not be justified with the higher contribution of ISWF in DM of E and EP samples compared to R. When the gas production profiles were separately analysed by fraction, higher $R_{max}S_1$ and $R_{max}S_2$ were observed for NWF in E and EP processed lupins, which was concomitant with a decreased NDF and elevated sugar content (Table 2). Although in SWF of lupins, E and EP increased the $R_{max}S_1$ (data not shown), such an increase was not concomitant with an elevated sugar content (Table 2). The elevated $R_{max}S_1$, however, might be due to the effect of E and EP processing on the soluble non-starch polysaccharides (NSP) content of the SWF. It is thought that E and EP processing might have broken down the soluble NSP to its monomer constituents thereby increasing the $R_{max}S_1$. Indeed, the increased $R_{max}S_1$ of SWF in lupins was accompanied with an increased residual unknown fraction (Table 2) that is thought to be soluble carbohydrates. The same tendency in increasing the residual unknown fraction of SWF was observed in E and EP treated barley (**Chapter 3**). It has been reported that soluble fibres increase after pelleting[39] and extrusion.[40,41] However, the authors believe that a further experiment has to be carried out to bear out this theory.

Some theories have been put forward by authors such as Dijkstra et al.;[42] Cone and van Gelder;[43] Groot et al.[20] that the first phase of gas production is presumably from the fermentation of highly soluble and easily fermentable fractions while the second phase is due to the fermentation of slowly fermenting insoluble fractions. Cone et al.[44] have recently stated that the gas production during the first 3 h of incubation can be regarded as fast initial fermentation of the water-soluble components and the gas production between 3 and 20 h as moderately fast fermentation of non-soluble components. In the current study, however, when the gas production profiles of the soluble fraction were fitted to a di-phasic model, we found that 49 to 74 % of the gas was produced in the second phase of fermentation with a C value ranging from 21.2 to 26.2 h. Moreover, some authors have tried to assign the fluctuation in gas production profiles to the fermentations of specific chemical components.[45] However, it was recently shown that

cyclic trends are an inevitable consequence of gas production methodology rather than the result of fermentation of a specific chemical component.[46] This observation is in line with our findings in the current study. Indeed, the multi-phasic manner of gas production observed with the fermentation of SWF of peas and faba beans in a previous study (**Chapter 2**) were not observed in the current study (data not shown).

Effect of processing on formation of fermentation end-products

Rumen micro-organisms convert feed organic matter into microbial biomass and end-products of fermentation mainly VFA, ammonia and fermentative gases. The VFA serve as an important sources of energy for the host animal (40-65 % of the digestible energy in lactating cows)[47] whilst ammonia plays an important role as a source of N for rumen micro-organisms. In the absence or shortage of highly available carbohydrates (rumen degradable carbohydrates), however, ammonia can not be utilised by rumen micro-organisms, resulting in unnecessary N-losses from the rumen, a situation that often happens when the ruminants are fed legume seeds.[18] Thermo-mechanical processes have the potential to alleviate such a problem by rendering carbohydrates into a more accessible form (gelatinised starch) and protein into a less available form for microbial degradation.[4] It is therefore of interest to study the changes on the pattern of fermentation occurring when a feed undergoes a technological process.

In peas, in spite of a higher content of gelatinised starch, the NGR was higher in E and EP than in R samples. The elevated NGR in E and EP is attributed to a lowered ISWF which has a higher starch content than NWF and SWF. Moreover, the increase in the degree of starch gelatinisation due to E and EP in peas was only 4.4 and 7.5 % which is presumably not high enough to cause a glucogenic type of fermentation. Therefore, in peas, the effect of E and EP on the formation of fermentation end-product can partly be attributed to changes in the distribution of DM over the different fractions. This is further confirmed by a marginally lower NH_3-N produced due to the fermentation of E and EP samples compared to R which was concomitant with a lower SWF. The importance of changes in the distribution of DM over the different fractions due to technological processing and its effect on degradative behaviour of feedstuffs has already been addressed by some authors.[18,34]

In general in lupins and faba beans, the NGR, BCR and NH_3-N tended to decrease with the processes E and EP. Process R showed higher BCR and NH_3-N as well as a higher NGR indicating that more protein is degraded and the VFA profile is more acetate dominated compared with processes E and EP. In both lupins and faba beans the

consistently lower BCR and NH_3-N with the processes E and EP compared to process R, indicates that processes E and EP provided a certain level of protection to dietary protein that is reflected in a lower CP content in the SWF of E and EP. These effects were in agreement with the findings of other researchers.[6,7,18] Compared to the R, a lowered NGR along with a higher propionate due to fermentation of E and EP in lupins and faba beans can be explained by an elevated $R_{max}S_1$. It is very well documented that a fast fermentation leads to production of more glucogenic VFA.[34,45,48] In faba beans, however, such an increased propionate and decreased NGR can also be explained by an elevated starch gelatinisation due to E and EP as compared to R as was shown in Table 3.

As is evident from the results in Tables 7 to 9, the fermentation of SWF with respect to fermentation end-product was distinctly different from that of NWF and ISWF. It is therefore expected that in whole seeds, E and EP treatment change the formation of end-products also by changing the contributions of the fractions NWF, ISWF and SWF that differ in chemical constituents (Table 2) and physical characteristics. This is also confirmed by a significant interaction of fraction and process on NGR, BCR and NH_3-N as presented in Tables 7 to 9. These results were in agreement with our earlier findings (**Chapter 3**) and findings of Yang et al.[21]

It is possible to determine the efficiency of microbial production (EMP) *in vitro*.[49,50] Since production of VFA and in vitro gas production are stoichiometrically related, the amount of substrate converted to the VFA-gas complex can be determined by multiplying the in vitro gas production by a stoichiometric factor.[51] The efficiency of microbial production is normally determined at a fixed time point.[52] However, the results in Tables 7 to 9 show that using a fixed time point, for example 24 h, to determine *in vitro* EMP might be misleading, since we observed a significant interaction between time of sampling, processing and fraction for production of some individual VFA. These results indicate that to improve the estimation of EMP, measurements of end-product formation along with the determination of substrate degradability has to be conducted at substrate-specific incubation times, based upon the type of feedstuffs.

Conclusions

Except in lupins, expander processing decreases the contribution of the truly soluble fraction (SWF) in legume seeds. Expander processing followed by pelleting only decreases the soluble fraction (SWF) in faba beans. In peas and faba beans, gelatinised starch serves as a binding agent reducing the size of ISWF. However, the pelleting after expander

processing disturbs the binding effect of gelatinised starch to some extent, and increases the size of ISWF.

In faba beans and lupins, thermo-mechanical processing (E and EP) shows a tendency to increase the fractional rate of substrate degradation and rate of gas production at the early stages of fermentation, thereby shift the pattern of fermentation towards a more glucogenic fermentation. The general tendency of a reduced CP and an elevated sugar content in SWF after E and EP treatment, accompanied with a lowered NH_3-N in all three legume seeds, indicates that the processing has the potential to provide a certain level of protection against ruminal breakdown of dietary protein, which could avoid unnecessary N losses and help to synchronise the utilization of fermentable carbohydrates and proteins.

Acknowledgements

First author thanks the Ministry of Science, Research and Technology of Iran for financing the project. The authors wish to express their gratitude to Saskia van Laar, Dick Bongers, Jane-Martine Muijlaert and Truus Post of the Laboratory of the Animal Nutrition Group; without their help this study would not have been possible. The contribution of Kuenga Namgay and Jan Mica to the experiment as part of their MSc thesis is highly appreciated, as is true for Tamme Zandstra for his co-operation in processing the feed samples.

References

1. Kozlowaska H, Nutrition, in *Carbohydrates in Grain Legume Seeds: Improving nutritional quality and agronomic characteristics*, Ed by Hedley CL. CABI publishing, Wallingford (2001).

2. van Straalen WM and Tamminga S, Protein degradability in ruminant diets, in *Feed Evaluation*, Ed by Wiseman J and Cole DJA. Butterworth, London, pp 55-72 (1990).

3. Goelema JO, Spreeuwenberg MAM, Hof G, van der Poel AFB and Tamminga S, Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. *Anim Feed Sci Technol* **76**:35-50 (1998).

4. van der Poel AFB, Prestløkken E and Goelema JO, Feed processing: effects on nutrient degradation and digestibility, in *Quantitative Aspects of Ruminant Digestion and Metabolism*, Ed by Dijkstra J, Forbes JM and France J. CABI Publishing, Wallingford, pp 734 (2005).

5. Goelema JO, Hof G, van der Poel AFB and Tamminga S, Effect of particle size, cold pelleting, steam pelleting and expander treatment on the rumen degradability of a compound feed for ruminants. *J Dairy Sci* **79 (Suppl. 1)**:142 (1996).

6. Prestløkken E, *In situ* ruminal degradation and intestinal digestibility of dry matter and protein in expanded feedstuffs. *Anim Feed Sci Technol* **77**:1-23 (1999).

7. Ljøkjel K, Skrede A and Harstad OM, Effects of pelleting and expanding on vegetable feeds on *in situ* protein and starch digestion in dairy cows. *J Anim Feed Sci* **12**:435-449 (2003).

8. Arieli A, Bruckental I, Kedar O and Sklan D, *In sacco* disappearance of starch, nitrogen and fat in processed grains. *Anim Feed Sci Technol* **51**:287-295 (1995).

9. Tóthi R, *Processed Grains as a Supplement to Lactating Dairy Cows*, PhD Thesis, Wageningen University, Wageningen (2003).

10. Weisbjerg MR, Hvelplund MR, Hellberg S, Olsson S and Sanne S, Effective rumen degradability and intestinal digestibility of individual amino acids in different concentrates determined *in situ*. *Anim Feed Sci Technol* **62**:179-188 (1996).

11. Michalet-Doreau B and Ould-Bah MY, *In vitro* and *in sacco* methods for estimation of dietary nitrogen degradability in the rumen: a review. *Anim Feed Sci and Technol* **40**:57-86 (1992).

12. Theodorou MK, Williams BA, Dhanoa MS, McAllan AB and France J, A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim Feed Sci Technol* **48**:185-197 (1994).

13. Williams BA, van der Poel AFB, Boer H and Tamminga S, The use of cumulative gas production to determine the effect of steam explosion on fermentability of two substrates with different cell wall quality. *J Sci Food Agric* **69**:33-39 (1995).

14. Davies ZS, Mason D, Brooks AE, Griffith GW, Merry RJ and Theodorou MK, An automated system for measuring gas production from forages inoculated with rumen fluid and its use in determining the effect of enzymes on grass silage. *Anim Feed Sci Technol* **83**:205-221 (2000).

15. Lowe SE, Theodorou MK, Trinci APJ and Hespel RB, Growth of anaerobic rumen fungi on defined and semi-defined media lacking rumen fluid. *J Gen Microb* **131**:2225-2229 (1985).

16. Williams BA, Bosch MW, Boer H, Verstegen MWA and Tamminga S, An *in vitro* batch culture method to assess potential fermentability of feed ingredients for monogastric diets. *Anim Feed Sci Technol* **123-124**:445-462 (2005).

17. Searle PL, The Berthelot or indophenol reaction and its use in the analytical chemistry. a review. *Analyst* **109**:549-568 (1984).

18. Goelema JO, *Processing of Legume Seeds: Effects on Digestive Behaviour in Dairy Cows*, PhD Thesis, Wageningen University, Wageningen (1999).

19. Rijnen MMJA, Verstegen MWA, Heetkamp MJW and Schrama JW, Effects of dietary fermentable carbohydrates on energy metabolism in group-housed sows. *J Anim Sci* **79**:148-154 (2001).

20. Groot JCJ, Cone JW, Williams BA, Debersaques FMA and Lantinga EA, Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. *Anim Feed Sci Technol* **64**:77-89 (1996).

21. Yang HJ, Tamminga S, Williams BA, Dijkstra J and Boer H, *In vitro* gas and VFA production profiles of barley and corn and their soluble washout fractions after feed processing. *Anim Feed Sci Technol* **120**:125-140 (2005).

22. Nocek JE and Tamminga S, The prediction of nutrient supply to dairy cows from the rate and extent of ruminal degradation of ration components. *J Dairy Sci* **74**:3598-3629 (1991).

23. Heimann M, Roller mill systems, in *Feed Manufacturing Technology*, Ed by McEllhiney RR. American Feed Industry Association, Arlington, pp 91-97 (1994).

24. Otto T, Baik B-K and Czuchajowska Z, Microstructure of seeds, flours, and starches of legumes. *Cereal Chem* **74**:445-451 (1997).

25. Masoero F, Pulimeno AM and Rossi F, Effect of extrusion, expansion and toasting on the nutritional value of peas, faba beans and lupins. *Ital J Anim Sci* **4**:177-189 (2005).

26. Slavin JL, Jacobs D and Marquart L, Grain processing and nutrition. *Crit Rev Food Sci Nutr* **40**:309-326 (2000).

27. Goelema JO, Smits A, Vaessen LM and Wemmers A, Effects of pressure toasting, expander treatment and pelleting on in vitro and in situ parameters of protein and starch in a mixture of broken peas, lupins and faba beans. *Anim Feed Sci Technol* **78**:109-126 (1999).

28. Klinger RW, Meuser F and Niediek E, Depolymerization of starch by high pressure extrusion. *Starch / Stärke* **38**:40-44 (1986).

29. Gomez MH and Aguilera JM, Changes in the starch fraction during extrusion-cooking of corn. *J Food Sci* **48**:378-381 (1983).

30. Svihus B, Uhlen AK and Harstad OM, Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: a review. *Anim Feed Sci Technol* **122**:303-320 (2005).

31. Tóthi R, Lund P, Weisbjerg MR and Hvelpund T, Effect of expander processing on fractional rate of maize and barley starch degradation in the rumen of dairy cows estimated using rumen evacuation and *in situ* techniques. *Anim Feed Sci Technol* **104**:71-93 (2003).

32. Offner A, Bach A and Sauvant D, Quantitative review of in situ starch degradation in the rumen. *Anim Feed Sci Technol* **106**:81-93 (2003).

33. Sveinbjornsson J, Murphy M and Uden P, *In vitro* evaluation of starch degradation from feeds with or without various heat treatments. *Anim Feed Sci Technol* **In press** (2006).

34. Sveinbjornsson J, Murphy M and Uden P, Effect of the proportions of neutral detergent fibre and starch, and their degradation rates, on *in vitro* ruminal fermentation. *Anim Feed Sci Technol* **In press** (2006).

35. Walhin PM, Foucant M and Thewis A, Influence of extrusion on ruminal and intestinal disappearance *in sacco* of pea (*Pisum sativum*) proteins and starch. *Anim Feed Sci Technol* **38**:43-55 (1992).

36. Nielsen I, Effect of expander treatment on protein quality in different raw materials and mixture, Intercoop Feedstuffs congress, Lofoten, Norway.

37. Wright DJ and Boulter D, Differential scanning calorimetry study of meals and constituents of some food grain legumes. *J Sci Food Agric* **31**:1231 - 1241 (1980).

38. Michalet-Doreau B and Cerneau P, Influence of food stuff particle size on in situ degradation of nitrogen in the rumen. *Anim Feed Sci Technol* **35**:69-81 (1991).

39. Graham H, Fadel JG, Newman CK and Newman RK, Effect of pelleting and β-glucanase supplementation on the illeal and faecal digestibility of a barley-based diet in the pig. *J Anim Sci* **67**:1293-1298 (1989).

40. Shinnick FL, Longacre MJ, Ink SL and Marlett JA, Oat fibre: composition versus physiological function in rats. *J Nutr* **144-151** (1988).

41. Vranjes MV and Wenk C, The influence of extruded vs. untreated barley in the feed, with and without dietary enzyme supplement on broiler performance. *Anim Feed Sci Technol* **54**:21-32 (1995).

42. Dijkstra J, Kebreab E, Bannink A, France J and Lopez S, Application of gas production technique to feed evaluation systems for ruminants. *Anim Feed Sci Technol* **123-124**:561-578 (2005).

43. Cone JW and Van Gelder AH, Influence of protein fermentation on gas production profiles. *Anim Feed Sci Technol* **76**:251-264 (1999).

44. Cone JW, Van Gelder AH and Chai WZ, Fermentation behaviour of the nylon bag washout and degradable fractions determined with the gas production technique. *Anim Feed Sci Technol* **127**:319-326 (2006).

45. van Laar H, Tamminga S, Williams BA and Verstegen MWA, Fermentation of the endosperm cell walls of monocotyledon and dicotyledon plants species by faecal microbes from pigs: The relationship between cell wall characteristics and fermentability. *Anim Feed Sci Technol* **88**:13-30 (2000).

46. Lopez S, Dhanoa MS, Dijkstra J, Bannink A, Kebreab E and France J, Some methodological and analytical considerations regarding application of the gas production technique. *Anim Feed Sci Technol* **In press** (2006).

47. France J and Dijkstra J, Volatile fatty acids production, in *Quantitaive Aspects of Ruminants Digestion and Metabolism*, Ed by Dijkstra J, Forbes JM and France J. CABI, Wallingford, pp 734 (2005).

48. Tóthi R, Babinszky L and Tamminga S, Effect of hydrothermal processing on the feed quality, the ruminal degradation of grains and the milk compositions in high producing dairy cows. *Krimva* **44**:203-210 (2002).

49. Blümmel M, Makkar HPS and Becker K, *In vitro* gas production: a technique revisited. *J Anim Physiol and Anim Nutr* **77**:24-34 (1997a).

50. Groot JCJ, Williams BA, Oostdam AJ, Boer H and Tamminga S, The use of cumulative gas production and volatile fatty acid production to predict *in vitro* fermentation kinetics of Italian ryegrass leaf cell walls and contents at various time intervals. *Br J Nutr* **79**:519-525 (1998).

51. Grings EE, Blümmel M and Sudekum KH, Methodological considerations in using gas production techniques for estimating ruminal microbial efficiencies for silage-based diets. *Anim Feed Sci Technol* **123-124**:527 (2005).

52. Blümmel M and Lebzien P, Predicting ruminal microbial efficiencies of dairy ration by in vitro techniques. *Livest Prod Sci* **68**:107-117 (2001).

Chapter 5

The effect of sample grinding procedures after processing on gas production profiles and end-product formation in expander processed barley and peas[♣]

Abstract

Grinding is a technological process widely applied in the feed manufacturing industry and is a prerequisite for obtaining representative samples for laboratory procedures (e.g. gas production analysis). When feeds are subjected to technological processes other than grinding (e.g. expander treatment), grinding afterwards may disturb the effect of processing, both in practice and when laboratory techniques are applied. Therefore, this study aimed to establish the possible effects of different grinding procedures and sample preparation on the degradative behaviour of expander processed barley and peas. Samples of expander processed barley and peas were subjected to six different sample preparation procedures (intact sample, dissolved sample, samples ground stepwise over 6 and 3 mm sieves, samples ground stepwise over 6, 3 and 1 mm sieves, samples ground stepwise over 6 and 1 mm sieves, samples ground over a 3 mm sieve and samples ground over a 1 mm sieve). The patterns of gas production in these samples were studied over a period of 72 h incubation using an automated in vitro gas production system. The particle size distribution determined by dry sieve analysis and the Coulter counter method changed due to the different grinding procedures. Grinding the samples of expander processed barley and peas changed the kinetics of gas production and led to a faster degradation, most pronounced after stepwise grinding. However, the formation of the fermentation end-product was not affected by the method of sample preparation. In expander processed barley, the difference in the degradation pattern due to the different grinding procedures was small.

[♣] Azarfar, A., vander Poel, A. F. B. and Tamminga, S. 2006. **Journal of the Science of Food and Agriculture**, Accepted for publication.

Introduction

Grinding feed samples prior to analysis allows the preparation of representative samples.[1] In *in vitro* and *in situ* digestibility studies, feeds are usually ground through a 1-4 mm sieve and then incubated with a rumen inoculum. However, stepwise grinding is sometimes used in the preparation of analytical samples.

In recent years the *in vitro* gas production technique has been widely used to characterise the degradative behaviour of feeds. As with all laboratory techniques, many factors have to be considered in the preparation of feed samples. It is generally recognised that a smaller particle size increases the surface area available for microbial degradation.[2] For gas production analysis most researchers grind the feed samples through a 1 mm screen,[3-5] in keeping with the other *in vitro* methods.[6,7] In technologically processed material, however, grinding the sample may disturb the effect of processing, resulting in confusion about the possible effects of processing on the degradative behaviour of the processed feeds. Therefore, this study aimed to establish the effects of different grinding procedures and sample preparation on the degradative behaviour of expander processed barley and peas using an automated gas production system.

Materials and Methods

Experimental setup

In a completely randomised design with three replicates, samples of expander processed barley and peas, representative of cereal grains and legume seeds, respectively, were subjected to six different sample preparation procedures (intact sample, dissolved sample; prepared by dissolving the original processed feeds into an appropriate quantity of water and freeze-drying, samples ground stepwise over 6 and 3 mm sieves, samples ground stepwise over 6, 3 and 1 mm sieves, samples ground stepwise over 6 and 1 mm sieves, samples ground over a 3 mm sieve and samples ground over 3 mm and 1 mm sieves). Subsequently, the patterns of gas production of these samples were studied over a period of 72 h incubation in two consecutive in vitro gas production runs using an automated in vitro gas production system (APES-IGER, Aberystwyth, Wales). After 72 h, samples were taken to determine the profile of volatile fatty acids (VFA) and ammonia (NH_3-N). The residue of the samples after terminating the incubation period were analysed for organic matter (OM) to determine the OM degradability of samples. Particle

size distribution of the samples was determined using dry sieve analysis and a Coulter counter.

Sample processing

Barley and peas were supplied by a commercial supplier (Research Diet Services, Wijk bij Duurstede, The Netherlands) and had already been ground through a 3 mm sieve using a hammer mill. Processing was carried out at the Wageningen Feed Processing Center (WFPC). An Almex expander (150 Φ, Almex BV Zutphen, the Netherlands), fitted with a 22 kW engine was used for the expander treatment. The temperature of the last mixing bolt before the exit of the expander was taken as the product temperature.

Steam at a pressure of 60 kPa was added to the mash materials in the conditioner. The processing temperature was 99 °C in the barley and 105 °C in the peas. The expander processed materials were then dried in a forced air oven for 16 h at 35 °C. Subsequently, the processed materials were subjected to the different methods of preparation described in the experimental setup. A centrifugal mill (Retsch ZM100, GmbH & Co, Hannover, Germany) was used to grind the samples. The temperature of the product after the grinding process was monitored using an infrared thermometer (Raytek ST3LX, Milton Keynes, UK).

Determination of particle size distribution

The particle size distribution of expander processed barley and peas was determined using dry sieve analysis and a Coulter counter.

In the dry sieve analysis, approximately 100 g of each sample was sieved through a Retsch AS 200 sieve analyser (GmbH & Co, Hannover, Germany) for 10 min using six sieves with mesh sizes of 2.5, 1.25, 0.63, 0.315, 0.160 and 0.071 mm. After finishing the sieving procedure, the sieve fractions were quantitatively weighed to determine the modulus of fineness (MF) and modulus of uniformity (MU). The size of the fraction < 0.071 mm was determined by weighing the particles collected in the pan underneath the last sieve (0.071 mm). The modulus of fineness and uniformity were calculated according to Pfost and Headly.[8] For the MU, the coarse, medium and fine fractions were calculated by pooling the material retained on sieves 1, 2 and 3 (2500, 1250 and 630 μm), sieves 4 and 5 (315 and 160 μm) and the last sieve (71 μm) and the pan, respectively.

In the Coulter counter method, the particle size distributions were determined by laser diffraction using a Coulter LS 230 particle size analyser (Beckman Coulter Inc.,

Hialeah, FL, USA). This equipment can measure particle sizes ranging from 0.04 to 2000 μm. In the particle analyser, laser light is scattered by the suspended particles and the generated diffraction pattern is measured. The Fraunhofer diffraction theory was used to calculate the particle size distribution of the expander processed barley and peas, variously ground, from the scattered light intensity pattern. The samples were added to a suspension of water in the sample vessel. The amount of sample transferred was adjusted to meet the minimum criterion of obscuration and polarisation intensity differential scattering (PIDS) as indicated by the instrument's software.

In vitro fermentation

The cumulative gas production technique of Theodorou et al.,[5] as modified by Williams et al.,[9] was used to determine the fermentation characteristics of the expander processed barley and peas prepared differently. Because of a limited number of places in the applied automated pressure system (APES),[10] the whole experiment had to be conducted in two runs; in the first run, treatment 6–1 mm was left out, whereas in run two treatment 6–3 mm was not included. Three replicates of each substrate were fermented in specific 100-ml APES bottles containing 0.5 g of each substrate, 82 ml of medium B[11] and 5 ml of rumen fluid inoculum in the automated pressure system for 72 h. The inoculum was a mixture of rumen fluid obtained from three non-lactating cows, fed once daily on a diet of moderate quality ryegrass hay (crude protein 100 g kg^{-1}; organic matter: 920 g kg^{-1} DM) ad libitum and 1 kg of a commercial concentrate. The sample of rumen fluid for the inoculum was taken at 08.00 h, prior to feeding. For VFA analysis, a sub-sample of 0.5 ml was withdrawn and added to a 1 ml Eppendorf tube containing 25 μl of phosphoric acid (850 g L^{-1}). A second sub-sample of 0.75 ml, to which 0.75 ml of trichloroacetic acid (TCA; 100 g L^{-1}) was added, was taken for measurement of NH_3-N. The remainder of the fermentation mixture was used to determine pH. After incubation, the amount of fermented organic matter (FOM) after 72 h incubation was calculated as the difference between incubated and residual organic matter (OM). The latter was determined by filtering the contents of the APES bottles through sintered glass crucibles (Schott Duran, porosity # 2, Mainz, Germany), and drying at 105 °C, followed by ashing at 530 °C.

