IMPACT OF NUTRITIONAL FACTORS ON EATING BEHAVIOR AND FEATHER DAMAGE OF LAYING HENS

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IMPACT OF NUTRITIONAL FACTORS ON EATING BEHAVIOR AND FEATHER DAMAGE OF LAYING HENS

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Abstract

Feather pecking remains one of the major problems facing the poultry industry. It is assumed that feather pecking behavior is a substitute for normal ground pecking or feeding behavior in the absence of adequate foraging incentives. This justifies a nutritional approach of this problem. Although energy and Non Starch Polysaccharides (NSP) concentrations and particle size of the added NSP source seem to reduce feather pecking behavior in laying hens, these nutritional factors were often confounded in experimental diets. Consequently, it's not clear which factor is most effective in causing these positive effects. In this project, the relationships between feed characteristics – satiety/ development of gut segments – feeding related behavior – feather pecking behavior are studied.

The objectives of the present study were:

1. To review the impact of nutritional factors and feeding strategies on feather pecking behavior in laying hens.
2. To determine the independent effects of nutrient density, NSP concentration, and particle sizes of NSP on eating behavior, feather pecking, performance and digesta mean retention time in laying hens between 18 and 40 wk of age.
3. To investigate the carry-over effects of nutrient density and NSP concentration in rearing diets on eating behavior, feather pecking and performance in laying hens.

From This study it could be concluded that increasing feeding related behavior and satiety by dietary manipulation are successful strategies in preventing feather pecking behavior, as long as this behavior is not developed in an earlier stage. In laying hens, nutrient dilution and addition of (coarse) insoluble NSP increase feeding related behavior, as expressed by prolonged eating time and decreased eating rate. Providing 15% diluted diets to rearing hens results in less feather damage during the laying period. Although dilution of the rearing diet does not prolong eating time in this stage, this might stimulate imprinting of pecks on feed, rather than on feathers of flock mates. Feeding related behavior and satiety of laying hens are mostly affected by eating diets with a high insoluble NSP content. Additive effects, however, are found if dietary energy content is reduced and the NSP source is coarsely ground. The most perspective feeding strategy to prevent feather damage is the supply of a 15% diluted diet during the rearing period, followed by a 10% diluted – coarsely ground – high NSP diet during the laying period.

Keywords: Dietary dilution, Energy concentration, NSP concentration, Particle sizes, Rearing Hens, Laying hens, Feather damage, Nutrition.

CONTENTS

Chapter 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Feather pecking remains one of the major problems facing the poultry industry because it is a significant welfare insult for the hens, an economic burden for the farmer, and a pressing societal concern. Hens peck and pull at the feathers of conspecifics, causing damage to the plumage and loss of feathers. This adversely affects the costs of egg production, since loss of feathers results in increased feed intake, feed conversion, and feeding costs (Leeson and Morrisson, 1978; Tullet et al., 1980; Keeling et al., 1988; Herremans et al., 1989; Peguri and Coon, 1993). Furthermore, feather pecking, which is painful in itself (Gentle and Hunter, 1990), can cause injury and bleeding, thereby increasing the risk of cannibalism (Allen and Perry, 1975; Hughes, 1982; Blokhuis et al., 2000). Thus, apart from these serious economic losses, there is also an ethical aspect to the problem. Clearly, feather pecking is extremely detrimental to the welfare of the birds.

Beak trimming is a common and effective precautionary measure practiced by poultry farmers to prevent serious feather damage and mortality. However, it has associated welfare problems. Beak trimming can cause acute and chronic pain (Gentle, 1986), and it has therefore already been prohibited in several European countries, such as Norway, Sweden, and Switzerland. In the Netherlands beak trimming is expected to be banned from 2011.

In 2012, changes in EU-legislation with regard to animal welfare and husbandry will be implemented that might increase the risk of feather pecking in layers. These changes include the ban on beak trimming and a ban on the use of traditional battery cages. The latter ban results from a societal debate that led to the conclusion that battery cages could not fulfill the birds' need to express their natural behavior. Clearly, it is imperative that we develop viable alternative housing systems for layers, e.g., free range or aviaries. However, a widespread introduction of such alternatives, specifically designed with the aim to improve poultry welfare, is hampered by the increased likelihood of outbreaks of feather pecking and cannibalism (Appleby and Hughes, 1991; Gunnarsson et al., 1999; Jones, 2001; Blokhuis et al., 2007). This may occur because the presence of a few feather peckers in a free housing system has a much greater impact because larger numbers of potential victims are present (Allen and Perry, 1975). Feather pecking in layers is still a very dominant welfare problem in non-cage housing systems. Results of an epidemiological survey in twenty-five commercial flocks showed that 40% developed already considerable frequencies of feather pecking by wk 5, which was risen to 77.3% at 14 wk of age (Huber Eicher and Sebo, 2001). Feather pecking became more damaging with increasing age of the birds. At 50 wk of age, 71% of birds had damaged tail feather. However, additional factors may include greater exposure to a wider range of stressors, increased difficulty of identifying the peckers and containing the problem, and higher levels of illumination. Despite more than 25 years of research and many efforts in practice to alleviate feather pecking, an adequate solution to this problem has not yet been found. This strongly suggests that this type of abnormal behavior cannot be completely prevented by simply changing the environment of a group of birds. Feather pecking in layers is a multi factorial problem, with a complex interaction between influential internal and external variables (Hughes and Duncan, 1972; Blokhuis and Van der Haar, 1989; Nørgaard-Nielsen et al., 1993; Leonard et al., 1995; Huber Eicher and Audige, 1999; Jones and Hocking, 1999; Nicol et al., 2001; Kjaer and Hocking, 2004).

Jungle fowl that are housed in a semi-wild environment have a high foraging motivation, spending 60% of their active time on feeding related behaviors, like eating and foraging (Table 1) (Dawkins, 1989). Feeding related behavior, however, is significantly reduced in laying hens housed in modern housing systems (Hansen, 1994).

Table 1. Time budgets (% of observations) of Red Jungle fowl in a semi-wild environment, and laying hens housed in cages and aviaries; adapted from Dawkins (1989) and Hansen (1994).

	Jungle fowl (Semi-wild)	Laying hens (Cage)	Laying hens (Aviary)
Feeding related behavior (%)	60	41[1]	37[1]
Non-feeding related behavior (%)	40	56[2]	59[2]

[1] Sum of walking, drinking, food pecking, and object pecking

[2] Sum of lying, standing/sitting, and comfort behavior

Feeding related behavior in modern laying hens, however, is strongly affected by housing and management conditions. Table 2 showed that feeding related behavior of laying hens housed in floor pens varied from 29% in hens that were fed pelletised feed without access to straw to 52% in hens that were fed mash with access to long-cut straw as foraging material (Aerni et al., 1999). These results confirm that the behavior of the domesticated laying hens is still very similar with that of their ancestors (Dawkins, 1989), but that the motivation to perform feeding related behavior could be reduced in the absence of the right stimuli. Furthermore, more feather pecking behavior was observed in hens that performed low levels of feeding related behavior (Table 2).

Table 2. Time budgets (% of observations) of laying hens in dependence of feed form and litter substrate housed in floor pens; adapted from Aerni et al. (1999).

Feed Form Litter substrate	Mash Straw	Pellets Straw	Mash No straw	Pellets No straw
Feeding related behavior (%)	52	48	41	29
Non-feeding related behavior (%)	48	52	59	71
Time spend feather pecking (%)	2	3	6	41

Feather pecking behavior has been hypothesized to arise from ground pecking behavior or feeding behavior that is redirected towards feathers in the absence of adequate foraging incentives (Hoffmeyer, 1969; Blokhuis, 1986). The level of feather pecking is inversely related to the time spent feeding and foraging (Huber Eicher and Wechsler, 1998b; Huber Eicher and Wechsler, 1998a; El lethey et al., 2001). Another hypothesis stated that redirected ground pecking behavior is associated with dust bathing motivation (Vestergaard and Lisborg, 1993). Indeed, the provision of high quality litter (i.e. dry and loose), preferably enriched with grains increased the time spent foraging and feeding, and as a consequence diminished the level of feather pecking (Blokhuis, 1991; Huber Eicher and Wechsler, 1998b; Huber Eicher and Wechsler, 1998a). Furthermore, the ontogeny of feather pecking behavior in mature hens seems to be influenced by early life experiences and rearing conditions. For instance, giving chicks appropriate substrates for feeding and foraging behavior, early in life, learns the birds to direct their pecks to the right materials and reduces the likelihood that they will perceive their companions' feathers as a suitable substrate for foraging or feeding (Blokhuis and Van der Haar, 1992; Chow and Hogan, 2005). But also the development of the digestive tract during the rearing period, by appropriate nutritional strategies, that results in an appropriate volume and digestive capacity of the gut at the beginning of lay, is thought to be of great importance in determining the expression of feather pecking behavior during the laying period (Hadorn and Wiedmer, 2001).

INCREASING FEEDING RELATED BEHAVIOR: ENERGY DILUTION AND NSP CONCENTRATION

Feed intake levels of laying hens may vary tremendously. Hens that were fed up to 30% diluted diets showed also up to 30% higher feed intake, resulting in a similar energy intake compared to hens fed the undiluted diets (Van der Meulen et al., 2006). Although it's generally accepted that feed intake capacity of modern layer strains is often not in balance with their energy needs, these findings showed that feed intake capacity of the used strains was not the factor that limits feed intake. Decreasing the dietary energy content of layer diets reduced mortality due to feather pecking and significantly improved feather condition (Elwinger, 1981; Van der Lee et al., 2001). Laying hens that were fed diluted diets will compensate for it by a higher feed intake, resulting in a similar energy intake, compared to hens fed a standard energy diet. Dilution levels up to 30%, by adding 30% sand to a control diet, were fully compensated by a higher feed intake (Van der Meulen et al., 2006).

A number of reports suggested that the addition of NSP to the diets of laying hens might reduce feather pecking and cannibalism (Miller and Bearse, 1937; Hughes, 1982; Aerni et al., 2000; El Lethey et al., 2000; Hartini et al., 2002; Hetland et al., 2004). Birds fed diets high in insoluble NSP spent more time feeding and appeared calmer than those fed low-NSP diets (Hetland and Choct, 2003). Insoluble NSP accumulates in the gizzard and is retained longer than other nutrients, probably because it has to be ground to a critical particle size before entering the small intestine (Hetland et al., 2002; Hetland et al., 2003a). Such accumulation of NSP in the gizzard may also indicate that increased levels of coarse NSP in the diet might lead to a slower rate of feed passage. It can be hypothesized that chickens prefer coarse NSP because when given the opportunity, birds fed low NSP diets will search for coarse materials (Hetland et al., 2003b).

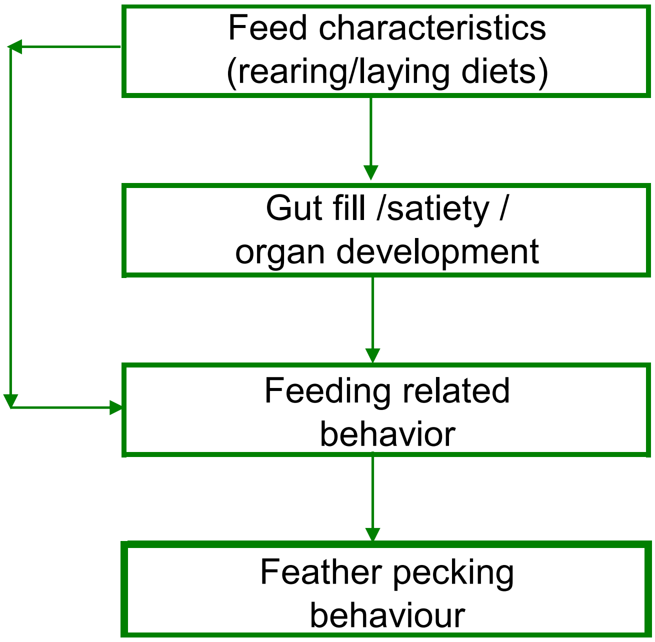
SCOPE OF THE STUDY

The assumption that feather pecking behavior is a substitute for normal ground pecking or feeding behavior in the absence of adequate foraging incentives justifies a nutritional approach of this problem. It is hypothesized here, that nutritional factors may reduce feather pecking behavior if these factors increase:

1) the time hens are spending on feeding related behavior, or
2) the (temporary) level of satiety by affecting retention time of digesta in the gut.

Increasing the amount of time laying hens are spending on exploratory and foraging behavior were found to be inversely related to the development of feather pecking behavior (Huber Eicher and Wechsler, 1998a). It is thought that accumulation of insoluble fiber in the gizzard triggers a temporary satiety, thereby reducing feather pecking behavior (Hetland and Choct, 2003). But once passed the gizzard, it passes through the gut rather quickly. Rearing conditions during the first 4 weeks of life have a major influence on the subsequent development of feather pecking in laying hens (Johnsen et al., 1998). Therefore, more measures are recommended to satisfy the needs of pullets in food-searching and food-ingestion to prevent feather-pecking in adult birds. Although energy and NSP concentrations and particle size of the added NSP source seem to reduce feather pecking behavior in laying hens, these nutritional factors were often confounded in experimental diets (Savory, 1980; Elwinger, 1981; Valkonen et al., 2008). Consequently, it's not clear which factor is most effective in causing these positive effects. In this project, the relationships between feed characteristics – satiety/development of gut segments – feeding related behavior – feather pecking behavior are studied (Figure 1).

Figure 1. Possible pathways between feed characteristics, satiety/development of gut segments, feeding related behavior, and feather pecking.



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3. To investigate the carry-over effects of nutrient density and NSP concentration in rearing diets on eating behavior, feather pecking and performance in laying hens.

The overall aim of the project is to provide the basis for the development of a new feeding strategy that meet the requirements of the modern layer breeds and help to solve the feather pecking problem in current husbandry systems.

OUTLINE OF THE THESIS

This thesis provides a literature review and describes the results of three experiments in which different nutritional factors were tested in hens of varying ages. For essential nutrients, like amino acids, minerals and vitamins, nutrients to energy ratio's were similar in all diets within an experiment. All experiments were carried out with ISA Brown hens.

Chapter 2 discusses the relative importance of specific deficiencies in layer diets, as well as the effectiveness and possible modes of action of certain nutritional factors and feeding strategies on feather pecking behavior in laying hens. From this review study it was decided to focus the following experiments on the effects of nutrient dilution, NSP concentration and particle sizes of NSP.

In Chapter 3, the effects of dietary dilution, particle sizes of NSP, and feed form on feed intake, eating behavior and performance of laying hens at early lay were investigated. Diets were diluted by low NSP (sand and grit), or by high NSP sources (oat hulls, straw, soya hulls, cellulose fiber, beet pulp, and sunflower meal). The high NSP sources differed in water solubility of the NSP fraction, which may affect feed intake, feeding related behavior, viscosity of the digesta and feed passage rate. Control diets and sand diluted diets were provided both in mash and crumble. Differences in particle sizes were created by adding finely ground versus whole oat hulls. Based on the results of Chapter 3, in the further experiments (Chapters 4 to 6) sand was chosen as dilution source in low NSP diets, whereas oat hulls were chosen to increase the NSP concentration.

Therefore, Chapter 4 describes an experiment in which the separate effects of energy concentration, NSP concentration and particle size of added NSP source on eating behavior, feather pecking behavior and hen performance of laying hens were investigated.

Chapter 5 provides mean retention time per gut segment (crop, proventriculus/ gizzard, small intestine, colon and caeca) that was determined by use of titanium capsules of diets varying in dilution level, NSP concentration and particle sizes of NSP. In this chapter, the weights and contents of the different gut segments are also presented.

To investigate the carry-over effects of nutrient density and NSP concentration in rearing diets on eating behavior, feather pecking and performance in laying hens, an experiment was performed (Chapter 6). In this experiment different levels of nutrient dilution and NSP concentration in rearing and laying diets were applied. Feed intake, eating behavior, feather pecking and development of gut segments in rearing and laying hens were measured.

In the General Discussion, the results reported in the Chapters 2-6 are discussed and evaluated with respect to theories on feather pecking behavior and nutrition. Prospects for further research are suggested. Practical implications for feeding strategies of rearing and laying hens, which could prevent feather pecking behavior, are presented.

REFERENCES

Aerni, V., H. El Lethey and B. Wechsler. 1999. Feather pecking by laying hens: effects of foraging material and food form. *Fachgruppe Verhaltensforschung vom 12. bis 14. November 1998 in Freiburg Breisgau. KTBL Schrift*:63-69.

Aerni, V., H. El Lethey and B. Wechsler. 2000. Effect of foraging material and food form on feather pecking in laying hens. *Br. Poult. Sci.,* 41:16-21.

Allen, J. and G. C. Perry. 1975. Feather pecking and cannibalism in a caged layer flock. *British Poultry Science,* 16:441-451.

Appleby, M. C. and B. O. Hughes. 1991. Welfare of laying hens in cages and alternative systems: environmental, physical and behavioural aspects. *World's Poultry Science Journal,* 47:109-128.

Blokhuis, H. J. 1986. Feather-pecking in poultry: its relation with ground-pecking. *Applied Animal Behaviour Science,* 16:63-67.

Blokhuis, H. J. 1991. Effects of rearing conditions on feather pecking in laying hens. *Applied Animal Behaviour Science. 1991, 30: 1 2, 186; 2 ref; Proceedings of the Summer Meeting of the Society for Veterinary Ethology, Montecatini Terme, Pistoia, Italy, (17 19 May 1990).*

Blokhuis, H. J. and J. W. Van der Haar. 1989. Effects of floor type during rearing and of beak trimming on ground pecking and feather pecking in laying hens. *Appl. Anim. Behav. Sci.,* 22:359-369.

Blokhuis, H. J. and J. W. Van der Haar. 1992. Effects of pecking incentives during rearing on feather pecking of laying hens. *British Poultry Science,* 33:17-24.

Blokhuis, H. J., E. D. Ekkel, S. M. Korte, H. Hopster and C. G. v. Reenen. 2000. Farm animal welfare research in interaction with society. *Veterinary Quarterly,* 22:217-222.

Blokhuis, H. J., T. Fiks-Van Niekerk, W. Bessei, A. Elson, D. Guémené, J. Kjaer, G. A. Maria Levrino, C. J. Nicol, R. Tauson, C. A. Weeks and H. A. Van de Weerd. 2007. The LayWel project: welfare implications of changes in production systems for laying hens. *World's Poult. Sci. J.,* 63:101-114.

Chow, A. and J. A. Hogan. 2005. The development of feather pecking in Burmese red junglefowl: the influence of early experience with exploratory-rich environments. *Appl. Anim. Behav. Sci.,* 93:283-294.

Dawkins, M. S. 1989. Time Budgets in Red Junglefowl as a Baseline for the Assessment of Welfare in Domestic-Fowl. *Appl. Anim. Behav. Sci.,* 24:77-80.