Chemical analysis

Feeds were analysed for dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF) and starch as described by Goelema et al. [12]

VFA were measured using gas liquid chromatography as described in **Chapter 2**. Acetic (HAc), propionic (HPr), *iso*-butyric (i-HBu), butyric (HBu), *iso*-valeric (i-HVal) and valeric (HVal) acid concentrations were expressed in mmol g^{-1} OM incubated. The NH_3-N concentration was expressed in mmol g^{-1} OM incubated. The ratio of non-glucogenic to glucogenic acids (NGR) was calculated using the approach of Ørskov.[13] Additionally, *iso*-butyric and *iso*-valeric acid were considered to originate primarily from the degradation of protein and were expressed as a proportion of the total VFA, called the branched chain ratio (BCR).

The concentration of NH_3-N (mmol g^{-1} OM) was determined by the indophenol method as described by Searle.[14]

Methane production (ml g^{-1} OM) and the efficiency of fermented feed carbohydrates incorporated into the microbial biomass (efficiency; expressed as percentage) were estimated using the stoichiometric equations described by Blümmel et al.[15] and Groot et al.[16]:

$$CO_2 = \frac{HAc}{2} + \frac{HPr}{4} + \frac{3HBu}{2} \quad (1)$$

$$CH_4 = HAc + 2HBu - CO_2 \quad (2)$$

$$Glucose_{biomass} = 0.8 Y_{ATP}(2HAc + 3HPr + 3HBu + CH_4) \quad (3)$$

$$Glucose_{products} = 162\frac{2HAc + 3HPr + 4HBu + CO_2 + CH_4}{6} \quad (4)$$

$$Efficiency = \frac{Glucose_{biomass}}{Glucose_{biomass} + Glucose_{products}} \times 100 \quad (5)$$

where CO_2 and CH_4 are fermentative carbon dioxide and methane (ml g^{-1} OM) produced, $glucose_{biomass}$ and $glucose_{products}$ represent the glucose consumed for production of microbial biomass and formation of end-products (mg), respectively.

Kinetic model analysis

Gas production profiles, obtained with the automated system, were fitted by iteration for individual incubation flasks to the multi-phasic model as described by Groot et al.[17] and shown in the following equation:

$$OMCV = \sum_{i=1}^{2} \frac{Ai}{1+\left(C_i \big/ (t-T)\right)^{Bi}} \tag{6}$$

where i is the number of phases, OMCV is the cumulative gas production at time t (ml g^{-1} OM incubated), A_i is the estimated asymptotic gas production in phase i (ml g^{-1} OM incubated) at time t, B_i represents the sharpness of the switching characteristic for the profile, C_i is the time (h) of incubation at which half of the asymptotic gas production has been formed and T is the lag time (h), the time elapsed between incubation and the start of gas production. The *in vitro* cumulative gas production profiles were further characterised by estimating the maximum rate of gas production ($R_{max}G$, ml h^{-1}) and the time at which this maximum rate was reached ($TR_{max}G$, h):

$$TR_{\max} G = C\left[\frac{B-1}{B+1}\right]^{1/B} + T \tag{7}$$

$$R_{\max} G = \frac{AC^B B\left[(TR_{\max} G - T)^{(-B-1)}\right]}{\left[1 + CB(TR_{\max} G - T)^{-B}\right]^2} \tag{8}$$

Maximum fractional rates of substrate degradation were also estimated according to Groot et al.[17]:

$$TR_{max}S = C(B-1)^{1/c} + T \tag{9}$$

$$R_{\max} S = \frac{B(TR_{\max} S - T)^{B-1}}{CB + (TR_{\max} S - T)^{B}} \tag{10}$$

where $R_{max}S$ (h^{-1}) is the maximum fractional rate of substrate degradation and $TR_{max}S$ (h) is the time at which the maximum fractional substrate degradation is reached.

Statistical analysis

Analysis of variance for cumulative gas production, parameter fittings of the results of cumulative gas production, VFA production, NH_3-N production, NGR, BCR and

disappearance of OM, were done for each run using the GLM procedure of SAS 9.1. The model applied was:

$$Y = \mu + T_i + \varepsilon_{ij}$$

where Y is the dependent variable under examination, μ is the overall mean, T_i is the treatment effect (i = 1, 2) and ε_{ijk} is the error term. Differences between individual substrates were analysed by a multiple comparison test (Duncan).

Results

Chemical composition of concentrate ingredients and their fractions

The chemical composition of the concentrate ingredients and their fractions are shown in Table 1.

Table 1. Chemical composition (g kg^{-1} DM) of expander processed barley and peas

	Barley	Peas
DM (g kg^{-1})	927.1	883.2
In dry matter (g kg^{-1})		
OM	904.7	850.8
Ash	22.4	32.4
CP	128.2	226.9
Starch	582.7	494.5
NDF	155.5	99.4

Particle size analysis

The results of the dry sieve analysis are shown in Table 2. The results indicate that in expander processed barley stepwise grinding significantly increased the pool size of particles smaller than 71 μm. This was further confirmed by a numerically lower MF, as

an indicator of the size of the fine particles pool. As indicated by the MU, stepwise grinding increased the proportion of fine particles mainly at the expense of coarse particles. Figure 1a shows how coarse, medium and fine particles were distributed in differently ground expander processed barley after passing through the final 1 mm sieve. It is evident that the pool of coarse particles had disappeared after stepwise grinding and the size of particles skewed towards more fine particles. When the expander processed barley was ground stepwise to finally pass through a 3 mm sieve, the pool size of fine particles did not change, whereas the pool size of medium particles increased and the pool size of coarse particles was decreased (Figure 1b).

In the expander processed peas, as is evident from Figure 1c and d, stepwise grinding increased the magnitude of the pool of fine particles. The effect of stepwise grinding on the change in the distribution of particle size was more evident in expander processed peas than for expander processed barley where the stepwise grinding skewed the distribution of the particle size towards having more fine particles and less coarse particles (Figure 1).

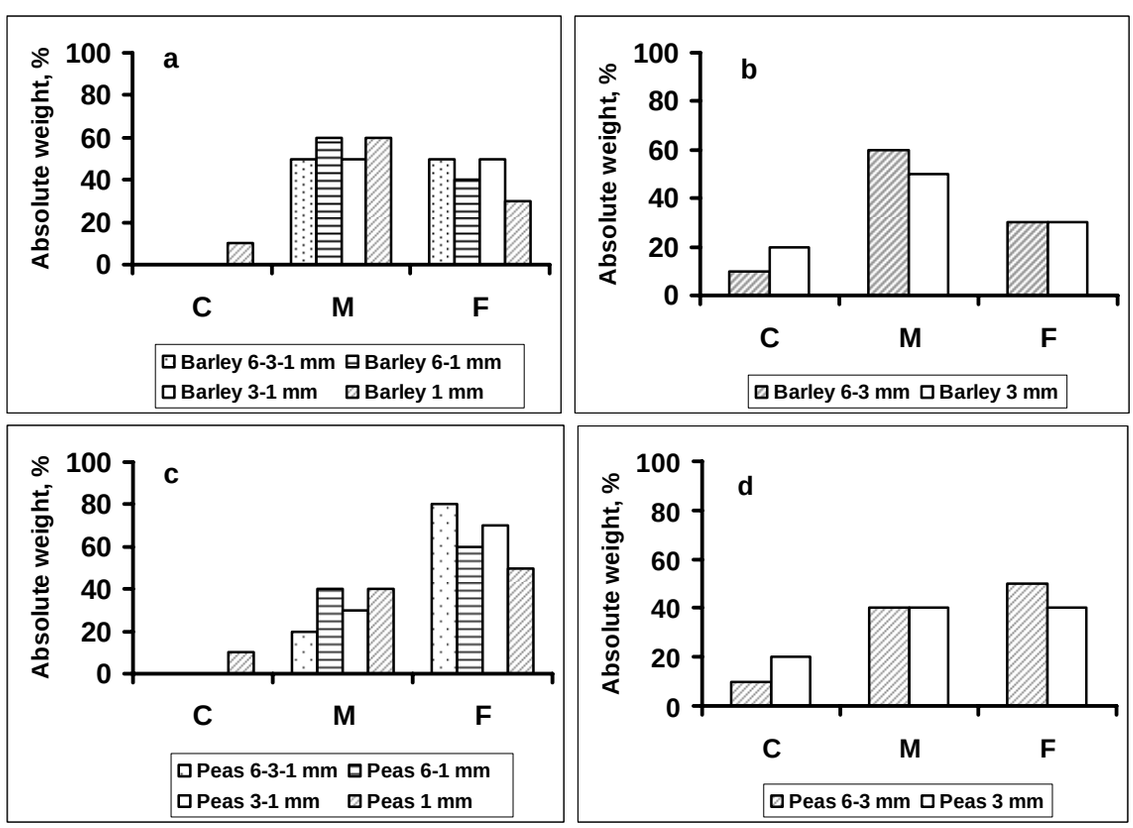


Figure1. The effects of different grinding procedures on the size (absolute weight, %) of fine (F), medium (M) and coarse (C) particle pools in the expander processed barley (a and b) and peas (c and d).

Table 2. Effects of different sample preparation on particle size distribution (fraction as % of air dry matter), modulus of fineness (MF) and modulus of uniformity (MU).

		Particle size (mm)								
Treatments	Temperature (°C)	>2.500	1.250 – 2.500	0.630 – 1.250	0.315 – 0.630	0.160 – 0.315	0.071 – 0.160	<0.071	MF	MU (C : M : F)
Barley 6-3-1 mm	34–36–41	0.2^{b}	0.2^{e}	1.8^{i}	20.3^{g}	29.1a	23.4^{ef}	25.0^{d}	2.5^{bcd}	0 : 5 : 5
Barley 6-1 mm	36–46	0.1^{c}	0.1^{f}	4.2^{g}	27.2^{c}	28.2^{a}	23.0^{ef}	17.2^{f}	2.8^{abc}	0 : 6 : 4
Barley 3-1 mm	39–45	0.2^{b}	0.1^{f}	2.3^{hi}	23.5^{f}	29.4^{a}	31.2^{a}	13.3^{g}	2.7^{abc}	0 : 5 : 5
Barley 1 mm	40	0.1^{c}	0.1^{f}	9.7^{d}	33.6^{a}	24.5^{b}	20.3^{g}	11.7^{g}	3.1^{ab}	1 : 6 : 3
Barley 6-3 mm	33–38	0.2^{b}	1.0^{c}	11.4^{c}	30.0^{b}	28.3^{a}	26.1^{cd}	3.0^{i}	3.3^{a}	1 : 6 : 3
Barley 3 mm	36	0.1^{c}	2.9^{a}	16.6^{a}	29.3^{b}	21.8^{c}	19.7^{g}	9.6^{h}	3.3^{a}	2 : 5 : 3
Peas 6-3-1 mm	31–36–40	0.1^{c}	0.1^{f}	0.9^{j}	9.4^{j}	14.1^{f}	28.6^{b}	46.8^{a}	1.9^{d}	0 : 2 : 8
Peas 6-1 mm	32–37	0.1^{c}	0.1^{f}	3.2^{g}	19.2^{h}	19.4^{de}	26.7^{c}	31.3^{c}	2.4^{bcd}	0 : 4 : 6
Peas 3-1 mm	33–40	0.1^{c}	0.1^{f}	2.1^{i}	14.6^{i}	17.9^{de}	27.7^{bc}	37.5^{b}	2.2^{cd}	0 : 3 : 7
Peas 1 mm	35	0.1^{c}	0.1^{f}	7.6^{e}	25.9^{d}	19.5^{d}	22.7^{f}	24.1^{de}	2.7^{abc}	1 : 4 : 5
Peas 6-3 mm	32–36	0.1^{c}	0.3^{d}	6.4^{f}	19.3^{h}	18.7^{de}	24.8^{de}	29.8^{c}	2.5^{bcd}	1 : 4 : 5
Peas 3 mm	35	1^{a}	1.2^{b}	13.1^{b}	24.4^{e}	17.6^{e}	20.2^{g}	22.7^{e}	2.9^{ab}	2 : 4 : 4
SEM		0.003	0.03	0.4	0.3	0.6	0.6	0.8	0.2	–

C, coarse; M, medium; F, fine; SEM, standard error of the mean.

To further confirm the results of dry sieve analysis we analysed the particle size distribution of differently ground expander processed barley and peas using a Coulter counter. The results are shown in Figure 2.

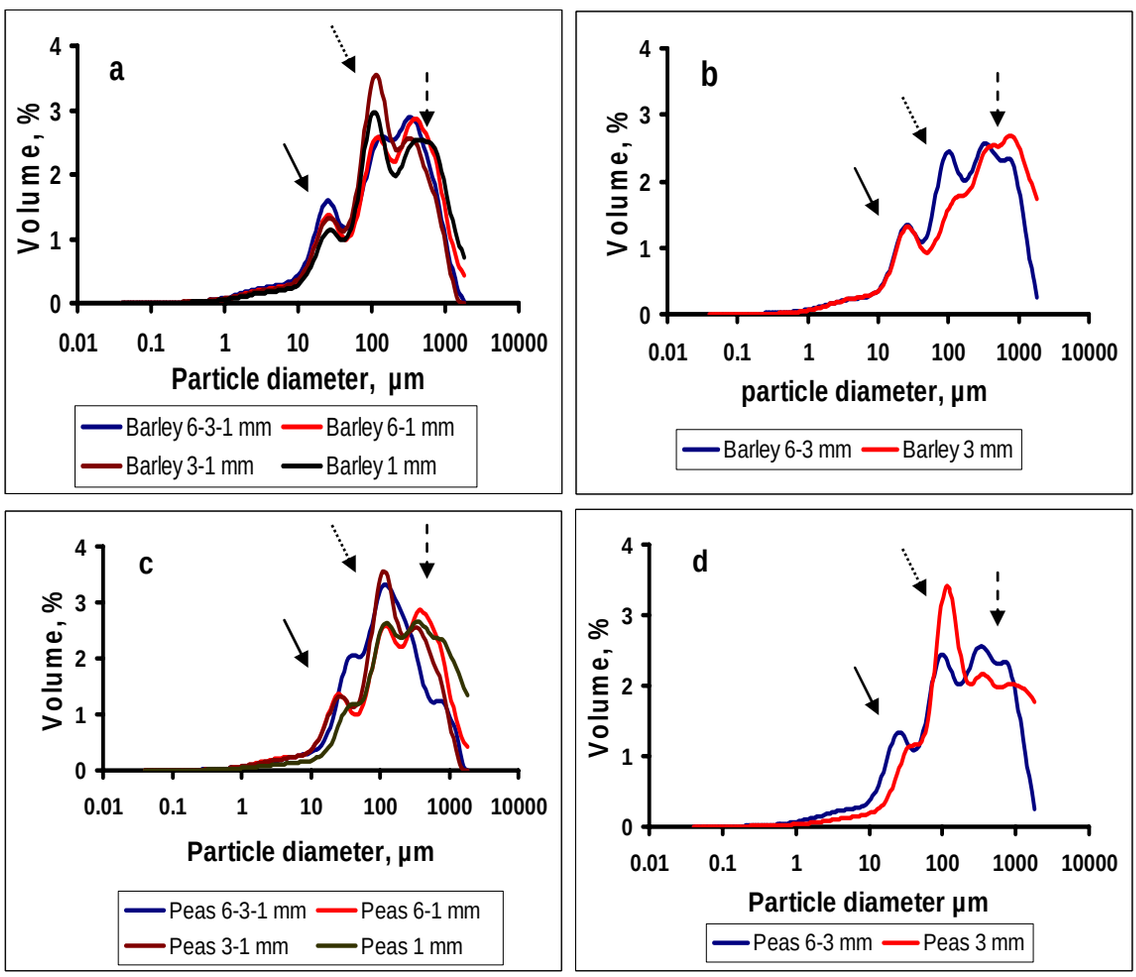


Figure 2. The changes in the size distribution of particles in the differently ground expander processed barley (a and b) and peas (c and d).

The particle size distribution curves are characterised by three peaks: (1) particle size in the range of 1–50 µm (solid arrows); (2) particle size in the range of 50–150 µm (dotted arrow); (3) particle size larger than 150 µm (dashed arrow). For the differently ground barley passing through a 1 mm sieve, the first peak (solid arrow) did not change much; however, it was slightly larger in the stepwise ground barley (Figure 2a). The most prominent effect of the different grinding procedures on the differently ground expander processed barley passing through a 1 mm sieve was observed in the distribution of particle size in the range of 50-150 µm (dotted arrow); there was a higher volume in the sample ground through a 3 mm sieve and then a 1 mm sieve, and the sample ground only through a 1 mm sieve (Figure 2a). As is evident from Figure 2a, the Coulter counter

curves for expander processed barley ground through a 1 mm sieve and ground stepwise through 3 and 1 mm sieves did not cross the horizontal axis indicating that these methods of grinding produced large particles that were out of the range of instrument measurement. A lower volume of particles out of the range of instrument measurement indicates that stepwise grinding of expander processed barley (even when the samples is ground through a 3 mm sieve) produces more fine particles (Figure 2b).

As can be seen from Figure 2c and d, particles larger than 1500 μm had almost disappeared after stepwise grinding of expander processed peas passed through a 1 mm as well as a 3 mm sieve.

Gas production profiles

The results of fitting the gas production profiles with a mono-phasic Groot model are shown in Tables 3 and 4. Between runs (run 1 vs. run 2) significant differences ($P < 0.001$) were observed with regard to C, T, $R_{max}S$, $TR_{max}S$, $R_{max}G$ and OMD (data are not shown).

In the first run, differences were observed between grains (barley vs. peas) with regard to A (peas 19.5 ml higher than barley, $P < 0.01$), OMD after 72 h (peas 9.9 units higher than barley, $P < 0.05$), C (peas 2.6 h longer than barley, $P < 0.001$), $R_{max}S$ (barley 0.034 h^{-1} higher than peas, $P < 0.001$) and $RmaxG$ (barley 5.8 ml h^{-1} higher than peas, $P < 0.001$). The controls (intact and dissolved) have a higher C, a lower T, a lower $R_{max}S$, a higher $TR_{max}S$, a lower $R_{max}G$ and a higher $TR_{max}G$ than the treatments (1, 6–3–1, 6–3, 3 and 1 mm). Differences among treatments (1, 6–3–1, 6–3, 3 and 1 mm) were not consistent.

In the second run, differences between grains (barley vs. peas) were also observed with regard to A (peas 17.1 ml higher than barley, $P < 0.05$), OMD after 72 h (peas 9.2 units higher than barley, $P < 0.001$), C (peas 6.2 h longer than barley, $P < 0.001$), T (barley 1.6 h longer than peas, $P < 0.01$), $R_{max}S$ (barley 0.057 h^{-1} higher than peas, $P < 0.001$), $TR_{max}S$ (peas 2.1 h later than barley, $P < 0.01$) and $R_{max}G$ (barley 13.8 ml h^{-1} higher than peas, $P < 0.001$). The controls (intact and dissolved) have a higher C, a lower T, a lower $R_{max}S$, a higher $TR_{max}S$, a lower $R_{max}G$ and a higher $TR_{max}G$ than the treatments (1, 6-3-1, 6-1, 3 and 1 mm). Differences among treatments (1, 6–3–1, 6–1, 3 and 1 mm) were also not consistent in the second run.

Table 3. Gas production characteristics of the differently prepared expander processed barley and peas in the first run

	Expander processed barley						Expander processed peas							Significance (*P*)	
	1 mm	6–3–1 mm	6–3 mm	3 mm	Dissolved	Intact	1 mm	6–3–1 mm	6–3 mm	3 mm	Dissolved	Intact	SEM	Barley vs. peas	Controls vs. others
A	289.4^{cde}	316.9^{bc}	260.1^{e}	296.3^{cde}	291.3^{cde}	316.2^{bc}	311.8^{bc}	293.2^{bc}	271.3^{de}	303.9^{bcd}	341.3^{ab}	367.7^{a}	11.2	<0.01	<0.001
C	5.0^{e}	4.9^{e}	5.1^{de}	5.5^{de}	9.9^{c}	8.4^{c}	8.5^{c}	6.7^{d}	6.8^{d}	6.5^{de}	12.1^{b}	14.1^{a}	0.5	<0.001	<0.001
T	4.6^{ab}	4.3^{ab}	4.7^{ab}	4.6^{ab}	3.8^{ab}	4.0^{ab}	4.0^{ab}	4.6^{ab}	5.0^{a}	4.3^{ab}	3.6^{b}	3.8^{ab}	0.4	NS	<0.01
$R_{max}S$	0.154^{a}	0.158^{a}	0.152^{a}	0.138^{ab}	0.091^{de}	0.099^{cd}	0.091^{de}	0.129^{bc}	0.118^{bc}	0.124^{b}	0.071^{ef}	0.056^{f}	0.007	<0.001	<0.001
$TR_{max}S$	6.5^{b}	6.6^{b}	6.2^{b}	6.7^{b}	11.7^{a}	10.1^{ab}	6.5^{b}	6.9^{b}	6.2^{b}	7.8^{ab}	8.9^{ab}	9.1^{ab}	1.2	NS	<0.001
$R_{max}G$	37.4^{ab}	40.4^{a}	33.0^{bc}	34.4^{abc}	18.8^{ef}	23.0^{def}	24.6^{de}	31.6^{bc}	29.3^{cd}	29.5^{cd}	20.2^{ef}	17.4^{f}	1.9	<0.001	<0.001
$TR_{max}G$	5.6^{b}	5.5^{b}	5.5^{b}	5.7^{b}	8.3^{a}	7.3^{ab}	5.3^{b}	5.8^{b}	5.6^{b}	5.5^{b}	6.5^{ab}	6.6^{ab}	0.6	NS	<0.001
OMD	87.4^{cd}	85.9^{e}	86.7^{cde}	86.3^{cdeq}	86.0^{de}	87.5^{c}	95.7^{b}	96.4^{ab}	96.6^{ab}	96.8^{ab}	97.3^{a}	96.1^{ab}	0.4	<0.001	NS

A, asymptotic gas production (ml g^{-1} disappeared organic matter); *C*, half-time of gas production (h); *T*, lag time (h); $R_{max}S$, maximum fractional rate of substrate degradation (h^{-1}); $TR_{max}S$, time at which the $R_{max}S$ is reached (h); $R_{max}G$, maximum rate of gas production (ml h^{-1}); $TR_{max}G$, time at which the $R_{max}G$ is reached; OMD, organic matter disappearance (%); SEM, standard error of mean; NS, not significant. Means with different superscripts within a row differ significantly ($P < 0.05$).

Table 4. Gas production characteristics of the differently prepared expander processed barley and peas in the second run

	Expander processed barley						Expander processed peas							Significance (*P*)	
	1 mm	6–3–1 mm	6–1 mm	3 mm	Dissolved	Intact	1 mm	6–3–1 mm	6–1 mm	3 mm	Dissolved	Intact	SEM	Barley vs. peas	Controls vs. others
A	302.6^{ab}	280.1^{b}	283.5^{ab}	309.5^{ab}	327.8^{ab}	306.9^{ab}	328.1^{ab}	323.9^{ab}	312.3^{ab}	330.3^{a}	311.5^{ab}	306.9^{ab}	13.1	<0.05	NS
C	5.3^{d}	5.2^{d}	5.4^{d}	6.2^{d}	9.6^{c}	10.2^{c}	13.9^{ab}	11.1^{bc}	11.7^{bc}	11.7^{bc}	15.4^{a}	15.4^{a}	0.9	<0.001	<0.001
T	4.8^{ab}	5.2^{a}	4.5^{abc}	4.0^{abcd}	3.2^{bcd}	2.8^{de}	3.8^{abcd}	3.1^{cd}	3.0^{cde}	2.5^{def}	1.1^{f}	1.5^{ef}	0.5	<0.001	<0.001
$R_{max}S$	0.148^{a}	0.149^{a}	0.151^{a}	0.126^{b}	0.089^{c}	0.087^{c}	0.059^{d}	0.072^{cd}	0.072^{cd}	0.069^{cd}	0.062^{d}	0.058^{d}	0.006	<0.001	<0.001
$TR_{max}S$	6.2^{e}	7.6^{de}	7.2^{de}	7.1^{de}	10.7^{cd}	11.5^{bc}	5.8^{e}	7.8^{cde}	10.0^{de}	9.3^{cde}	15.5^{cd}	14.2^{ab}	1.0	<0.01	<0.001
$R_{max}G$	39.9^{a}	34.4^{a}	35.6^{a}	31.8^{a}	21.1^{b}	18.7^{b}	17.9^{b}	19.2^{b}	17.9^{b}	18.0^{b}	12.8^{b}	12.8^{b}	3.5	<0.001	<0.001
$TR_{max}G$	5.5^{ef}	6.5^{cde}	6.0^{def}	5.7^{ef}	7.4^{bcd}	7.6^{cd}	4.8^{f}	5.6^{ef}	6.6^{cde}	6.2^{cdef}	9.3^{a}	8.7^{ab}	0.5	NS	<0.001
OMD	88.4^{c}	87.4^{c}	88.2^{c}	87.4^{c}	87.8^{c}	88.1^{c}	95.7^{b}	97.0^{ab}	97.1^{ab}	97.4^{a}	97.8^{a}	97.7^{a}	0.5	<0.001	NS

Gas production parameters explained in Table 3. Means with different superscripts within a row differ significantly ($P < 0.05$).

The coefficient of variation (CV) of asymptotic gas production of differently prepared samples of expander processed barley and peas was calculated to indicate the effect of different sample preparations on the uniformity of gas production results (Table 5). The results show that stepwise grinding of expander processed barley in both the first and second runs resulted in a higher CV of asymptotic gas production compared to the other treatments. Interestingly, in the first run when the expander processed barley was ground stepwise to pass through a 3 mm sieve, the coefficient of variation of asymptotic gas production did not change, whereas in the expander processed peas it was dramatically decreased when the sample was ground stepwise to pass through a 3 mm sieve (Table 5). In the second run, when a third step was involved (treatment 6–3–1 mm) in grinding the samples of expander processed peas to pass through a 1 mm sieve, the CV of asymptotic gas production increased to almost twice that when the sample was ground through a 6 and then a 1 mm sieve. In contrast, grinding the samples of expander processed barley in three steps (6–3–1 mm) instead of two steps (6–1 mm) decreased the CV of asymptotic gas production (Table 5).

Table 5. The coefficient of variation (%) of asymptotic gas production in differently prepared expander processed barley and peas

	Expander processed barley		Expander processed peas	
	Run 1	Run 2	Run 1	Run 2
1 mm	3.2	3.1	6.5	8.1
6–3–1 mm	8.7	8.1	6.9	10.9
6–1 mm	NE	17.1	NE	5.2
6–3 mm	7.9	NE	8.4	NE
3 mm	7.8	3.3	3.5	2.5
Dissolved	2.5	1.0	6.4	1.3
Intact	3.0	5.6	6.9	7.1

NE, not examined.

Formation of fermentation end-products in the samples of differently prepared expander processed barley and peas

Statistical analysis revealed that different grains resulted in different VFA patterns with higher total VFA production (TVFA, mmol g^{-1} OM), NGR and BCR in peas than in barley, but that the different types of sample preparation did not affect the proportions of individual fatty acids or TVFA, NGR and BCR after 72 h of incubation (Tables 6 and 7).

The estimated methane produced, NH_3-N and the efficiency of fermented carbohydrate incorporation in the microbial biomass were also not affected by the different procedures of sample preparation.

Discussion

Nowadays, *in vitro* gas production techniques as a tool to study the degradative behaviours of unprocessed as well as processed feeds have attracted the attention of researchers in animal nutrition fields.[18] These techniques were developed to predict fermentation of ruminant feeds[19] and are widely used to study the degradative behaviour of feeds, evaluate microbial fermentation of ruminant feeds,[20] estimate short chain fatty acid production,[21] estimate the production of methane,[22] and rank the fermentability of feedstuffs.[23] As with other laboratory methods, many factors, such as the pressure of the head space of the fermentation vessels,[24] atmospheric pressure,[25] the type of apparatus,[19] the sample size and preparation,[1,4,5] hydrating the samples with the medium before inoculation,[26] and the source of inoculum,[27,28] may affect the gas production profiles. In the upcoming paragraphs we have tried to elaborate on whether different grinding procedures and sample preparations in the technologically processed feeds have any effect on particle size distribution and consequently on the gas production profiles and formation of fermentation end-products.

The different grinding procedures indeed changed the distribution of particle size. In the expander processed barley and pea samples ground stepwise, the pool of coarse particles totally disappeared after applying the second and third grinding steps (Figure 1a and c). This was further confirmed by using a Coulter counter where the volume of particles larger than can be measured by the instrument were reduced when the samples were ground stepwise. Consequently, in the chemical determination of neutral detergent fibre (NDF) or in determining the disappearance of organic matter *in vitro*, where the size of leftover material is determined gravimetrically after filtering (through a filter paper or sintered glass crucibles), stepwise grinding might increase the chance of underestimating the size of leftover material. Moreover, as indicated in Figure 1, grinding the samples in only one step produced a more homogenous sample compared to the samples ground stepwise. However, the effect of different grinding procedures on the distribution of particle size was more profound in the expander processed pea samples than in those of barley.

Table 6. VFA proportions (mmol mol^{-1} of acetic acid, HAc; propionic acid, HPr; butyric acid, HBu), total VFA production (TVFA, mmol g^{-1} OM), non-glucogenic ratio (NGR), branched chain ratio (BCR), estimated methane production (ml g^{-1} OM), ammonia production (NH_3-N, mmol g^{-1} OM) and efficiency of fermented feed carbohydrate incorporation in microbial biomass (efficiency, %) in differently prepared expander processed barley and peas in the first run

	Expander processed barley							Expander processed peas						Significance (*P*)	
	1 mm	6–3–1 mm	6–3 mm	3 mm	Dissolved	Intact	1 mm	6–3–1 mm	6–3 mm	3 mm	Dissolved	Intact	SEM	Barley vs. peas	Controls vs. others
HAc	428.9^{d}	431.9^{d}	438.3^{d}	435.6^{d}	435.3^{d}	421.4^{d}	484.9^{c}	497.1^{bc}	516.7^{a}	496.4^{bc}	507.9^{ab}	522^{a}	5.3	<0.001	NS
HPr	422.4^{ab}	430.6^{a}	427.1^{ab}	407.5^{ab}	405.3^{b}	420.3^{ab}	324.6^{c}	318.1^{cd}	296.9^{de}	301.4^{cde}	314.3^{cd}	285.6^{e}	7.4	<0.001	NS
HBu	87.5^{d}	83.0^{d}	79.0^{d}	91.5^{d}	101.3^{bcd}	96.1^{cd}	121.8^{ab}	122.3^{ab}	122.4^{ab}	136.5^{a}	115.0^{abc}	121.4^{ab}	7.3	<0.001	NS
TVFA	10.3^{bcd}	10.7^{abc}	9.8^{de}	10.1^{cde}	9.4^{e}	10.0^{cde}	11.0^{ab}	10.7^{ab}	11.2^{a}	10.7^{abc}	10.7^{abc}	11.2^{a}	0.2	<0.001	NS
NGR	1.38^{bcd}	1.36^{cd}	1.34^{d}	1.39^{bcd}	1.43^{abcd}	1.43^{abcd}	1.48^{ab}	1.47^{ab}	1.45^{abc}	1.52^{a}	1.43^{abcd}	1.45^{abc}	0.03	<0.001	NS
BCR	0.033^{bc}	0.030^{c}	0.030^{c}	0.033^{bc}	0.030^{c}	0.033^{bc}	0.044^{a}	0.040^{ab}	0.040^{ab}	0.040^{ab}	0.040^{ab}	0.045^{a}	0.002	<0.001	NS
CH_4	40.1^{c}	40.8^{c}	38.0^{c}	41.5^{c}	40.2^{c}	39.3^{c}	62.5^{b}	62.7^{b}	69.8^{a}	65.6^{b}	63.3^{b}	71.5^{a}	1.2	<0.001	NS
NH_3-N	3.3^{d}	3.6^{d}	3.2^{d}	3.4^{d}	3.2^{d}	3.3^{d}	4.6^{abc}	4.1^{c}	4.3^{bc}	4.8^{a}	4.2^{bc}	4.7^{ab}	0.2	<0.001	NS
Efficiency	19.6^{a}	19.7^{a}	19.7^{a}	19.6^{a}	19.5^{a}	19.6^{a}	19.2^{b}	19.2^{b}	19.1^{b}	19.0^{b}	19.2^{b}	19.1^{b}	0.0	<0.001	NS

Means with different superscripts within a row differ significantly ($P < 0.05$).

Table 7. VFA proportions (mmol mol^{-1} of acetic acid, HAc; propionic acid, HPr; butyric acid, HBu), total VFA production (TVFA, mmol g^{-1} OM), non-glucogenic ratio (NGR), branched chain ratio (BCR), estimated methane production (ml g^{-1} OM), ammonia production (NH_3-N, mmol g^{-1} OM) and efficiency of fermented feed carbohydrate incorporation in microbial biomass (efficiency, %) in differently prepared expander processed barley and peas in the second run

	Expander processed barley						Expander processed peas							Significance (*P*)	
	1 mm	6–3–1 mm	6–1 mm	3 mm	Dissolved	Intact	1 mm	6–3–1 mm	6–1 mm	3 mm	Dissolved	Intact	SEM	Barley vs. peas	Controls vs. others
HAc	447.0^{b}	428.9^{b}	435.9^{b}	436.2^{b}	435.2^{b}	425.5^{b}	494.7^{a}	481.9^{a}	497.1^{a}	495.3^{a}	497.6^{a}	501.3^{a}	10.6	<0.001	NS
HPr	400.0^{a}	433.5^{a}	417.9^{a}	406.1^{a}	389.3^{ab}	412.5^{a}	328.4^{c}	343.8^{bc}	320.2^{c}	325.9^{c}	334.5^{c}	325.2^{c}	17.2	<0.001	NS
HBu	96.9^{abc}	89.7^{bc}	83.7^{c}	96.3^{abc}	111.2^{a}	97.4^{abc}	107.9^{a}	108.4^{a}	113.2^{a}	111.3^{a}	103.9^{ab}	106.6^{ab}	5.9	<0.001	NS
TVFA	9.9^{d}	9.9^{d}	10.5^{bcd}	10.5^{bcd}	10.1^{cd}	10.6^{bcd}	11.2^{ab}	11.1^{abc}	11.2^{ab}	11.9^{a}	10.3^{bcd}	10.5^{bcd}	0.4	<0.001	NS
NGR	1.6^{cd}	1.4^{d}	1.4^{d}	1.5^{d}	1.7^{bcd}	1.5^{d}	2.1^{a}	1.9^{abc}	2.2^{a}	2.2^{a}	2.0^{ab}	2.1^{a}	0.13	<0.001	NS
BCR	0.032^{c}	0.032^{c}	0.035^{bc}	0.037^{a}	0.036^{bc}	0.036^{bc}	0.044^{a}	0.042^{abc}	0.045^{ab}	0.047^{a}	0.039^{abc}	0.043^{ab}	0.003	<0.001	NS
CH_4	43.6^{b}	38.1^{b}	41.5^{b}	44.4^{b}	45.3^{b}	43.0^{b}	62.7^{a}	59.2^{a}	63.8^{a}	66.9^{a}	57.1^{a}	59.8^{a}	3.2	<0.001	NS
NH_3-N	3.6^{bcde}	3.4^{cde}	3.3^{de}	3.7^{bcde}	3.4^{de}	3.1^{e}	4.1^{abc}	4.2^{ab}	3.7^{bcde}	3.8^{abcde}	3.9^{abcd}	4.3^{a}	0.2	<0.001	NS
Efficiency	19.5^{ab}	19.6^{a}	19.6^{a}	19.5^{ab}	19.4^{bc}	19.5^{ab}	19.3^{bc}	19.3^{bc}	19.2^{c}	19.2^{c}	19.4^{bc}	19.3^{bc}	0.0	<0.001	NS

Means with different superscripts within a row differ significantly ($P < 0.05$).

During the incubation of expander processed barley and peas samples, dissolved and intact samples showed a slower pattern of fermentation, as indicated by a lower and later occurring $R_{max}S$ and $R_{max}G$, than the ground samples. Since the intact and dissolved samples showed a similar pattern of gas production, it can be concluded that grinding the technologically processed samples by a laboratory mill can change the degradative behaviour of processed feeds thereby resulting in misunderstanding the effects of processing on the degradative behaviour of technologically processed feeds. Grinding the technologically processed feeds most likely reduces the size of the particles to a larger extent than the technological processing does, thereby exposing them more to microbial attachment, colonisation and attack.[29,30] It has been demonstrated that damaging wheat, barley and maize kernels by grinding greatly increased both the rate and extent of degradation *in situ*.[31] It has also been shown with processed maize that investigating the effect of processing may require a larger mesh size to ensure that the zein protein is not disrupted.[19]

The absence of a discrepancy between the results of total gas production and the disappearance of organic matter and the formation of fermentation end-products due to grinding, might be related to the fact that most of the substrate would have been exhausted by 72 h incubation and OM residue and a proportion of fermentation end-products might originate from microbial biomass and microbial lysis rather than from feed particles.[32]

Whereas grinding remarkably changed the gas production characteristics of the intact expander processed barley and peas, the differences between the differently ground samples were not that apparent.

The results show that the variation in gas produced in the replicates, as represented by the coefficient of variation of asymptotic gas production, was affected by the type of sample preparation (Table 5). An elevated coefficient of variation for asymptotic gas production was observed in both the first and the second run when the samples of expander processed barley and peas were ground stepwise to pass through a 1 mm screen. This can most likely be explained by the fact that the stepwise grinding produces a less homogenous sample (Figures 1 and 2) thereby increasing the variation in gas produced in the replicates. Surprisingly, the dissolved and intact samples in both the expander processed barley and peas show a reasonably comparable CV of the asymptotic gas production with ground samples.

Conclusions

Our data show that grinding the samples of technologically processed barley and peas changes their degradative behaviour and leads to a faster, but not higher degradation. The different grinding procedures change the particle size distribution in the samples of processed material. As a consequence, the kinetics of gas production change. However, the formation of fermentation end-product was not affected by the different methods of sample preparation. When monitoring the effects of feed processing by using an *in vitro* gas production technique, no grinding prior to *in vitro* incubation should be applied.

Acknowledgement

A.A. thanks the Ministry of Science, Research and Technology of Iran for financing the project. The authors wish to express their gratitude to Saskia van Laar, Dick Bongers, Jane-Martine Muijlaert, Truus Post and Meijke Booij of the Laboratory of the Animal Nutrition Group; without their help this study would not have been possible. Tamme Zandstra is thanked for his co-operation in processing the feed samples. The help of Wilbert Pellikaan during the gas production runs is highly appreciated.

References

1. Lowman RS, Theodorou MK and Cuddeford D, The effect of sample processing on gas production profiles obtained using the pressure transducer technique. *Anim Feed Sci Technol* **97**:221-237 (2002).

2. Williams BA, Cumulative gas production techniques for forage evaluation, in *Forage Evaluation in Ruminant Nutrition*, Ed. by Givens DI, Owen E, Axford RFE and Omed HM. CABI Publishing, Bangor, pp. 189-213 (2000).

3. Menke KH, Rabb L, Salewski A, Steingass H, Fritz D and Schneider W, The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. *J Agric Sci* **93**:374-379 (1979).

4. Pell AN and Schofield P, Computerized monitoring of gas production to measure forage digestion in vitro. *J Dairy Sci* **76**:1063-1073 (1993).

5. Theodorou MK, Williams BA, Dhanoa MS, McAllan ADB and France J, A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim Feed Sci Technol* **48**:185-197 (1994).

6. Tilley JMA and Terry RA, A two stage technique for the *in vitro* digestion of forage crops. *J Br Grassl Soc* **18**:104-111 (1963).

7. Georing HK and Van Soest PJ, *Forage Fibre Analysis (Apparatus, Reagents, Procedures and Some Applications)*, Vol. 379, ARS, USDA, Washington, DC (1970).

8. Pfost H and Headley V, Methods for determining and expressing particle size, in *Feed Manufacturing Technology*, Ed. by Pfost H and Pickering D. American Feed Industry, Arlington, VA, pp. 512-520 (1976).

9. Williams BA, van der Poel AFB, Boer H and Tamminga S, The use of cumulative gas production to determine the effect of steam explosion on fermentability of two substrates with different cell wall quality. *J Sci Food Agric* **69**:33-39 (1995).

10. Davies ZS, Mason D, Brooks AE, Griffith GW, Merry RJ and Theodorou MK, An automated system for measuring gas production from forages inoculated with rumen fluid and its use in determining the effect of enzymes on grass silage. *Anim Feed Sci Technol* **83**:205-221 (2000).

11. Lowe SE, Theodorou MK, Trinci APJ and Hespel RB, Growth of anaerobic rumen fungi on defined and semi-defined media lacking rumen fluid. *J Gen Microb* **131**:2225-2229 (1985).

12. Goelema JO, Spreeuwenberg MAM, van der Poel AFB and Tamminga S, Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. *Anim Feed Sci Technol* **76**:35–50 (1998).

13. Ørskov ER, Manipulation of rumen fermentation for maximum food utilization. *World Rev Nutr Dietet* **22**:152-182 (1975).

14. Searle PL, The berthelot or indophenol reaction and its use in the analytical chemistry. A review. *Analyst* **109**:549-568 (1984).

15. Blümmel M, Makkar HPS and Becker K, In vitro gas production: a technique revisited. *J Anim Physiol Anim Nutr* **77**:24-34 (1997).

16. Groot JCJ, Williams BA, Oostdam AJ, Boer H and Tamminga S, The use of cumulative gas production and volatile fatty acid production to predict *in vitro* fermentation kinetics of Italian ryegrass leaf cell walls and contents at various time intervals. *Br J Nutr* **79**:519-525 (1998).

17. Groot JCJ, Cone JW, Williams BA, Debersaques FMA and Lantinga EA, Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. *Anim Feed Sci Technol* **64**:77-89 (1996).

18. Krishnamoorthy U, Rymer C and Robinson PH, The *in vitro* gas production technique: limitations and opportunities. *Anim Feed Sci Technol* **123-124**:1-7 (2005).

19. Rymer C, Huntington JA, Williams BA and Givens DI, *In vitro* cumulative gas production techniques: history, methodological considerations and challenges. *Anim Feed Sci Technol* **123-124**:9-30 (2005).

20. Getachew G, DePeters EJ, Robinson PH and Fadel JG, Use of an *in vitro* rumen gas production technique to evaluate microbial fermentation of ruminant feeds and its impact on fermentation products. *Anim Feed Sci Technol* **123-124**:547-559 (2005).

21. Fievez V, Babayemi OJ and Demeyer D, Estimation of direct and indirect gas production in syringes: a tool to estimate short chain fatty acid production that requires minimal laboratory facilities. *Anim Feed Sci Technol* **123-124**:197-210 (2005).

22. Getachew G, Robinson PH, DePeters EJ, Taylor SJ, Gisi DD, Higginbotham GE and Riordan TJ, Methane production from commercial dairy rations estimated using an *in vitro* gas technique. *Anim Feed Sci Technol* **123-124**:391-402 (2005).

23. van Gelder AH, Hetta M, Rodrigues MAM, De Boever JL, Den Hartigh H, Rymer C, van Oostrum M, van Kaathoven R and Cone JW, Ranking of *in vitro* fermentability of 20 feedstuffs with an automated gas production technique: results of a ring test. *Anim Feed Sci Technol* **123-124**:243-253 (2005).

24. Theodorou MK, Lowman RS, Daives ZS, Cuddeford D and Owen E, Principles of techniques that rely on gas measurements in ruminant nutrition, in *In vitro Techniques for Measuring Nutrient Supply to Ruminants*, Ed. by Deaville ER, Owen E, Adesogen AT, Rymer C, Huntington JA and Lawrence TLJ. BSAS, Edinburgh, pp. 55-64 (1998).

25. Pell AN, Pitt RE, Doane PH and Schofield P, The development, use and application of the gas production technique at Cornell University, in *In vitro Techniques for Measuring Nutrient Supply to Ruminants*, Ed. by Deaville ER, Owen E, Adesogen AT, Rymer C, Huntington JA and Lawrence TLJ. BSAS, Edinburgh, pp. 45-54 (1998).

26. Rymer C, Huntington JA and Givens DI, Effects of inoculum preparation method and concentration, method of inoculation and pre-soaking the substrate on the gas production profile of high temperature dried grass. *Anim Feed Sci Technol* **78**:199-213 (1999).

27. Bueno ICS, Cabral Filho SLS, Gobbo SP, Louvandini H, Vitti DMSS and Abdalla AL, Influence of inoculum source in a gas production method. *Anim Feed Sci Technol* **123-124**:95-105 (2005).

28. Calabro S, Lopez S, Piccolo V, Dijkstra J, Dhanoa MS and France J, Comparative analysis of gas production profiles obtained with buffalo and sheep ruminal fluid as the source of inoculum. *Anim Feed Sci Technol* **123-124**:51-65 (2005).

29. Emanuele SM and Staples CR, Effect of forage particle size on *in situ* digestion kinetics. *J Dairy Sci* **71**:1947-1954 (1988).

30. Bowman JGP and Firkins, J. L., Effects of forage species and particle size on bacterial cellulolytic activity and colonisation. *J Anim Sci* **71**:1623-1633 (1993).

31. McAllister TA, Rode LM, Major DJ, Cheng K-J and Buchanan-Smith JG, Effect of ruminal microbial colonisation on cereal grain digestion. *Can J Anim Sci* **70**:571-579 (1990).

32. Cone JW, van Gelder AH and Driehuis F, Description of gas production profiles with a three-phasic model. *Anim Feed Sci Technol* **66**:31-45 (1997).

General Discussion

General discussion

Introduction

During the past decade intensive genetic selection for higher milk production and improved husbandry practices in dairy cows have resulted in a higher demand for nutrients to be supplied by feedstuffs. Despite being transferred into more productive animals, dairy cows are still ruminants. Before nutrients are delivered to the organs and tissues of a ruminant animal, feedstuffs are subjected to a microbial fermentation in the rumen. Hence, feedstuffs in ruminants, often distinguished in forages and concentrates, have two major roles:

1. To meet the requirements of micro-organisms inhabiting the rumen.

2. To meet the requirements of the animal (maintenance, growth, (re-)production).

The first role of feeds in ruminants is to fulfil the nutrient requirements of rumen micro-organisms. Rumen micro-organisms grow in the rumen and provide the host animal with valuable nutrients, notably microbial protein and B-vitamins. Microbial production in the rumen follows similar principles as do other biological processes that produce biomass. Rumen microbes have a need for nutrients to deliver fuel for their maintenance and to deliver precursors for their production. They utilise the nutrients in the feed and convert them into microbial biomass (MB), with the concomitant inevitable production of waste products; volatile fatty acids (VFA) and fermentation gases. Of the microbial products, MB and VFA play an important role in providing the energy and protein for the host animal.[1] To have an optimal production of MB and an efficient nutrient utilisation, rumen microbes require both rumen degradable carbohydrates and protein, preferably in a ratio of around 25 g of N kg^{-1} of fermentable OM or a ratio of rumen degradable protein to rumen degradable carbohydrates around 0.20.[2,3]

In a high producing dairy cow, feedstuffs should deliver the essential nutrients so that they fulfil both the requirements of maintenance and production. At the maintenance level, feed serves primarily to provide energy for fuel and in addition some proteins to provide essential amino acids, needed to replace protein that is lost in organs and tissues. In high producing dairy cows, however, they should provide not only energy and protein for maintenance, but they also must deliver aminogenic, ketogenic and lipogenic nutrients as precursors for the synthesis of the milk components fat, protein and lactose.[4] In most situations, when they are offered as the sole feed, forages not only fail to fulfil the demand of high producing dairy cows for energy and essential nutrients in the desired

ratio, they also fail to provide the optimal ratio of rumen degradable carbohydrates to proteins.[5,6] Hence, diets for high producing dairy cows have to be supplemented with concentrates. Although feeding the dairy cows with concentrates in their ration is an extra financial burden on dairy farmers, to maintain the milk production in a profitable way and to have an optimal ruminal utilisation of nutrients, it is inevitable. Cereal grains and legume seeds are without any doubt important potential concentrate ingredients that can be used to provide the required nutrients to ruminants. Prior to their inclusion in the diet, cereal grains and legume seeds often undergo technological processes such as expander/expander-pelleting. These processes are applied to guarantee a durable feed form (pellets), and to achieve an optimal balance between rumen fermentation and post-ruminal digestion. Such a hydrothermal processing has been reported to be beneficial in dairy cows since it synchronizes the energy and protein utilisation.[5,7] Such beneficiary effects are normally evaluated in a frame work of a so-called feedstuff evaluation system. Nowadays feedstuffs for ruminants are not only characterised by their chemical composition and digestibility, but they are also characterised by their distribution (see Figure 1) between non-washable (NWF), insoluble washable (ISWF) and soluble (SWF) fractions, because these fractions differ in rumen degradation rate and rumen residence time.[3]

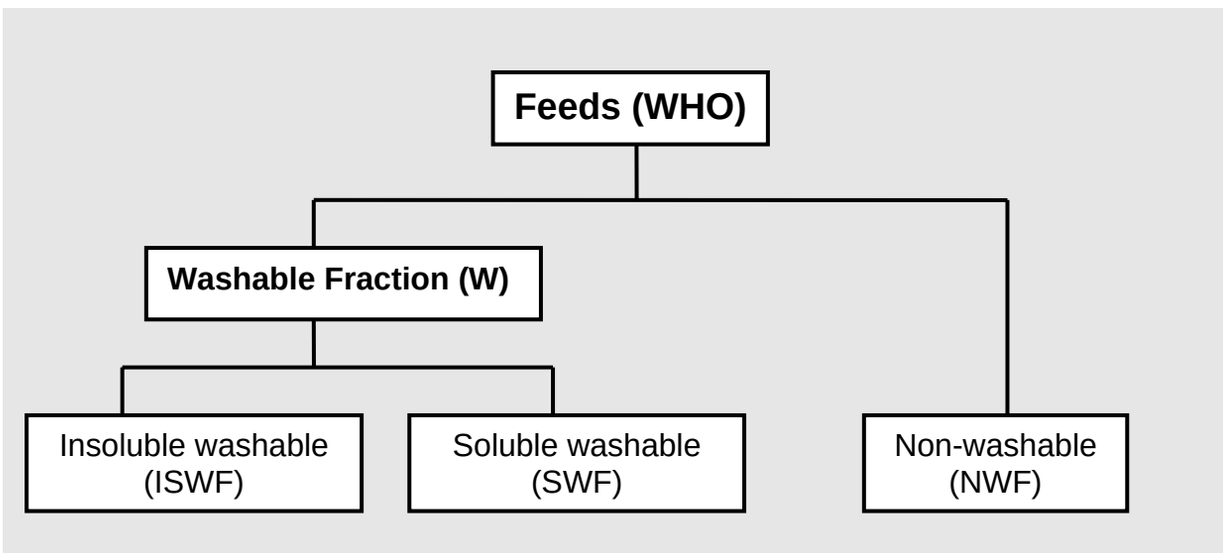


Figure1. Schematic representation of feed fractions

The distribution of these fractions in the dry matter of feedstuffs, their chemical composition and their rate of ruminal degradation and outflow plays an important role in supplying nutrients for microbial growth and maintenance, and in delivering the nutrients into the small intestine to be digested and utilised by host animal.