El lethey, H., T. W. Jungi and B. Huber Eicher. 2001. Effects of feeding corticosterone and housing conditions on feather pecking in laying hens (Gallus gallus domesticus). *Physiology and Behavior,* 73:243-251.

El Lethey, H., V. Aerni, T. W. Jungi and B. Wechsler. 2000. Stress and feather pecking in laying hens in relation to housing conditions. *Br. Poult. Sci.,* 41:22-28.

Elwinger, K. 1981. Different energy levels and restricted feeding to three strains of SCWL hybrids. 1. Effects on egg production. *Swedish J. Agric. Res.,* 11:149-157.

Gentle, M. J. 1986. Neuroma formation following partial beak amputation (beak trimming) in the chicken. *Research in Veterinary Science,* 41:383-385.

Gentle, M. J. and L. N. Hunter. 1990. Physiological and behavioural responses associated with feather removal in *Gallus gallus var domesticus*. *Research in Veterinary Science,* 50:95-101.

Gunnarsson, S., L. J. Keeling and J. Svedberg. 1999. Effect of rearing factors on the prevalence of floor eggs, cloacal cannibalism and feather pecking in commercial flocks of loose housed laying hens. *British Poultry Science,* 40:12-18.

Hadorn, R. and H. Wiedmer. 2001. Effect of whole wheat feeding and different aviary systems on the development of white and brown growing pullets. *Proceedings of the 6th European symposium on poultry welfare.*, Eds.: H. Oester &C. Wyss.

Hansen, I. 1994. Behavioral Expression of Laying Hens in Aviaries and Cages - Frequencies, Time Budgets and Facility Utilization. *British Poultry Science,* 35:491-508.

Hartini, S., M. Choct, G. Hinch, A. Kocher and J. V. Nolan. 2002. Effects of light intensity during rearing and beak trimming and dietary fiber sources on mortality, egg production, and performance of ISA brown laying hens. *J. Appl. Poult. Res.,* 11:104-110.

Herremans, M., E. Decuypere and O. Siau. 1989. Effects of feather wear and temperature on prediction of food intake and residual food consumption. *British Poultry Science,* 30:15-22.

Hetland, H. and M. Choct. 2003. Role of insoluble non-starch polysaccharides in poultry nutrition. *Proceedings of the 14 th European Symposium of Poultry Nutrition*, Lillehammer, Norway, 64-69.

Hetland, H., B. Svihus and V. Olaisen. 2002. Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *Br. Poult. Sci.,* 43:416-423.

Hetland, H., B. Svihus and A. Krogdahl. 2003a. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *Br. Poult. Sci.,* 44:275-282.

Hetland, H., M. Choct and B. Svihus. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poult. Sci. J.,* 60:415-422.

Hetland, H., B. Svihus, S. Lervik and R. Moe. 2003b. Effect of feed structure on performance and welfare in laying hens housed in conventional and furnished cages. *Acta Agric. Scand., Section A, Animal Science,* 53:92-100.

Hoffmeyer, I. 1969. Feather pecking in pheasants - an ethological approach to the problem. *Danish review of game biology,* 6:1-36.

Huber Eicher, B. and B. Wechsler. 1998a. The effect of quality and availability of foraging materials on feather pecking in laying hen chicks. *Animal Behaviour,* 55:861-873.

Huber Eicher, B. and B. Wechsler. 1998b. The influence of quality and availability of foraging material on the development of feather pecking in domestic chicks. *KTBL Schrift*:21-29.

Huber Eicher, B. and L. Audige. 1999. Analysis of risk factors for the occurrence of feather pecking in laying hen growers. *British Poultry Science,* 40:599-604.

Huber Eicher, B. and F. Sebo. 2001. The prevalence of feather pecking and development in commercial flocks of laying hens. *Appl. Anim. Behav. Sci.,* 74:223-231.

Hughes, B. O. 1982. Feather pecking and cannibalism in domestic fowl. In: *Disturbed Behaviour in Farm Animals*. Ed. W. Bessei. Verlag Eugen Ulmer 121:138-146.

Hughes, B. O. and I. J. H. Duncan. 1972. The influence of strain and environmental factors upon feather pecking and cannibalism in fowls. *British Poultry Science,* 13:525-547.

Johnsen, P. F., K. S. Vestergaard and G. Norgaard Nielsen. 1998. Influence of early rearing conditions on the development of feather pecking and cannibalism in domestic fowl. *Applied Animal Behaviour Science,* 60:25-41.

Jones, R. B. 2001. Environmental enrichment for poultry welfare. In: *Integrated Management Systems for Livestock*. Ed. C. M. Wathes. British Society of Animal Science Occasional Publication 28:125-131.

Jones, R. B. and P. M. Hocking. 1999. Genetic selection for poultry behaviour: big bad wolf or friend in need? *Animal Welfare,* 8:343-359.

Keeling, L. J., B. O. Hughes and P. Dun. 1988. Performance of free-range laying hens in a polythene house and their behaviour on range. *Farm Building Progress,* 94:21-28.

Kjaer, J. B. and P. M. Hocking. 2004. The genetics of feather pecking and cannibalism. In: *Welfare of the Laying Hen*. Ed. G. C. Perry. CABI Publishing 109-122.

Leeson, S. and W. D. Morrisson. 1978. Effect of feather cover on feed efficiency in laying birds. *Poultry Science,* 57:1094-1096.

Leonard, M. L., A. G. Horn and R. W. Fairfull. 1995. Correlates and consequences of allopecking in White Leghorn chickens. *Applied Animal Behaviour Science,* 43:17-26.

Miller, M. W. and G. E. Bearse. 1937. The cannibalism preventing properties of oats. *Poultry Science,* 16:314-321.

Nicol, C. J., A. C. Lindberg, A. J. Phillips, S. J. Pope, L. J. Wilkins and L. E. Green. 2001. Influence of prior exposure to wood shavings on feather pecking, dustbathing and foraging in adult laying hens. *Applied Animal Behaviour Science,* 73:141-155.

Nørgaard-Nielsen, G., K. S. Vestergaard and H. B. Simonsen. 1993. Effects of rearing experience and stimulus enrichement on feather damage in laying hens. *Applied Animal Behaviour Science,* 38:345-352.

Peguri, A. and C. Coon. 1993. Effect of feather coverage and temperature on layer performance. *Poultry Science,* 72:1318-1329.

Savory, C. J. 1980. Meal occurrence in Japanese quail in relation to particle size and nutrient density. *Anim. Behav.,* 28:160-171.

Tullet, S. G., M. G. Macleod and T. R. Jewitt. 1980. The effects of partial defeathering on energy metabolism in the laying fowl. *British Poultry Science,* 21:241-245.

Valkonen, E., E. Venäläinen, L. Rossow and J. Valaja. 2008. Effects of Dietary Energy Content on the Performance of Laying hens in Furnished and Conventional Cages. *Poult. Sci.,* 87:844-852.

Van der Lee, A. G., G. Hemke and R. P. Kwakkel. 2001. Low density diets improve plumage condition in non-debeaked layers. *Proc.13th Eur. Symp. poult. nutr.*, Blankenbergen, 244-245.

Van der Meulen, J., C. Kwakernaak and C. A. Kan. 2006. Sand intake by laying hens and its effect on egg production parameters. *J. Anim. Nutr. Phys.,* DOI:_10.1111/j.1439-0396.2007.00732.x.

Vestergaard, K. S. and L. Lisborg. 1993. A model of feather pecking development which relates to dustbathing in the fowl. *Behaviour,* 126:291-308.

Chapter 2

IMPACT OF FEEDING MANAGEMENT ON FEATHER PECKING IN LAYING HENS

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ABSTRACT

In the near future EU-legislation will ban the use of conventional battery cages, while national legislation in some countries in Western Europe will ban beak trimming as well. The ban on battery cages and beak trimming causes an increased risk of feather pecking and cannibalism in laying hens. Many factors influence feather pecking behavior, but in this paper we will focus on nutritional factors. Nutritional factors can have positive and negative effects on feather pecking behavior in laying hens. Severe feather pecking has been demonstrated in birds that were fed a too low mineral level in the diet, a too low protein level or a too low amino acid level (methionine, arginine). Sometimes somewhat more feather pecking was found when layers were fed diets with mainly vegetable protein sources as compared with diets with protein from animal origin. Also more feather pecking may occur when the diets were fed restrictedly, fed coarsely ground, or fed as pellets. Feeding high-fiber diets, low energy diets, or roughages reduced feather pecking. Providing additional grain or straw in the litter during rearing could result in lower levels of feather pecking behavior in adult stages. Some of these positive effects on feather pecking seem to be related to the time birds spend on feed intake and foraging. This paper gives an overview of the relationships between the occurrence of feather pecking behavior and nutritional factors, such as diet composition and feeding strategies in laying hens.

Keywords: nutrition; feather pecking; pullets; laying hens; diet composition; feeding management

INTRODUCTION

In 2012, changes in EU-legislation with regard to animal welfare and husbandry will be implemented that might increase the level of feather pecking in layers. These changes include a ban on traditional battery cages as the current housing system for layers in Western Europe. This ban is the result of a societal debate from which the conclusion was drawn that battery cages could not fulfill the birds' need to express their natural behavior. This stressed the need to develop alternative housing systems for layers, such as furnished cages, free range systems, or aviary systems. These systems, however, show much higher incidences of feather pecking and cannibalism compared to cage systems (Morgenstern, 1995). In organic farming, mortalities of even up to 30%, as a result of cannibalism, have been reported (Wouw, 1995). The most effective tool to prevent feather pecking and subsequent cannibalism is beak trimming, but in some West-European countries (e.g. Great Britain and The Netherlands) a general ban on beak trimming can be expected in the near future too. The bans on battery cages and beak trimming increase the risk of feather pecking and cannibalism.

Feather pecking in layers is a multi factorial problem, which can be caused by environmental, genetic or nutritional factors (Blokhuis, 1989). In this paper, the focus will be on nutritional factors. The objective of the current study is to provide an overview of the relationship between feather pecking behavior and nutritional factors, such as diet composition and feeding strategies in laying hens. It has been demonstrated many times that dietary deficiencies stimulate explorative behavior (Bessei, 1983) and may increase feather pecking (e.g. (Ambrosen and Petersen, 1997). Some authors have shown that the addition of fiber to the diet or feeding roughages could decrease feather pecking and cannibalism (e.g. (Steenfeldt et al., 2001). The relative importance of specific deficiencies in layer diets, as well as the effectiveness and possible modes of action of certain nutritional factors, will be examined and discussed in this review.

DEFINITIONS

Feather pecking in laying hens can be characterized as pecking at and pulling out of feathers of conspecifics. Five different types of bird-to-bird pecking can be distinguished, based on both cause and its effect (Savory, 1995). These are:

(1) aggressive pecking,

(2) gentle feather pecking without removal of feathers,

(3) severe feather pecking leading to feather loss,

(4) tissue pecking in denuded areas and
(5) vent pecking.

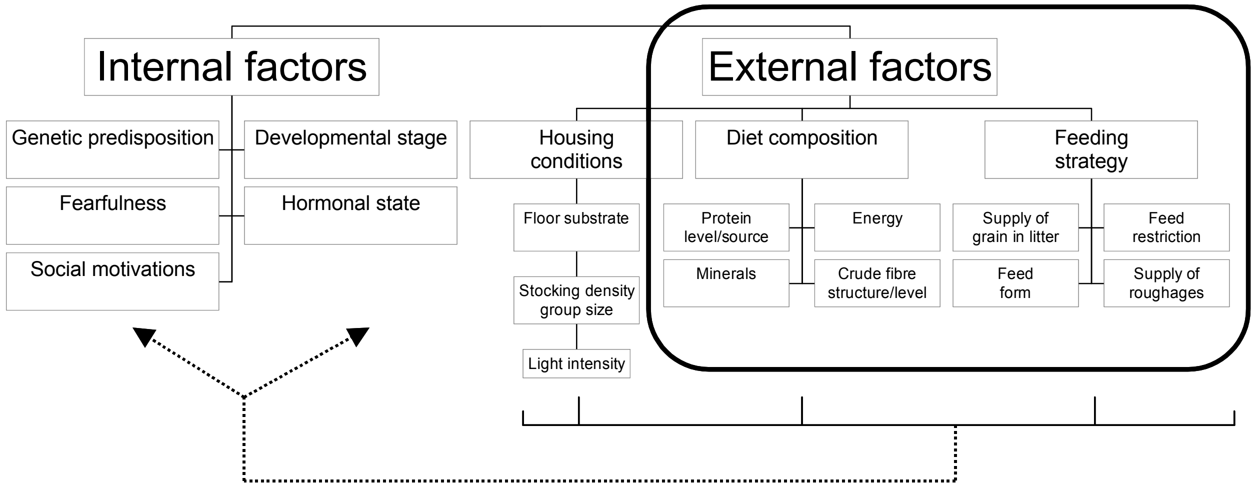
Aggressive pecking among chickens is used to establish a stable dominance hierarchy. It may lead to some damage to the neck and neck region, but should not be confused with feather pecking behavior. Gentle feather pecking without the removal of feathers sometimes appears to be directed at litter particles on the plumage. However, it can also develop into stereotypic pecking with a high frequency at the same spot on another bird (McAdie and Keeling, 2002), which can cause damage. Gentle feather pecking is often ignored by the recipient. Severe feather pecking, or feather pulling, is characterized by forceful pecking at or pulling out of feathers, to which the victim usually reacts. Feather removal has been shown to be painful (Gentle and Hunter, 1990), cause feather damage and can lead to bald patches. These bald patches may attract tissue pecking, which can result in wounding of the victim and eventually to cannibalism. Vent pecking may start as investigative pecking, but it can also lead to cannibalism when the oviduct is damaged or the internal organs are pulled out. The distinction between gentle feather pecking, severe feather pecking and tissue pecking is not always clear and the different pecking forms may transform into each other (Savory, 1995).

Feather pecking, especially the severe type, negatively affects the welfare of laying hens (Blokhuis and Wiepkema, 1998). Moreover, feather pecking causes feather loss of pecked birds resulting in higher feed intake, worse feed conversion ratio, and as a consequence higher feed costs (Tauson and Svensson, 1980; Herremans et al., 1989; Peguri and Coon, 1993).

FACTORS AFFECTING FEATHER PECKING BEHAVIOR

Many factors that affect feather pecking behavior are related either to internal factors like the genetic nature or the physiological status of the birds, or to external factors like housing conditions of the birds or nutritional factors or to a combination of these factors. The interaction between internal and external factors also can increase feather pecking behavior. It appears that feather pecking is initially performed by frustrated birds (Lindberg and Nicol, 1994). An overview of factors that affect feather pecking behavior is given in Figure 1. This paper is mainly focused on the circled external factors 'diet composition' and 'feeding strategy'.

Figure 1. Factors affecting feather pecking behavior.



Internal factors

A large variation in the level of feather pecking behavior exists between strains of laying hens. Some studies indicate the possibility for breeding programmes and behavior-genetic experiments to reduce the feather pecking problem (Sørensen and Christensen, 1997; Craig and Muir, 1998). Often the results of such programmes are inconsistent, with heritability estimates ranging from 0.04 – 0.56 (Bessei, 1984; Rodenburg et al., 2003) depending on age and method of recording.

The role of fear in relation to feather pecking behavior is unclear. Some authors have suggested that feather pecking is more likely to be initiated by fearful birds (Vestergaard et al., 1993; Johnsen et al., 1998). Observations in an open-field test show that laying hens that were more fearful and less social as young pullet showed higher levels of feather pecking as adult hens (Rodenburg et al., 2004). Based on the same data a quantitative trait loci study (QTL) was performed, which indicated that there may be a common gene or a set of genes that affect both open-field behavior and feather pecking behavior (Buitenhuis et al., 2003; Buitenhuis et al., 2004). Most studies indicate that fearfulness is a consequence of feather pecking, induced by feather damage and pain, rather than the other way around (Lee and Craig, 1991; Hansen and Braastad, 1994; Jones and Hocking, 1999).

It appears that feather pecking is initially performed by a restricted number of birds in a flock. Such behavior (and in particular cannibalism) can escalate into a great number of birds in a flock showing feather pecking (Zeltner et al., 2000). (McAdie and Keeling, 2002) found some evidence that gentle feather pecking was transmitted in laying hens housed in cages. However, they found no evidence for the spreading of severe feather pecking. Social learning has been found to facilitate and accelerate outbreaks of feather pecking (Cloutier et al., 2000; Zeltner et al., 2000; McAdie and Keeling, 2002). Severe feather pecking should never be confused with normal gentle feather pecking, which plays an important functional role in the building and maintenance of social relationships between birds (Riedstra and Groothuis, 2002).

The intensity and severity of feather pecking seems to depend on age (Rodenburg and Koene, 2003). Gentle feather pecking is mostly observed in young chickens (Kjaer and Sørensen, 1997; Wechsler et al., 1998) and severe feather pecking is more often seen at a later age (Huber Eicher and Sebo, 2001). In addition, the nature of environmental conditions given to young birds plays an important role in the development or occurrence of feather pecking later in life (Blokhuis and Van der Haar, 1992; Huber Eicher and Wechsler, 1998). (McAdie and Keeling, 2002) suggest that severe feather pecking and stereotyped gentle feather pecking can develop from gentle feather pecking, either by increased severity or increased intensity of pecks.

The increase in feather pecking around onset-of-lay is hormonally mediated, and can either be stimulated by administering a combination of oestrogen and progesterone or be blocked by giving testosterone (Hughes, 1973).

External factors

Provision of litter at early ages substantially reduces feather pecking at later age (Blokhuis and Van der Haar, 1989)). This is consistent with the theory that feather pecking is a form of redirected behavior, developing either from ground pecking (Blokhuis, 1986) or pecking during dust-bathing (Vestergaard et al., 1993). According to these theories, exposing chickens to litter early in life would prevent them from perceiving feathers as a substrate for either foraging or dust-bathing.

Increasing group size (Keeling, 1994; Bilcik and Keeling, 1999) or increasing stocking density (Appleby et al., 1988; Savory and Mann, 1999) have been linked to an increase in feather pecking behavior. Because group size and stocking density are confounded, the role of each individual factor can not be distinguished (Nicol et al., 1999; Savory and Mann, 1999).

Increasing light intensity seems to increase the level of severe feather pecking (Allen and Perry, 1975; Kjaer and Vestergaard, 1999). Laying hens that were reared in 3 lux developed stereotypic gentle feather pecking, showing about 20 times more gentle pecking than hens that were reared at 30 lux. Severe pecks were 2 - 3 times more frequent in laying hens that were reared at 30 than at 3 lux. During the laying period, the immediate effects of the two light intensities on pecking behavior were less pronounced than during rearing (Kjaer and Vestergaard, 1999). Possibly, low light levels during rearing impairs the bird's ability to identify environmental cues and consequently increases exploratory pecking in order to compensate. Light color may also play a role in social recognition in laying hens (D' Eath and Stone, 1999).