Factors affecting the distribution of DM between fractions

Washing procedure

In **Chapter 1** of this dissertation we developed a laboratory method to fractionate the feed's DM into its fractions, and we elaborated on factors that affect the distribution of DM between fractions. Theses factors were: washing procedure, grain type, particle size and dilution ratio (proportion of consumed water (ml) to sample weight (g)).

The results presented in **Chapter 1** revealed that regardless grain type and particle size, both washing procedure and washing duration play an important role on the distribution of DM between the fractions. The results indicate that if the duration of washing exceeds 1 h, the washing procedure leads to an increased washout (W) fraction The effects of these two factors on the size of fractions depend in turn highly on other factors such as chemical composition of the substrate (grain type), dilution ratio and pre-treatment before applying the washing procedure (fineness, type of grinding and grinding procedure).

Chemical composition of substrate and dilution ratio

The results of **Chapter 1** indicated that different grains differ in the contribution of their DM over the different fractions. These differences are not only due to differences in their seed structure and microstructure [8,9] but it is also due to differences in their chemical constituents (e.g. higher soluble protein in peas and faba beans than in the other grains[10]). It could be expected that when different grains differ in the distribution of DM over the different fractions, they also respond differently to changes in the washing procedure parameters (e.g. dilution ratio). Indeed our experiment showed that increasing the dilution ratio in legume seeds increased the SWF, whilst its effect on the SWF of grains was not remarkable (**Chapter 1**). Therefore, it can be concluded that in addition to the dilution ratio the nature of a chemical constituent, for example protein, may play a prominent role in determining the size of the SWF in feeds. This implies that besides the factors mentioned, the type of solvent (osmolality and pH) is also of great importance for the size of the fractions, at least for the SWF.

In **Chapter 1** we demonstrated that carbohydrates have only a minor effect on the size of the SWF in feeds and that water soluble proteins play a key role in the magnitude of the SWF. Indeed, a high correlation was observed between the SWF and STP (R^2 = 0.91), indicating that the magnitude of SWF is very much dependent on the quantity of

soluble true proteins (e.g. albumins) in feedstuffs. This means that factors such as temperature, pH, osmolality and solvent composition are of great importance in determining the magnitude of the SWF and soluble crude protein (CP). In a recent study, De Jonge et al. (2006, unpublished data) studied the effect of pH, osmolality and temperature on the magnitude of the soluble CP in some feeds including peas, soybean meal, sunflower seed meal and faba beans. They found that increasing the temperature from 18 to 38 ºC did not have a significant effect on the solubility of CP. However, both changes in pH and osmolality changed the solubility of CP. Their results indicate that decreasing the pH from 7.5 to 5.0 drastically decreased the solubility of CP whilst the solubility of CP was increased by increasing the osmolality (from 9 to 286 mOsm). They found that pH had a greater effect on the change in solubility of CP than osmolality did. The conditions of rumen liquid differ distinctly from those of tap water[11] with regard to pH (5.5-7.0 in rumen liquid and 7.5 in tap water) and osmolality (250-400 mOsm in rumen liquid and 9 mOsm in tap water).Therefore, the size of the SWF determined in our experiments may change if we use a solvent more similar to rumen liquid with respect to pH and osmolality. A further development in a laboratory method that mimics the results of the washing procedure in the *in situ* method should, therefore, focus on using a water base solvent, other than tap water, whose characteristics (pH and osmolality) are more similar to those of rumen liquid.

Pre-treatment before washing procedure

Fineness of grinding

To obtain homogenous and representative samples, feeds are normally ground with a laboratory mill before fractionation. The results presented in **Chapter 1** indicate that the fineness of grinding notably affects the distribution of DM between the fractions. As the fineness of grinding increased (3 vs. 1 mm sieve), both the size of ISWF and the SWF were increased. Fine grinding increases the size of the W fraction either by rupturing cell walls and releasing more soluble nutrients or by making particles small enough to be washed out.[12] The nutritional importance of the fine grinding (particle size reduction) becomes more important when we realise that in both cereal grains and legume seeds, the ISWF contributes considerably to the DM of the entire grains (**Chapter 2**). Of this fraction it is now assumed that its protein is degraded at the same rate and its starch at a faster rate as in NWF.[3] However, one should realise that the extent to which a feed is effectively degraded in the rumen not only depends on the rate of degradation but also on

the rate of its outflow from the rumen.[13] France et al.[14] proposed a mathematical approach to estimate the extent of degradation using the data of the *in vitro* gas production technique as below:

$$E = \frac{S_0 I}{(S_0 + U_0)} \quad (1)$$

$$I = BC^B \int_T^\infty \{t^{B-1} e^{-kpt} / [(t^B + C^B)^2]\} dt \quad (2)$$

where E is the extent of degradation, S_0 is the degradable fraction (1- U_0), U_0 is the undegradable fraction (organic matter residue after 72 h incubation), I is the integration factor, B is the switching factor, C is the half time of the gas production, kp is the fractional passage rate and t is the incubation time. Using this approach and based on the C and B values of the mono-phasic Groot model (**Chapter 2**), the E was estimated for the ISWF and NWF. The E value for NWF was estimated assuming a kp of 0.06 h^{-1} (accepted fractional passage rate of particulates in the DVE/OEB system)[3] whilst for the ISWF the E value was estimated at both kp of 0.06 and 0.08 h^{-1} (the latter being the suggested value for the fractional passage rate of fine particles).[3] The results indicate that the extent of degradation of the ISWF, estimated based on the *in vitro* gas production parameters, is very similar to that of the NWF when a fractional passage rate of 0.06 h^{-1} is assumed (45 and 40 % in ISWF and NWF, respectively). However, at the fractional passage rate of 0.08 h^{-1} for ISWF, the extent of degradation is 17.5 % lower in ISWF than in NWF (33 vs. 40 %). Therefore, it seems that a considerable quantity of small particles that are usually rich in starch (except in lupins) can escape form ruminal degradation. This escape may affect the proportion of rumen degradable nitrogen to rumen degradable carbohydrates and as a consequence the supply of nutrients for the animal. The fine grinding as well as technological processes that involve shear forces, therefore, have the potential to change the extent of degradation, and therefore the supply of indispensable nutrients for both rumen micro-organisms and the host animal either by reducing the size of particles (higher ISWF) or by changing the size of the truly soluble fraction (SWF) (**Chapter 1**, **3** and **4**), the fraction of which it is assumed that it can be effectively degraded in the rumen up to 95 %.[3]

Type of grinding and grinding procedure

In **Chapters 3** and **4** we demonstrate that not only the sample fineness but also the type of grinding (hammer mill vs. centrifugal mill) affects the distribution of DM between the different fractions (Figure 3). As it is evident from the results in Figure 3, a centrifugal

mill (Retsch ZM1, 3 mm) produces more fine particles compared to a hammer mill. A further grinding with a centrifugal laboratory mill (Retsch), in all the concentrate ingredients drastically increased the size of both the ISWF and the SWF fractions.

One of the most important aims of *in vitro* and *in situ* techniques are to measure the intrinsic rate of digestion of chemical components in (un)processed feedstuffs.[13] To ensure that particle size of the sample does not hamper the digestion, normally representative samples of feeds stuffs are ground to pass through a 1-4 mm sieve and then their degradative behaviour will be evaluated by either an *in vitro* or an *in situ* technique. As mentioned by Goelema et al.[15] in the standard *in situ* procedures [16,17], samples are ground (normally over a 3 mm sieve) to mimic the mastication and to obtain a representative sample[18]. In the *in vitro* gas production analysis most researchers grind the feed samples through a 1 mm screen. [19-21] It has been demonstrated that the effect of hydrothermal processes that involve shear forces on the ruminal degradability is largely due to changes in the distribution of DM over the different particle sizes.[7] Some researchers,[15] therefore, did not grind the processed samples before evaluating their degradative behaviour *in situ*. In most research, however, as a part of the standard operating procedure, the samples of technologically processed feedstuffs were ground before evaluating their degradative behaviour *in situ* [22-26] or *in vitro*. [27-29]

In **Chapter 5,** it is investigated if the different sample grinding procedures have any effect on the distribution of particles, and if in a technologically processed material a further grinding would disturb the effect of processing. The particle size distribution was determined using the dry sieve analysis[30] and the Coulter counter technique in samples of differently ground expander processed barley and peas as model feed ingredients. The different grinding procedures indeed changed the distribution of particle sizes. In the samples of expander processed barley and peas the pool of coarse particles totally disappeared after applying the second and third grinding steps. However, the processed barley and peas responded differently to the different sample grinding. Stepwise grinding produced more fine particles in the expander processed barley than it did in peas (**Chapters 5**). Whilst stepwise grinding remarkably changed the distribution of fine particles, the gas production characteristics of differently ground samples were not remarkably different. This implies that grinding the samples over a sieve of up to 3 mm with a laboratory mill most likely does not hamper the digestion of the samples *in vitro*. Interestingly, elevated variation in gas produced in the replicates was observed in both the first and the second run when the samples of expander processed barley and peas were ground stepwise to pass through a 1 mm screen. This elucidates that stepwise grinding

clearly produces less homogenous samples. Indeed, both dry sieve analysis and Coulter counter particle size analysis revealed that stepwise grinding skewed the pool of particles towards more fine particles.

In the study presented in **Chapter 5,** samples of barley and peas had already been ground through a 3 mm sieve using a hammer mill before subjecting them to the further thermo-mechanical processing. It was of interest to see if a further grinding still leads to a representative sample that resembles the degradative behaviour of the original technologically processed samples. For this purpose samples of expander processed barley and peas were either fermented without any further grinding (intact) or they were dissolved in water, freeze-dried (dissolved), and then their degradative behaviour was evaluated using the *in vitro* gas production system. The lack of difference between the parameters of gas production in both the intact and dissolved samples indeed indicates that the dissolved samples were still representative for the original processed ones (**Chapter 5**). The results presented in **Chapter 5** indicate that the further grinding does change the degradative behaviour of the technologically processed samples. Further grinding of the E and EP barley and peas with a centrifugal mill (Retsch) indeed caused a faster fermentation (**Chapter 5**) which was mainly due to a higher ISWF and SWF of the Retsch mill ground samples compared to the hammer mill ground samples (Figure 3). It can therefore be concluded that the degradative behaviour of the processed samples, especially those involving shear forces, not only depends on the pre-processing operations[7] (e.g. ground with hammer mill vs. roller mill), but it also depends on the post-processing laboratory operations applied to prepare the sample for a further *in vitro* or *in situ* evaluation. Should these samples be ground? Findings presented in **Chapter 5** say, no they should not. However, when a further grinding is inevitable it would be recommended to grind both the processed and unprocessed sample in the same way.

Why processing?

The suitability of feedstuffs as a substrate for microbial degradation and growth and to supply the indispensable nutrients for ruminants depends on two factors:

1. The ratio of rumen degradable protein to the rumen fermentable carbohydrate.

2. The distribution of DM between the different fractions that may differ in chemical composition, degradation and passage rates.

Our hypothesis was that thermo-mechanical processing of concentrate ingredients (**Chapter 2**), that are used as a supplement next to forages, is a suitable way to help to

create an optimal rumen fermentation as well as to improve the quality of the nutrients provided to the host-animal. To test this hypothesis we used an approach described below.

In this approach, the ratio of fermented CP (FCP) to fermented OM (FOM) is calculated using the chemical components of the concentrate ingredients (**Chapter 2**) and the tabulated rate of degradation for the different fractions given in DVE/OEB, assuming an undegradable fraction (U fraction) after 336 h of ruminal incubation.[3] The parameters that are used to calculate the ratio of FCP to FOM in this approach are presented in Table 1.

Table 1. Fractional rates of degradation (*kd*, h^{-1}) used in our approach

		Protein	Starch	NDF[1]	Sugars	RNSP[2]
Barley	NWF	0.120	0.200	0.080	2.000	0.150
	ISWF	0.120	0.780	0.080	2.000	0.380
	SWF	2.000	NA	NA	2.000	0.380
Maize	NWF	0.035	0.056	0.039	2.000	0.100
	ISWF	0.035	0.490	0.039	2.000	0.250
	SWF	2.000	NA	NA	2.000	0.250
Milo	NWF	0.035	0.036	0.025	2.000	0.080
	ISWF	0.035	0.450	0.025	2.000	0.190
	SWF	2.000	NA	NA	2.000	0.190
Peas	NWF	0.089	0.103	0.150	2.000	0.250
	ISWF	0.089	0.580	0.150	2.000	0.630
	SWF	2.000	NA	NA	2.000	0.630
Lupins	NWF	0.141	NA	0.060	2.000	0.130
	ISWF	0.141	NA	0.060	2.000	0.310
	SWF	2.000	NA	NA	2.000	0.310
Faba beans	NWF	0.106	0.101	0.150	2.000	0.180
	ISWF	0.106	0.580	0.150	2.000	0.440
	SWF	2.000	NA	NA	2.000	0.440

[1] Neutral detergent fibre. [2] Residual non-starch polysachharides.

The ratio of FCP_t to FOM_t for each fraction was then calculated as below:

$$FOM_t = \sum_{i=1}^{5} kd \times componenet \times (1 - e^{-kdt}) \quad (3)$$

$$FCP_t = kd \times CP \times (1 - e^{-kdt}) \quad (4)$$

$$FCP_t / FOM_t = \frac{FCP_t}{FOM_t - FCP_t} \quad (5)$$

where the FOM_t and FCP_t are the calculated fermented OM and crude protein at each time point, *kd* is the fractional rate of degradation (h^{-1}) and component is the chemical components including, protein, starch, NDF, sugars and the residual NSP. The FOM_t and FCP_t are calculated in WHO on the basis of the contributions of different fractions in WHO. The results of this approach are displayed in Table 2.

Table 2. The estimated ratio of fermented CP (FCP) to fermented OM (FOM)

		Time frame (h)				
Ingredient	Fraction	6	12	24	48	72
Barley	WHO	0.12	0.13	0.14	0.14	0.14
	NWF	0.12	0.14	0.15	0.15	0.15
	ISWF	0.05	0.07	0.08	0.09	0.09
	SWF	0.24	0.23	0.23	0.23	0.23
Maize	WHO	0.07	0.07	0.08	0.09	0.09
	NWF	0.07	0.07	0.08	0.09	0.09
	ISWF	0.01	0.02	0.04	0.05	0.05
	SWF	0.22	0.20	0.20	0.20	0.20
Milo	WHO	0.13	0.13	0.13	0.14	0.14
	NWF	0.15	0.16	0.16	0.16	0.16
	ISWF	0.01	0.01	0.02	0.03	0.03
	SWF	0.42	0.34	0.32	0.31	0.31
Peas	WHO	0.34	0.36	0.38	0.39	0.39
	NWF	0.13	0.15	0.17	0.18	0.18
	ISWF	0.07	0.11	0.14	0.16	0.16
	SWF	1.19	1.18	1.18	1.18	1.18
Lupins	WHO	0.52	0.51	0.47	0.44	0.44
	NWF	0.52	0.49	0.45	0.41	0.41
	ISWF	0.61	0.68	0.69	0.65	0.65
	SWF	0.45	0.42	0.41	0.41	0.41
Faba beans	WHO	0.54	0.56	0.59	0.60	0.60
	NWF	0.25	0.25	0.28	0.29	0.29
	ISWF	0.18	0.25	0.32	0.34	0.34
	SWF	1.93	1.86	1.86	1.86	1.86

It becomes apparent from the results in Table 2, that as such, both the cereal grains and the legume seeds fail to provide an appropriate ratio of FCP to FOM. The ratios in the cereal grains are below and those in the legume seeds are far above the ratio considered optimal to create a synchronised fermentation of protein and carbohydrates. It is clear that the ratio of FCP to FOM not only differs between the concentrate ingredients but it also differs between the fractions of the same concentrate ingredients. Such differences can be attributed to differences in the chemical constituents as well as differences in the distribution of DM between the different fractions. The high ratio of FCP to FOM in lupins is not only attributed to its high protein content (34.5 %) but it is also caused by a lack of starch. In peas and faba beans when the WHO was fractionated in its fractions (NWF and ISWF), it is revealed that the ratio of FCP to FOM is lower in the NWF and ISWF than in WHO. Taking into account that SWF contributed in as much as 49 and 37 % of WHO protein of peas and faba beans, it can be concluded that the high ratio of FCP to FOM is largely due to the high SWF of these ingredients. Since most of the SWF (95 %)[3,31] can be degraded in the rumen, it would result in a surplus of N in comparison with available energy for microbial protein synthesis, when the raw legume seeds are included in the diet. This is confirmed in **Chapter 2** by a higher NH_3-N and a lower non-glucogenic ratio (NGR) in the legume seeds compared to the cereal grains. Indeed, the produced NH_3-N presented as mg g^{-1} FOM were higher in the legume seeds (WHO) compared to the cereal grains (Figure 2) throughout a 72 h incubation period.

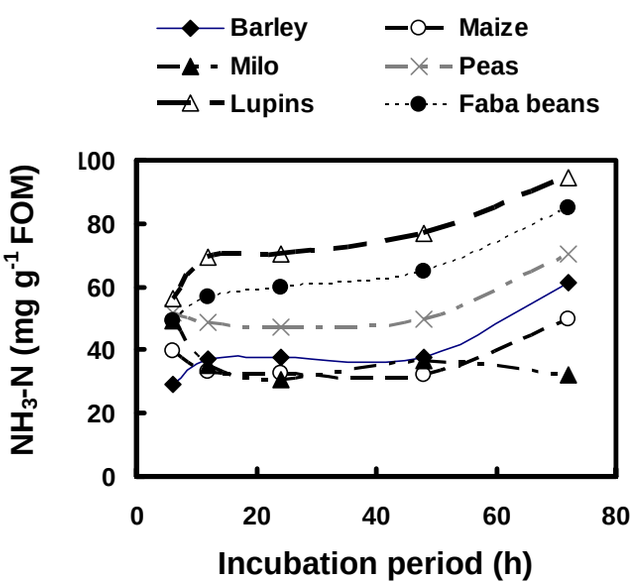


Figure 2. NH_3-N production (mg g^{-1} fermented OM) throughout 72 h incubation

The results mentioned in the previous paragraph indicate that in order to have an optimal ratio of FCP to FOM, cereal grains have to be included in the ration of dairy cows next to a source of rumen degradable protein whereas legume seeds have to be either supplemented with a source of readily fermentable carbohydrates or they should be subjected to a technological process (e.g. expander) so that their protein is fermented in the rumen to a lesser extent.

In the Netherlands, the diet of dairy cows consists on average of two-third roughage and one-third concentrate feed. The main roughages in dairy cows' diets in the Netherlands are grass, grass silage and maize silage.[32] Recently, the ratio of rumen degradable protein (RDP) to rumen degradable carbohydrates (RDCHO) was calculated for the different composed diets including only fresh grass, winter diets contain either grass silage or maize silage supplemented with 50% of a concentrate. The results showed that, except for the high maize silage diet, all diets exceed the recommended ratio of 0.2, notably shortly after feed intake.[3] In Iran, the most abundant roughages used in the industrialised dairy farms are maize silage and lucerne hay, respectively.[33] Using the same approach of Tamminga et al.[3] the ratio of RDP to RDCHO was calculated for diets containing only maize silage or only lucerne hay. The lucerne hay resulted in a ratio far exceeding the recommended ratio of 0.2 whereas in the diet containing only maize silage the ratio was far below the recommended ratio (data are not shown). In such a situation, the inclusion of processed legume seeds and cereal grains in the diet may alleviate a deficiency (in case of maize silage) or a surplus (in case of lucerne hay) of nitrogen to the total available carbohydrates. Tóthi [5] demonstrated that supplementation of a dairy cows' diet, grazing only on fresh grass, with processed barley and maize (pelleted and expander-pelleted) resulted in more synchronised substrates with regard to the ratio of RDP to RDCHO, and that processed maize was more effective than was barley. He concluded that in maize, pelleting or expander-pelleting has more advantages to provide an appropriate ratio of RDP to RDCHO than simple mechanical treatment (grinding) does. In those industrialised dairy farms in Iran, where they feed dairy cows with lucerne as the sole roughage, therefore, supplementation of diets with processed cereal grains would provide more synchrony with respect to the utilisation of N. On the contrary, in those dairy farms where the main source of the roughage is maize silage, supplementing the diets with proteinaceous concentrate ingredients would provide more synchronised diets for the dairy cows. Statistics[33] show that soybean is the most abundant proteinaceous concentrate ingredient that has been used in the diets of dairy cows in Iran, which is mostly imported from all over the world. The other pulses such as feed grade peas, lupins and faba beans that can be cultivated under the Iran's climate conditions, therefore,

would be appropriate alternatives for soybean. However, since these concentrate ingredients are relatively rich in soluble proteins, for reasons discussed; they should undergo a thermo-mechanical process such as expander treatment, prior to the inclusion in the ruminant diets.

In choosing an appropriate supplementation strategy to create an optimal rumen fermentation (e.g. choosing an appropriate technological process) for dairy cows, therefore, factors such as type of basal diet and supplement feed (cereal grains vs. legume seeds) play a critical role. Next to these two factors, it is demonstrated that the level of supplementation is also of great importance.[5] The small level of supplementation may alleviate the deficiencies of the basal diet whereas a large level of supplementation (for instance more than one-third of basal diet) may cause substitution of the basal diet with usually more expensive components that may cause a disturbed rumen fermentation. Tóthi[5] showed that supplementing the grazing dairy cows with 3 kg of pelleted or expander-pelleted maize per day resulted in a more nutrient-synchronised diet with regard to the ratio of RDP to the RDCHO. However, he found that processed barley was effective in providing an appropriate ratio of RDP to RDCHO when it was fed to the grazing dairy cows in a higher inclusion level compare to the maize (6 and 9 kg/day).

Processing of cereal grains and grains of legume seeds

The six different concentrate ingredients used in the present study (**Chapters 3** and **4)** were selected as model feedstuffs based on the fact that they represent a wide range of concentrate ingredients used in dairy cattle rations with regard to their chemical constituents (**Chapters 2**), type of proteins (soluble vs. insoluble) [10] and structure of starch granules[34]. The *in vitro* gas production technique along with the fractionation method (**Chapters 1**) was used to characterise the kinetics of gas production and formation of end-products in samples of the concentrate ingredients. This study aimed to elucidate:

1. How expander processing (E) and the ensuing pelleting process (EP) change the distribution of DM over the different fractions.

2. What would be the consequences of these changes for the nutrient supply to both micro-organisms and animal.

Effect of processing on the distribution of DM between different fractions

In line with the practical situation before further processing, all the samples were ground over a 3 mm sieve using a hammer mill. Interestingly, a further grinding with a centrifugal laboratory mill (Retsch ZM1 centrifugal mill) over a sieve of 3 mm, in all the concentrate ingredients drastically increased the size of the W fraction with a more profound effect on the ISWF (Figure 3**)**.

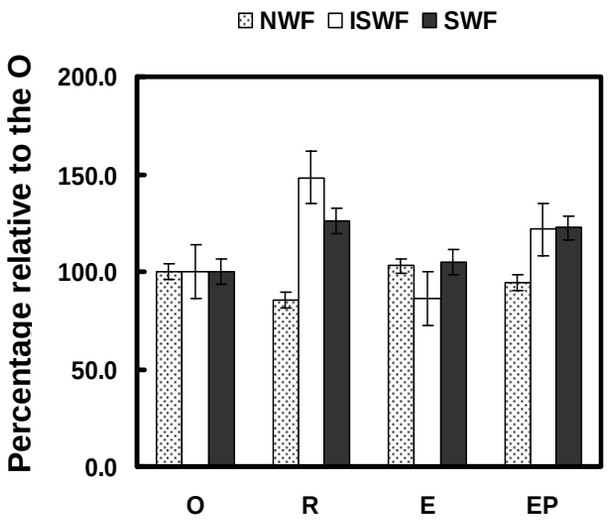


Figure 3. Distribution of DM between the different fractions in R (Retsch milled), and expander processed (E) and expander-pelleted (EP) samples relative to O (Hammer milled).

The decrease in the size of ISWF (< 40 μ) in maize, milo, peas and faba beans due to expander treatment reported in **Chapters 3** and **4** was inconsistent with the results of Goelema et al.[15] They reported an elevated number of small particles (< 71 μ) in samples of an expander processed in a mixture of broken legume seeds. The processing variables used in our study were almost similar to those used in the study of Goelema et al.[15] The discrepancy between the results of the current study with the findings of Goelema et al.[15], therefore, must be the result of different pre-processing methods used prior to subjecting the samples to further processing. Goelema et al.[15] ground the samples using a roller mill whilst in the present study (**Chapters 3** and **4**) samples were ground in a hammer mill. These findings imply that the effects of hydrothermal processes that involve shear forces, on the physico-chemical properties of the final products, largely depend on the way they

are prepared (e.g. grinding procedure) prior to that specific thermo-mechanical processing.

Figure 3 indicates that both processes E and EP changed the distribution of DM between the fractions. Process E decreased the ISWF which was concomitant with an increase in gelatinised starch in all concentrate ingredients except in lupins. This resulted in a more synchronised fermentation with regard to the fermentation of protein and carbohydrates. Nevertheless, Figure 3 shows that subjecting the expander processed samples to further pelleting caused a higher yield of fine particles, as represented by a higher ISWF, and further elevated the degree of starch gelatinisation (**Chapters 3** and **4**). As it stands, the pelleting process, especially die forces, is of crucial importance in determining the size of particles, phyiso-chemical properties of the final agglomerate and therefore, in the supply of nutrients for both rumen micro-organisms and host animal. This will be discussed further in the upcoming paragraphs.

Effect of processing on the ratio of fermented CP to fermented carbohydrates

Chapters 3 and **4** demonstrate that technological processes that involve shear forces may change the chemical constituents of WHO and their fractions. For instance, a lower NDF content was observed with the WHO and NWF of expander-pelleted samples, which was always paralleled by an elevated not chemically determined residue in the SWF. This shift between the unknown residual fraction of SWF and NDF might be the result of partly solubilising the hemicellulose due to processing. As it is stated in **Chapter 4** it is found that expander and extrusion processing increase the ratio of soluble to insoluble dietary fibre.[35]

In all the concentrate ingredients except lupins, SWF of E and EP contained higher sugar levels than did the R samples (**Chapters 3** and **4**). Starch dextrinisation and depolymerisation that occur due to shear forces in expander processing,[36] may explain such higher sugar contents of SWF of processed samples compared to the unprocessed sample. The fact that the elevated sugar content is most probably due to the starch gelatinisation and depolymerisation is fortified in **Chapter 4** where in lupins with a negligible quantity of starch such an elevated sugar content of SWF was not observed with the processed samples.

These shifts between chemical components of the different fractions due to the processing change the ratio of CP to the total carbohydrates (TCARB), an important

factor that influences the efficiency of microbial production.[3] From the results presented in **Chapters 3** and **4,** shifts in the ratio of CP to the TCARB in the different fractions of R, E and EP is calculated and presented in Table 3.

Table 3. The ratio of CP to the total carbohydrates (TCARB)

		CP/TCARB			
		WHO	NWF	ISWF	SWF
Barley	R	0.18	0.16	0.12	0.60
	E	0.17	0.20	0.06	0.28
	EP	0.17	0.20	0.06	0.32
Maize	R	0.13	0.12	0.09	0.39
	E	0.13	0.13	0.12	0.24
	EP	0.12	0.14	0.08	0.24
Milo	R	0.14	0.15	0.07	0.75
	E	0.15	0.14	0.15	0.36
	EP	0.15	0.16	0.07	0.35
Peas	R	0.35	0.23	0.22	1.73
	E	0.34	0.28	0.16	1.39
	EP	0.37	0.30	0.14	1.60
Lupins	R	1.09	0.66	3.53	0.99
	E	1.04	0.89	2.80	0.76
	EP	1.08	0.98	3.15	0.76
Faba beans	R	0.48	0.34	0.22	3.38
	E	0.49	0.34	0.50	2.32
	EP	0.50	0.34	0.20	2.95

As shown in Table 3, E and EP drastically decreased the ratio of CP to TCARB in SWF of all the concentrate ingredients. The magnitude of the decrease in CP to TCARB ranged from 8.1 to 53% and was higher in the SWF of cereal grains than in that of legume seeds. The decreased CP to TCARB in the SWF can be explained by a lowered CP and an elevated sugar content of this fraction due to the E and EP. Despite the SWF, a consistent effect of E and EP on the ratio of CP/TCARB was not observed in NWF and ISWF.

The ratio of FCP to FOM in the processed concentrate ingredients calculated based on the approach described earlier, are presented in Table 4. The time frame of 6 h was

chosen based on the fact that values after a time frame of some 6 to12 h have little practical relevance, as dairy cows usually have a new meal well within that time frame.[3]

Table 4. The estimated ratio of FCP to FOM (g N kg^{-1} FOM) after 6 h incubation

		FCP/FOM			
		WHO	NWF	ISWF	SWF
Barley	R	0.13	0.13	0.06	0.30
	E	0.11	0.14	0.02	0.16
	EP	0.12	0.16	0.04	0.16
Maize	R	0.08	0.08	0.02	0.26
	E	0.08	0.08	0.02	0.16
	EP	0.08	0.09	0.02	0.14
Milo	R	0.13	0.14	0.01	0.37
	E	0.12	0.12	0.02	0.20
	EP	0.13	0.15	0.02	0.18
Peas	R	0.38	0.14	0.09	1.43
	E	0.29	0.15	0.06	1.04
	EP	0.34	0.19	0.08	1.10
Lupins	R	0.63	0.48	1.69	0.53
	E	0.58	0.57	0.89	0.38
	EP	0.77	0.77	1.30	0.38
Faba beans	R	0.73	0.22	0.11	2.88
	E	0.49	0.20	0.19	1.82
	EP	0.57	0.30	0.15	1.98

The fractional degradation rates of the chemical components of the processed concentrate ingredients were derived from available literature (Tamminga, S.; personal communication). In agreement with the results of Table 3, both E and EP drastically decreased the ratio of FCP to FOM of SWF. However, a consistent effect of processing on the FCP to FOM ratio was not observed in the other fractions (NWF and ISWF). Overall, as shown in Fig 5, both the E and EP decreased the NH_3-N (mg g^{-1} FOM) in WHO, NWF and ISWF compared to the R samples. The effect of processing in reducing the produced NH_3-N, however, was more profound in the early stage of incubation (up to 12 h). The results indicate that in a batch culture where enough N is provided for the

micro-organisms, both E and EP are shifting the fermentation towards a more synchronised fermentation with regard to the fermentation of protein and carbohydrates.

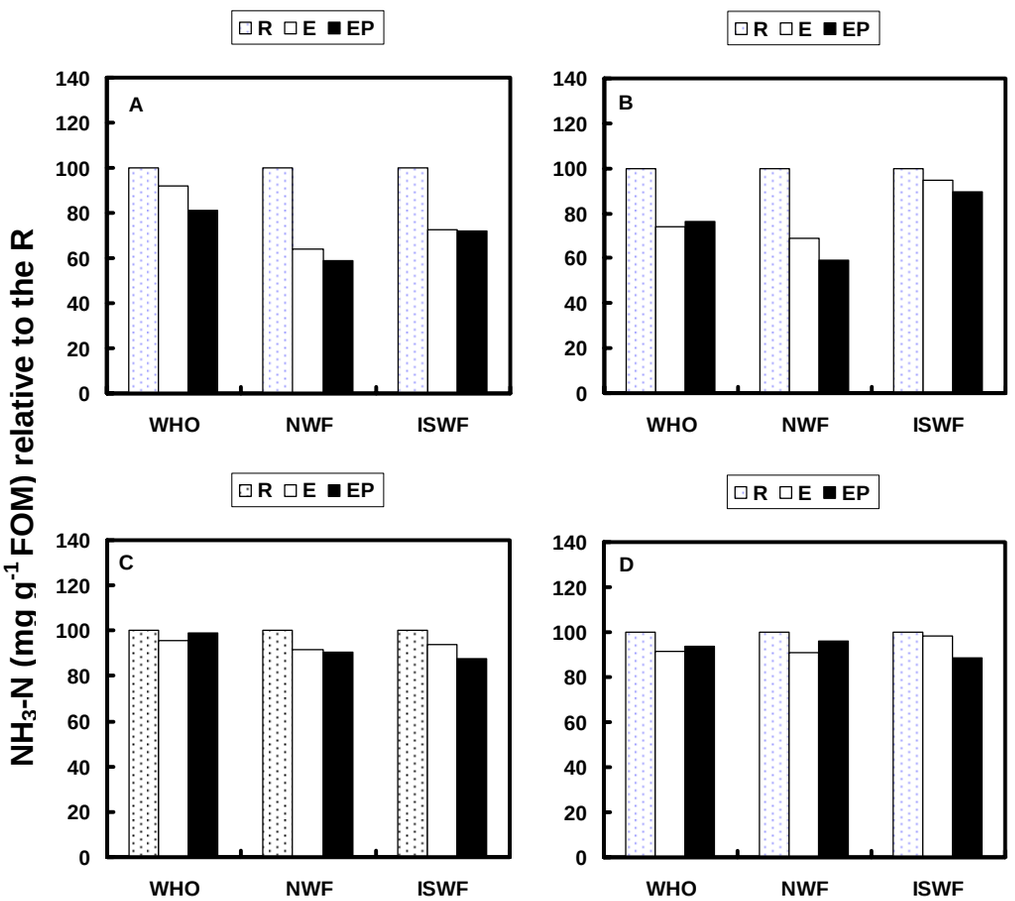


Figure 4. Overall NH_3-N (mg g^{-1} FOM) in WHO, NWF and ISWF after 6 (A), 12(B), 24 (C) and 48 (D) h incubation

This shift was mainly achieved by changes in the distribution of DM between the fractions (Figure 3) that differ drastically in their chemical composition (e.g. the ratio of CP to TCARB) and degradative behaviour (**Chapters 2** to **4**), and by rendering the carbohydrates (mainly starch) into a more accessible form and protein in a less available form for micro-organisms (**Chapters 3** and **4**).

Degradative behaviour of processed cereal grains and legume seeds

In **Chapters 3** and **4** we characterised the kinetics of gas production and formation of fermentation end-products in samples of processed cereal grains and legume seeds.

When a feedstuff is incubated with buffered rumen fluid, it is first degraded and the degraded fraction may either be fermented to produce gas and fermentation end-products (VFAs and NH_3-N), or is incorporated into microbial biomass. When combined with measures of organic matter disappearance, gas production techniques may provide a measure of the proportion of feed that is fermented as opposed to the proportion that is partitioned to microbial growth.[37] Therefore, we used three different approaches to characterise the effect of the degradative behaviour of some processed concentrate ingredients. These three approaches were:

1) Characterising the profiles of gas production
2) Determining the organic matter disappearance at the different time points
3) Measuring the formation of fermentation end-products

Gas production

Introducing the automated gas production system, such as those described by Cone et al.[38] and Davies et al.,[39] enables us to fit the gas production profiles with enormous number of data points to a multi-phasic model as described by Groot et al.[40] Each phase of gas production profiles is characterised by the asymptotic gas production, half time of gas production and a switching factor which determines the shape of curve. Using these parameters we are able to estimate the maximum rate at which a substrate is being degraded ($R_{max}S$).[14,40] The benefit of using such an approach is that we are able to characterise the behaviour of a fraction of feed namely W fraction, whose degradative behaviour can not be measured with the routine *in situ* method. More importantly, when combined with a fractionation method described in **Chapter 1**, the gas production technique enables us to study the kinetics of gas production of SWF, the fraction whose degradative behaviour is masked by the other fractions in the entire feedstuffs (WHO) especially in the cereal grains (**Chapter 2**).

The results presented in **Chapters 3** and **4** suggest that both E and EP changed the degradative behaviour of feed samples in the early stages of fermentation, as indicated by an elevated maximum fractional rate of degradation of all concentrate ingredients in the first phase of fermentation. The results of **Chapters 3** and **4** outlined that not only different grains but also different fractions (NWF, ISWF and SWF) responded differently to the processing. For instance both E and EP samples showed a faster fermentation compared to the R samples in barley like the other cereal grains, as a result of a higher gelatinised starch content. However, in the NWF of barley, expander processing and

ensuing pelleting led to a slower fermentation as described in **Chapter 3**. This provokes the debate that probably the inconsistent results on expander treatment on ruminal degradation of feeds like barley, may not have been only caused by different treatment conditions[41] but also by the different fractions used in the different studies (WHO vs. NWF).[22]

As outlined in **Chapter 3** the ISWF contributes significantly (up to 26 %) in the DM of cereal grains (R, E and EP). Since this fraction in the cereal grains is very rich in starch, its fermentation characteristics would be expected to resemble those of starch. Compared to the R samples, both ISWF of E and EP samples had a higher maximum fractional rate of substrate degradation in the first phase of fermentation caused by a higher content of gelatinised starch in WHO. Ljøkjel et al. [23] and Tóthi et al.[22], however, reported a decreased fractional rate of degradation of starch in expander treated barley. Offner et al.[42] in a review on *in situ* starch degradation also reported a decreased *in situ* fractional rate of starch degradation with expander processed samples of barley and sorghum. In the *in situ* method, pores of nylon bags allow escape of fine particles (mostly starch) during the course of incubation.[13] Therefore, the rate of degradation measured *in situ* is accounted for by the larger particles that have a different degradation rate than the particles that escape nylon bags (**Chapter 2**). Indeed in our experiment we demonstrated that the ISWF of E and EP samples of barley had degradation rates that were significantly higher than those in the E and EP of NWF. More importantly, it is likely that starch in ISWF responds to the processing in a different way than it does in the NWF. This is further confirmed by the fact that whilst the E and EP decreased the degradation rate of NWF, it was increased in the ISWF. Further *in vitro* studies should therefore focus on elaborating the differences between the degradation characteristics of starches in NWF and ISWF.

Interestingly, in the cereal grains when the gas production parameters are statistically analysed by fractions, it is revealed that compared to the R samples, the SWF of the EP samples of cereal grains fermented faster, as shown by a higher fractional rate of substrate degradation in the first phase of fermentation. These elevated rates were always reflected by a higher chemically not determined fraction, so-called residual NSP.[3] In line with our findings, Yang et al.[29] also reported an elevated chemically not determined fraction in the samples of pressure toasted barley and maize. They proposed that this fraction might be soluble NSP. Since this fraction remarkably contributed to SWF of cereal grains (up to 42 %), its fermentative characteristics would be expected to have a great effect on

fermentative characteristics of SWF. Further research, therefore, should aim at chemical characterisation of this fraction.

Like in the cereal grains, the expander processing and ensuing pelleting increased the maximum fractional rate of substrate degradation in the first phase of fermentation in pulses. This means that the factors that initially offer some resistance to microbial degradation such as the presence of large particles are modified by applying these two technological processes. Moreover, these two processes facilitate the initiation of fermentation by providing the micro-organisms with a readily available source of energy namely gelatinised starch. Indeed, in all concentrate ingredients except lupins, the higher $R_{max}S$ in the first phase of fermentation was accompanied by a higher content of gelatinised starch (**Chapters 3** and **4**). This was in a good agreement with the findings of Gallant et al.[43] and Thomas[44] who found that gelatinised starch degrades faster than native starch does. In lupins, however, the elevated $R_{max}S$ can neither be justified by particle size reduction nor by increased gelatinised content. The elevated $R_{max}S_1$ due to the E and EP in lupins was more related to a lowered NDF of NWF and an elevated residual NSP[3] of SWF. This implies that to have a better insight in the effect of such processing not only the entire feed samples (WHO) but also their constituent fractions have to be evaluated for their chemical composition and degradative behaviour. As a tool, the *in vitro* gas production technique provided us the opportunity to examine the degradative behaviour of entire feed samples (WHO) as well as their fractions. The resulting gas production profiles can be easily fitted to the available multi-phasic models,[14,40] which provide us with a great deal of valuable information on the fermentation of (un)processed feed samples, their fractions and chemical constituents. However, after fitting the gas productions profiles to a multi-phasic model, finding an appropriate biological meaning of these phases is an important obstacle to explain the significance of these phases (**Chapters 3** and **4)**. Moreover, since the presence of proteins hamper the indirect gas production,[45] the produced gas alone can not be an appropriate criterion for evaluating the fermentability of protein-rich substrates.

Organic Matter disappearance

The time at and the extent to which feedstuffs are effectively degraded in the rumen is of great importance for supplying the indispensable nutrients for both rumen micro-organisms and host animal. Therefore, attempts have been made to estimate the extent of degradation using the kinetics of gas production parameters[14] or by relating the *in situ* parameters (e.g. *kd*) to the gas production parameters.[46] In our study with the processed

concentrate ingredients (**Chapters 3** and **4**) the disappearance of OM was measured at different time points (6, 12, 24, 48 and 72 h). The fractional rate of degradation for different concentrate ingredients and their fractions were then calculated fitting the data to the model described by Ørskov and McDonald.[47] There was no close relationship between the calculated *kd* using the Ørskov and McDonald[47] model and the maximum fractional rates of degradation ($R_{max}S1$, R = 0.41; $R_{max}S2$, R = 0.15) calculated using a di-phasic Groot's model.

The extent of degradation was estimated for the ISWF and NWF of processed samples using their *kd* values, and assuming a *kp* of 6 % for NWF, and 6 and 8 % for ISWF. Interestingly, in accordance with our calculation using the approach of France et al.[14] the extent of degradation of the ISWF, calculated based on the *kd* of OM, is very similar to that of NWF when assuming a fractional passage rate of 6 % (60.5 and 59.7 % in the ISWF and NWF, respectively). However, at the fractional passage rate of 8 %, the extent of ISWF degradation is 10.4 % lower than that of the NWF (53.5 vs. 59.7 %). The ISWF contributed significantly in the DM of WHO, therefore, it is likely that the process EP decreases the extent of degradation compared to the original mash samples (hammer milled, 3 mm sieve) partly by increasing the size of ISWF, the fraction which is rich in starch (except in lupins). Prestløkken and Harstad,[41] however, could not find any effect of expander-pelleting processing on the ruminal outflow of dry matter in a barley- based diet.

The average fractional degradation rates of OM are presented in Table 5, and calculated as given below:

$$kd = \frac{-\left[(\ln(1-(OMDt_j/1000)) - \ln(1-(OMDt_i/1000))\right]}{t_j - t_i} \qquad (6)$$

where *kd* is the fractional rate of degradation at a specific interval time (*i* to *j*) and OMD is the organic matter disappearance (g kg^{-1} OM) at a specific time point. The results show that in the early stages of fermentation the fractional rates of degradation were higher in ISWF than in NWF. This would explain why E and EP elevated the maximum fractional degradation rate of OM at the first phase ($R_{max}S1$) of fermentation. The results presented in **Chapters 3** and **4** also show that both the E and EP process had a more profound effect on the first phase of fermentation in particular by elevating the maximum fractional rate of OM degradation (half time of gas production ranging from 7.6 to 11.4 h).

Table 5. Average fractional rates of OM degradation in all concentrate ingredients

		Fractional rate of degradation, h^{-1}				
Class		0-6 h	6-12 h	12-24 h	24-48 h	48-72 h
Fraction	ISWF	0.155^{a}	0.120^{a}	0.061^{a}	0.023^{a}	0.009^{a}
	NWF	0.063^{b}	0.066^{b}	0.051^{a}	0.019^{a}	0.022^{a}
	WHO	0.102^{b}	0.082^{b}	0.052^{a}	0.024^{a}	0.011^{a}
Process	R	0.086^{a}	0.093^{a}	0.060^{a}	0.021^{a}	0.012^{a}
	E	0.109^{a}	0.084^{a}	0.051^{a}	0.019^{a}	0.020^{a}
	EP	0.126^{a}	0.090^{a}	0.053^{a}	0.026^{a}	0.010^{a}

Means with different superscripts within class and column differ significantly ($P < 0.05$).

Based on the approach described above, the fractional rates of OM disappearance are calculated for different concentrate ingredients used in **Chapters 3** and **4** and presented in Figure 5.

It is evident that different concentrate ingredients respond differently to the E and EP process. In most concentrate ingredients, the effects of different treatments become apparent only shortly after incubation. Interestingly in lupins, no differences were observed between the E and EP processed samples compared to the R samples. This implies that based on the OMD approach, the effect of E and EP on the degradative behaviour of feed samples very much depends upon the lack or presence of starch in feed samples.

In the interpretation of the effect of processing based on the disappearance of OM care should be taken since the filtration method involved in the OMD determination may lead to overestimation of OMD at the early stage and underestimation of it as incubation progresses. At the early stage of incubation, during the filtration procedure some fine particles may escape through the pores of the crucibles causing an overestimation of OMD whereas in the later stage, the OM residue can be contaminated with microbial biomass causing an underestimation of OMD. This becomes practically more important since some researchers[48] use the OMD as a criterion to estimate the efficiency of microbial production in (un)processed feedstuffs *in vitro*. An alternative approach to estimate the disappearance of OM would be the appearance of VFAs as proposed by Groot et al.[49] and Blümmel et al.[50]

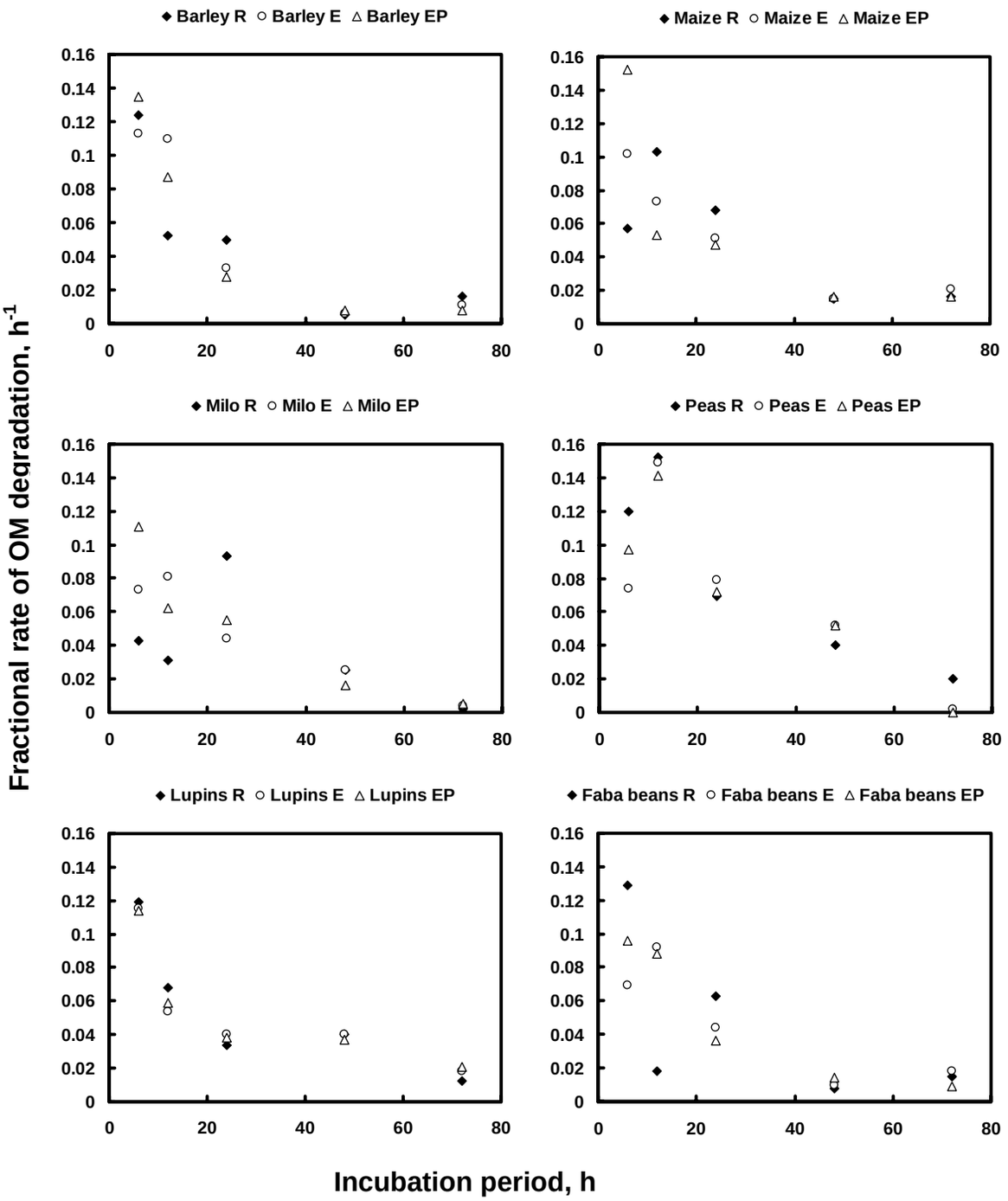


Figure 5. Fractional rates of OM degradation in the different entire concentrate ingredients (WHO) based on the OMD approach.