Numerous other housing conditions can also influence feather pecking behavior. An appropriate housing design, resulting in no competition or increased activity at feeders, drinkers and nest boxes, and the availability of perches may prevent feather pecking (Savory, 1995). Feather damage, caused by abrasion against other birds at high density or against equipment in the system or the side of cages, has also been found to facilitate and accelerate outbreaks of feather pecking (Savory and Mann, 1997; McAdie and Keeling, 2000). Also the availability of short feathers on the floor can influence feather pecking behavior. Based on an experiment with layer pullets (McKeegan and Savory, 1999) concluded that once feather eating has become established, a too low availability of short feathers on pen floors may cause feather eating and pecking to be redirected to other birds.

In the above section genetic, physiological and some management factors related to feather pecking were briefly discussed. In the following paragraphs an overview is given of the impact of nutritional factors, such as diet composition and feeding strategies, on feather pecking behavior in laying hens. Furthermore, the possible modes of action of these nutritional factors related to feather pecking behavior are discussed.

DIET COMPOSITION

Protein and amino acid content

Crude protein: For decades, it has been known that protein-deficient diets may increase feather pecking and cannibalism in birds (Schaible et al., 1947). A protein deficiency, especially a methionine deficiency (Elwinger et al., 2002), might play a significant role in organic poultry production, because of the ban on particular protein sources and synthetic amino acids in organic layer diets (European Commission 1139/98, 1988). The addition of protein supplements, such as casein, gelatin, liver meal, blood meal, soybean oil meal, cotton seed meal and other protein sources to basal diets low in crude protein (CP) (135 g/kg), as well as in phosphorus (5.3 g/kg) and in fiber (26 g/kg) reduced the incidence of feather pecking and cannibalism in pullets from 0 to 8 weeks of age (Schaible et al., 1947). A low protein diet (111 g/kg CP) without the addition of synthetic amino acids, that was tested in 7 layer strains, resulted in 17.6% cannibalism mortality compared to 2.5% cannibalism mortality in layers that were fed a diet of 193 g/kg crude protein (Ambrosen and Petersen, 1997). Mortality, however, was not significantly affected by dietary protein contents in two experiments of (Al Bustany and Elwinger, 1987b). The crude protein content in Experiment 1 ranged from 124 g/kg to 176 g/kg (total lysine intake 487 to 919 mg/hen/day) and in Experiment 2 from 134 g/kg to 177 g/kg (total lysine intake 703 to 1024 mg/hen/day) respectively. The results of (Ambrosen and Petersen, 1997) and (Al Bustany and Elwinger, 1987b) seem to contradict. However, in the experiment of (Ambrosen and Petersen, 1997) only significant effects of CP on mortality were found at CP levels of 126 g/kg or lower, while (Al Bustany and Elwinger, 1987b) did their experiments at CP levels above 124 g/kg. Furthermore, in Experiment 2 of (Al Bustany and Elwinger, 1987b) a treatment with 120 g/kg crude protein was excluded because of a high rate of cannibalism and mortality. Increasing the dietary protein and amino acid contents in these experiments resulted in improved plumage condition (3 points on a scale that ranged from 5 to 20) (Al Bustany and Elwinger, 1987b). In an earlier experiment of (Al Bustany and Elwinger, 1986) experimental diets were fed with crude protein contents of 124 g/kg, 150 g/kg and 176 g/kg and lysine contents of 4.6 g/kg, 6.6 g/kg and 8.7 g/kg, resulting in a total lysine intake of 461, 709, and 919 mg/hen/day. In that experiment no effect of protein and lysine content on plumage condition and mortality was found, but the strains of layers used in that experiment had been selected for several generations to perform well on a low protein and low energy diet.

Methionine and cysteine: Since feathers are 89-97% protein, dietary amino acids play a critical role in feather development. Feather development is related to the incidence of feather pecking (McAdie and Keeling, 2000). Ruffled or trimmed feathers encourage feather pecking behavior, and even cannibalism, and this stresses the need of good feather development. The major amino acids involved in the synthesis of feather keratin are the sulphur-containing amino acids, methionine and cysteine. Marginal deficiencies of these amino acids will often be initially manifested in abnormal feathering (Robel, 1977; Deschutter and Leeson, 1986). Feeding an organic diet low in protein and amino acids (135 g/kg crude protein, 5.9 g/kg lysine and 5.1 g/kg methionine + cysteine) to laying hens resulted in an inferior plumage condition and a higher incidence of peck injuries of the comb and the rear body parts compared to feeding a standard organic diet with 169 g/kg CP, 8.7 g/kg lysine and 6.7 g/kg methionine + cysteine (Elwinger et al., 2002). Hens fed the organic diet had a daily intake of 649 mg lysine and 561 mg methionine + cysteine, compared to 940 mg and 724 mg respectively in hens fed the standard diet. In

Table 1. Effect of dietary crude protein, lysine and/or methionine levels (g/kg) on plumage condition, occurrence of feather pecking and mortality in birds.

Type of bird [1]	Period of age (weeks)	Beak-trimmed	Crude Protein	Total Lysine	Total Methionine + Cysteine	Plumage Condition [2]	Level of feather pecking [3]	Mortality (%)	Authors
Laying hens; LSL and SH	16 - 58	No	135 and 169	5.9 and 8.7	5.1 and 6.7	4.0 and 6.6	3.3 and 3.9 [4]	2.3 and 3.9 (n.s.)	Elwinger *et al.*, 2002
Laying hens; ISA brown, NH, WL, NH x WL	16 - 43	No	154	8.1	4.2 and 8.2	9.0 and 9.3 (n.s.)	Not recorded	Not available	Kjaer and Sorensen, 2002
Laying hens; WL and Brown layer strain	20 - 60	No	111 - 193	4.2 - 9.5 [4]	4.2 - 7.9 [5]	4.5 - 7.0	Not recorded	17.6 - 2.5	Ambrosen and Petersen, 1997
Laying hens; 3 x WL (Hisex, LSL, Shaver), WL x RIR, WL x (WL x RIR)	20 - 80	Yes	124 - 176	4.6 - 8.7	Not recorded	4.1 - 6.1	Not recorded	12.4 - 10.0 (n.s.)	Al Bustany and Elwinger, 1987a Experiment 1
Laying hens: 2 x WL (LSL, Shaver), RIR, WL x RIR	20 - 80	Yes	134 - 177	5.8 - 8.8	Not recorded	4.1 - 6.3	Not recorded	8.4 - 6.9 (n.s.)	Al Bustany and Elwinger, 1987a Experiment 2
Laying hens: RIR, Shaver, WL x RIR	20 - 80	Yes	123 - 177	5.6 - 9.4	Not recorded	4.5 - 6.2	Not recorded	7.5 - 10.4 (n.s.)	Al Bustany and Elwinger, 1987b
Laying hens: WL and RIR	32 - 72	Yes	124 - 176	4.6 - 8.7	Not recorded	7.5 - 7.7 (n.s.)	Not recorded	Average 8.4 (n.s.)	Al Bustany and Elwinger, 1986

[1] Explanation of abbreviations: NH = New Hampshire, WL = White Leghorn, LSL = Lohmann Selected Brown, RIR = Rhode Island Red

[2] Original data recalculated to a scale of 0 to 10 where 0 indicates almost naked and 10 an intact plumage

[3] Original data recalculated to a scale of 0 to 10 where 10 indicates no observed injuries

[4] Peck injuries to cloacae/rear body parts at 58 weeks ranging from score 1 to 4 where 4 indicates no observed injuries

[5] Based on own recalculation of the diets

contrast with these results, Kjaer and Sørensen (2002) found no effect of a low (4.2 g/kg) versus a high (8.2 g/kg) level of methionine + cysteine in organic diets on the plumage conditionof laying hens. However, in the experiment of (Elwinger et al., 2002), CP and lysine content changed in addition to methionine + cysteine content, so these experiments are not fully comparable.

Lysine: Adding lysine to a diet low in CP, such that the consumption of lysine increased from 485 to 587 mg per hen per day, improved plumage condition of laying hens considerably (Al Bustany and Elwinger, 1987a). In a dose – response trial, in which the total lysine content varied from 5.6 to 9.4 g/kg (resulting in increased crude protein contents), no further improvement of plumage condition was found from a lysine level of 8.2 g/kg onwards. Based on three experiments of (Al Bustany and Elwinger, 1987a; Al Bustany and Elwinger, 1987b), a total lysine content of about 8,0 g/kg, corresponding with in a total lysine intake of 850 to 950 mg/hen/day, seems to be sufficient for an optimal plumage condition. The effect of the different protein, lysine and/or methionine levels on feather pecking behavior is summarized in *Table 1*.

Arginine: Reducing the dietary level of arginine from 6.9% to 3.9% of the total protein in diets of 4-week old cockerels increased the level of cannibalism from 0 to 21% (Sirén, 1963). Cannibalism in 8-week old cockerels, fed a diet with 3.9% arginine, could subsequently be cured by feeding a diet with 6.9% (of the total protein) arginine. Madsen (1966) completed similar experiments with pheasants and partridges. He found no evidence that arginine influenced pecking at the back and wings, tail feathers, or vent of pen mates.

Tryptophan: Dietary supplementation with tryptophan in growing bantams, ranging from 2.6 to 22.6 g/kg, resulted in a suppression of pecking damage with the higher doses compared to the control dose (2.6 g/kg), at 4 and 6 weeks of age (Savory, 1998; Savory et al., 1999). This lower level of pecking damage is probably caused by a lower level of severe feather pecking behavior. In line with this observation reported reduced frequencies of gentle feather pecking in young chickens that were fed a diet with a very high tryptophan level (21 g/kg) compared to a diet with a standard tryptophan level (1.6 g/kg). Tryptophan is a precursor for serotonin synthesis (5-HT) and chickens from a high feather pecking line were found to display lower 5-HT turnover levels in response to acute stress than chickens from a low feather pecking line (Van Hierden et al., 2002). Increased dietary tryptophan stimulates serotonergic neurotransmission, resulting in a higher turnover of tryptophan to 5-HT in the brains (Van Hierden et al., 2004). Thus feather pecking behavior seems to be triggered by low serotonergic neurotransmission, because increasing serotonergic tone (higher levels of dietary tryptophan) decreases feather pecking behavior. The effect of dietary tryptophan content on feather pecking behavior is summarized in *Table 2*.

It can be concluded that marginal levels of CP and amino acids can result in feather pecking behavior, whereas high levels of dietary tryptophan might decrease feather pecking behavior. In most of the above mentioned cases of increased feather pecking the CP and amino acid levels of the control groups were below NRC requirements for laying hens. NRC requirements for layer diets are 150 g/kg CP, 5.8 g/kg methionine + cysteine, 6.9 g/kg lysine, 7.0 g/kg arginine and 1.6 g/kg tryptophan, and based on daily intake per hen daily 15 g CP, 580 mg/kg methionine + cysteine, 609 mg lysine, 700 mg arginine and 160 mg tryptophan (NRC, 1994).

Animal versus vegetable protein

To prevent feather pecking behavior, feed producers often add some animal protein (e.g. fish meal, meat and bone meal or milk protein sources) to the diet (Hadorn et al., 1998). It has been suggested that any suppressive effect on feather pecking induced by animal protein is due to something beneficial found only in these protein sources, for instance vitamin B_{12} (McKeegan et al., 2001). However, it is also conceivable that a detrimental compound in plant protein sources could increase feather pecking behavior. As an example, phytoestrogens could elevate plasma oestradiol concentrations and affect bird behavior (McKeegan et al., 2001). Since the ban on meat and bone meal in Europe, the diets of laying hens contain mainly vegetable proteins. In practice, farmers expect a higher occurrence of cannibalism as a result of using vegetable diets; some examples were given by (Curtis and Marsh, 1992). Diets based on animal (fish meal) or plant (soybean meal) protein were fed to layer pullets up to 24 weeks of age (McKeegan et al., 2001). Greater numbers of vigorous pecks/pulls were observed in the plant protein groups throughout the experiment, although they were only significantly higher from week 13 to 16. Pecking damage scores, plasma oestradiol and progesterone, and egg production, however, were unaffected by diet. Laying hens that were fed diets with exclusively vegetable protein sources, such as extracted soybean meal, peas, faba beans and extracted sunflower seed tended to a higher mortality rate due to feather pecking compared with laying hens fed a diet with 4% meat and bone meal (Richter and Hartung, 2003). In contrast with these results, no differences in plumage condition were found in laying hens that were fed diets with either a mixture of vegetable and animal protein or only vegetable protein sources, while the plumage condition of laying hens that were fed exclusively animal protein sources was markedly

Table 2. Effect of dietary tryptophan levels (g/kg) on plumage condition, occurrence of feather pecking and mortality in birds.

Type of bird	Period of age (weeks)	Beak-trimmed	Tryptophan content	Plumage Condition [1]	Level of feather pecking	Mortality (%)	Authors
Pullets:	1 – 7	No	1.6	Not recorded	137.7/16.2 [2]	Not recorded	Van Hierden *et al.*, 2003
White Leghorns (WL)			21.0		65.0/9.4[2]		
(Low and high feather pecking line)							
Bantams	1 - 6	Unknown	2.6	3.8	Not recorded	0	Savory *et al.*, 1999
			12.6	5.3		0	
			22.6	5.9		0	

[1] Original data recalculated to a scale of 0 to 10 where 0 indicates almost naked and 10 an intact plumage

[2] Respectively number of gentle and severe feather pecks in 30 minutes.

Table 3. Effect of protein source (animal versus vegetable protein) on plumage condition, occurrence of feather pecking and mortality in birds.

Type of bird	Period of age (weeks)	Beak-trimmed	Protein source	Plumage Condition [1]	Level of feather pecking	Mortality (%)	Authors
Laying hens:	21 – 72	Unknown	Soyabean meal	9.2	Not recorded	3.3	Hadorn *et al.*, 1998;
LSL White			Fish meal/meat meal	9.2		2.2	Hadorn *et al.*, 1999
Bantams	0 - 6	Unknown	Soyabean meal (30%)	6.1	Not recorded	Not recorded	Savory *et al.*, 1999
			Fish meal (6%), Blood meal (3.2%), Feather meal (5.2%)	4.7			
			Casein (8.4%)	5.7			
Laying hens:	Period of 40 weeks	Unknown	Soyabean meal (7.5%), potato protein (6%)	6.7	Not recorded	Not recorded	Pfirter and Walser, 1998
ISA brown, Lohmann brown			Soyabean meal (3%), potato protein (6%), Meat meal (4%)	7.7			
			Potato protein (3%), meat meal (3%), blood meal (3%), fish meal (1.5%)	5.6			

[1] Original data recalculated to a scale of 0 to 10 where 0 indicates almost naked and 10 an intact plumage

worse (Pfirter and Walser, 1998). Performance and mortality (including cannibalism) were unaffected by feeding diets with either animal (herring and meat meal) or vegetable (soybean meal extracted) protein sources (Hadorn et al., 1998; Hadorn et al., 1999). Also feeding diets based on either plant (soybean meal), animal (blood meal, fish meal and hydrolyzed feather meal) or semi-purified (casein) protein to growing bantams did not result in differences in pecking damage scores between treatments (Savory, 1998; Savory et al., 1999).

The effect of different protein sources on feather pecking behavior is summarized in *Table 3*. Although practical evidence suggests a higher incidence of feather pecking in laying hens fed vegetable protein diets, no conformation of this hypothesis can be found in literature.

Minerals

Magnesium: High magnesium content in layer diets is suggested to reduce feather pecking and cannibalism. Supplementation of 7 g/kg $MgSO_4$ (1.4 g/kg Mg) to a diet low in protein (135 g/kg), as well as in phosphorus (5.3 g/kg) and fiber (26 g/kg) reduced pecking behavior and mortality due to cannibalism (Schaible et al., 1947). A doubling of the magnesium content (from 1.35 to 2.70 g/kg), however, did not affect mortality due to cannibalism and feather quality (Hadorn et al., 2001). The NRC requirement (NRC, 1994) for magnesium in diets of laying hens is 0.5 g/kg.

Zinc: Supplementation of micro elements, such as aluminium, barium, chromium, copper had no effect on plumage condition and cannibalism of laying hens (0-44 weeks of age) (Willimon and Morgan, 1953). Zinc, however had an effect: adding 0.1 g/kg $ZnCl_2$ (48 ppm zinc) to a zinc-deficient pullet diet (9.5 ppm zinc) improved the feather score from poor to good (Supplee et al., 1958). Supplementing a zinc-deficient diet, containing about 40 ppm zinc, with 52 ppm extra zinc during the first week age reduced the incidence of feather abnormalities of pullets from 5-20 percent to very low levels (Sunde, 1972). Adding 200 ppm $ZnCO_3$ (104 ppm zinc) to a high rice bran (81.5%) layer diet that contained no specific zinc source markedly improved the feather score of the progeny of the layers at 2 weeks of age (Piliang et al., 1984). Thus, in view of today's fast developing pullets, the NRC requirement (NRC, 1994) for zinc in diets of pullets of about 40 ppm seems to be marginal for optimal feather development and to avoid feather pecking behavior.

Sodium: Feeding a low sodium diet (0.4 g/kg) to 2 year old laying hens for only a period of four weeks showed no increase in feather pecking, toe pecking, pecking activity or general activity, compared to a control group fed a diet with 2.3 g/kg sodium, although egg production of the low sodium group almost completely ceased (Hughes and Whitehead, 1974). These authors investigated the effect of different dietary sodium (0.03 g/kg, 0.3 g/kg and 1.3 g/kg) and calcium (29 g/kg, 33 g/kg and 39 g/kg) levels on behavior and plumage condition of 90-week old laying hens (Hughes and Whitehead, 1979). While the increased calcium levels did not affect cannibalism or feather pecking, cannibalism was seen in birds receiving low (0.03 g/kg) or intermediate (0.3 g/kg) levels of sodium. Cannibalism was not seen in birds receiving the control diet (1.3 g/kg Na). Plumage condition was unaffected by sodium content in the diet, but the birds showed an increased awareness of the environment, resulting in more general pecking. The NRC requirement (NRC, 1994) for sodium in diets of laying hens (1.5 g/kg) seems to give no reason for increased feather pecking behavior.

The effect of dietary mineral contents on feather pecking behavior is summarized in *Table 4*. Unfortunately, only a few investigations have been reported on the relationship between mineral contents in diets of laying hens and their feather pecking behavior. The scarcely available literature, however, shows that deficiencies of dietary minerals can increase feather pecking behavior and feather abnormalities.