Appearance of VFAs

Rumen micro-organisms derive their energy mainly from fermenting carbohydrates.[51] As a result of carbohydrate fermentation, energy (ATP) is made available for microbial maintenance and growth. Moreover, as an important fermentation end-

product, VFAs are produced which account for 80 % of energy disappearing in the rumen and serve as an important source of energy in ruminants.[52,53] Therefore, it is of crucial importance to know the way processing might affect the partitioning of OM to the VFAs. Using the stoichiometric equations described by Blümmel et al.[50] and Groot et al.[49] it is possible to calculate the quantity of fermented hexose to produce a certain quantity of VFAs. In this approach it is assumed that proteins only contribute in producing *iso*-acids, therefore, the main VFAs (acetic, propionic and butyric acid) are assumed to originate from fermentation of carbohydrates. Then the organic matter disappearance (OMD) can be calculated as:

$$OMD = \left(\frac{HAc}{2} + \frac{H\,\mathrm{Pr}}{2} + HBu \right) \times 162 \qquad (7)$$

where 162 is the molecular weight of 1 mol of hexose in a polysaccharide. The VFAs appearance is then calculated back to the OM they originate from, using the equation 7. The results (Table 6) indicate that there is a discrepancy between the disappearance of OM and appearance of VFAs. The OM disappears much faster than VFAs appear. Indeed, there is no good linear relationship between OMD and VFAs appearance (R^2 = 0.19). This is in line with the findings of Yang et al.[29] who found that the average profile of gas production in samples of (un)processed barley and maize reached its asymptote sooner than that of VFAs. The discrepancy between the disappearance of OM and appearance of VFAs might have been arisen form the fact that the *in vitro* gas production technique overestimates the OMD. This overestimation is mainly due to loss of fine particles during the filtration procedure.[54] Indeed, the magnitude of discrepancy between OMD and VFAs appearance was higher in the ISWF (fine particles smaller than 40 μ) than in the NWF and WHO. Therefore, it can be concluded that determined OMD in the *in vitro* gas production technique would not be an appropriate criterion for evaluating the degradative behaviour of feedstuffs containing a substantial quantity of fine particles.

Processes E and EP (Tables 5 and 6) increase the fractional rate of VFAs appearance as EP > E > R in the first 12 h of incubation. In the early stages of fermentation, processes E and EP would provide more energy for both micro-organisms (higher OMD) and energy yielding precursors (VFAs) for the host animals. Moreover, as presented in **Chapters 3** and **4,** the process E and EP not only provide more energy yielding precursors (VFAs), but they also would provide more glucogenic precursors for milk component synthesis as indicated with an overall higher NGR in the E and EP samples compared to the R samples (2.38, 2.25 and 2.20 in R, E and EP samples, respectively).

Table 6. Average fractional rates of OMD in all concentrate based on VFAs appearance approach

		Fractional rate of degradation, h^{-1}				
Class		0-6 h	6-12 h	12-24 h	24-48 h	48-72 h
Fraction	ISWF	0.026^{b}	0.051^{b}	0.060^{a}	0.029^{a}	0.016^{a}
	NWF	0.027^{b}	0.058^{ab}	0.045^{b}	0.027^{a}	0.010^{a}
	WHO	0.035^{a}	0.066^{a}	0.051^{ab}	0.030^{a}	0.006^{a}
Process	R	0.028^{a}	0.045^{c}	0.055^{a}	0.032^{a}	0.006^{a}
	E	0.029^{a}	0.059^{b}	0.051^{a}	0.023^{a}	0.017^{a}
	EP	0.031^{a}	0.070^{a}	0.050^{a}	0.030^{a}	0.010^{a}

Means with different superscripts within class and column differ significantly ($P < 0.05$).

Fractional rates of OMD are calculated based on appearance of VFAs for the entire concentrate ingredients (WHO) and presented in Figure 6. From Figure 6 two conclusions can be drawn. Firstly, the effect of a specific technological process on degradative behaviour (e.g. fractional rates of OMD) is a time dependent and substrate specific phenomenon. Secondly, in thermo-mechanical processes such as expander and expander-pelleting, the lack or presence of starch plays a critical role on degradative behaviours of final agglomerate. The results indicate that in maize, milo, peas and faba beans fractional rate of OMD, when estimated from VFAs appearance, were increased by E and EP in the first 12 h of incubation whilst in lupins there was a good agreement with the OMD approach, in that both E and EP had no remarkable effects on elevating the fractional rates.

Although by measuring the VFAs appearance and using the stoichiometry of VFAs production we are able to estimate the OMD, one should realise that these stoichiometries may not be valid for all substrates especially those are rich in CP.[45]

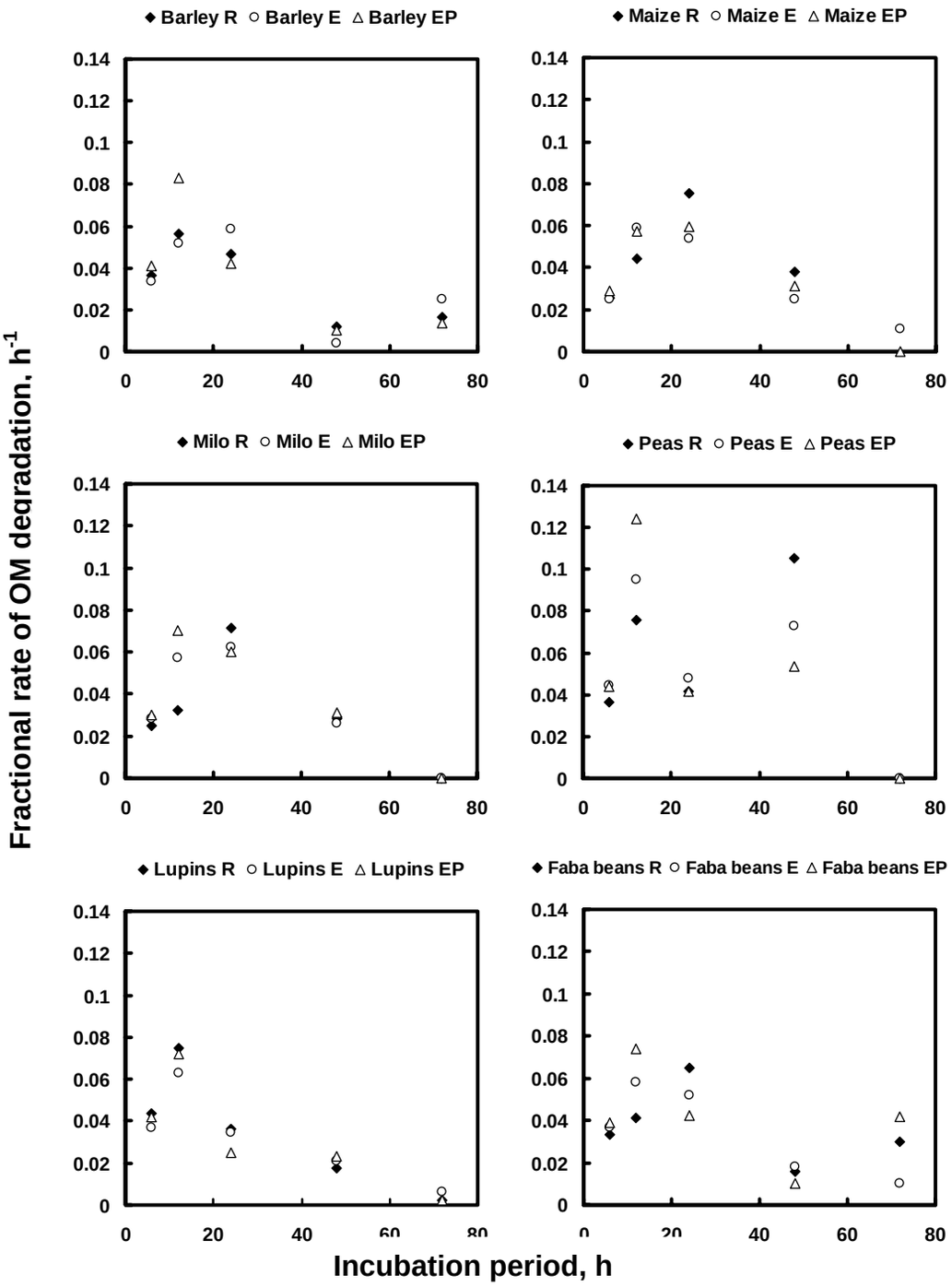


Figure 6. Fractional rates of OM degradation in the different entire concentrate ingredients (WHO) based on the appearance of VFAs.

General conclusions

- The fractionation methods developed in this study allow us to study the degradative behaviour of a fraction (soluble washable fraction) whose behaviour is masked by the other fractions in the entire feedstuff.
- The degradative behaviour of SWF differs distinctly from that of the non-washable (NWF) and insoluble washable fraction (ISWF) mainly because its chemical constituents differ distinctly from those in NWF and ISWF.
- Rumen degradative behaviour differs between concentrate ingredients because of differences in the distribution of the DM between their non-washable, insoluble washable and soluble washable fractions.
- The degradation rate of OM in ISWF is higher than that of NWF. However, its extent of degradation is lower than that of the NWF when assuming a ruminal outflow rate of 8 % per hour.
- Changes in the degradative behaviour of feedstuffs subjected to a hydrothermal processing that involve shear forces (e.g. expander processing) is mainly a result of changes in the distribution of DM between the different fractions that differ in their chemical constituents, digestion and ruminal outflow rate.
- The expander processing and ensuing pelleting help to create a more optimal rumen fermentation by reducing the ratio of rumen degradable protein to rumen degradable carbohydrate.
- Overall, both expander processing and the ensuing pelleting process provide animals with more glucogenic precursors, as indicated by a higher non-glucogenic ratio (NGR), which is mainly due to an elevated gelatinised starch and fractional rate of substrate degradation.
- The discrepancy between OM disappearance and VFAs appearance at the early stage of fermentation is mainly due to the loss of fine particles during the filtration procedure.
- The physico-chemical properties of a thermo-mechanical processed feedstuff (e.g. expander/expander-pelleting) depend largely upon the milling procedure they have undergone prior to the thermo-mechanical processing.

- The degradative behaviour of a technologically processed feed sample is very much influenced by the way it is prepared for the further *in situ* or *in vitro* studies.
- The effects of expander processing and ensuing pelleting on the degradative behaviour of the final agglomerate depends very much upon the lack or presence of starch in feedstuffs.
- The OMD approach is fraud with methodological error. Measuring the appearance of fermentation end-products mainly VFAs along with measuring the gas production through the course of incubation seems to be an appropriate method for evaluating the degradative behaviour of feed samples *in vitro*.

Further research era

In this study it was found that a considerable proportion of OM in the SWF constitutes a chemically not determined fraction so-called residual NSP.[3] It is of interest that the further research identifies its chemical entity. Moreover, in this study we mainly focused on characterising the degradative behaviour of OM in two groups of concentrate ingredients and their fractions. The further research, therefore, should focus more on characterising the degradative behaviour of different chemical constituents of theses fractions (e.g. starch and protein).

References

1. France J and Dijkstra J, Volatile Fatty Acids production, in *Quantitaive Aspects of Ruminants Digestion and Metabolism*, Ed by Dijkstra J, Forbes JM and France J. CABI, Wallingford, pp 734 (2005).

2. Van Vuuren AM, Tamminga S and Ketelaar RS, Ruminal availability of nitrogen and carbohydrates from fresh and preserved herbage in dairy cows. *Neth J Agric Sci* **38**:490-512 (1990).

3. Tamminga S, Brandsma G, Dijkstra J, van Duinkerken G, van Vuuren AM and Blok MC, Protein evaluation for ruminants: The DVE/OEB-System: Update 2007. CVB-Documentation report (2007).

4. Tamminga S and Hof G, Feeding Systems for Dairy Cows, in *Feeding Systems and Feed Evaluation Models*, Ed. by Theodorou MK and France J. CABI, Wallingford, pp 481 (2000).

5. Tóthi R, *Processed Grains as a Supplement to Lactating Dairy Cows*, PhD Thesis, Wageningen, University, Wageningen (2003).

6. Taweel ZH, *Perennial Ryegrass for Dairy Cows: Grazing Behaviour, Intake, Rumen Function and Performance*, PhD Thesis, Wageningen University, Wageningen, pp 156 (2004).

7. Goelema JO, *Processing of Legume Seeds: Effects on Digestive Behaviour in Dairy Cows*, PhD Thesis, Wageningen University, Wageningen (1999).

8. Otto T, Baik B-K and Czuchajowska Z, Microstructure of seeds, flours, and starches of legumes. *Cereal Chem* **74**:445-451 (1997).

9. Pomeranz Y and Czuchajowska Z, Structure of coarse and fine fractions of corn samples ground on the Severt hardness tester. *Food Microstructure* **4**:213-219 (1985).

10. Van Straalen WM and Tamminga S, Protein degradability in ruminant diets, in *Feed Evaluation*, Ed. by Wiseman J and Cole DJA. Butterworth, London, pp 55-72 (1990).

11. Dehority BA, *Rumen Microbiology*, Seventh edn, Nottingham University, Nottingham, pp 372 (2003).

12. De Boever JL, Cottyn BG, Vanacker JM and Boucque CV, Potential of solubility, enzymatic methods and NIRS to predict *in situ* rumen escape protein. *Neth J Agric Sci* **45**:291-306 (1997).

13. Mertens DR, Rate and extent of digestion, in *Quantitative Aspects of Ruminant Digestion and Metabolism*, Ed. by Dijkstra J, Forbes JM and France J. CABI Publishing, Wallingford, pp 734 (2005).

14. France J, Dijkstra J, Dhanoa MS, Lopez S and Bannink A, Estimating the extent of degradation of ruminants feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. *Br J Nutr* **83**:143-150 (2000).

15. Goelema JO, Smits A, Vaessen LM and Wemmers A, Effects of pressure toasting, expander treatment and pelleting on in vitro and in situ parameters of protein and starch in a mixture of broken peas, lupins and faba beans. *Anim Feed Sci Technol* **78**:109-126 (1999).

16. Michalet-Doreau B and Cerneau P, Influence of foodstuff particle size on in situ degradation of nitrogen in the rumen. *Anim Feed Sci Technol* **35**:69-81 (1991).

17. CVB, Centraal Veevoeder Bureau, Voorloping protocol voor *In situ* pensincubatie voor het meten van eiwitbestendigheid, Centraal Veevoeder Bureau, Lelystad, The Netherlands (2003).

18. Lowman RS, Theodorou MK and Cuddeford D, The effect of sample processing on gas production profiles obtained using the pressure transducer technique. *Anim Feed Sci Technol* **97**:221 (2002).

19. Theodorou MK, Williams BA, Dhanoa MS, McAllan AB and France J, A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim Feed Sci Technol* **48**:185-197 (1994).

20. Menke KH, Raab L, Salewski A, Steingass H, Fritz D and Schneider W, The estimation of the digestibility and metabolisable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *J Agric Sci* **93**:374-379 (1979).

21. Pell AN and Schofield P, Computerized monitoring of gas production to measure forage digestion *in vitro*. *J Dairy Sci* **76**:1063-1073 (1993).

22. Tóthi R, Lund P, Weisbjerg MR and Hevlpund T, Effect of expander processing on fractional rate of maize and barley starch degradation in the rumen of dairy cows estimated using rumen evacuation and *in situ* techniques. *Anim Feed Sci Technol* **104**:71-93 (2003).

23. Ljøkjel K, Skrede A and Harstad OM, Effects of pelleting and expanding on vegetable feeds on *in situ* protein and starch digestion in dairy cows. *J Anim Feed Sci* **12**:435-449 (2003c).

24. Prestløkken E, *In situ* ruminal degradation and intestinal digestibility of dry matter and protein in expanded feedstuffs. *Anim Feed Sci Technol* **77**:1-23 (1999).

25. Arieli A, Bruckental I, Kedar O and Sklan D, *In sacco* disappearance of starch nitrogen and fat in processed grains. *Anim Feed Sci Technol* **51**:287-295 (1995).

26. Masoero F, Pulimeno AM and Rossi F, Effect of extrusion, expansion and toasting on the nutritional value of peas, faba beans and lupins. *Ital J Anim Sci* **4**:177-189 (2005).

27. Sveinbjornsson J, Murphy M and Uden P, *In vitro* evaluation of starch degradation from feeds with or without various heat treatments. *Anim Feed Sci Technol* **In press** (2006).

28. Smith D, Removing and analysing total nonstructural carbohydrates from plant tissue. *Wisconsin Agric Exp Sep* **41**:1-10 (1969).

29. Yang HJ, Tamminga S, Williams BA, Dijkstra J and Boer H, *In vitro* gas and VFA production profiles of barley and corn and their soluble washout fractions after feed processing. *Anim Feed Sci Technol* **120**:125-140 (2005).

30. Pfost H and Headley V, Methods for determining and expressing particle size, in *Feed Manufacturing Technology*, Ed. by Pfost H and Pickering D. Arlington, VA: American Feed Industry, pp 512-520 (1976).

31. Volden H, Myland LT and Olaisen V, Apparent ruminal degradation and rumen escape of soluble nitrogen fractions in grass and grass silage administered intraruminally to lactating dairy cows. *J Anim Sci* **80**:2704-2716 (2002).

32. Tas B, *Perennial Ryegrass for Dairy Cows: Intake, Milk Production and Nitrogen Utilisation*, PhD Thesis, Wageningen University, Wageningen (2005).

33. Statistical Centre of Iran, The amount and value of consumed feedstuffs in the industrialised dairy farms. Available at: http://amar.sci.org/Detail.aspx?Ln=F&no=81619&S=GW (2006)

34. Nocek JE and Tamminga S, The prediction of nutrient supply to dairy cows from the rate and extent of ruminal degradation of ration components. *J Dairy Sci* **74**:3598-3629 (1991).

35. Slavin JL, Jacobs D and Marquart L, Grain processing and nutrition. *Crit Rev Food Sci Nutr* **40**:309-326 (2000).

36. Gomez MH and Aguilera JM, Changes in the starch fraction during extrusion-cooking of corn. *J Food Sci* **48**:378-381 (1983).

37. Rymer C, Huntington JA, Williams BA and Givens DI, *In vitro* cumulative gas production techniques: History, methodological considerations and challenges. *Anim Feed Sci Technol* **123-124**:9 (2005).

38. Cone JW, Van Gelder AH, Visscher GJW and Oudshoorn L, Use of a new automated time related gas apparatus to study the influence of substrate concentration and source of rumen fluid on fermentation kinetics. *Anim Feed Sci Technol* **61**:113-128 (1996).

39. Davies ZS, Mason D, Brooks AE, Griffith GW, Merry RJ and Theodorou MK, An automated system for measuring gas production from forages inoculated with rumen fluid and its use in determining the effect of enzymes on grass silage. *Anim Feed Sci Technol* **83**:205-221 (2000).

40. Groot JCJ, Cone JW, Williams BA, Debersaques FMA and Lantinga EA, Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. *Anim Feed Sci Technol* **64**:77-89 (1996).

41. Prestløkken E and Harstad OM, Effects of expander-treating a barley-based concentrate on ruminal fermentation, bacterial N synthesis, escape of dietary N, and performance of dairy cows. *Anim Feed Sci Technol* **90**:227- 246 (2001).

42. Offner A, Bach A and Sauvant D, Quantitative review of *in situ* starch degradation in the rumen. *Anim Feed Sci Technol* **106**:81-93 (2003).

43. Gallant DJ, Bouchet B, Buelon A and Perez S, Physical characteristics of starch granules and susceptibility to enzymatic degradation. *Eur J Clin Nutr* **46**:3-16 (1992).

44. Thomas M, *Physical Quality of Pelleted Feed. A Feed Model Study*, PhD Thesis, Wageningen University, Wageningen (1998).

45. Cone JW and Van Gelder AH, Influence of protein fermentation on gas production profiles. *Anim Feed Sci Technol* **76**:251-264 (1999).

46. Cone JW, Van Gelder AH, Bachmma H and Hindle VA, Comparison of organic matter degradation in several feedstuffs in the rumen as determined with nylon bag and gas production techniques. *Anim Feed Sci Technol* **96**:56-67 (2002).

47. Ørskov ER and McDonald I, The estimation of protein degradation in the rumen from incubation measurements weighted according to rate of passage. *J Agric Sci* **92**:499-503 (1979).

48. Blümmel M and Lebzien P, Predicting ruminal microbial efficiencies of dairy ration by *in vitro* techniques. *Livest Prod Sci* **68**:107-117 (2001).

49. Groot JCJ, Williams BA, Oostdam AJ, Boer H and Tamminga S, The use of cumulative gas production and volatile fatty acid production to predict *in vitro* fermentation kinetics of Italian ryegrass leaf cell walls and contents at various time intervals. *Br J Nutr* **79**:519-525 (1998).

50. Blümmel M, Makkar HPS and Becker K, *In vitro* gas production: a technique revisited. *J Anim Physiol and Anim Nutr* **77**:24-34 (1997a).

51. France J and Siddons RC, Volatile Fatty Acid Production, in *Quantitative Aspects of Ruminant Digestion and Metabolism*, Ed. by Forbes JM and France J. CABI, Wallingford, pp 107-121 (1993).

52. Sutton JD, *In vivo* Measurement of Energy Supply and Protein Synthesis in the Rumen, in *Tracer Studies on Non-Protein Nitrogen for Ruminants*, Ed. by Agency IAE. International Atomic Energy Agency, Vienna, pp 35-42 (1972).

53. Sutton JD, Digestion and absorption of energy substrates in lactating cow. *J Dairy Sci* **68**:3376-3393 (1985).

54. Udén P, Recovery of insoluble fibre fractions by filtrations and centrifugation. *Anim Feed Sci Technol* **129**:316-328 (2006).

Appendixes

Summary

Nowadays, feedstuffs to be included in ruminant diets are not only characterised by their chemical composition and digestibility, but they are also characterised by their distribution in non-washable (NWF), insoluble washable (ISWF) and soluble washable (SWF) fractions, because these fractions differ in rumen degradation rate and rumen residence time. The distribution of these fractions in the dry matter of feedstuffs, their chemical composition and their rate of ruminal degradation and outflow play an important role in the supply of nutrients for microbial growth and maintenance, as well as in delivering the nutrients into the small intestine to be digested and utilised by the host animal. A widely adopted procedure to characterise the degradation in the rumen and its dynamics is the *in situ* incubation technique. Since the ISWF and SWF are washed out of nylon bags, their degradative behaviour cannot directly be measured, and an alternative method has to be applied. Since the washable fraction (ISWF + SWF) quantitatively constitutes an important part of the feedstuffs' dry matter (up to 50 %), characterising its degradative behaviour is of great importance. This dissertation, therefore, aimed to characterise the degradative behaviour of some important unprocessed and processed concentrate ingredients and their fractions, using a methodology that fractionates feed samples into its inherent constituents (NWF, ISWF and SWF) in combination with an *in vitro* gas production technique.

The objective of **Chapter 1** was to further develop a laboratory method that mimics the washing procedure in the routine application of the *in situ* technique. The effects of washing procedure, particle size distribution and dilution (the ratio of consumed water to sample weight) on the distribution of non-washable (NWF), insoluble washable (ISWF) and soluble washable (SWF) fractions were studied. The effects of three described washing procedures (Yang (Y), Melin (M) and *in situ* (IS)) on the distribution of dry matter between NWF, ISWF and SWF in six concentrate ingredients (maize, barley, milo, peas, lupins and faba beans), ground through different sieve sizes (1 and 3 mm; Retsch mill), were compared. Subsequently, method M was further developed (method SM) by reducing the dilution ratio and its effect on NWF, ISWF and SWF was compared. Finally, a new washing method was developed (method AA), and its effect on NWF, ISWF and SWF at different dilutions with water was compared with the IS, M, SM and Y methods. Differences and similarities between the different washing procedures are summarised in Table 1. It was concluded that method AA20 gave the best results and this was adopted as our standard procedure.

Table 1. Washing methods to simulate washing in *in situ* (IS) technique

Method	Sample weight (g)	Dilution[1]	Step 1	Step 2 (NWF)	Step 3 (WF)	Step 4 (ISWF)	Step 5 (SWF)
IS	5.0	> 100 mL g^{-1}	Washing machine	Leaking			
Y	100.0	30 mL g^{-1}	plunging in and out water with an excentric rod		Centrifugation (20 min; 715 × g)	Filtration	Filtrate
M	3.0	25 mL g^{-1}	Dispersion of sample in a glass beaker ; shaking at 150 rpm for 1 h	Filtration through nylon cloth in funnel	Centrifugation (20 min; 715 × g)	Filtration	Filtrate
SM	3.0	12.5 mL g^{-1}					
AA10[2]	5.5	10 mL g^{-1}	continuous washing of nylon bags in a centrifuge beaker with a shaking bath at 150 rpm for 1 h		Centrifugation (20 min; 715 × g)	Filtration	Filtrate
AA20[2]	5.5	20 mL g^{-1}					
AA30[2]	5.5	30 mL g^{-1}					

[1] Defined as the proportion of consumed water to sample weight. [2] Method AA at dilutions of 10, 20 and 30.

The effects of different dilutions on SWF and soluble true protein (STP) in six concentrate ingredients were also studied. The effects of grain, washing method and particle size on the size of NWF and ISWF were significant, with significant interactions between grain and particle size, grain and washing method, particle size and washing method, but no interaction between grain type, washing method and particle size. In method Y the size of NWF was smaller than in the other methods. The results showed that, except in lupins, NWF in cereal grains was significantly higher than in legume seeds. Increasing the particle size significantly increased NWF, whereas ISWF was decreased. The size of SWF in legume seeds was higher than in the cereal grains. Increasing the dilution, increased STP in legume seeds, but not in cereal grains.

Chapter 2 aimed to characterise the degradative behaviour and the formation of fermentation end-products of the NWF, ISWF and SWF in the concentrate ingredients maize, barley, milo, yellow peas, lupins and round-seeded brown faba beans. The procedure that mimics washing in the *in situ* incubation technique, as adopted in chapter 1, was combined with an *in vitro* gas production (Automated Pressure Evaluation System, APES) and a volatile fatty acids (VFA) production technique, and used to compare the behaviour of the fractions NWF, ISWF and SWF and to verify the assumption that rumen degradation behaviour of material washed out of nylon bags is instantaneous and complete. In a manually operated *in vitro* fermentation system, the same substrates as used in the APES were fermented for 0, 3, 6, 12, 24 and 48 h in order to collect samples for volatile fatty acids (VFA) and ammonia (NH_3-N) analysis.

Except in lupins, ISWF of the concentrate ingredients was very rich in starch. SWF was relatively rich in ash, crude protein, soluble sugars, and a residual fraction (chemically not determined) but contained only a negligible quantity of starch. The fermentation characteristics of ISWF were more similar to those from WHO and NWF rather than SWF. Total gas production of SWF was considerably lower than that of the other fractions. A very rapidly degradable fraction was seen in the first phase of degradation of SWF. It is concluded that the nature of the VFA profile resulting from the fermentation of the different fractions differs between fractions and changes with time of fermentation.

In **Chapters 3** and **4** the effects of using a pre-compacting device (expander) alone or in combination with a pelleting process on the degradative behaviour of barley, maize, milo, peas, lupins and faba beans and their different fractions were studied using the *in vitro* gas production. The aim was to establish whether the processing changes the contribution of NWF, ISWF and SWF to the dry matter (DM), changes the chemical constituents within DM and changes the degradative behaviour of the fractions within the concentrate

ingredients. The samples were fractionated into SWF, ISWF, and NWF fractions. The samples of the 3 fractions of the entire concentrate ingredients (WHO) were subjected to three processes (R, Retsch mill ground samples; E, expander treated samples; EP, expander-pelleted samples), and their fermentation characteristics were evaluated using the *in vitro* incubation technique (APES) for 72h. In the manually operated *in vitro* fermentation system, the same substrates were fermented for 6, 12, 24 and 48 h in order to collect samples for volatile fatty acids (VFA) and ammonia (NH_3-N) analysis. The ISWF of the cereal grains, peas and faba beans was very rich in starch. In all concentrate ingredients, fraction SWF was relatively rich in ash, crude protein, soluble sugars, and a residual (chemically not determined, but contributing 3.7-42 %) fraction, which was elevated by expander-pelleting and the ensuing pelleting process (except in lupins). In maize and milo ($P < 0.05$), the E process significantly (in barley numerically) increased the size of NWF compared to process R, while it was decreased again by ensuing pelleting. In peas and faba beans, both the E and EP process significantly ($P < 0.05$) increased the size of NWF compared to process R. Fraction SWF in the processed samples E of cereal grains was significantly ($P < 0.05$) lower than in the R samples. In the legume seeds, the SWF in the processed samples E and EP was lower than in the R samples. Gas production profiles were fitted to a di-phasic model. Compared to the R samples in the cereal grains, the EP samples gave a faster fermentation, as presented by a significantly (in barley numerically) higher maximum fractional rate of substrate degradation ($P < 0.05$) and a significantly (in maize numerically) higher maximum rate of gas production ($P < 0.05$) in the first phase of fermentation. In the legume seeds compared to the R samples; both the E and EP samples had a significantly higher maximum fractional rate of gas production in the first phase of fermentation ($P < 0.05$). In barley, maize, lupins and faba beans; E and EP samples shifted the pattern of fermentation towards a more glucogenic fermentation as represented by a lower non-glucogenic to glucogenic ratio (NGR). In all cereal grains, the NH_3-N and branched chain ratio (BCR), representing protein degradation, for E and EP samples were significantly ($P < 0.05$) lower than in R samples, whereas in the legume seeds, the NH_3-N for E and EP samples were significantly ($P < 0.05$) lower than in R samples.

Chapter 5 aimed to establish the possible effects of different grinding procedures and sample preparation on the degradative behaviour of processed concentrate ingredients. Grinding is a technological process that is widely applied in the feed manufacturing industry and it is a prerequisite for obtaining representative samples for laboratory procedures (e.g. gas production analysis). When feeds are subjected to technological processes other than grinding (e.g. expander treatment), grinding afterwards may disturb

the effect of processing, both in practice and when laboratory techniques are applied. Therefore, samples of expander processed barley and peas were subjected to six different sample preparation procedures (intact sample, dissolved sample, samples ground stepwise over 6 and 3 mm sieves, samples ground stepwise over 6, 3 and 1 mm sieves, samples ground stepwise over 6 and 1 mm sieves, samples ground over a 3 mm sieve and samples ground over a 1 mm sieve). The particle size distribution of ground samples, determined by dry sieve analysis and analysis according to the Coulter counter method differed due to the different grinding procedures. The patterns of gas production in these samples were studied over a period of 72 h incubation using the automated in vitro gas production system. Grinding the samples of expander processed barley and peas changed the kinetics of gas production and led to a faster degradation that was most pronounced after stepwise grinding. However, the formation of the fermentation end-product was not affected by the method of sample preparation. In expander processed barley, the difference in the degradation pattern due to the different grinding procedures was small.

In chapter 6 the findings of the previous chapters were reviewed in a general discussion. This thesis leads to the conclusions that:

- The degradative behaviour of SWF in all concentrate ingredients differs distinctly from that of the NWF and ISWF mainly because its chemical constituents differ distinctly from those in NWF and ISWF.
- Rumen degradative behaviour differs between concentrate ingredients because of differences in the distribution of the DM between their non-washable, insoluble washable and soluble washable fractions.
- Changes in the degradative behaviour of feedstuffs subjected to a hydrothermal process that involves shear forces (e.g. expander processing) is mainly a result of changes in the distribution of DM between the different fractions that differ in their chemical constituents, rumen degradative behaviour and ruminal outflow rate.
- The expander processing and ensuing pelleting create a more optimal rumen fermentation by reducing the ratio of rumen degradable protein to rumen degradable carbohydrates, and provide animals with more glucogenic precursors, as indicated by a higher NGR, which is mainly due to an elevated gelatinised starch content and fractional rate of substrate degradation.

- The degradative behaviour of a technologically processed feed sample is very much influenced by the way it is prepared for the further chemical analysis, *in situ* or *in vitro* studies.

In retrospect with the subjects pointed out in this dissertation, further research should mainly focus on characterising the degradative behaviour of different inherent constituents in NWF, ISWF and SWF fractions, notably starch, proteins and non-starch polysaccharides.

Samenvatting

Voeders in rantsoenen voor herkauwers worden tegenwoordig niet alleen beoordeeld op basis van hun chemische samenstelling en verteerbaarheid, maar ook op basis van hun uitwasbaarheid uit in de pens gehangen nylon zakjes. Ze worden dan verdeeld in een niet uitwasbare (NWF), een wel uitwasbare maar niet oplosbare (ISWF) en een zowel uitwasbare als oplosbare (SWF) fractie, waarbij oplosbaarheid wordt bepaald door filtreren of centrifugeren. Deze indeling vindt plaats omdat de fracties verschillen in hun verblijftijd en snelheid van afbraak in de pens. De verdeling van deze fracties over de drogestof van voeders, hun chemische samenstelling en hun snelheid van afbraak en passage door de pens spelen een belangrijke rol bij zowel het aanbod van voedingsstoffen (nutriënten) voor onderhoud en groei van de microben in de pens als voor het aanbod van nutriënten in de dunne darm teneinde daar verteerd en door het gastheerdier benut te worden.

Een wereldwijd aanvaarde methode om de dynamiek van afbraak in de pens te karakteriseren is de *in situ* incubatie techniek. Omdat ISWF en SWF vrijwel onmiddellijk na hun incubatie in de pens uit de nylon zakjes worden gewassen, kan hun afbraak in de tijd zo niet worden gemeten en is daarvoor een alternatieve methode nodig. Omdat de uitwasbare fracties (ISWF + SWF) een aanzienlijk (tot aan 50%) deel uitmaken van de drogestof in een voedermiddel is het karakteriseren van hun afbraakgedrag erg belangrijk. Dit proefschrift had mede tot doel een methode te ontwikkelen voor het karakteriseren van de afbraak in de pens van een aantal technologisch wel en niet bewerkte krachtvoergrondstoffen en hun fracties. Hiervoor werd een methode ontwikkeld waarbij voedermiddelen werden opgedeeld in hun onderdelen (NWF, ISWF en SWF) in combinatie met een *in vitro* gas productie techniek

De doelstelling van **hoofdstuk 1** was om een methode te ontwikkelen die de bij de routinematig uitgevoerde *in situ* techniek gebruikte uitwasprocedure nabootst. Hierbij werden de invloed van wasprocedure, deeltjesgrootte verdeling en verdunning (hoeveelheid water per g voer) op de verdeling tussen niet uitwasbaar (NWF), niet oplosbaar uitwasbaar (ISWF) en oplosbaar uitwasbaar (SWF) bestudeerd. Eerst werd van 6 krachtvoer grondstoffen (mais, gerst, milo, erwten, lupines en faba bonen) de invloed nagegaan van een drietal in de literatuur reeds beschreven uitwasprocedures (*in situ* (IS), Melin (M) en Yang (Y)), op de verdeling van de droge stof tussen NWF, ISWF en SWF, na malen (Retsch molen) over verschillende zeefgroottes (1 en 3 mm). Vervolgens werd

Tabel 1. Procedures voor het simuleren van de bij de *in situ* (IS) techniek gebruikte wasprocedure

Methode	Gewicht monster (g)	Verdunning[1]	Stap 1	Stap 2 (NWF)	Stap 3 (WF)	Stap 4 (ISWF)	Step 5 (SWF)
IS	5.0	> 100 ml g^{-1}	Wasmachine	Uitlekken			
Y	100.0	30 ml g^{-1}	In en uit het water laten plonsen m.b.v. een excentrische staaf		Centrifugeren (20 min; 715 × g)	Filtreren	Filtrate
M	3.0	25 ml g^{-1}	Dispersie van het voermonster in een bekerglas met water; schudden bij 150 rpm gedurende 1 uur	Filtreren door nylon doek in een trechter	Centrifugeren (20 min; 715 × g)	Filtreren	Filtrate
SM	3.0	12.5 ml g^{-1}					
AA10[2]	5.5	10 ml g^{-1}	Continue wassen van nylon zakjes in een centrifugebuis in een schudwater-bad bij 150 rpm gedurende 1 uur		Centrifugeren (20 min; 715 × g)	Filtreren	Filtrate
AA20[2]	5.5	20 ml g^{-1}					
AA30[2]	5.5	30 ml g^{-1}					

[1] Gedefinieerd als de hoeveelheid water per g voermonster. [2] Methode AA bij verdunningen van 10, 20 and 30.

methode M verder ontwikkeld (methode SM) door de verdunning met water te verminderen en de invloed daarvan op NWF, ISWF en SWF werd nagegaan. Ten slotte werd een nieuwe wasprocedure ontwikkeld (methode AA) en de effecten ervan op NWF, ISWF en SWF bij verschillende verdunningen met water vergeleken met de IS, M, SM en Y methode.

Verschillen en overeenkomsten tussen de verschillende wasprocedures worden weergegeven in tabel 1. Geconcludeerd werd dat methode AA20 de beste resultaten gaf en deze methode werd verder gebruikt als standaard methode.De effecten van de verschillende verdunningen op SWF en oplosbaar werkelijk eiwit (STP) bij de zes krachtvoer grondstoffen werd ook nagegaan. De effecten van grondstof, wasprocedure en deeltjesgrootte op de grootte van NWF en ISWF bleken significant, met eveneens significante interacties tussen grondstof en deeltjesgrootte, grondstof en wasprocedure, deeltjesgrootte en wasprocedure, maar geen interacties tussen type grondstof, wasprocedure en deeltjesgrootte. Bij methode Y was de omvang van NWF kleiner dan bij de andere methodes. Uit de resultaten bleek ook dat, uitgezonderd lupine, NWF bij granen significant groter was dan bij zaden van vlinderbloemigen. Het vergroten van de deeltjesgrootte deed de omvang van NWF significant toenemen, waarbij ISWF afnam. De omvang van SWF was groter bij zaden van vlinderbloemigen dan bij granen. Het verhogen van de verdunning deed STP in zaden van vlinderbloemigen toenemen, maar dit deed zich niet voor bij granen.

Hoofdstuk 2 had als doel het karakteriseren van het afbraakgedrag in de pens en de vorming van eindproducten van fermentatie van NWF, ISWF en SWF van de krachtvoer grondstoffen mais, gerst, milo, gele erwten, lupines en ronde bruine faba bonen. De wasprocedure die ontwikkeld was in hoofdstuk 1, werd gecombineerd met een geautomatiseerde *in vitro* gasproductie techniek (APES) en een handmatige techniek voor het meten van de productie van vluchtige vetzuren (VFA). Deze benadering werd enerzijds gebruikt om het gedrag van de fracties NWF, ISWF en SWF te vergelijken en anderzijds om de aanname dat materiaal dat uit nylon zakjes wordt gewassen ogenblikkelijk en compleet wordt afgebroken, te verifiëren. In een handmatig bediend *in vitro* fermentatie systeem werden dezelfde grondstoffen als die welke gebruikt werden in de APES, gefermenteerd gedurende 0, 3, 6, 12, 24 en 48 uur met het doel monsters te verzamelen voor de analyse van vluchtige vetzuren (VFA) en ammoniak (NH_3-N)

Met uitzondering van lupines was de ISWF van de krachtvoer grondstoffen zeer rijk aan zetmeel. SWF bevatte verwaarloosbaar kleine hoeveelheden zetmeel, maar was relatief rijk aan as (mineralen), ruw eiwit, oplosbare suikers en een (chemisch niet nader bepaalde)

restfractie. De fermentatie karakteristieken van ISWF kwamen meer overeen met die van de oorspronkelijke hele grondstof (WHO) en NWF dan met die van SWF. De totale gasproductie van SWF was aanzienlijk lager dan die van de andere fracties. Een zeer snel afbreekbare fractie werd waargenomen in de eerste fase van de afbraak van SWF. Geconcludeerd werd dat de aard van het VFA profiel, dat resulteerde van de fermentatie van de verschillende fracties, verschilt tussen fracties en in de loop van de fermentatie verandert.

In de **hoofdstukken 3 en 4** werd het effect van het gebruik van een voorverdichtings apparaat (expander) alleen (E) of in combinatie met een brokjespers (EP) op het afbraakgedrag in de pens van maïs, gerst, milo, erwten, lupines en faba bonen en hun onderscheiden fracties bestudeerd met behulp van de *in vitro* gas productie techniek. Het doel was na te gaan of deze processen veranderingen veroorzaken in de verdeling van NWF, ISWF en SWF in de drogestof, of de chemische samenstelling van de fracties er door verandert en of het afbraakgedrag in de pens van deze fracties binnen een krachtvoergrondstof er door wordt beïnvloed.

Monsters van de krachtvoer grondstoffen werden onderworpen aan 3 technologische processen, malen over een Retsch molen (R), expanderen (E) of expanderen gevolgd door pelleteren (EP) en vervolgens gefractioneerd in SWF, ISWF en NWF. De fermentatie karakteristieken van deze fracties werden daarna beoordeeld in de *in vitro* gas productie techniek APES gedurende 72 uur. Tegelijkertijd werden in een handmatig bediend *in vitro* systeem monsters verzameld na incubatietijden van 0, 6, 12, 24 en 48 uren voor het bepalen van de gehaltes aan VFA en NH_3-N.

De ISWF van de granen en van erwten en faba bonen waren zeer rijk aan zetmeel. In alle krachtvoergrondstoffen was de fractie SWF relatief rijk aan as (mineralen), ruw eiwit, oplosbare suikers en een (chemisch niet nader gekarakteriseerde) restfractie (3.7 - 42%). Met uitzondering van lupines werd deze restfractie vergroot door de processen E en EP. Behandeling E vergrootte de omvang van NWF in maïs en milo significant ($P<0.05$) en in gerst numeriek, in vergelijking met behandeling R, terwijl de omvang weer werd verkleind door het er op volgende pelleteren (proces EP). In erwten en faba bonen werd de omvang van fractie NWF significant ($P<0.05$) vergroot door zowel E als EP ten opzichte van R. In de granen was als gevolg van behandeling E, de omvang van fractie SWF significant ($P<0.05$) kleiner dan na behandeling R, terwijl in de zaden van de vlinderbloemigen SWF na zowel behandeling E als behandeling EP kleiner was dan na behandeling R. Gasproductie profielen werden gefit met een twee-fasen model. In vergelijking met de volgens R behandelde monsters granen, resulteerde behandeling EP in

een snellere fermentatie wat werd afgeleid uit de significant (in gerst numeriek) snellere ($P<0.05$) maximale fractionele afbraaksnelheid van het substraat in de eerste fase van de fermentatie en een significant (in maïs numeriek) hogere ($P<0.05$) maximale snelheid van gasproductie in de eerste fermentatie fase. In vergelijking met behandeling R gaven zowel E als EP bij de zaden van vlinderbloemigen een significant hogere ($P<0.05$) maximale gasproductie snelheid in de eerste fermentatiefase. In gerst, maïs, lupinen en faba bonen deden de behandelingen E en EP het fermentatiepatroon verschuiven in de richting van een meer glucogene fermentatie, dat werd afgeleid uit een lagere non glucogenic to glucogenic ratio (NGR) in de VFA. In de granen waren de gehaltes aan NH_3-N en de "branched chain ratio" (BCR) die beiden worden beschouwd als een afspiegeling van de eiwitafbraak, significant lager ($P<0.05$) na de behandelingen E en EP vergeleken met behandeling R. In de zaden van vlinderbloemigen waren alleen de NH_3-N gehaltes significant lager ($P<0.05$) na behandelingen E en EP in vergelijking met behandeling R.

Het onderzoek in **hoofdstuk 5** had als doel een mogelijke invloed vast te stellen van verschillende procedures van malen en monster voorbereiding op het afbraakgedrag in de pens, van procestechnologisch bewerkte krachtvoer grondstoffen. Malen is een technologisch proces dat in de mengvoerindustrie veelvuldig wordt toegepast en het is bovendien een voorwaarde om representatieve monsters te krijgen voor laboratorium onderzoek (bv. gas productie analyse). Wanneer voeders worden blootgesteld aan andere technologische processen dan malen (bv. expanderen), kan malen achteraf het effect van procestechnologie verstoren. Dit geldt zowel voor de praktijk van mengvoerbereiding als bij laboratoriumwerk. Daarom werden geëxpandeerde monsters gerst en erwten blootgesteld aan zes verschillende behandelingen (intacte monsters, in oplossing gebrachte monsters, monsters die stapsgewijs waren gemalen over een zeef van 6 en 3 mm, monsters die stapsgewijs waren gemalen over een zeef van 6 en 1 mm, monsters die waren gemalen over een zeef van 3 mm en monsters die waren gemalen over een zeef van 1 mm). De deeltjesgrootte verdeling van de gemalen monsters, bepaald volgens een droge zeef analyse en volgens de Coulter counter methode, verschilde afhankelijk van de gebruikte maalmethode. Gasproductie patronen in de monsters werden gemeten met APES over een periode van 72 h. Het malen van monsters gerst en erwten die behandeling E hadden ondergaan veranderde de kinetiek van gasproductie en leidde tot een snellere afbraak, die het meest uitgesproken was na stapsgewijs malen. Echter, de vorming van fermentatie eindproducten werd niet beïnvloed door de wijze waarop de monsters waren voorbehandeld. In geëxpandeerde gerst waren de veranderingen als gevolg van de verschillende maalprocedures betrekkelijk klein.

In **hoofdstuk 6** worden de waarnemingen uit de eerdere hoofdstukken besproken in een algemene discussie. Het proefschrift leidt dan tot de conclusies dat:

- Het afbraakgedrag van SWF in alle krachtvoergrondstoffen verschilt behoorlijk van dat van NWF en ISWF, vooral omdat de chemische samenstelling van SWF verschilt van die van NWF en ISWF.

- Het afbraakgedrag in de pens verschilt tussen krachtvoer grondstoffen als gevolg van verschillen in de verdeling van drogestof over NWF, ISWF en SWF.

- Veranderingen in het afbraakgedrag van voeders, die zijn blootgesteld aan een technologisch proces waarin naast vocht en warmte, ook krachten als gevolg van wrijving een rol spelen (bv expanderen), zijn voornamelijk het gevolg van veranderingen in de verdeling van de drogestof over de verschillende fracties (NWF, ISWF en SWF) die op hun beurt verschillen in chemische samenstelling, afbraakgedrag in de pens en passagesnelheid uit de pens.

- Expanderen gevolgd door pelleteren leidt tot een meer optimale pensfermentatie doordat de verhouding tussen pensafbreekbaar eiwit en pensafbreekbare koolhydraten kleiner wordt en voorzien de dieren bovendien met meer glucogene nutriënten, vooral als gevolg van een hogere graad van zetmeel ontsluiting en een verhoging van de fractionele afbraaksnelheid van hun substraat.

- Het afbraakgedrag in de pens van procestechnologisch bewerkte voeders wordt sterk beïnvloed .door de wijze waarop de voeders zijn behandeld als voorbereiding op chemische analyse, *in vitro* of *in situ* studies.

Terugkijkend op de onderwerpen die in dit proefschrift zijn behandeld, wordt geconcludeerd dat verder onderzoek zich vooral zou moeten richten op het karakteriseren van afbraakgedrag in de pens van de chemische componenten binnen de NWF, ISWF en SWF fracties, met name zetmeel, eiwitten en de niet zetmeel polysachariden (NSP).

Acknowledgement

Almighty God, who has the power to thank you for what you bestow upon him or her. I would like, however, to thank you for keeping me and my family healthy. Of course, those who do not thank the creature; they do not thank the creator. I therefore, would like to express my deepest gratitude to the Ministry of Science, Research and Technology of Iran and University of Lorestan, for funding my PhD project that allowed me to accomplish this thesis.

With deep gratitude, I wish to express my sincere thanks to my promotor Prof. Seerp Tamminga. Dear Seerp, I thank you for your invaluable comments, suggestions and encouragements throughout my PhD research. I will never forget how you challenged me in writing my lengthy General Discussion.

I wish to extend my sincere gratitude to my co-promotors. Dear Thomas, thank you for introducing me to the world of "Feed Technology". Thomas, I will always remember your excellent performance in our famous, but abolished Play Back Show. You were the only one who defeated the invincible Ajay. For that, I am proud of you. Huug, I am very glad to have you on podium seated next to Thomas and Seerp. I must admit without your guidance developing the famous washing procedure "Method AA" would have not been possible.

Dear Barbara, out the blue you decided to leave our group around the same time when Huug was retired. Indeed, it was a great shock for somebody like me who just decided to start working with APES. Somehow, however, I managed to survive, and I am glad to have you on podium as one of my opponents!!!

My dear highly esteemed co-author, Dr. Pellikaan, I would like to express my profound thanks to you for your invaluable comments, suggestions and corrections on my two last papers. It was really a great pleasure talking to you about different topics and sometimes about animal sciences!!! After all, I still do not know whether the model has to be fitted to data or vice versa.

I cannot imagine how it would be possible to accomplish my PhD programme without the helps of skilful staffs of the laboratory of the Animal Nutrition Group. Dear Dick, Jane-Martine, Meijke, Saskia and Truus so many thanks for what you did for me. Dear Tamme, I should thank you for your co-operation in processing the feed samples at WFPC (Wageningen Feed Processing Centre!!!).

Dear Betty, I thank you very much for your help in all my bureaucratic affairs and translating Dutch letters for me that sometimes was even so difficult for you!!

I would like to express my gratitude to Prof. Verstegen and his lovely wife Marite. Martin, I will never forget the memory of the short cycling trip we had together around Wageningen. After that, I must admit that you are younger than I am.

I take the opportunity to express my sincerely thanks to Dr. Jan Dijkstra and Dr. Walter Gerrits for answering my "Jump into office and barge in" questions.

I would like to thanks my officemates, Quang and Khoa who provided me a very pleasant atmosphere in the office. Dear Quang, I tried to be as tidy as you are, but I must admit I failed. Dear Khoa, you are still young enough to learn from Quang to be a tidy person, do not be disappointed. My dear PhD fellows, Bernardo (Dr. Suarez), Guido, Joost (Dr. van der Borne) and Sander, it was a great pleasure working with you. I will miss you all.

I would like to extend my thanks to all the students who worked with me, as their MSc thesis. Kuenga, I will never forget the nights we spent together in the *in vitro* lab. Indeed, it was a very heavy work, but you did a fantastic job, well done.

I am deeply indebted to my parents for their support and encouragement that allowed me to pursue my study. Dear father, I would give up all I have to meet you once again to express my sincere thanks to you. I sincerely appreciate my parents-in-law for their kind and supportive attention. I also thank them for looking after my wife and my sons for a while in Iran. I would like to express my appreciation to my brothers, brothers and sisters-in-law for their supports throughout my PhD study. Dear Masoud, what you did for me worth millions of Dollars.

I would like also to express my special gratitude to my Iranian PhD fellows in Wageningen for their invaluable supports and friendship. My special thanks to my colleagues and dear friends, Farhad and Hossein. It is hard to imagine how I could bear to be far from my family without having your accompany. You two, however, must confess that I am the best of us at cooking.

I was blessed very much with few very valuable friendships during my stay in Wageningen. Dear Mahmoud, you and your family were so kind to me and my family that I do not know how to compensate what you did for us. Dear Hadi, I enjoyed a lot being with you and your family. You are not only a great scientist but also a professional baker. If you decide to open a bakery I will be ready to be your assistance provided that you use your famous shear cell to process the dough.