Energy content

The energy content of the diet may also affect feather pecking behavior. Increasing the dietary energy content of layer diets (10.7, 11.2, 11.7 and 12.2 MJ/kg) resulted in increased energy consumption, a tendency to higher mortality and a significant decrease in feather condition (Elwinger, 1981). Feeding non-debeaked laying hens a low density diet (11.05 MJ ME/kg, 51 g/kg crude fat), in which all nutrients were decreased by 5%, improved plumage condition compared to hens that were fed a standard diet (11.55 MJ ME/kg, 65 g/kg crude fat) (Van der Lee et al., 2001). Laying performance was not adversely affected by the lower density diet. Feed intake of the low density diet was higher, resulting in an almost equal energy intake in both diets. This suggests that laying hens fed diets with a lower energy density spent more time on feed intake, and so less time is remaining for feather pecking behavior. This is in accordance with the results of (Savory, 1980) who fed male Japanese quail diluted (with 40% cellulose) and undiluted diets. Those receiving the diluted mash consumed about 40% more feed (14.9 vs. 10.8 g/d), spent a higher proportion of total time (24 h) on feed intake (23.8 vs. 9.1%), had a longer meal length (1.54 vs. 0.87 min), a shorter inter-meal interval length (4.98 vs. 8.92 min) and more meals per day (128 vs. 86). Despite meal length being longer with diluted mash, the weight eaten per meal (av. 0.116 g) was equal to the amount with undiluted mash. However, the two diets had different densities and a much greater volume per meal was consumed with diluted mash than with undiluted mash (0.409 cm^3 vs. 0.182 cm^3); this suggests that the difference in meal length was related to dietary bulk. The passage rate through the digestive tract and the emptying of the crop were both about 1.5 times faster with diluted compared to undiluted mash. The undiluted mash was 1.5 times better digestible than the diluted mash (Savory, 1980). The length of the inter-meal interval was closely associated with the difference in rate of feed passage. suggested that gut-emptying, and particularly filling and emptying of the gizzard or duodenum, could be the main activating mechanism in meal initiation and termination.

The effect of dietary energy content on feather pecking behavior is summarized in *Table 5*. A low energy content of the diet seems to reduce feather pecking behavior and to improve plumage condition. However, the different energy levels are confounded with changes in other ingredients, protein and fiber levels, and with differences in meal length and frequency, as well as in passage rate and emptying of gut segments. The optimal dietary energy level for reducing feather pecking while maintaining laying performance remains unknown. However, we expect that a reduction of the dietary energy content of about 10%, compared to a standard level of about 10.6 MJ/kg will markedly reduce the incidence of feather pecking, without negatively affecting the egg production of the hen. Research should be initiated to measure the interaction between the pure effect of energy dilution of a diet and eating time on feather pecking behavior in laying hens.

Fiber content

Fiber helps to maintain normal structure and function of the gastrointestinal tract and prevents cannibalism, and should therefore be included in poultry rations (Esmail, 1997). For decades it is known that an increase in crude fiber content in diets for growing and laying pullets can markedly reduce feather pecking and cannibalism. Increasing the crude fiber content from 29 to 123 g/kg (by substituting corn with oat hulls) decreased feather pecking and cannibalism (Bearse et al., 1940). The oat hull fiber fraction (obtained by dilute acid digestion of the hulls) was as effective as the oat hulls themselves in preventing feather pecking and cannibalism, while the ash of the dilute acid extract and the water extract of oat hulls were of little value in preventing cannibalism. Increasing the crude fiber content in diets of chickens up to 180 g/kg,

Table 4. Effect of dietary mineral levels on plumage condition, occurrence of feather pecking and mortality in birds.

Type of bird	Period of age (weeks)	Beak-trimmed	Mineral	Plumage Condition [1]	Level of feather pecking	Mortality (%)	Authors
Laying hens:	21 - 62	No	Magnesium / Crude fibre	n.s.	Not recorded	n.s.	Hadorn *et al.*, 2001
Isa brown			1.35 g/kg/ 40 g/kg	5.7		17.3	
			2.7g/kg / 40 g/kg	5.5		15.7	
			1.35 g/kg/ 25 g/kg	4.9		18.6	
			2.7 g/kg / 25 g/kg	5.7		14.9	
Pullets;	0 – 8	Unknown	0 and 7 g/kg $MgSO_4$	3.3 and 3.3	27 and 8% [2]	31 and 21% [3]	Schaible *et al.*, 1947
Leghorn							
Breeder layers:	2	Unknown	0 and 200 ppm $ZnCO_3$	4.1 and 9.2	Not recorded	Not recorded	Piliang *et al.*, 1984
feather development scored on progeny							
Laying hens:	93 – 98	Unknown	0.03 g/kg Sodium	9.0	9.2%		Hughes and Whitehead, 1979
(Babcock and Warren SSL)			0.3 g/kg Sodium	8.7	9.5%		
			1.3 g/kg Sodium	8.7 n.s.	2.3% [4]		

[1] Original data recalculated to a scale of 0 to 10 where 0 indicates almost naked and 10 an intact plumage; [2] Percentage of chickens picked, but alive; [3] Percentage of chickens killed by picking; [4] Percentage of birds that had to be removed because of injurious pecking

Table 5. Effect of dietary energy levels (MJ/kg) on plumage condition, occurrence of feather pecking and mortality in birds.

Type of bird [1]	Period of age (weeks)	Beak-trimmed	Energy content Exp. 1	Energy content Exp. 2	Plumage Condition [2] Exp. 1	Plumage Condition [2] Exp. 2	Level of feather pecking	Mortality (%) Exp. 1	Mortality (%) Exp. 2	Authors
Laying hens:	18-70	Unknown	10.7	10.7	4.4	3.7	Not recorded	8.8	7.4	Elwinger, 1981
SCWL, Hisex, LSL			11.2	11.2	3.4	4.2		13.0	10.5	
			11.7	11.7	4.0	2.9		12.0	10.4	
			12.2	12.2	3.3	3.0		11.7	11.0	
Laying hens:	30 - 52	No	11.05		8.1		Not recorded	Not recorded		Lee *et al.*, 2001
(LSL and Bovans Goldline)			11.55		7.3					

[1] Explanation of abbreviations: SCWL = Single Comb White Leghorns, LSL = Lohmann Selected Leghorn;

[2] Original data recalculated to a scale of 0 to 10 where 0 indicates almost naked and 10 an intact plumage

by substituting oat mill feed by corn, reduced feather pecking rate and mortality, and also improved plumage condition. At a crude fiber content of over 130 g/kg a low incidence of feather pecking and cannibalism was recorded, whereas a high incidence of feather pecking and cannibalism was shown at a crude fiber content of below 80 g/kg (Esmail, 1997).

A number of studies have confirmed that the insoluble fiber fraction in the diets of laying hens is beneficial in preventing pecking behavior (Aerni et al., 2000; El Lethey et al., 2000; Hartini et al., 2002; Hetland and Choct, 2003). One experiment showed that both insoluble (mill run) and soluble (barley) fiber were effective in reducing and controlling cannibalism in laying hens (Hartini et al., 2002).

No effects of substitution of corn by wheat in diets for growing and laying pullets on feather pecking and cannibalism were found (Miller and Bearse, 1937). Feather pecking and cannibalism were reduced slightly by substituting barley for corn, and markedly when substituting oats for corn (Miller and Bearse, 1937; Al Bustany and Elwinger, 1988; Abrahamsson et al., 1996; Wahlstrom et al., 1998). The crude fiber content of barley (46 g/kg) and oat (105 g/kg) is substantially higher than corn (22 g/kg) and wheat (24 g/kg) (CVB, 2003)

Birds fed diets high in insoluble fiber spent more time eating and appear calmer than those fed low-fiber diets (Hetland and Choct, 2003). Insoluble fiber plays an important role in modulating gut development and digestive function. Feeding a supplement of wood shavings (an insoluble fiber-rich raw material) to laying hens fed wheat-based diets increased starch digestibility (Hetland and Choct, 2003). The improvement of starch digestibility may, in part, be due to enhanced emulsification of lipids as a result of a higher content of bile acids in the gizzard. The total content of bile acids in the gizzard increased in proportion to the amount of wood shavings retained in the gizzard. Consumption of 4% of feed as wood shavings resulted in a 50% percent heavier gizzard of broiler chickens, whereas including 40% whole wheat in a wheat-based mash diet increased the gizzard weight by only 10% (Hetland et al., 2002), indicating that wood shavings has a higher impact on gizzard weight than whole wheat. The insoluble fiber content in the gizzard of chickens fed food shavings was twice as much as the content in the feed (Hetland et al., 2004). This suggests that insoluble fiber accumulates in the gizzard and is retained longer than other nutrients, probably because it has to be ground to a critical particle size before entering the small intestine (Hetland et al., 2002; Hetland et al., 2004). The fact that feeding a mash diet that was diluted with 10% powdered cellulose (an insoluble fiber source) to growing bantams did not affect pecking damage scores compared with an undiluted mash (Savory et al., 1999) could possibly be explained by the small particle size of the powder. Coarse fiber also decreases the passage time of fine particles when it is fed to broiler chickens (Hetland and Svihus, 2001; Svihus et al., 2002). The fact that insoluble fiber accumulates in the gizzard may also indicate a slower feed passage rate when the level of coarse fiber is increased in the diet. This confirms that the gizzard is almost like a point of regulation for digestion, selectively retaining different feed particles and letting nutrients pass for further digestion. It is thought that accumulation of insoluble fiber in the gizzard triggers a temporary satiety, but once passed the gizzard, it passes through the gut quickly. This could make the bird feel more satisfied between feeding bouts, but more hungry after gizzard emptying (Hetland and Choct, 2003). It can be hypothesized that chickens prefer not just fiber, but coarse fiber. The attractiveness for coarse fiber, such as wood shavings and paper seems to be considerably higher for birds fed a wheat-based diets than for those fed an oat-based diet (Hetland et al., 2002). Since oats contain considerably more coarse fiber than wheat, the data indicate that the birds needed some coarse fiber in their diets, perhaps for gizzard activity (Hetland and Choct, 2003). In line with this, birds fed an oat-based diet had a significantly heavier gizzard and a larger content of the gizzard compared with those fed a wheat-based diet when housed in cages. The reverse was true for the gizzard weight when the birds were reared under a free range system (Hetland et al., 2003). These results support the hypothesis that, given the opportunity, birds fed low fiber diets will search for coarse materials to satisfy their fiber need. The amount of feathers in the gizzard of individual housed laying hens was higher in laying hens fed a low-structure diet based on rice and casein than in hens fed a diet based on wheat or enriched with coarse fibers. The gizzard content of the birds fed the rice-based diet, however, was markedly less than in hens fed the wheat-based or coarse fiber diets. Until now no causal factors for feather eating are known (McKeegan and Savory, 1999; McKeegan and Savory, 2001), but these results indicate that feather eating and pecking behavior may be partly related to feed structure, which play a major role in the volume of gizzard contents (Hetland et al., 2004).

The effect of dietary fiber content on feather pecking behavior is summarized in *Table 6*. Both soluble and insoluble fiber sources seem to affect feather pecking behavior, although possibly other properties of the fiber-rich raw materials (mostly barley or oats) were determinative for the positive effects. The relationship between fiber content of the ration and prevention of feather pecking is only partially understood. Conceivably, it may be related to the increased consumption of feed resulting in a higher level of satiety, or the time occupied in eating. It was also postulated that ingestion of insoluble dietary fiber would increase gut viscosity and gut fill (Hartini et al., 2002). However, the ideal dietary fiber content and fiber source for reducing feather pecking results while maintaining laying performance remains unknown. It is suggested here that an increase of at least 25% of the dietary insoluble fiber content, compared to a standard NSP (Non Starch Polysaccharides) level of about 140 g/kg, might markedly reduce the incidence of feather pecking due to a possible effect on satiety. This hypothesis should be tested in a trial, in which the effect of different NSP sources and levels on feather pecking behavior will be measured.

FEEDING STRATEGY

Feeding strategy in the rearing period

The development of the digestive tract during the rearing period, resulting in an appropriate volume and digestive capacity of the gut at the beginning of lay, was suggested to be of great importance in the occurrence of feather pecking and cannibalism during the laying period (Hadorn and Wiedmer, 2001). The volume of the digestive tract (mainly the gizzard) can be increased by feeding coarse particles and/or fiber-rich diets. Similarly feeding whole wheat during the rearing period is thought to increase the digestive capacity of laying hens at the beginning of the lay. Supplementing extra straw or spreading 10% of the estimated feed intake as whole wheat into the litter had no effect on the development of body weight, plumage condition and mortality rate of the pullets (Hadorn and Wiedmer, 2001), but markedly reduced feather damage in the layer period (Blokhuis and Van der Haar, 1992). Distributing grain in the litter during rearing also directed foraging-related behaviors like ground scratching and ground pecking, suggesting that the incentive value of the ground, and the substrate covering it, might be increased with grain during the rearing period (Blokhuis and Van der Haar, 1992). Although feeding strategy during rearing seems to be of importance for feather pecking behavior in the laying period, few investigations studied this kind of nutritional carry-over effect. In diets of pullets an energy dilution or an increase of (coarse) insoluble fiber may stimulate their feed related behavior during the rearing period, resulting in less feather pecking behavior of the laying hens.

Table 6a. Effect of dietary fiber levels (g/kg) on plumage condition, occurrence of feather pecking and mortality in birds.

Type of bird [1]	Period of age (weeks)	Beak trimmed	Fiber Source	Fiber content (g/kg)	Plumage Condition [2]	Level of feather pecking [3]	Mortality (%)	Authors
Laying hens:	17 – 20	50% yes	Control (73% wheat)	29.3	Not recorded	Not recorded	13.2	Hartini *et al.*, 2002
ISA brown		50% no	Millrun [3]) (32%) and Sorghum (48%)	43.4			3.9	
			Barley (76%)	51.6			5.8	
			Barley + enzyme (76%)	51.6			4.1	
Laying hens:	21 – 24	50% yes	Control (73% wheat)	29.3	Not recorded	Not recorded	28.9	Hartini *et al.*, 2002
ISA brown		50% no	Millrun [4] (32%) and Sorghum (48%)	43.4			14.3	
			Barley (76%)	51.6			15.9	
			Barley + enzyme (76%)	51.6			17.8	
Laying hens:	20 – 80	No	Wheat diet (25% wheat, 10% oats)	44	Not recorded	Not recorded	18.4	Wahlstrom *et al.*, 1998a
LSL and			Oats diet (0% wheat, 33% oats)	64			13.4	(exp. 1)
Lohmann brown								
Laying hens:	20 – 80	No	Oats/wheat ratio:			Not recorded		Wahlstrom *et al.*, 1998b
LSL, Lohmann brown			0/60	21.8	7.8		8.9	(exp. 2)
and SLU			12/48	38.3	8.1		10.8	
			24/36	48.6	8.3		8.4	
			36/24	58.8	8.9		10.9	
			48/12	68.9	9.0		10.8	
			60/0	79.2	9.1		12.8	
Laying hens:	18 – 80	Unknown	Wheat/barley ratio			Not recorded	n.s.	Abrahamsson *et al.*, 1996
ISA brown			50/13.7	36.0	3.6		15.8	
and LSL			25/38.7	40.0	5.1		16.5	
Laying hens:	20 – 73	Unknown	Wheat diet (72% wheat)	25.5	3.8	Not recorded	8.8	Al Bustany and Elwinger, 1988
LSL and L324			Barley diet (74% barley)	39.0	4.1		8.8	
			Oats diet (75% oats)	69.0	5.1		7.2	

Table 6b. Effect of dietary fiber levels (g/kg) on plumage condition, occurrence of feather pecking and mortality in birds.

Type of bird [1]	Period of age (weeks)	Beak trimmed	Fiber Source	Fiber content (g/kg)	Plumage Condition [2]	Level of feather pecking [3]	Mortality (%)	Authors
Laying hens: SCWL)	2 – 40	Unknown	Corn (81% corn)	29.1	Not recorded	100 [5]	3.9 / 8.7 [6]	Bearse *et al.*, 1940
			Corn /oat hull fiber (23% oat hull fiber)	110.7		5.8	4.0 / 13.4	
			Corn/oat hulls (34.5% oat hulls)	122.6		2.3	0.8 / 3.7	
Laying hens: SCWL	2 – 40	Unknown	Wheat (80%)	32.9 [7]	Not recorded	92.3 [5]	0.0	Miller and Bearse, 1937
			Corn (80%)	31.2		97.0	1.7	
			Barley (78%)	51.2		73.2	0.0	
			Oats (81%)	100.1		0.0	0.0	

[1] Explanation of abbreviations: LSL = Lohmann Selected Leghorn, SLU = cross-bred of Leghorn x Rhode Island Red, SCWL = Single Comb White Leghorn, L324 = cross-bred of White Leghorn x Rhode Island Red

[2] Original data recalculated to a scale of 0 to 10 where 0 indicates almost naked and 10 an intact plumage

[3] Number of pecking interactions per bird per hour

[4] (2/3 wheat bran, 1/3 wheat pollard)

[5] Percentage of birds pecked at 40 weeks of age

[6] Percentage mortality in growing period and after 16 weeks of laying period respectively

[7] Based on own recalculation of the diets

Feed form

The physical form of the diet, e.g. mash, crumble or pellet, and also the distribution of particle size in mash diets, can affect feather pecking behavior, possibly due to differences in time spending on feed intake. More feather pecking was found in laying hens fed a coarsely ground meal (33-55% of particles > 2mm) compared with laying hens fed a finely ground meal (0-13% of particles > 2mm) (Walser and Pfirter, 2001). Based on the results of this experiment an optimal mash structure should have a normal distribution pattern of fine particles between 0.25 and 2 mm. Addition of whole cereals to mash diets enlarges the average particle size of the diet, which may cause an increasing risk of feather pecking. The type of whole cereal seems to be of importance in affecting feather pecking behavior: laying hens fed diets containing whole wheat or barley had poorer performance, inferior plumage condition and a higher mortality rate than laying hens fed mash diets, (Al Bustany and Elwinger, 1988). Whole oats or mixtures of whole oats, whole barley and whole wheat resulted in better plumage than did mash diets with ground barley or ground wheat. Possibly, the favorable effects of the high insoluble fiber content of whole oats compensate amply for the adverse effects of whole wheat and barley.

A number of studies have confirmed that laying hens fed pellets are more likely to develop feather pecking than birds fed on mash (Heywang and Morgan, 1944; Bearse et al., 1949; Jensen et al., 1962; Savory, 1974; El Lethey et al., 2000; Walser and Pfirter, 2001). Providing pellets may also decrease the age when feather pecking behavior is initiated. Incorporating more coarse structure into pellets by adding whole wheat in the mixer before pelletising, however, positively affects plumage condition, gizzard weight and gizzard contents of laying hens, all indicators of better welfare (Hetland et al., 2004). The coarse wheat particles seem to accumulate in the gizzard, which possibly trigger a temporary satiety. In contrast, when pullets were kept in pens with litter-covered floors, feed form (mash or pellet) exhibited no significant effect on feather pecking (Savory and Mann, 1997). In another study, feather pecking behavior was equal in laying hens fed on crumbles or mash (Wahlstrom et al., 2001). Since feeding pellets had dissimilar effects on feather pecking in different studies, interaction effects of pellets with other factors, e.g. housing conditions, is highly probable.