Last and most of all, I offer a special word of thanks to my wife, Shabnam, and our sons, Arsalan and Amir. My darling Shabnam, words can not express my felling to you. You are my dearest and you will be forever. My life had so many ups and downs. When I was down you were the only one who provided me the peace of mind, for that I thank you. My dear sons Arsalan and Amir; when I came home from work absolutely

exhausted, you recharged me with your smiles and sweet words. I doubt if I was able to accomplish this dissertation without having my family beside me. I, therefore, dedicate this book to them.

Arash Azarfar

January 2007

Training and Supervision Plan		**Graduate School WIAS**	
Name	Arash Azarfar		
Group	Animal Nutrition		
Period	March 2003 - January 2007		
Supervisor	Prof. Dr. Ir. S. Tamminga		
Daily supervisors	Dr. Ir. A.F.B. van der Poel, Dr. Ir. H. Boer		
The Basic Package		year	credits *
WIAS Introduction Course (mandatory)		2003	
Course on philosophy of science and ethics (mandatory)		2006	
SUBTOTAL			**3**
Scientific Exposure		year	
International conferences			
55th Annual Meeting of the EAAP in Bled, Slovenia		2004	
56th Annual Meeting of the EAAP in Uppsala, Sweden		2005	
BSAS, York, UK		2005	
31st Meeting NVO, Dutch Speaking Nutrition Researchers, The Netherlands		2006	
ADSA, ASAS, Joint Annual Meeting, Minneapolis, Minnesota, USA		2006	
Seminars and workshops			
WIAS Science Day 2003, 2004, 2005 and 2006		2003-06	
Perennial ryegrass for dairy cows, WIAS Seminar		2005	
Methane emissions in cattles, WIAS Seminar		2005	
Farewell seminar prof. dr. ir. Martin Verstegen		2006	
Presentations			
Oral presentation in 55th Annual Meeting of the EAAP in Bled, Slovenia		2004	
Poster presentation in56th Annual Meeting of the EAAP in Uppsala, Sweden		2005	
Oral presentation in BSAS, York, UK		2005	
Oral presentation in WIAS Science Day		2006	
Oral presentation in ADSA, ASAS, Minneapolis, Minnesota, USA		2006	
SUBTOTAL			**12**
In-Depth Studies		year	
Disciplinary and interdisciplinary courses			
WIAS Debating Course		2003	
Advances in Feed Evaluation Science, Wageningen Business School Course		2006	
Undergraduate courses			
Case studies of Animal Nutrition		2003	
Nutritional Physiology of Farm Animals		2004	
Feed Technology		2003	
Nutrient Dynamics		2004	
SUBTOTAL			**27**
Professional Skills Support Courses		year	
Techniques for writing and presenting a scientific paper		2003	
English language course, upper intermediate, WUR Language Centre		2003	
PhD competence assesment		2004	
Scientific Writing, WUR Language Centre		2006	
SUBTOTAL			**5**
Research Skills Training (apart from carrying out the PhD project, optional)		year	
Preparing own PhD research proposal		2003	
SUBTOTAL			**3**
Didactic Skills Training		year	
Supervising theses			
Romulo Alverado Gomez		2004	
Kuenga Namgay		2006	
Jan Mica		2006	
SUBTOTAL			**6**
Education and Training Total			**56**

* one ECTS credit equals a study load of approximately 28 hours

List of Publications

Peer-reviewed articles:

1. Azarfar A, Tamminga S and Boer H, Effects of washing procedure, particle size and dilution on the distribution between non-washable, insoluble washable, and soluble washable fractions in concentrate ingredients. *J Sci Food Agric* **In press** (2006).

2. Azarfar A, Williams BA, Boer H and Tamminga S, *In vitro* gas production profile and the formation of end products from non-washable, insoluble washable and soluble washable fractions in some concentrate ingredients. *J Sci Food Agric* **In press** (2006).

3. Azarfar A, van der Poel AFB and Tamminga S, The effect of sample grinding procedures after processing on gas production profiles and end-product formation in expander processed barley and peas. *J Sci Food Agric* **In press** (2006).

4. Azarfar A, Namgay K, Pellikaan WF, Tamminga S and van der Poel AFB, *In vitro* gas production profiles and formation of fermentation end-product in processed barley, maize and milo. *Anim Feed Sci Technol* **Submitted** (2006).

5. Azarfar A, Pellikaan WF, Tamminga S and van der Poel AFB, *In vitro* gas production profiles and formation of fermentation end-product in processed peas, lupins and faba beans. *Anim Feed Sci Technol* **Submitted** (2006).

6. Khosravinia H and Azarfar A, Corn-based plant by-products and dried rumen contents as broiler litter under heat stress condition. *Indian Vet J* **83**: 191-195 (2006).

Farsi research papers:

1. Azarfar A and Ghorbani G, *In situ* starch degradability of different varieties of sorghum and barley. *Agricultural Sciences*.

2. Azarfar A, Ghorbani G and Ebadi MR, Utilisation of sorghum varieties in lamb's finishing diets. *Agricultural Sciences*.

About the Author

Arash Azarfar was born in 1969 in Tehran, Iran. He accomplished his high school in biology science in 1987. He started his higher education studies in University of Shahid Chamran (Ahwaz, Iran) and received a BSc degree (with distinguished degree) in the filed of Animal Sciences in 1993. He continued his studies in Tabriz University (Tabriz, Iran) and accomplished his MSc in the same field in 1996. The topic of his master thesis was on the "Utilisation of sorghum varieties in lamb's finishing diets". After completing the master studies, He was appointed as a lecturer at the department of Animal Sciences in University of Lorestan and worked there for 7 years. In 2002, he was awarded a scholarship from the Ministry of Science, Research and Technology (MSRT) of Iran, allowing him to do a PhD in abroad. In March 2003, he started his PhD project in Animal Nutrition Group of Wageningen University, The Netherlands. He is now returning to his duty as an academic staff.

Author's address:

Food Science and Technology Group, College of Agriculture, University of Lorestan, Khorramabad, PO Box 46551664, I.R. Iran. *e-mail:* Arash.Azarfar@gmail.com

This research was financially supported by the Ministry of Science, Research and Technology (MSRT) of Islamic Republic of Iran.

- **ب**دلیل تفاوت در ترکیبات شیمیایی رفتار تجزیه پذیری جز محلول قابل شتشو کاملا" از اجزای غیر قابل شتشو و غیر محلول قابل شتشو متمایز می باشد.

- بدلیل تفاوت در نحوهٔ توزیع اجزای غیر قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشوی مادهٔ خشک ترکیبات تشکیل دهندهٔ کنسانتره ها نحوهٔ تجزیه پذیری آنها در شکمبه با یکدیگر تفاوت دارد.

- تغییرات حاصله در نحوهٔ تجزیه پذیری ترکیبات خوراکی فرآوری شده با روشهای آبی- حرارتی[1] (نظیر اکسپندر کردن) که توام با اعمال نیروهای برشی[2] می باشند عمدتا" ناشی از تغییر در نحوهٔ توزیع اجزای تشکیل دهندهٔ مادهٔ خشک بوده که از نظر ترکیبات شیمیایی، نحوهٔ تجزیه پذیری در شکمبه و میزان عبور از شکمبه متفاوت می باشند.

- با کاهش نسبت پروتیین قابل تجزیه به کربوهیدراتهای قابل تجزیه در شکمبه، فرآیندهای اکسپندر و پلت کردن متعاقب آن سبب تخمیرشکمبه ای مطلوبتری می گردند. بعلاوه این فرآوریها در نتیجهٔ افزایش میزان نشاستهٔ ژلاتینی شده و سرعت نسبی تجزیه پذیری در شکمبه ای برای دام میزبان پیش سازهای گلوکز زای بیشتری فراهم می نمایند.

- نحوهٔ تجزیه پذیری مواد خوراکی فرآوری شده به میزان زیادی بستگی به چگونگی آماده سازی آنها جهت آنالیز شیمیایی، مطالعات بر روی حیوان[3] و در شرایط آزمایشگاهی[4] دارد.

با توجه به نکاتی که در این رساله بدآنها اشاره گردید مطالعات آینده عمدتا" می باید بر چگونگی تجزیهٔ پذیری ترکیبات شیمیایی تشکیل دهندهٔ اجزای غیر قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشو نظیر نشاسته و پروتیین تمرکز نماید.

[1] Hydrothermal
[2] Shear forces
[3] *In situ*
[4] *In vitro*

به سمت یک تخمیر گلوکززا سوق داد. در تمامی غلات تولید آمونیوم و نسبت اسیدهای چرب انشعاب دار[1] به عنوان شاخص میزان پروتئین تجزیه شده در نمونه های اکسپندر و اکسپندر- پلت شده به طور معنی داری(P <۰/۰۵) کمتر از نمونه های آسیاب شده با آسیاب رچ بود. در حالیکه در لگومها تنها میزان آمونیوم تولیدی به طور معنی دار (P <۰/۰۵) کمتر بود.

هدف **فصل ٥** بررسی تاثیر روشهای متفاوت آسیاب کردن و آماده نمودن نمونه های فرآوری شده مواد کنسانتره ای بر نحوهٔ تجزیه پذیری آنها بود. آسیاب کردن فرآوری است که به طور گسترده در ساخت مواد خوارکی مورد استفاده قرار گرفته و همچنین روشی است که از آن جهت تهیهٔ نمونه های آزمایشگاهی (به عنوان مثال تهیه نمونه های مورد استفاده در روش تولید گاز در شرایط آزمایشگاهی) استفاده می شود. آسیاب کردن مواد خوراکی که تحت یک فرآوری تکنولوژیکی به غیر از آسیاب کردن قرار گرفته اند هم در شرایط عملی و هم در هنگام تهیهٔ نمونه های آزمایشگاهی ممکن است سبب تخریب اثر فرآوری مورد نظر گردد. به همین دلیل نمونه های اکسپندر شده جو و نخود توسط شش روش متفاوت (نمونهٔ دست نخورده، نمونهٔ حل شده، آسیاب کردن مرحله ای نمونه توسط الکهای ٦ و ٣ میلیمتری، آسیاب کردن مرحله ای نمونه توسط الکهای ٦، ٣ و ١ میلیمتری، آسیاب کردن مرحله ای نمونه توسط الکهای ٦ و ١ میلیمتری، آسیاب کردن نمونه توسط الک ٣ میلیمتری و آسیاب کردن نمونه توسط الک ١ میلیمتری) آماده گردیدند. نحوهٔ توزیع اندازه ذرات نمونه های آسیاب شده توسط روشهای الک کردن خشک[2] و شمارشگر کولتر[3] ارزیابی شد. نتایج روش الک خشک و شمارشگر کولتر در ارتباط با نحوهٔ پراکنش ذرات نمونه های آسیاب شده نشانگر آن بود که روشهای متفاوت آسیاب کردن نحوهٔ پراکنش ذرات را تغییر می دهد. الگوی تولید گاز در این نمونه ها با استفاده از سیستم تولید گاز خودکار در شرایط آزمایشگاهی در طول یک دورهٔ انکوباسیون ٧٢ ساعته بررسی شد. آسیاب کردن نمونه های جو و نخود اکسپندر شده کینتیک تولید گاز را تغییر داده و منجر به تخمیر سریعتری شد که در نمونه های آسیاب شده در چند مرحله مشهودتر بود. در صورتیکه تولید فرآورده های نهایی تخمییر تحت تاثیر شیوهٔ تهیهٔ نمونه قرار نگرفت. در جو اکسپندر شده تفاوت در نحوهٔ تجزیه پذیری در اثر اعمال روشهای متفاوت آسیاب کردن بسیار ناچیز بود.

در **فصل ٦** یافته های سایر فصلها به عنوان بحث عمومی مرور گردید. پایان نامه حاضر منتج به نتایج ذیل گردید:

[1] Branched chain ratio
[2] Dry sieve analysis
[3] Coulter counter

در **فصلهای ۳ و ٤** با استفاده از روش تولید گاز در شرایط آزمایشگاهی اثرات استفاده از اکسپندر[1] به تنهایی یا با تلفیقی از فرآیند پلت کردن بر نحوهٔ تجزیه پذیری جو، ذرت، سورگوم، نخود، باقلا و لوبیا و اجزای آنها بررسی گردید. هدف از این مطالعه بررسی چگونگی نحوهٔ تاثیر فرآوریهای ذکر شده بر نحوهٔ توزیع اجزای تشکیل دهندهٔ مادهٔ خشک (غیر قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشو)، تغییرات حاصله در ترکیبات شیمیایی و نحوهٔ تجزیه پذیری اجزای تشکیل دهندهٔ مادهٔ خشک مواد مورد استفاده در کنسانتره ها بود. نمونه های مورد استفاده به اجزای غیر قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشو تجزیه گردید. نمونه های سه جز تشکیل دهندهٔ مادهٔ خشک مواد کنسانتره ای با سه روش متفاوت (آسیاب کردن با آسیاب رچ[2]، اکسپندر و اکسپندر- پلت کردن) فرآوری گردید و نحوهٔ تخمیر آنها با استفاده از روش تولید گاز در شرایط آزمایشگاهی مورد بررسی قرار گرفت. با استفاده از سیستم تخمیر غیر خودکار در شرایط آزمایشگاهی نمونه ها بمدت ٦، ١٢، ٢٤ و ٤٨ ساعت تخمیر شده ونمونه های مورد نیاز جهت آنالیز اسیدهای چرب فرار و آمونیوم گردآوری گردید. جز غیر محلول قابل شتشو در غلات، نخود و لوبیا غنی از نشاسته بود. در تمام ترکیبات کنسانتره ای تحت مطالعه جز محلول قابل شتشو حاوی مقادیر قابل توجه ای از خاکستر، پروتیین خام، قندهای محلول و جز باقیمانده ای (غیر قابل شناسایی با روشهای متداول شیمیایی، ٣/٧ تا ٤٢٪ مادهٔ خشک) بود که میزان آن توسط اکسپندر و اکسپندر- پلت کردن افزایش (به استثنای باقلا) یافت. در مقایسه با آسیاب کردن با آسیاب رچ، فرآیند اکسپندر در ذرت و سورگوم به طور معنی داری (در جو از نظر عددی) میزان جز غیر قابل شتشو را افزایش داد در حالیکه میزان این جز با پلت کردن متعاقب اکسپندر نمودن مجددا" کاهش یافت. اکسپندر و اکسپندر- پلت کردن در نخود و لوبیا در مقایسه با آسیاب کردن با آسیاب رچ به طور معنی داری جز غیر قابل شتشو را افزایش داد. جز محلول قابل شتشوی غلات در نمونه های فرآوری شده توسط اکسپندر به طور معنی داری کمتر از نمونه های فرآوری شده با آسیاب رچ بود. جز محلول لگومهای فرآوری شده توسط اکسپندر و دستگاه پلت کننده کمتر از نمونه های آسیب شده توسط آسیاب رچ بود. پروفیل های گاز تولیدی با استفاده از یک مدل دو فازی برازش گردید. در غلات حداکثر میزان تجزیه پذیری نسبی[3] و تولید گاز[4] بیشتر نمونه های اکسپندر شده در مقایسه با سایر نمونه ها (P <۰/۰۵؛ در ذرت از نظر عددی بالاتر) در فاز اول تخمیر نشانگر آن بود که اکسپندر- پلت کردن در مقایسه با سایر روشهای فرآوری سبب تخمیر سریعتر نمونه ها شد. با توجه به نسبت کمتر اسیدهای چرب غیر گلوکز زا به گلوگز زا در جو، ذرت، باقلا و لوبیا، فرآیند اکسپندر و اکسپندر- پلت کردن الگوی تخمیر را

[1] Expander
[2] Retsch mill
[3] Maximum fractional rate of substrate degradation
[4] Maximum rate of gas production

قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشو با روش کیسه های نایلونی، میلین، میلین اصلاح شده و یانگ مورد مقایسه قرار گرفت. تفاوتها و شباهتهای میان روشهای ذکر شده در جدول ۱ نشان داده شده است. نتایج نشان داد روش آآ۲۰ بهترین روش بوده و به همین دلیل به عنوان روش استاندارد انتخاب گردید. همچنین در این مطالعه اثر رقتهای متفاوت بر اندازهٔ جز محلول قابل شتشو و میزان پروتیین حقیقی محلول در شش کنسانتره بررسی گردید. اثرات نوع دانه، روش شتشو، اندازهٔ ذرات و اثرات متقابل نوع دانه و اندازهٔ ذرات، نوع دانه و روش شتشو، اندازهٔ ذرات و روش شتشو بر اندازهٔ اجزای غیر قابل شتشو و غیر محلول قابل شتشو معنی دار بود. در حالیکه اثر متقابل نوع دانه، روش شتشو و اندازهٔ ذرات معنی دار نبود. اندازهٔ جز غیر قابل شتشو در روش یانگ از سایر روشها کمتر بود. نتایج نشانگر آن بود که به استثنای باقلا اندازهٔ جز غیر قابل شتشو در غلات به طور معنی داری از لگومها بیشتر بود. افزایش اندازهٔ ذرات به طور معنی داری اندازهٔ جز غیر قابل شتشو را افزایش داده در حالیکه باعث کاهش اندازهٔ جز غیر محلول قابل شتشو گردید. اندازهٔ جز محلول قابل شتشو در لگومها از غلات بیشتر بود. افزایش رقت در لگومها میزان پروتیین حقیقی محلول را افزایش داد در حالیکه در غلات اثری بر آن نداشت.

در **فصل ۲** نحوهٔ تجزیه پذیری و تولید فرآورده های نهایی حاصل از تخمیر اجزای غیر قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشوی شش مادهٔ خوراکی مورد استفاده در کنسانتره ها شامل جو، ذرت، نخود زرد، باقلا و لوبیای قهوه ای دانه گرد بررسی گردید. روش شتشوی ابداعی در **فصل ۱** با روش اندازه گیری خودکار گاز و تولید اسیدهای چرب فرار تلفیق گردید و جهت مطالعهٔ نحوهٔ تجزیه پذیری اجزای مادهٔ خشک و بررسی صحت این فرضیه که اجزای قابل شتشو به طور کامل و بسیار سریع در شکمبه تجزیه می گردند مورد استفاده قرار گرفت. با استفاده از سیستم غیر خودکار تخمیر در شرایط آزمایشگاهی، همان مواد مورد استفاده در سیستم خودکار جهت گردآوری نمونه های مورد نیاز اندازه گیری اسیدهای چرب فرار و آمونیوم به مدت ۰، ۳، ۶، ۱۲، ۲۴، ۴۸ و ۷۲ ساعت تخمیر شدند. به استثنای باقلا، جز غیر محلول قابل شتشو در تمام مواد مورد مطالعه غنی از نشاسته بود. جز محلول قابل شتشو بطورنسبی غنی از خاکستر، پروتیین خام، قندهای محلول، و یک جز باقیمانده (با روشهای متداول شیمیایی شناسایی نگردید)بود در حالیکه این بخش حاوی مقادیر بسیار اندکی از نشاسته بود. خصوصیات تخمیری جز غیر محلول قابل شتشو شباهت بیشتری به کل مواد کنسانتره ای و جز غیر قابل شتشو داشت تا جز محلول قابل شتشو. میزان گاز تولیدی ناشی از تخمیر جز محلول قابل شتشو به طور قابل ملاحظه ای از سایر اجزا کمتر بود. در مرحلهٔ نخست تخمیر جز محلول قابل شتشو، یک جز بسیار سریع التخمیر مشاهده گردید. از این بخش نتیجه گیری گردید که پروفیل اسیدهای چرب فرار ناشی از تخمیر اجزای متفاوت مادهٔ خشک با یکدیگر تفاوت داشته و در طول مدت تخمیر تغییر می یابند.

جدول ۱. روشهای شتشو جهت شبیه سازی شتشو در روش کیسه های نایلونی

روش	وزن نمونه (گرم)	رقت[۱]	اول مرحله	دوم مرحله (WF)[۲]	سوم مرحله (NWF)[۳]	چهارم مرحله (ISWF)[۴]	پنجم مرحله (SWF)[۵]
کیسه های نایلونی	۵	بیشتر از ۱۰۰	شتشو با ماشین لباسشویی	تراوش کردن	سانتریفوژ کردن (۲۰ دقیقه، ۷۵۰ جی)	فیلتر کردن	مایع فیلتر شده
یانگ	۱۰۰	۳۰	خارج از فرو بردن و خارج نمودن از آب توسط یک میله مرکز				
میلین	۳	۲۵	حل نمودن نمونه توسط آب در بشر، تکان دادن به مدت یک ساعت در ۱۵۰ دور در دقیقه	پارچه فیلتر کردن بوسیله نایلونی بر روی قیف	سانتریفوژ کردن (۲۰ دقیقه، ۷۵۰ جی)	فیلتر کردن	مایع فیلتر شده
میلین توسعه یافته	۳						
آآ۱۰[۶]	۵/۵	۱۰	شتشوی مداوم کیسه های نایلونی به مدت یک ساعت درون بشر سانتریفوژ در ۱۵۰ دور در دقیقه		سانتریفوژ کردن (۲۰ دقیقه، ۷۵۰ جی)	فیلتر کردن	مایع فیلتر شده
آآ۲۰[۶]	۵/۵	۲۰					
آآ۳۰[۶]		۳۰					

[۱] نسبت آب مصرفی به وزن نمونه (میلی لیتر به ازای هر گرم). [۲] جز قابل شتشو. [۳] جز غیر قابل شتشو. [۴] جز غیر محلول قابل شتشو. [۵] جز محلول قابل شتشو. [۶] روش آآ در رقتهای ۱۰، ۲۰ و ۳۰.

خلاصه

امروزه مواد خوراکی مورداستفاده در جیرهٔ نشخوارکنندگان نه تنها بر اساس ترکیبات شیمیایی و قابلیت هضم، بلکه بدلیل ماهیت متفاوت تجزیه پذیری و میزان عبور از شکمبه بر اساس نحوهٔ توزیع اجزای غیر قابل شتشو[1]،غیر محلول قابل شتشو[2] و محلول قابل شتشو[3] ارزشیابی می گردند. نحوهٔ توزیع این اجزا در مادهٔ خشک مواد خوراکی، ترکیبات شیمیایی، میزان تجزیه پذیری و میزان عبور آنها از شکمبه نقش بسیار مهمی در تامین نیازهای رشد و نگهداری میکروارگانیزمهای شکمبه و دام میزبان به مواد مغذی دارد. روش کیسه های نایلونی[4] از متداولترین روشها جهت اندازه گیری میزان تجزیه پذیری و دینامیک هضم مواد خوراکی در شکمبه می باشد. بدلیل اینکه اجزای غیر محلول قابل شتشو و محلول قابل شتشو به هنگام شتشو از کیسه های نایلونی عبور می کنند، اندازه گیری میزان هضم شکمبه ای آنها با این روش امکان پذیر نمی باشد. به همین دلیل می باید از روشهای دیگری جهت مطالعهٔ رفتار تجزیه پذیری آنها در شکمبه بهره جست. جز قابل شتشو[5] بخش قابل توجه ای از مادهٔ خشک (تا ۵۰ درصد مادهٔ خشک)مواد خوراکی را تشکیل می دهد. بنا بر این ارزشیابی نحوهٔ تجزیه پذیری آن در شکمبه از اهمیت قابل توجه ای برخوردار است.دراین رساله نحوهٔ تجزیه پذیری برخی از مهمترین کنسانتره ها و اجزای تشکیل دهندهٔ آنها مورد بررسی قرار گرفت. جهت نیل به این هدف از روشی که مواد خوراکی را به اجزای غیر قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشو تجزیه نموده همراه با روش اندازه گیری تولید گاز[6] استفاده گردید.

هدف **فصل ۱** ابداع روشی آزمایشگاهی بود که نتایج روش متدوال شتشو را در روش کیسه های نایلونی شبیه سازی می نماید. اثرات شیوهٔ شتشو،اندازهٔ ذرات و رقت (نسبت آب مصرفی به وزن نمونه) بر نحوهٔ توزیع اجزای غیر قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشو مورد مطالعه قرار گرفت.اثرات سه روش متفاوت شتشو(یانگ، میلین و کیسه های نایلونی) بر نحوهٔ توزیع مادهٔ خشک در میان اجزای غیر قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشو شش نوع کنسانتره (جو، ذرت، سورگوم، نخود، باقلا و لوبیا) آسیاب شده توسط دو اندازهٔ متفاوت الک(۱و۳ میلیمتر) مورد بررسی قرار گرفت.روش میلین متعاقبا" با کاهش میزان رقت توسعه یافت و اثر آن بر میزان اجزای غیر قابل شتشو، غیر محلول قابل شتشو و محلول قابل شتشو با سایر روشها مورد مقایسه قرار گرفت. در نهایت روش جدیدی جهت شتشوی کیسه های نایلونی ارائه گردید(روش آآ)واثرآن در رقتهای متفاوت بر اندازهٔ اجزای غیر

[1] Non-washable fraction
[2] Insoluble washable fraction
[3] Soluble washable fraction
[4] *In situ*
[5] Washable fraction
[6] *In vitro* gas production technique

تقدیم به :

همسر عزیزم شبنم

و

پسران دلبندم ارسلان و امیر

اجزای خوراک نشخوارکنندگان: رفتار تجزیه پذیری در شرایط آزمایشگاهی

آرش آذرفر

زمستان 1385

پایان نامه دوره دکتری تخصصی

گروه تغذیه دام
دانشکده علوم دامی
دانشگاه واگنینگن
هلند


بسم الله الرحمن الرحیم

Design and lay out: by the author

Printing:

Ponsen & Looijen BV Grafischbedrijf, Postbus 68, 6700 AB, Wageningen, the Netherlands