There may be an interaction between feed form and available floor space: in pullets, feather pecking was only observed in two of the six groups receiving a pelleted diet (Heywang and Morgan, 1944) and feather pecking stopped when these two groups were removed from the houses to yards where they had more floor space. According to the authors space explained the reduction of feather pecking. However, apparently other environmental factors, like changes in temperature and the availability of daylight were confounded with space. Also a significant interaction was shown between foraging material (with or without long straw) and food form (mash or pellet) (Aerni et al., 2000). High rates of feather pecking and pronounced feather damage were only found in laying hens housed without straw and fed on pellets, indicating that laying hens (especially when fed pellets) should be provided with an adequate amount of foraging material. Laying hens with access to foraging material also had a lower hetrophiles-to-lymphocyte ratio and an increased immune response to immunization than those without access to such materials, indicating lower stress in these birds (El Lethey et al., 2000).

Chickens engage in more feed directed behavior when fed finely ground mash than when fed coarsely ground mash, crumbles or pellets (Savory, 1974; Savory, 1995; Aerni et al., 2000; Walser and Pfirter, 2001). Similarly, laying hens in individual cages spent more time on feed intake as the particle size of the diet decreased (100 minutes per day for pellets, 154 for crumbles and 234 for mash) (Tanaka et al., 1983). The frequency of feed pecking also increased with decreasing particle size: 9,723 times per day for pellets, 15,874 for crumbles and 22,845 for mash, with an average feed intake of 11.8, 7.4 and 5.2 gram per peck. Laying hens that were fed a high volume mash pecked feed more frequently and feathers less than birds fed a low volume mash (Bessei, 1983). Feeding pelletised diets resulted in two times more pecks directed to a bundle of feathers (Bessei et al., 1999), or more time spent on perching, whereas more feeding directed behaviors (sum of time spent on feeding and foraging) were recorded in hens fed on mash (Aerni et al., 2000). Spending more time eating will fulfil the need of the foraging behavior of the laying hens, which may lead to a decrease in feather pecking (Blokhuis and Arkes, 1984).

The effect of feed form on feather pecking behavior is summarized in *Table 7*. It seems that a too high amount of coarse particles or pellets in the diet may cause an increasing risk of feather pecking behavior compared to mash diets, possibly due to spending less time on feed intake. Feeding strategies that result in laying hens spending more time on feed intake and foraging could decrease the risk of feather pecking behavior.

Table 7. Effect of feed structure on plumage condition, occurrence of feather pecking and mortality in birds.

Type of birds [1]	Period of age (weeks)	Beak-trimmed	Feed structure	Plumage Condition [2]	Level of feather pecking [3]	Mortality (%)	Authors
Laying hens: LSL and Shaver	16 – 68	No	Pellets	2.4	Not recorded	8.8	Hetland *et al.*, 2003a
			Pellets; whole wheat (40%) in mixer before pelleting	3.7		8.8	
Laying hens: LSL	19 - 27	No	Pellets	7.8	3.9	Not recorded	Aerni *et al.*, 2000
			Mash	9.4	0.7		
Bantams	1 – 6	Unknown	Pellets	6.5	Not recorded	Not recorded	Savory *et al.*, 1999
			Mash	9.4			
			Diluted Mash (40% cellulose powder)	9.6			
Laying hens: LSL and L324	20 – 73	Unknown	Mash and whole cereals	Mash/whole	Not recorded	Mash / whole	Al Bustany and Elwinger, 1988
			Barley	4.9 / 3.3		6.6 / 10.9	
			Wheat	4.6 / 3.1		8.1 / 9.4	
			oats	n.r./5.1		n.r. / 7.2	

[1] Explanation of abbreviations: LSL = Lohmann Selected Leghorn, L324 = cross-bred of White Leghorn x Rhode Island Red

[2] Original data recalculated to a scale of 0 to 10 where 0 indicates almost naked and 10 an intact plumage

[3] Number of pecking interactions per bird per hour

Feed restriction

Freezing the feed consumption of cage housed pullets at the ad libitum level of intake at six weeks of age until the age of sixteen weeks resulted in the same amount of time spending on pecking at food and non-food objects as pullets fed ad libitum (Savory and Fisher, 1992). There was no evidence that this 'freeze-feeding' was associated with increased bird-to-bird pecking, either aggressively or non-aggressively. Thus, the time freeze-fed birds spent pecking at non-food objects appeared to substitute the time they would otherwise spent on feeding. However, laying hens housed as pairs in cages with no access to feed from 07:30 to 15:30 h each day, spent 23% of their time on stereotypic behavior like cage-pecking, feather pecking and pacing when feed was unavailable, whereas ad libitum fed hens spent 7% of their time on these behaviors (Preston, 1987). Hens fed ad libitum showed a tendency for more feather- and cage pecking before 07:30 h than the hens with limited access to feed. Hens fed ad libitum and those fed 6% less feed showed no difference in plumage condition (Elwinger and Andersson, 1978). Feed intake was expected to affect feather pecking behavior, with more feather pecking in birds that spent less time on feed intake. However, the changeable effects of feed restriction on feather pecking has been shown to vary in the literature.

Supplying roughages

Roughage supplements may reduce feather pecking in birds (Hoffmeyer, 1969; Köhler et al., 2001; Steenfeldt et al., 2001). Supplements of cut green clover and branches with green leaves as roughage sources, given to young pheasants (five and ten weeks old), led to significantly less feather pecking than in the controls (Hoffmeyer, 1969). Mutual comparison of the two roughage sources (branches with green leaves and cut green clover spread on the floor) in pheasants of ten weeks old showed markedly less feather pecking in the clover group. The amount of feather pecking was inversely correlated with the amount of pecking directed at the supplemented source. The pheasants treated the leaves and other roughages in the same way as feathers, indicating a great similarity between the behavior shown in feather pecking and the normal feeding behavior (Hoffmeyer, 1969). Roughages, which are a normal target for the pheasant food pecking activity in natural habitats, must provide a sign for stimulating feeding behavior. Feathers may provide some of the sensory stimuli (optical, tactile) to which the (innate) feeding response mechanisms of pheasants are specially attuned (Hoffmeyer, 1969). Based on these experiments (Hoffmeyer, 1969) concluded that feather pecking is a substitute for normal feeding behavior.

Carrots, maize-silage and barley-pea-silage were supplied to laying hens from 20-54 weeks of age to examine the effect of supplementing roughages on performance, gastro-intestinal health and feather pecking behavior (Steenfeldt et al., 2001). At 24 weeks of age, treatments differed significantly in the incidence of feather pecking, with less gentle and severe feather pecking in hens fed carrots or maize-silage compared to the control group. At 53 weeks of age, differences in feather pecking were non-significant but similar tendencies were still observed. Hens fed the silage had the best plumage condition at 53 weeks of age. In line with this, hens given ad libitum access to fresh grass had better plumage condition than those without (Köhler et al., 2001). Roughage supplementation did not affect egg production (except for barley-pea-silage) and feed efficiency, but significantly decreased mortality rate (Steenfeldt et al., 2001). Roughage supplementation significantly decreased pH in the caeca, probably caused by a higher fermentation rate in this part of the gastro intestinal tract (Steenfeldt et al., 2001). The positive effects of roughage supplementation could possibly be explained by a lower dietary density and/or an increased crude fiber content of the diet. Supplementing the diets with carrots (in the experiment of (Steenfeldt et al., 2001) decreased the density of the diet by about 40%. This could be an explanatory factor, especially since the roughages increased the total consumption of the laying hens, which could be an indication of spending more time on feed intake. Regrettably, (Steenfeldt et al., 2001) showed no data concerning distribution of time spent on different types of behavior. Conceivably, the positive effects may be related to other nutrients than dietary density and/or crude fiber.

The effect of roughage supply on feather pecking behavior is summarized in *Table 8*. Supplying roughages to laying hens seems to be a promising approach to reduce feather pecking behavior (though there is scarce literature on this). The relationship of roughage intake and feather pecking, however, is only partially understood.

Table 8. Effect of roughage supply on plumage condition, occurrence of feather pecking and mortality in birds.

Type of bird	Period of age (weeks)	Beak-trimmed	Source of Roughage	Plumage Condition [1]	Level of feather pecking [2]	Mortality (%)	Authors
Laying hens:	20 – 54	Unknown	Control diet (pellet)	5.9	1.08	15.3	Steenfeldt *et al.*, 2001
ISA brown			Maize-silage	8.9	0.36	1.5	
			Barley-pea-silage	9.5	0.71	2.5	
			Carrots	7.5	0.69	0.5	
Laying hens:	20 – 33	Unknown	Control (all-mash)	6.6	Not recorded	Not recorded	Köhler *et al.*, 2001
Lohmann brown			Fresh grass	7.2			
Laying hens:	19 - 27	No	Without straw	7.8	4.2	Not recorded	Aerni *et al.*, 2000
LSL (Lohmann Selected Leghorns)			Long-cut straw (foraging material)	9.4	0.8		
Pheasant chickens	3	Unknown	Control vs. clover	6.5 vs.10.0	Not recorded	Not recorded	Hoffmeyer, 1969
	5		Control vs, clover	6.1 vs. 8.8			
	10		Branches with leaves vs. clover	5.5 vs. 1.8			
	5		Green plastic band	4.8			
			Branches with green leaves	6.8			
			Green clover	8.4			

[1] Original data recalculated to a scale of 0 to 10 where 0 indicates almost naked and 10 an intact plumage

[2] Pecks per bird per hour

Summarizing Conclusion

Nutritional factors may positively or negatively affect feather pecking behavior in laying hens. Some investigations, indeed, show that feather pecking behavior is a substitute for normal feeding behavior. Until now, the mode of action of these nutritional factors is not fully understood. Dietary deficiencies, resulting in a marginal supply of nutrients, such as protein, amino acids, or minerals, may increase feather pecking behavior and cannibalism. Nutritional factors seem to reduce feather pecking behavior in laying hens if these factors increase the time spent on feeding behavior, by affecting foraging and feed intake. Laying hens may spend more time on these feeding behaviors when they are fed 1) mash diets in stead of crumbles or pellets, 2) low energy diets, 3) high (in-)soluble fiber diets or 4) roughages. Further research, especially directed to the role of dietary density and (coarse) insoluble fiber, is needed to better understand the impact of nutritional factors on feather pecking behavior and thus, welfare of layers. Future research should focus on the interaction effects between energy level, insoluble fiber and particle sizes of the insoluble fiber on foraging time and passage rate, as being assumed indicators for developing feather pecking behavior.

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References

Abrahamsson, P., R. Tauson and K. Elwinger. 1996. Effects on production, health and egg quality of varying proportions of wheat and barley in diets for two hybrids of laying hens kept in different housing systems. *Acta Agriculturae Scandinavica. Section A, Animal Science,* 46:173-182.

Aerni, V., H. El Lethey and B. Wechsler. 2000. Effect of foraging material and food form on feather pecking in laying hens. *Br. Poult. Sci.,* 41:16-21.

Al Bustany, Z. and K. Elwinger. 1986. Effect of dietary protein concentration on performance of hens selected on low protein diet and grouped according to their early production. *Acta Agriculturae Scandinavica,* 36:264-274.

Al Bustany, Z. and K. Elwinger. 1987a. Response of laying hens to different dietary lysine intakes. A comparison of some commercial hybrids with strains selected on a low protein diet. *Acta Agric. Scand.,* 37:27-40.

Al Bustany, Z. and K. Elwinger. 1987b. Comparison between barley/fish meal- and maize/soybean meal-based diets with various lysine and protein levels fed to different strains of laying hens. *Acta Agriculturae Scandinavica,* 37:41-49.

Al Bustany, Z. and K. Elwinger. 1988. Whole grains, unprocessed rapeseed and beta-glucanase in diets for laying hens. *Swedish Journal of Agricultural Research,* 18:31-40.

Allen, J. and G. C. Perry. 1975. Feather pecking and cannibalism in a caged layer flock. *British Poultry Science,* 16:441-451.

Ambrosen, T. and V. E. Petersen. 1997. The influence of protein level in the diet on cannibalism and quality of plumage of layers. *Poult. Sci.,* 76:559-563.

Appleby, M. C., G. S. Hogarth, J. A. Anderson, B. O. Hughes and C. T. Whittemore. 1988. Performance of a deep litter system for egg production. *British Poultry Science,* 29:735-751.

Bearse, G. E., V. L. Miller and C. F. McClary. 1940. The cannibalism preventing properties of fibre fraction of oat hulls. *Poultry Science,* 19:210-215.

Bearse, G. E., L. R. Berg, C. F. McClary and V. L. Miller. 1949. The effect of pelleting chicken rations on the incidence of cannibalism. *Poultry Science,* 28:756.

Bessei, W. 1983. Zum Problem des Federpickens und Kannibalismus. *DGS Magazin,* 24:656-665.

Bessei, W. 1984. On the heritability of feather pecking in pullets. 1st report. *Archiv fur Geflugelkunde,* 48:224-231.

Bessei, W., K. Reiter, T. Bley and F. Zeep. 1999. Measuring pecking of a bunch of feathers in individually housed hens: first results of genetic studies and feeding related reactions. *Lohmann Information*:27-31.

Bilcik, B. and L. J. Keeling. 1999. Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. *Br. Poult. Sci.,* 40:444-451.

Blokhuis, H. J. 1986. Feather-pecking in poultry: its relation with ground-pecking. *Applied Animal Behaviour Science,* 16:63-67.

Blokhuis, H. J. 1989. The development and causation of feather pecking in the domestic fowl, *Vakgroep Ethologie*, Wageningen, Thesis Landbouw Universiteit Wageningen,

Blokhuis, H. J. and J. G. Arkes. 1984. Some observations on the development of feather-pecking in poultry. *Applied Animal Behaviour Science,* 12:145-157.

Blokhuis, H. J. and J. W. Van der Haar. 1989. Effects of floor type during rearing and of beak trimming on ground pecking and feather pecking in laying hens. *Appl. Anim. Behav. Sci.,* 22:359-369.

Blokhuis, H. J. and J. W. Van der Haar. 1992. Effects of pecking incentives during rearing on feather pecking of laying hens. *British Poultry Science,* 33:17-24.

Blokhuis, H. J. and P. R. Wiepkema. 1998. Studies of feather pecking in poultry. *Veterinary Quarterly,* 20:6-9.

Buitenhuis, A. J., T. B. Rodenburg, Y. M. v. Hierden, M. Siwek, S. J. B. Cornelissen, M. G. B. Nieuwland, R. Crooijmans, M. A. M. Groenen, P. Koene, S. M. Korte, H. Bovenhuis and J. J. v. d. Poel. 2003. Mapping quantitative trait loci affecting feather pecking behavior and stress response in laying hens. *Poultry Science,* 82:1215-1222.

Buitenhuis, A. J., T. B. Rodenburg, M. Siwek, S. J. B. Cornelissen, M. G. B. Nieuwland, R. Crooijmans, M. A. M. Groenen, P. Koene, H. Bovenhuis, Y. M. v. Hierden, S. M. Korte and J. J. v. d. Poel. 2004. Identification of QTLs involved in open-field behavior in young and adult laying hens. *Poultry Science,* 82:1215-1222.

Cloutier, S., R. C. Newberry, C. T. Forster and K. M. Girsberger. 2000. Does pecking at inanimate stimuli predict cannibalistic behaviour in domestic fowl? *Applied Animal Behaviour Science,* 66:119-133.

Craig, J. and W. Muir. 1998. Genetic influences on the behavior of chickens associated with welfare and productivity. In: *Genetics and the behavior of domestic animals*. Ed. T. Grandin. Academic Press 265-299.

Curtis, P. E. and N. W. A. Marsh. 1992. Cannibalism in laying hens. *Veterinary Record,* 131:424.

CVB. 2003. Veevoedertabel 2003, Centraal Veevoederbureau. Lelystad, The Netherlands.

D' Eath, R. and R. J. Stone. 1999. Chickens use visual cues in social discrimination: an experiment with coloured lighting. *Applied Animal Behaviour Science,* 62:233-242.

Deschutter, A. and S. Leeson. 1986. Feather growth and development. *World's Poultry Science Journal,* 42:259-267.

El Lethey, H., V. Aerni, T. W. Jungi and B. Wechsler. 2000. Stress and feather pecking in laying hens in relation to housing conditions. *Br. Poult. Sci.,* 41:22-28.

Elwinger, K. 1981. Different energy levels and restricted feeding to three strains of SCWL hybrids. 1. Effects on egg production. *Swedish J. Agric. Res.,* 11:149-157.

Elwinger, K. and K. Andersson. 1978. Restricted feeding and different protein levels to two strains of SCWL hybrids. 1. Effects on egg production. *Swedish J. Agric. Res.,* 8:231-240.

Elwinger, K., R. Tauson, M. Tufvesson and C. Hartmann. 2002. Feeding of layers kept in an organic feed environment. *11th. European Poultry Conference*, Bremen,

Esmail, S. H. M. 1997. Fibre Nutrition. *Poultry International,* 36:31-34.

European Commission 1139/98. 1988. Council Regulation (EC) No 1139/98, amended by Commission Regulation (EC) No 49/2000 and Commission Regulation (EC) No 50/2000.

Gentle, M. J. and L. N. Hunter. 1990. Physiological and behavioural responses associated with feather removal in *Gallus gallus var domesticus*. *Research in Veterinary Science,* 50:95-101.

Hadorn, R. and H. Wiedmer. 2001. Effect of whole wheat feeding and different aviary systems on the development of white and brown growing pullets. *Proceedings of the 6th European symposium on poultry welfare.*, Eds.: H. Oester &C. Wyss.

Hadorn, R., H. Wiedmer and A. Gloor. 1998. Effect of the exclusion of animal proteins from layer diets. *Agrarforschung,* 5:409-412.

Hadorn, R., A. Gloor and H. Wiedmer. 1999. Exclusion of animal protein from layer diets. *12th European Symposium of Poultry Nutrition*, Veldhoven (NL), 349 - 351.

Hadorn, R., A. Gloor and H. Wiedmer. 2001. Effect of dietary magnesium and crude fibre content on performance, mortality rate and plumage condition of brown laying hens. *Proceedings of the 6th European symposium on poultry welfare.*, Ed. H. O. a. C. Wyss.

Hansen, I. and B. O. Braastad. 1994. Effect of rearing density on pecking behaviour and plumage condition of laying hens in two types of aviary. *Applied Animal Behaviour Science,* 40:263-272.

Hartini, S., M. Choct, G. Hinch, A. Kocher and J. V. Nolan. 2002. Effects of light intensity during rearing and beak trimming and dietary fiber sources on mortality, egg production, and performance of ISA brown laying hens. *J. Appl. Poult. Res.,* 11:104-110.

Herremans, M., E. Decuypere and O. Siau. 1989. Effects of feather wear and temperature on prediction of food intake and residual food consumption. *British Poultry Science,* 30:15-22.

Hetland, H. and B. Svihus. 2001. Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *Br. Poult. Sci.,* 42:354-361.

Hetland, H. and M. Choct. 2003. Role of insoluble non-starch polysaccharides in poultry nutrition. *Proceedings of the 14 th European Symposium of Poultry Nutrition*, Lillehammer, Norway, 64-69.

Hetland, H., B. Svihus and V. Olaisen. 2002. Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *Br. Poult. Sci.,* 43:416-423.

Hetland, H., B. Svihus and A. Krogdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *Br. Poult. Sci.,* 44:275-282.

Hetland, H., M. Choct and B. Svihus. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poult. Sci. J.,* 60:415-422.

Heywang, B. W. and R. B. Morgan. 1944. A comparison of a pelleted and unpelleted all-mash diet for growing chickens. *Poultry Science,* 23:16-20.

Hoffmeyer, I. 1969. Feather pecking in pheasants - an ethological approach to the problem. *Danish review of game biology,* 6:1-36.

Huber Eicher, B. and B. Wechsler. 1998. The effect of quality and availability of foraging materials on feather pecking in laying hen chicks. *Animal Behaviour,* 55:861-873.

Huber Eicher, B. and F. Sebo. 2001. The prevalence of feather pecking and development in commercial flocks of laying hens. *Appl. Anim. Behav. Sci.,* 74:223-231.

Hughes, B. O. 1973. The effect of implanted gonadal hormones in feather pecking and cannibalism in pullets. *British Poultry Science,* 14:341-348.

Hughes, B. O. and C. C. Whitehead. 1974. Sodium deprivation, feather pecking and activity in laying hens. *British Poultry Science,* 15:435-439.

Hughes, B. O. and C. C. Whitehead. 1979. Behavioural changes associated with the feeding of low-sodium diets to laying hens. *Appl. Anim. Ethology,* 5:255-266.

Jensen, L. S., L. H. Merill, C. V. Reddy and J. McGinnis. 1962. Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. *Poultry Science,* 41:1414-1419.

Johnsen, P. F., K. S. Vestergaard and G. Norgaard Nielsen. 1998. Influence of early rearing conditions on the development of feather pecking and cannibalism in domestic fowl. *Applied Animal Behaviour Science,* 60:25-41.

Jones, R. B. and P. M. Hocking. 1999. Genetic selection for poultry behaviour: big bad wolf or friend in need? *Animal Welfare,* 8:343-359.

Keeling, L. J. 1994. Feather pecking - who in the group does it, how often and under what circumstances? *Proceedings of the 9th European Poultry Conference*, Glasgow, United Kingdom, 288-289.

Kjaer, J. B. and P. Sørensen. 1997. Feather pecking behaviour in White Leghorns, a genetic study. *British Poultry Science,* 38:333-341.

Kjaer, J. B. and K. S. Vestergaard. 1999. Development of feather pecking in relation to light intensity. *Applied Animal Behaviour Science,* 62:243-254.

Kjaer, J. B. and P. Sørensen. 2002. Feather pecking and cannibalism in free-range laying hens as affected by genotype, dietary level of methionine+cystine, light intensity during rearing and age at first access to the range area. *Applied Animal Behaviour Science,* 76:21-39.

Köhler, B., J. Föhlsch, J. Strube and K. Lange. 2001. Influences of green forage and lighting conditions on egg quality and hen welfare. *Proceedings of the 6th European symposium on poultry welfare.*, Eds.: H. Oester &C. Wyss.

Lee, H. Y. and J. V. Craig. 1991. Beak trimming effects on behavior patterns, fearfulness, feathering, and mortality among three stocks of White Leghorn pullets in cages or floor pens. *Poultry Science,* 70:211-221.

Lindberg, A. C. and C. J. Nicol. 1994. An evaluation of the effect of operant feeders on welfare of hens maintained on litter. *Applied Animal Behaviour Science,* 41:211-227.

McAdie, T. M. and L. J. Keeling. 2000. Effect of manipulating feathers of laying hens on the incidence of feather pecking and cannibalism. *Applied Animal Behaviour Science,* 68:215-229.

McAdie, T. M. and L. J. Keeling. 2002. The social transmission of feather pecking in laying hens: effects of environment and age. *Applied Animal Behaviour Science,* 75:147-159.

McKeegan, D. E. F. and C. J. Savory. 1999. Feather eating in layer pullets and its possible role in the aetiology of feather pecking damage. *Applied Animal Behaviour Science,* 65:73-85.

McKeegan, D. E. F. and C. J. Savory. 2001. Feather eating in individually caged hens which differ in their propensity to feather peck. *Applied Animal Behaviour Science,* 73:131-140.

McKeegan, D. E. F., C. J. Savory, M. G. MacLeod and M. A. Mitchell. 2001. Development of pecking damage in layer pullets in relation to dietary protein source. *British Poultry Science,* 42:33-42.

Miller, M. W. and G. E. Bearse. 1937. The cannibalism preventing properties of oats. *Poultry Science,* 16:314-321.

Morgenstern, R. 1995. Alternative Haltung in der Schweiz: Woran erkranken die Legehennen? *DGS Magazin,* 4-2-1995:11-14.

Nicol, C. J., N. G. Gregory, T. G. Knowles, I. D. Parkman and L. J. Wilkins. 1999. Differential effects of increased stocking density, mediated by increased flock size, on feather pecking and aggression in laying hens. *Applied Animal Behaviour Science,* 65:137-152.

NRC. 1994. Nutrient requirements of poultry. *Ninth Revised Edtion.* Washington DC.

Peguri, A. and C. Coon. 1993. Effect of feather coverage and temperature on layer performance. *Poultry Science,* 72:1318-1329.

Pfirter, H. P. and P. Walser. 1998. Wieviel und welches protein? *UFA-Reveu,* 11:26-28.

Piliang, W. G., H. R. Bird, M. L. Sunde and D. J. Pringle. 1984. Rice bran as the major energy source of laying hens. *Poultry Adviser,* 17:21-31.

Preston, A. P. 1987. Restricted feeding time and the behaviour of caged laying hens. *British Poultry Science,* 28:387-396.

Richter, G. and H. Hartung. 2003. Pflanzliche Rationen im Vergleich. *DGS Magazin,* 1:20-24.

Riedstra, B. and T. G. G. Groothuis. 2002. Early feather pecking as a form of social exploration: the effect of group stability on feather pecking and tonic immobility in domestic chicks. *Applied Animal Behaviour Science,* 77:127-138.

Robel, E. J. 1977. A feather abnormality in chicks fed diets deficient in certain amino acids. *Poultry Science,* 56:1968-1971.

Rodenburg, T. B. and P. Koene. 2003. Comparison of individual and social feather pecking tests in two lines of laying hens at ten different ages. *Applied Animal Behaviour Science,* 81:133-148.

Rodenburg, T. B., A. J. Buitenhuis, B. Ask, K. A. Uitdehaag, P. Koene, J. J. Van der Poel and H. Bovenhuis. 2003. Heritability of feather pecking and open-field response in laying hens at two different ages. *Poultry Science,* 82:861-867.

Rodenburg, T. B., A. J. Buitenhuis, B. Ask, K. A. Uitdehaag, P. Koene, J. J. v. d. Poel, A. J. A. M. van and H. Bovenhuis. 2004. Genetic and phenotypic correlations between feather pecking and open-field response in laying hens at two different ages. *Behavior Genetics,* 34:407-415.

Savory, C. J. 1974. Growth and behaviour of chicks fed on pellets or mash. *British Poultry Science,* 15:281-286.

Savory, C. J. 1980. Meal occurrence in Japanese quail in relation to particle size and nutrient density. *Anim. Behav.,* 28:160-171.

Savory, C. J. 1995. Feather pecking and cannibalism. *World's Poultry Science Journal,* 51:215-219.

Savory, C. J. 1998. Feather pecking damage in growing bantams is influenced by dietary tryptofphan concentration but not dietary protein source. *Supplement Britisch Poultry Science,* 39:S17-S18.

Savory, C. J. and R. D. A. Fisher. 1992. Influence of a period of 'freeze-feeding' on behaviour of growing layers pullets. *Applied Animal Behaviour Science,* 35:55-66.

Savory, C. J. and J. S. Mann. 1997. Behavioural development in groups of pen-housed pullets in relation to genetic strain, age and food form. *British Poultry Science,* 38:38-47.

Savory, C. J. and J. S. Mann. 1999. Feather pecking in groups of growing bantams in relation to floor litter substrate and plumage colour. *British Poultry Science,* 40:565-572.

Savory, C. J., J. S. Mann and M. G. Macleod. 1999. Incidence of pecking damage in growing bantams in relation to food form, group size, stocking density, dietary tryptophan concentration and dietary protein source. *British Poultry Science,* 40:579-584.

Schaible, P. J., J. A. Davidson and S. L. Bandemer. 1947. Cannibalism and feather pecking in chicks as influenced by certain changes in a specific ration. *Poultry Science,* 26:651-656.

Sirén, M. J. 1963. Cannibalism in cockerels and pheasants. *Acta Veterinaria Scandinavica,* 4:1-47.

Sørensen, P. and L. G. Christensen. 1997. Laying hens may have lost important genes. *Animal Genetic Resources Information*:81.

Steenfeldt, S., R. M. Engberg and J. B. Kjaer. 2001. Feeding roughage ro laying hens affects egg production, gastro-intestinal parameters and mortality. *Proceedings of the 13th European symposium on poultry nutrition.*, Blankenbergen,

Sunde, M. L. 1972. Zinc requirement for normal feathering of commercial Leghorn-type pullets. *Poultry Science,* 51:1316-1322.

Supplee, W. C., G. F. Combs and D. L. Blamberg. 1958. Zinc and potassium effects on bone formation, feathering and growth of poults. *Poultry Science*:63-67.

Svihus, B., H. Hetland, M. Choct and F. Sundby. 2002. Passage rate through the anterior digestive tract of broiler chickens fed on diets with ground and whole wheat. *British Poultry Science,* 43:662-668.

Tanaka, T., T. Yoshimoto and K. Mimura. 1983. Feeding behaviour of laying hens fed pellets, crumbles or mash feed. *Japanese Journal of Zootechnical Science,* 54:433-438.

Tauson, R. and S. A. Svensson. 1980. Influence of plumage conditions on the hen's feed requirement. *Swedish Journal of Agricultural Research,* 10:35-39.

Van der Lee, A. G., G. Hemke and R. P. Kwakkel. 2001. Low density diets improve plumage condition in non-debeaked layers. *Proc.13th Eur. Symp. poult. nutr.*, Blankenbergen, 244-245.

Van Hierden, Y. M., S. M. Korte and J. M. Koolhaas. 2003. Chronic increase of dietary L-Tryptophan decreases feather pecking behaviour. *Applied Animal Behaviour Science*:Submitted for publication.

Van Hierden, Y. M., S. F. d. Boer, S. M. Korte and J. M. Koolhaas. 2004. The control of feather pecking by serotonin. *Behavioural Neuroscience,* 118:575-583.

Van Hierden, Y. M., S. M. Korte, E. W. Ruesink, C. G. v. Reenen, B. Engel, G. A. H. Korte Bouws, J. M. Koolhaas and H. J. Blokhuis. 2002. Adrenocortical reactivity and central serotonin and dopamine turnover in young chicks from a high and low feather-pecking line of laying hens. *Physiology and Behavior,* 75:653-659.

Vestergaard, K. S., J. P. Kruijt and J. A. Hogan. 1993. Feather pecking and chronic fear in groups of red junglefowl: their relations to dustbathing, rearing environment and social status. *Animal Behaviour,* 45:1127-1140.

Wahlstrom, A., R. Tauson and K. Elwinger. 1998. Effects on plumage condition, health and mortality of dietary oats/wheat ratios to three hybrids of laying hens in different housing systems. *Acta Agriculturae Scandinavica. Section A, Animal Science,* 48:250-259.

Wahlstrom, A., R. Tauson and K. Elwinger. 2001. Plumage condition and health of aviary-kept hens fed mash or crumbled pellets. *Poultry Science,* 80:266-271.

Walser, P. and H. P. Pfirter. 2001. Feed structure influences behaviour of laying hens. *Proceedings of the 6th European symposium on poultry welfare.*, Eds.: H. Oester &C. Wyss.

Wechsler, B., B. Huber Eicher and D. R. Nash. 1998. Feather pecking in growers: a study with individually marked birds. *British Poultry Science,* 39:178-185.

Willimon, C. P. and C. L. Morgan. 1953. The effect of minor nutrient mineral elements in the diet of chickens on feather pulling and cannibalism. *Poultry Science,* 32:309-313.

Wouw, S. v. d. 1995. Kannibalisme bij biologische legkippen. *Landbouw Universiteit Wageningen.* 109.

Zeltner, E., T. Klein and B. Huber Eicher. 2000. Is there social transmission of feather pecking in groups of laying hen chicks? *Animal Behaviour,* 60:211-216.

Chapter 3

EFFECT OF NUTRIENT DILUTION ON FEED INTAKE, EATING TIME AND PERFORMANCE OF HENS IN EARLY LAY

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EFFECT OF NUTRIENT DILUTION ON FEED INTAKE, EATING TIME AND PERFORMANCE OF HENS IN EARLY LAY

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ABSTRACT

1. An experiment with 480 ISA Brown layer strains was conducted to measure the effect of dietary energy (11.8, 11.2 and 10.6 MJ/kg) and NSP (128, 146 and 207 g/kg) concentration, soluble NSP content (64 and 85 g/kg), particle size distribution of the NSP fraction (fine and coarse) and feed form (mash and crumble) on feed intake, eating time and egg-performance of laying hens in early lay (from 18 to 26 weeks of age). Twelve experimental diets were tested, each replicated four times.

2. Laying hens in early lay that were fed low- or high-NSP diets were able to compensate for 10% dietary dilution by a 9.5 and 4.9% higher feed intake, respectively. Feeding crumble or coarsely ground mash did not affect feed intake.

3. Eating time of the hens fed the undiluted diets increased over the experimental period from 16.4 to 24.6% of the observation period, but was not affected by sand or grit addition, particle size distribution or feed form. Feeding high-NSP diets increased eating time by 22%.

4. Egg performance and body gain of the hens that were fed low-NSP or high-NSP diets were similar or better compared to the undiluted diets, whereas coarse grinding of the diets showed 7-10% lower egg performance and body gain. Egg performance and body gain was not affected by feed form.

5. It is concluded that hens in early lay, that were fed energy diluted diets, as a result of addition of sand or grit (low-NSP) or NSP-rich raw materials (high-NSP) to the control diet, were able to increase their feed intake, resulting in a comparable energy intake and egg performance as the control group. Supplementing diets with insoluble NSP also decreased eating rate. Prolonged eating time using insoluble NSP could be useful in reducing feather pecking behavior.

INTRODUCTION

Feather pecking in layers, that is often seen in modern alternative housing systems (Morgenstern, 1995; Mollenhorst, 2005), is a multi factorial problem, which can be caused by environmental, genetic or nutritional factors (Blokhuis, 1989). Nutritional factors may positively or negatively affect feather pecking behavior in laying hens (Van Krimpen et al., 2005). Some researchers reported that feather pecking behavior is a substitute for normal feeding behavior (Hoffmeyer, 1969; Blokhuis, 1989). Dietary deficiencies, resulting in a marginal supply of nutrients, such as protein (Ambrosen and Petersen, 1997), amino acids (Al Bustany and Elwinger, 1987a; Al Bustany and Elwinger, 1987b; Elwinger *et al.*, 2002), or minerals (Schaible *et al.*, 1947; Hughes and Whitehead, 1979), may increase feather pecking behavior and cannibalism. In addition, laying hens seem to spend more time on feeding and foraging behavior when they are fed mash diets in stead of crumbles or pellets, low energy diets, diets with high (in-)soluble Non Starch Polysaccharides (NSP) or roughages (Van Krimpen et al., 2005). Furthermore, diets high in insoluble NSP content increased the rate of digesta passage (Hartini *et al.*, 2003). Both an increased feeding and foraging time and/or an increased digesta passage rate may reduce feather pecking behavior (Hartini et al., 2003; Van Krimpen et al., 2005).

Laying hens, which are fed low nutrient density diets, will normally compensate for the lower nutrient concentration by increased feed intake (Savory, 1980; Van der Lee et al., 2001). However, because of the decreased feed intake capacity in young modern layer strains, we postulate that these birds at the onset of lay are not able to fully compensate for the dietary dilution by increased intake. Therefore, a reduction in nutrient density could result in a low nutrient intake during early lay, resulting in reduced laying performance.

The nutrient density of the diet can be decreased by addition of Low-NSP raw materials, like sand and grit, or by High-NSP raw materials, like oat hulls, soya hulls, beet pulp and straw. High-NSP raw materials may differ in water solubility of the NSP fraction, which can affect feed intake, viscosity of the chymus and feed passage rate (Hartini *et al.*, 2003). The combined effect of energy content, NSP content, water solubility of the NSP-sources and particle size of the NSP fraction on feed intake behavior of hens at early lay is unknown. We hypothesize that eating time will be increased by feeding diets with low energy levels and/or high contents of coarsely ground insoluble NSP's. Therefore, an experiment was conducted to investigate the effect of certain nutritional factors (nutrient density, NSP-content, solubility and particle size of NSP-fraction, as well as feed form) on feed intake behavior, eating time and performance of young layers.

MATERIALS AND METHODS

Housing, birds and management

A total of 480 beak trimmed 16 wk old layers (Isa Brown strain) were housed in two climate controlled rooms, both measuring 9 x 9 m. Each room had 24 floor pens (90 x 150 cm.), while a laying nest was placed at the outside of the pen. The pens were built of wire and hens could see their flock mates in other pens. Each pen contained 2 perches, a feeding trough (length of 100 cm), nipple drinkers. Initially, hens were housed with ten birds per pen, and sand was used as litter. Average body weight at arrival at 16 weeks of age was 1281 g (±17.3). Because hens were allotted to the pens on the basis of weight, initial body weight was similar for all treatments. For 1.5 weeks, hens were fed a commercial diet (ME = 10.9 MJ/kg) for rearing birds. From an age of 17.5 weeks hens received the experimental laying diets until the end of the experiment, 8 weeks later. Birds were fed *ad libitum* and had free access to water. Room temperature was set at 20°C and two times a day, health status of the hens, room temperature and air humidity were monitored.

At 16 weeks, the light schedule was set at 10L : 14D (10 Lux). Weekly, the light period was extended by one hour, while light intensity was increased three times, till the birds had a 16L: 8D (50 Lux) light schedule at the age of 22 weeks. Photoperiod lasted from 1:00 to 17:00h. Throughout the experiment, litter quality was maintained monthly by adding new sand.

Experimental design

A randomized block design, of twelve treatments x two replicates (blocks) in each of the two rooms was used. The control diets in mash (diet 1) and crumble (diet 2) met the NRC requirements of laying hens (NRC, 1994). Sand and grit as low-NSP dilution materials were tested in diet 3 (sand, mash), diet 4 (sand, crumble) and diet 5 (grit, mash). Diet 6 to 11 contained five different NSP-sources (oat hulls, beet pulp, arbocel, soya hulls, and straw), differing in the content of (in-) soluble NSP and particle size. Arbocel is a pure alpha cellulose source in powdered form. Finally, a positive control diet was tested (treatment 12). This diet was balanced for the NRC requirements (NRC, 1994), but the energy content was 5% lower and the NSP content 22% higher than the level of the negative controls. In this diet sunflower meal was used as the main NSP source. Diets 3 to 11 were 10% diluted, adding 100 grams diluents to 900 grams of control diet. The characteristics and classification of the different treatments are shown in Table 1.

Table 1. Characteristics and classification of the dietary treatments.

No.	Diet	Feed form	Nutrient dilution (%)	NSP level	Level of soluble NSP	Particle size
1	Negative Control – M	Mash	0	Intermediate	Low	Coarse
2	Negative Control – C	Crumble	0	Intermediate	Low	Fine
3	Sand – M	Mash	10	Low	Low	Fine
4	Sand – C	Crumble	10	Low	Low	Fine
5	Grit	Mash	10	Low	Low	Coarse
6	Oat hulls (fine)	Mash	10	High	Low	Fine
7	Oat hulls (coarse)	Mash	10	High	Low	Coarse
8	Beet pulp	Mash	10	High	High	Coarse
9	Arbocel	Mash	10	High	High	Coarse
10	Soya hulls	Mash	10	High	High	Coarse
11	Straw	Mash	10	High	High	Coarse
12	Positive control	Mash	5	High	High	Fine

Most of the NSP sources were added to the diet after grinding. The oat hulls added to diet 6 were hammer milled, along with the other raw materials. The diet composition and the chemical contents are shown in Table 2a and 2b, respectively.

Table 2a. Diet composition (g/kg).

Treatment nr.	1,2	3 to 11	12
Diet	Negative control	Diluted diets	Positive control
Dilution level (%)	0	10	0
Diet composition (%)			
Maize	351	316	350
Wheat	300	270	165
Soybean meal	173	155	124
Peas	—	—	125
Sunflower seed, extracted	—	—	100
Oyster shells	73	66	70
Rapeseed, extracted	36	33	09
Soybean oil	29	26	23
Limestone	20	18	18
Monocalcium phosphate	8	7	7
Premix laying hen	5.0	4.5	4.8
Salt	3.7	3.3	3.3
DL-Methionine	1.4	1.3	0.9
L-Lysine	0.8	0.7	—
Source of dilution	—	100	—

Table 2b. Composition (%) and analyzed and calculated chemical contents of the diet (g/kg as-fed basis).

Treatment No.	1[1]	2[2]	3	4	5	6	7	8	9	10	11	12[3]
Dilution source	—	—	Sand	Sand	Grit	Oat hulls	Oat hulls	Beet pulp	Arbocel	Soya hulls	Straw	—
Feed Form	Mash	Crumble	Mash	Crumble	Mash	(fine) Mash	(coarse) Mash	Mash	Mash	Mash	Mash	Mash
Dilution level (%)	0		10	10	10	10	10	10	10	10	10	0
Analyzed chemical contents (g/kg as-fed basis)												
Ash	125	113	209	185	194	109	105	108	103	106	117	104
Crude protein	157	161	145	149	147	148	147	154	150	156	151	173
Crude fat	39	44	39	38	36	29	29	30	29	37	33	27
Crude fibre	25	27	21	23	23	51	53	42	68	56	54	41
Starch	370	370	338	342	345	358	351	335	340	332	334	359
Sugar	37	42	35	37	35	35	35	43	34	37	36	39
Insoluble NSP [4])	78	79	71	70	71	138	144	113	144	123	131	101
Soluble NSP [4])	69	65	53	60	61	61	67	100	80	89	79	78
ADF	31	33	29	29	29	68	69	53	108	70	66	47
Lignin	10	12	12	11	9	19	18	11	12	11	12	16
Cellulose [4])	21	21	16	18	20	49	51	41	96	59	54	32
Hemi cellulose [4])	47	46	43	41	42	70	75	60	35	53	64	54
Calcium	38.6	33.3	32.9	30.5	33.7	33.8	31.8	33.9	32.2	33.5	32.8	32.1
Calculated chemical content (g/kg as-fed basis)												
ME (MJ/kg)	11.8	11.8	10.6	10.6	10.6	10.8	10.8	11.1	10.6	10.6	10.6	11.2
dig. Lysine	6.7	6.7	6.0	6.0	6.0	6.1	6.1	6.3	6.0	6.0	6.0	6.5
dig. Meth.+Cys.	5.8	5.8	5.2	5.2	5.2	5.3	5.3	5.4	5.2	5.2	5.2	5.2

[1]) Negative control Mash

[2]) Negative control Crumble

[3]) Positive control Mash

[4]) Cellulose = ADF – ADL; hemi cellulose = NDF – ADF; NSP (DM base) = 1000 – ash – crude protein – fat – starch – sugar; soluble NSP = NSP – NDF.

Addition of 100 g/kg sand or grit to the control diet increased the ash content from 69 (grit) to 84 g/kg (sand, mash), while the other chemical components were diluted up to 10%. Addition of 100 g/kg high-NSP raw materials to the control diet decreased the contents of ash, protein, fat and starch up to 10%, whereas the contents of crude fibre, NSP, (hemi-) cellulose and lignin increased.

OBSERVATIONS

Particle size distribution

The particle size distribution of the diets was analyzed by using the wet sieve method (Goelema *et al.*, 1999). The seven particle size fractions were separated by using six sieves with diameters of 0.25, 0.50, 1.25, 2.50, 3.15 and 5.0 mm respectively.

Body weight, feed intake and egg production

All hens were weighed individually in the pre-experimental period (at 16 wks of age) and per pen at 17, 21, 24 and 26 wks of age. Feed consumption and egg production per pen were recorded weekly. Egg weight per pen was based on the amount of 'normal' egg mass, i.e. all clean and dirty (blood- or fecal-stained), normal graded eggs. The remaining 'abnormal' egg mass consisted of broken, cracked, shell-less, double-yolked and very small (< 30 g) eggs. For the trait 'total egg mass' the entire egg mass production was calculated, assuming shell-less and cracked eggs to weigh the mean 'normal' egg weight of that specific pen and week.

Eating time

In week 4, 7 and 9, video observations were made from which eating time per pen could be calculated. The day was divided in three blocks, from 9.00 until 11.00 hrs, 11.30 until 13.30 hrs and from 14.00 until 16.00 hrs. In each block on every day, eight pens were observed using 4 cameras. Each observation lasted one hour. The number of eating hens (between 0 and 10), was recorded continuously until the end of each observation by using Observer 4.1/5.0 software (Noldus, 1993). Then, the percentage of eating time per hen per pen per observation period was calculated. Eating rate on a weight base was calculated as feed intake (g/d) divided by daily eating minutes . Eating minutes per day were not determined, but calculated as the number of hours with light on (16 h) multiplied by the percentage of observed eating time.

CURVE-FITTING PROCEDURE AND STATISTICAL ANALYSIS

Performance data from each experimental unit were generated over time at regular intervals as longitudinal data. These data normally show a nonlinear pattern that can be described by exponential or logistic functions. An appropriate method to process such data is the use of general, nonlinear mixed effects models for repeated measures data (Lindstrom and Bates, 1990). The choice for a type of model was presumed on the knowledge of the development of the specific performance parameters.

A REML procedure in (Genstat 8 Committee, 2002) was used to estimate curve parameters per pen. The nonlinear parameters were estimated by using a two-step iterative procedure, starting from a first order Taylor approach (Lindstrom and Bates, 1990; Engel *et al.*, 2003). Following curve-fitting, the REML procedure tested which model parameters of the experimental factors differed from the base-level. Contrast statements were made with the control group (mash diet, average NSP-level, finely ground) versus low and high NSP level, coarse grinding, crumbles, and – within the high NSP treatments – soluble vs. insoluble NSP). In this experimental design the factor 'dilution' is confounded with 'NSP'. The models included random block effects and week effects per pen (both negligibly small), random pen effects, heterogeneity of the variance over time and dependency within time per pen (first order power) (Lindstrom and Bates, 1990; Engel *et al.*, 2003). Egg weight was corrected for number of weighed eggs, because average weight of the first eggs per pen varied highly as a result of low number of eggs. A residual term was added to account for records with no eggs. For each video observation week (week 4, 7 and 9) a REML analysis was performed to test the effects of feed form, grinding, NSP-level on eating time. The model was corrected for the effect of period of the day.

Feed intake, egg mass and body weight of the hens usually start at an initial value and increase over time to a maximum asymptotic value, following an exponential course. Mean body weight of the hens at the start of the experiment was similar for all treatments. An exponential function [1] was used to model feed intake, egg mass and body weight.

$$Y = A + B\left(1 - e^{-\alpha t}\right) \qquad [1]$$

where *Y* is the expected value of the performance parameter; *A* is performance value at $t = -\infty$; *B* is the increase of performance value over time; *t* is point in time (week number-1); α is velocity of increase of the performance parameter.

The water supply was disrupted over two days in room 2 in the fourth week of the experiment, resulting in a reduced average feed intake in that week. Because the experimental design was balanced per room, all treatments were equally affected by this. A factor was added to the model that corrected predicted feed intake for this effect. The average effect of it on feed intake is estimated as -36.4 (±2.71) g/hen/d. Feed intake values during that week were corrected for this.

Egg production and egg weight usually start at a low level, but increase over a number of weeks to an asymptotic value, which for egg production is near to 100%, following a S-shape pattern. Therefore, a logistic curve [2] was used to model egg production and egg weight. At $t = -\infty$ (hatch) egg production of the hens is zero, which means that the value of the intercept (A) for egg production was set at zero.

$$Y = A + \frac{B}{1 + e^{-\alpha(t-\mu)}} \qquad [2]$$

where *Y, A, B, t* and α are as in curve [1]; μ is point of inflection.

Curves for egg mass per treatment were generated by multiplying the values for rate of lay and egg weight and dividing these values by 100.

RESULTS

Particle size distribution

Particle size distributions of the diets, analyzed with the wet sieve method (Goelema *et al.*, 1999), are presented in Table 3.

Table 3. Particle size distribution of the diets.

No.	Diet	Feed Form	Average particle size (mm) [1]	Modulus of Uniformity [2]
1	Negative Control	Mash	0.87	5.4 : 1.2 : 3.4
2	Negative Control	Crumble	0.66	4.7 : 1.3 : 4.0
3	Sand	Mash	0.71	4.6 : 1.7 : 3.7
4	Sand	Crumble	0.59	3.8 : 1.9 : 4.3
5	Grit	Mash	1.00	5.9 : 0.8 : 3.3
6	Oat hulls (fine)	Mash	0.78	5.3 : 1.1 : 3.6
7	Oat hulls (coarse)	Mash	0.87	5.4 : 1.0 : 3.6
8	Beet pulp	Mash	0.88	5.7 : 0.8 : 3.5
9	Arbocel	Mash	0.82	5.1 : 1.6 : 3.3
10	Soya hulls	Mash	0.84	5.5 : 1.1 : 3.4
11	Straw	Mash	0.85	5.5 : 1.0 : 3.5
12	Positive control	Mash	0.81	5.5 : 1.1 : 3.4

[1] Calculated as (Fraction < 0.25mm * 0.125) + (Fraction 0.25 – 0.50mm * 0.375) + (Fraction 0.50 – 1.25mm * 0.875) + (Fraction 1.25 – 2.50mm * 1.875) + (Fraction 2.50 – 3.15mm * 2.830) + (Fraction 3.15 – 5.00mm * 4.07) + (Fraction > 5.00mm * 6.50)/100.

[2] Ratio of particle size fractions; coarse (> 1.25mm) : intermediate (1.25 – 0.16mm) : fine (< 0.16mm)

Average particle size varied from 0.59 mm (sand, crumble) to 1.00 mm (grit, mash). Diets in crumble form had lowest average particle size and largest amount of fine particles, whereas the grit diet showed highest average particle size and largest amount of coarse particles. Crumbling reduced the average particle size (± sd) of both the control and the sand diets with 20.8% (± 3.9). Average particle size of the high-NSP diets was 9% higher than the how-NSP diets (0.77 ± 0.17 versus 0.83 ± 0.03 mm). Average particle size and modulus of uniformity of the high-NSP diets showed little variation.

Feed intake

For a useful comparison between the treatments, particular combinations of treatments (contrast) are presented in the results (mash versus crumble, intermediate-NSP versus low-NSP and high-NSP, soluble versus insoluble NSP, and fine versus coarse grinding). The estimates of the intake parameters per treatment, as described by an exponential curve, are given in Table 4. Over the first week of the experiment hens of the control group consumed on average 68 (± 3.2 g/d, while the asymptotic feed intake of this treatment was estimated as 125 (± 3.6) g/d. Initial feed intake of hens that were fed the high-NSP diets was 9.4 (± 3.7) g/d higher than the control, whereas hens that were fed crumbles consumed 9.3 (± 3.1) g/d less. The initial feed intake of the hens that were fed the soluble high-NSP diets was 5.7 (± 2.8) g/d less compared with the hens that were fed the insoluble high-NSP diets. Feeding low-NSP diets (NSP-) or coarse ground diets did not affect the initial feed intake. Hens

Table 4. Parameter estimates (A, B and α; standard error within brackets) of feed intake (g/hen/d) as described by an exponential curve for Control, Low NSP, High NSP, Coarse grinding, Crumble and High soluble NSP treatment groupings.

Treatment grouping	Diet	Initial feed intake (g) (A)	Increase in feed intake (g) (B)	Rate of increase (α)	Asymptotic feed intake (g) A+B)
Control	1	67.96 (3.22)	57.03 (3.57)	0.478 (0.08)	124.99
		Differences in parameter estimates compared with the control group			
NSP-Low	3-5	3.425 (3.13)	**8.391^{***} (3.32)**	0.075 (0.06)	11.816
NSP-High	6-12	**9.407^{*} (3.72)**	-3.306 (3.97)	0.056 (0.08)	6.101
Coarse grinding	5, 7-11	0.484 (2.41)	1.431 (2.58)	-0.075 (0.05)	1.915
Crumble form	2,4	**-9.257^{**}(3.14)**	**7.448^{*} (3.31)**	0.071 (0.06)	-1.809
Level NSP-High class	6-12	77.37 (3.72)	53.72 (3.97)	0.534 (0.08)	131.09
		Differences in parameter estimates compared with the NSP-High class			
NSP-High Soluble	8-12	**-5.710^{*} (2.84)**	**$5.415^{\#}$ (3.04)**	0.011 (0.06)	-0.295

= $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ (all in bold)

that were fed the low NSP diets, however, had a higher (8.4 ± 3.3 g/d) increase in feed intake than the control. Although the hens that were fed crumble had a lower initial value of feed intake, their increase in feed intake was higher (7.4 ± 3.3 g/d) than that of the control hens. As a result, maximum feed intake of the crumble fed hens was similar to that of the control hens. The increase in feed intake of hens that were fed soluble high-NSP diets was numerically 5.4 (± 3.0) g/d higher ($P < 0.10$), compared with hens that were fed insoluble-rich diets. The rate of increase in feed intake over time was not affected by the treatments.

Eating time and eating rate

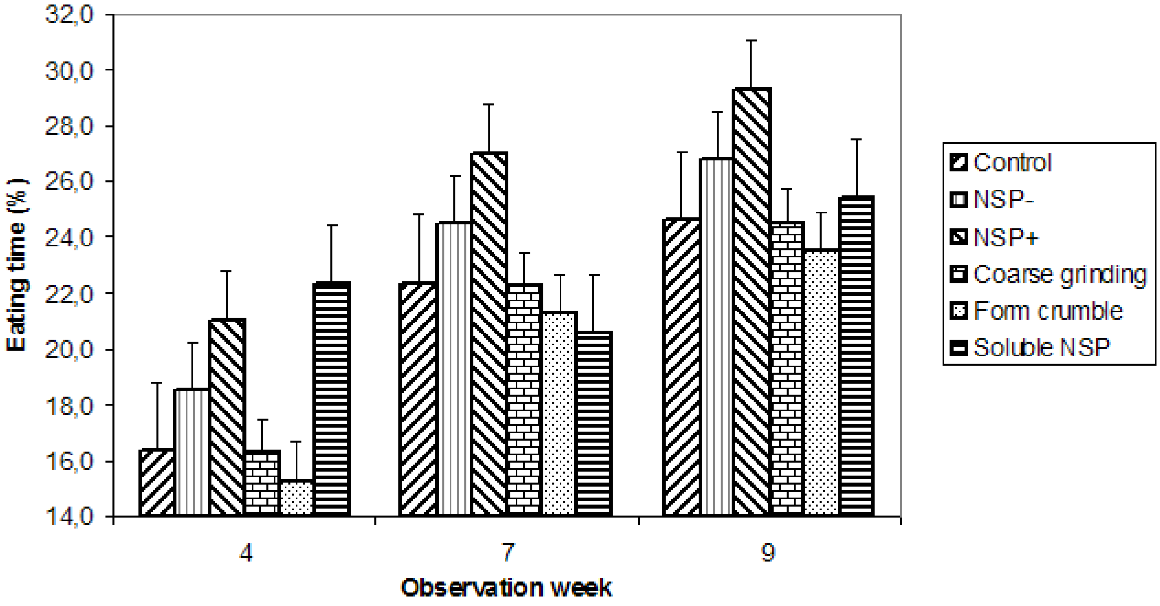
Based on video observations the percentage of time the hens were spending on feed intake was recorded. Eating behavior was observed during week 4, 7 and 9 of the experiment. The results are summarized in Table 5 and Figure 1a.

Table 5. Effect of week and period of the day on eating time (% of observation period) of the control group (standard error within brackets).

	Week		
Treatment grouping	4	7	9
Control group (%), week 4, period 1 (9.00 – 11.00h)	13.21 (2.457)		
Effect of week	—	**7.27 (2.284)** ***	**9.21 (2.446)** ***
Effect of Period 2 (11.30 - 13.30h)	-0.48 (2.166)	3.05 (2.930)	3.95 (2.930)
Effect of Period 3 (14.00 - 16.00h)	**10.00 (2.249)** **	-6.92 (3.662)	-6.82 (3.662)
Total level Control group (%)	16.4	22.4	24.6

** = *P*< 0.01; *** = *P*< 0.001 (all in bold

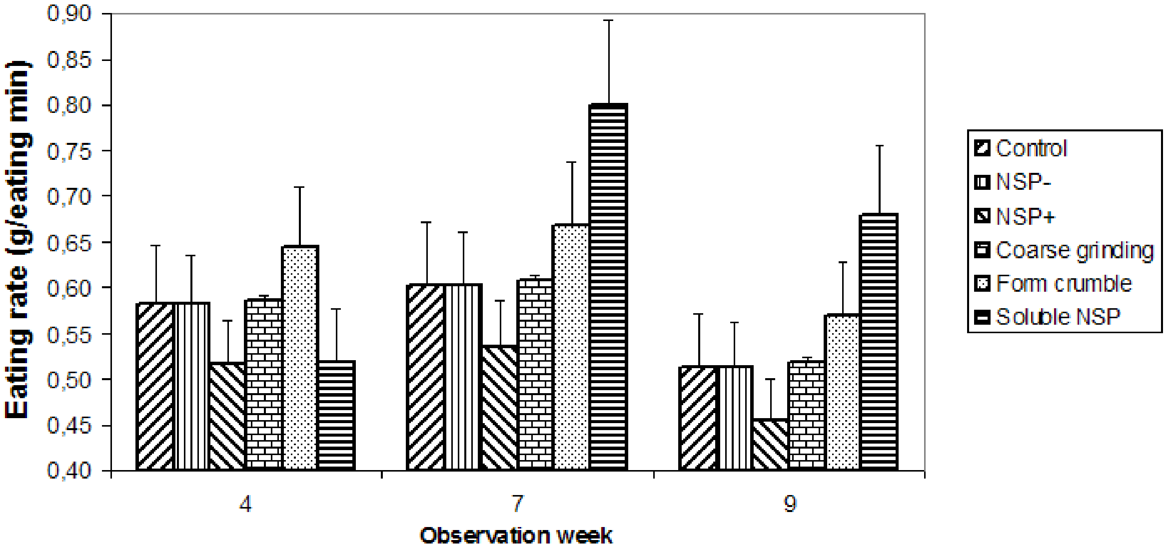
Figure 1a. Eating time (%) of the treatment groups Control, Low NSP, High NSP, Coarse grinding, Crumble and Soluble NSP over week 4, 7, and 9.



Eating time increased over the experimental period. In week 4, 7, and 9 the control group spent respectively 16.5%, 22.4% and 24.6% (± 2.4) of their time on feed intake. In week 4, eating time was significantly (*P*=0.002) affected by period of the day; over period 3 eating time was 10.0% (± 2.2) higher than over period 1 and 2. Eating time was not affected by feeding low-NSP, coarsely ground or crumbled diets. Hens that were fed high-NSP diets on average spent 4.6% (± 1.8) more time on feed intake (*P*=0.06) over the whole experimental period. Over week 4, eating time of the soluble NSP treatment did not differ from the level of the high-NSP treatment. Eating time, however, significantly (*P*=0.007) reduced over week 7 (-7.7% ± 2.45) and week 9 (-5;2% ± 2.45), compared to the high-NSP treatment.

Eating rate (feed intake (g)/eating minute) data is shown in Figure 1b.

Figure 1b. Eating rate (g feed intake/eating minute/) of the treatment groups Control, Low NSP, High NSP, Coarse grinding, Crumble and Soluble NSP over week 4, 7, and 9.



Eating rate significantly differed per observation week (*P*<0.001). Eating rate of the control group was similar for week 4 and 7 (0.58–0.60 g/min ± 0.065), but decreased to 0.51 g/min (± 0.057) over week 9. During week 7 and 9, eating rate was significantly (*P*=0.001) affected by period of the day; in period 3 eating rate was respectively 0.59 and 0.54 g/min (± 0.10) higher than in period 1 and 2. Hens that were fed low-NSP, coarsely ground or crumbled diets had similar eating rates as the control group. Dilution with NSP-rich raw materials numerically (*P*=0.08) decreased eating rate with 0.06 g/min. Eating rate of the soluble NSP-group showed an interaction effect with observation week (*P*=0.004), resulting in a similar eating rate over week 4, but an increased eating rate over week 7 (0.20 g/min ± 0.092) and week 9 (0.17 g/min ± 0.078), compared to the control group.

Rate of lay

The estimates of the rate of lay parameters per treatment, as described by a logistic curve, are given in Table 6.

Table 6. Parameter estimates (A, B, α and μ; standard error within brackets) of rate of lay (%) as described by a logistic curve for Control, Low NSP, High NSP, Coarse grinding, Crumble and High soluble NSP treatment groupings.

Treatment grouping	Diet	Increase in hen-day egg production (%) (B)	Rate of increase (α)	Point of inflection (wk – 1) (μ)
Control	1	97.35 (2.02)	1.531 (0.12)	3.032 (0.13)
		Differences in parameter estimates compared with the control group		
NSP-Low	3-5	1.824 (1.89)	**0.334**** **(0.12)**	-0.211 (0.13)
NSP-High	6-12	-1.239 (2.23)	**0.411**** **(0.16)**	-0.150 (0.15)
Coarse grinding	5, 7-11	-1.034 (1.28)	**-0.155**$^{\#}$ **(0.09)**	0.075 (0.08)
Crumble form	2,4	-2.129 (1.87)	0.137 (0.12)	-0.043 (0.12)
Level NSP-High class	6-12	96.11 (2.23)	1.941 (0.16)	2.882 (0.15)
		Differences in parameter estimates compared with the NSP-High class		
NSP-High Soluble	8-12	1.018 (1.72)	-0.111 (0.13)	0.090 (0.11)

= $P < 0.10$; ** = $P < 0.01$ (all in bold)

Rate of lay of the control group increased from 0% to 97.4%. The asymptotic rate of lay value was not affected by any of the treatments. Increase in rate of lay, however, differed significantly between treatments. Rate of increase was higher in hens that were fed low-NSP diets (0.334 ± 0.12) or high-NSP diets (0.411 ± 0.16) than the control hens, which means that these hens reached their maximum egg production earlier. This resulted in more eggs over the experimental period. The rate of increase in egg production was enhanced by both the soluble and insoluble NSP sources. The rate of increase of the hens that were fed coarse ground diets was numerically lower (-0.155 ± 0.09; $P < 0.10$) compared to the control. The point of inflection of the control group was reached in week 4 ($t - 1 = 3$), which means that from week 4 the rate of increase of rate of lay shifts to lower values. Point of inflection was not affected by treatments.

Egg weight

The estimates of the egg weight parameters per treatment, as described by a logistic curve, are given in Table 7.

Table 7. Parameter estimates (A, B, alpha and mu; standard error within brackets) of egg weight (g) as described by a logistic curve for Control, Low NSP, High NSP, Coarse grinding, Crumble and High soluble NSP treatment groupings.

Treatment grouping	Diet	Initial egg weight (g) (A)		Increase in egg weight (g) (B)		Rate of increase (α)		Point of inflection (wk – 1) (μ)		Asymptotic egg weight (g) (A+B)
Control	1	46.72	(1.55)	12.35	(1.68)	1.569	(0.29)	4.015	(0.20)	59.07
		Differences in parameter estimates compared with the control group								
NSP-Low	3-5	-0.091	(1.95)	1.034	(2.29)	-0.346	(0.27)	0.185	(0.25)	0.943
NSP-High	6-12	-4.134	(3.85)	7.037	(4.59)	**-0.730***	**(0.32)**	-0.345	(0.49)	2.903
Coarse grinding	5, 7-11	**-3.269$^{\#}$**	**(2.02)**	3.689	(2.40)	**-0.237$^{\#}$**	**(0.14)**	-0.242	(0.25)	0.420
Crumble form	2,4	-1.002	(2.60)	2.333	(3.24)	-0.252	(0.31)	-0.060	(0.32)	1.331
Level NSP-High class	6-12	42.59	(3.85)	19.39	(4.59)	0.839	(0.32)	3.67	(0.49)	61.97
		Differences in parameter estimates compared with the NSP-High class								
NSP-High Soluble	8-12	-2.225	(4.21)	3.540	(5.04)	-0.045	(0.16)	-0.041	(0.52)	1.32

= $p < 0.10$; * = $P < 0.05$ (all in bold)

Initial egg weight of the control group was 46.7 g (± 1.6). It increased by 12.4 g (± 1.7) to an asymptotic egg weight of 59.1 g (± 1.7). Feeding coarse ground diet reduced initial egg weight numerically ($P < 0.10$) by 3.3 g (± 2.0) compared with the control. Rate of increase of egg weight was lower in hens that were fed high-NSP (-0.730 ± 0.32; $P<0.05$) or coarse ground (-0.237 ± 0.14; $P<0.10$) diets compared to the control hens. Therefore the maximum egg weight of these treatments was reached at a later time compared with the control. Egg weight parameters were not affected by low-NSP diets, feed form or solubility of the NSP sources. In conclusion, coarse grinding of the diets negatively affected initial egg weight, whereas the rate of increase of egg weight decreased when the hens were fed high-NSP or coarse ground diets.

Body weight

The estimates of the body weight parameters per treatment, as described by an exponential curve, are given in Table 8.

Table 8. Parameter estimates (A, B and alpha; standard error within brackets) of body weight (kg) as described by an exponential curve for Control, Low NSP, High NSP, Coarse grinding, Crumble and High soluble NSP treatment groupings.

Treatment grouping	Diet	Initial body weight (kg) (A)	Increase in body weight (kg) (B)	Rate of increase (wk – 1) (α)	Asymptotic body weight (kg) (A+B)
Control	1	1.382 (0.01)	0.502 (0.03)	0.288 (0.03)	1.890
		Differences in parameter estimates compared with the control group			
NSP-Low	3-5	0	-0.031 (0.03)	0.045 (0.03)	-0.031
NSP-High	6-12	0	-0.044 (0.03)	**0.095* (0.05)**	-0.044
Coarse grinding	5, 7-11	0	**-0.035* (0.02)**	0.016 (0.03)	-0.035
Crumble form	2,4	0	0.007 (0.03)	-0.016 (0.03)	-0.007
Level NSP-High class	6-12	1.382 (0.01)	0.458 (0.03)	0.383 (0.05)	1.845
		Differences in parameter estimates compared with the NSP-High class			
NSP-High Soluble	8-12	0	-0.006 (0.02)	0.005 (0.04)	-0.006

* = $P < 0.05$ (all in bold)

Body weight of the hens at the start of the observation period was 1.382 kg (± 0.01). The increase in body weight was 0.502 kg (± 0.03) for the control hens. As a consequence of the relatively short observation period, maximum body weight in these birds was not reached during this experiment. Coarse grinding of the diet reduced the increase of body weight by 0.035 kg (± 0.02). Addition of (in-)soluble High-NSP sources to the diet enhanced the rate of increase of body weight (0.095 ± 0.05) development compared with the control.

DISCUSSION

EFFECT OF NUTRIENT DILUTION IN LOW-NSP DIETS

The reduction in dietary density is always confounded with changes in the concentration of other ingredients and nutrients, like amino acid and NSP concentration. Until now, the pure effects of energy dilution and NSP supplementation on feed intake behavior are unknown. In this experiment the effect of energy dilution with or without NSP addition on feed intake behavior and performance was investigated. We conclude that hens in early lay have a large ability to compensate for dietary dilution. Feed intake of hens that were fed diets diluted with 10% low-

NSP raw materials (on a weight base) was increased by 10%, resulting in an similar nutrient intake compared to undiluted diets. Furthermore, feeding these low-NSP diets numerically (but not significantly) prolonged eating time by 10%; eating rate, however, was not affected. This is in accordance with the results of Savory (1980) who fed male Japanese quail diluted (with 40% cellulose) and undiluted diets. Those receiving the diluted mash consumed about 40% more feed (14.9 vs. 10.8 g/d) and spent a higher proportion of time on feed intake (23.8 vs. 9.1%). An increase in feed intake and eating time may compensate for redirected foraging behavior, resulting in decreased feather pecking behavior.

Feeding low-NSP diets to hens in early lay resulted in similar or even better egg production compared with hens that were fed standard diet. These results are in accordance with other experiments with hens of higher age. Feeding laying hens (30 – 52 weeks of age) a 5% nutrient diluted diet did not affect egg performance compared to hens that were fed a standard diet (Van der Lee et al., 2001). In a recent trial with laying hens (34-37 weeks of age) in our facilities, a reduced dietary energy concentration (by adding 10, 20, 25 or 30% sand) did not affect egg performance of the hens (Meulen *et al.*, Submitted). The hens fully compensated for the effect of added sand in the diet by increasing their daily feed intake. The increase in body weight, however, was less for the hens that were fed the diets with the lowest densities. Even nutrient to egg conversion ratio's of the hens that were fed the diluted diets were improved, indicating that the presence of sand may have had a beneficial effect on performance. However, the mode of action of this effect is not clear. Such positive effects may be explained by sand being useful in degradation of the feed particles, as well as stimulating gut motility. Laying hens that were fed roughages are able to increase their daily feed consumption (as-fed base) by 70% compared with the control group, without negatively affecting productivity (Steenfeldt *et al.*, 2001). We can conclude that laying hens in early lay are able to compensate for dietary dilution with low-NSP raw materials by a higher feed intake.

EFFECT OF NUTRIENT DILUTION IN (IN-)SOLUBLE HIGH-NSP DIETS

Feed intake of hens that were fed diets diluted with 10% high-NSP raw materials was on average increased by 8%, resulting in an almost similar nutrient intake compared to undiluted diets. Furthermore, eating time was on average prolonged by 22% on feeding these high-NSP diets, whereas eating rate was decreased by 10%. The effect of high-NSP diets on eating time and eating time was larger than the effect of low-NSP diets, possibly due to differences in specific gravity of the raw materials. For instance, sand has a specific gravity of 1600 kg/m^3, against 780 kg/m^3 for oak wood (Jansen, 1977). Therefore, less volume of feed has to be consumed for reaching the same amount of feed intake when the hens are supplemented with sand-rich low-NSP diets, compared with high-NSP diets. (Savory, 1980) also suggested that the difference in meal length was related to dietary bulk.

Feed intake and eating time were clearly affected by NSP-source. Feed intake of hens that were fed insoluble NSP-rich diets, was only 6.4% higher than the control group, whereas eating time only differed significantly from the control group over week 4. In contrast to insoluble NSP-rich diets, diets high in soluble NSP increase digesta viscosity and reduce digesta passage (Hartini et al., 2003), which may cause birds to have a higher gut fill, resulting in a reduced feed intake. Birds fed diets high in insoluble NSP spent more time eating and appear calmer than those fed low-fiber diets (Hetland *et al.*, 2004).

NSP source had only a minor effect on egg production traits. This was confirmed by (Hartini et al., 2003) who performed a number of feeding experiments in which they substituted wheat by millrun, barley, rice hulls or oats as (in)soluble NSP sources on an isocaloric and isonitrogenous basis, and also found no detrimental effects on performance. A better performance may be due to an increased nutrient digestibility. Feeding diets supplemented with insoluble NSP's increased nutrient digestibility, possibly due to more reflux activity in the fore-gut (Hetland *et al.*, 2004). Possibly this phenomenon explains the improved rate of lay of the hens fed high-NSP diets in the current experiment.

We can conclude that laying hens in early lay are able to compensate for dietary dilution with high-NSP raw materials by a higher feed intake.

EFFECT OF PARTICLE SIZE DISTRIBUTION OF THE DIET

Particle size distribution had no effect on feed intake, eating time and eating rate. Particle size distribution as such did not improve performance. Moreover, feeding coarsely ground diets negatively affected egg performance of the young hens. These results were not expected. Coarse particles accumulate in the gizzard, stimulating gizzard weight and activity, like an increased reflux of bile acids, resulting in an improved starch digestibility and an enhanced emulsification of liberated lipids (Hetland *et al.*, 2003). Fine oat hulls will pass the gizzard immediately after intake, whereas coarse oat hulls were still found in the gizzard 48 hours post feeding. The fact that insoluble fiber accumulates in the gizzard may indicate a slower feed passage rate when the coarse fiber content of the diet increased. It is thought that accumulation of insoluble fiber in the gizzard triggers a temporary satiety, but once passed the gizzard, it passes through the gut quickly. This could make the bird feel more satisfied between feeding bouts, but more hungry after gizzard emptying (Hetland *et al.*, 2004). In conclusion, coarsely ground NSP sources do not stimulate feed intake behavior, but reduce egg performance in early lay. More insight on the long term effects of particle size of NSP sources on performance and feed intake behavior of layers is necessary.

EFFECT OF FEED FORM

Because it was unclear whether hens consumed sand added to mash diets voluntarily or that they refused to eat it, sand-rich diets were tested both in mash and crumble form and compared with the negative control diets. Feed form, however, had no effect on average feed intake, eating time and egg performance in the current experiment. Initial feed intake of the hens fed the crumble diets, however, was lower than the control (mash) diet, but the increase in feed intake was higher, indicating that it takes time for the hens to adapt to the crumble form. In contrast to our findings, (Tanaka *et al.*, 1983; Aerni *et al.*, 2000) showed that feeding crumbles decreased eating time. The results of the current experiment may imply that the hens had no selective intake of raw materials when they were fed the sand diets in mash form. Supplementing sand diets in mash form is an appropriate strategy for future experiments directed to the role of nutrition in controlling feather pecking behavior.

CONCLUSIONS

It is concluded that 10% dietary dilution with low-NSP or high-NSP sources increased asymptotic feed intake by 9.5% and 4.9% respectively, whereas eating time was increased by 10.1% and 22.0%. Asymptotic feed intake and eating time were not affected by feed form and particle size distribution. Dietary dilution with low-NSP or high-NSP sources did not affect performance of young layers. Feeding diets diluted with insoluble NSP-rich raw materials was the most effective dietary method to increase eating time and might play a role in decreasing feather pecking behavior.

REFERENCES

Aerni, V., H. El Lethey and B. Wechsler. 2000. Effect of foraging material and food form on feather pecking in laying hens. *Br. Poult. Sci.,* 41:16-21.

Al Bustany, Z. and K. Elwinger. 1987a. Response of laying hens to different dietary lysine intakes. A comparison of some commercial hybrids with strains selected on a low protein diet. *Acta Agric. Scand.,* 37:27-40.

Al Bustany, Z. and K. Elwinger. 1987b. Comparison between barley/fish meal- and maize/soybean meal-based diets with various lysine and protein levels fed to different strains of laying hens. *Acta Agriculturae Scandinavica,* 37:41-49.

Ambrosen, T. and V. E. Petersen. 1997. The influence of protein level in the diet on cannibalism and quality of plumage of layers. *Poult. Sci.,* 76:559-563.

Blokhuis, H. J. 1989. The development and causation of feather pecking in the domestic fowl, *Vakgroep Ethologie*, Wageningen, Thesis Landbouw Universiteit Wageningen,

Elwinger, K., R. Tauson, M. Tufvesson and C. Hartmann. 2002. Feeding of layers kept in an organic feed environment. *11th. European Poultry Conference*, Bremen,

Engel, B., K. v. Reenen and W. Buist. 2003. Analysis of correlated series of repeated measurements: application to challenge data. *Biometrical Journal,* 45:866-886.

Genstat 8 Committee. 2002. Genstat 8 Reference Manual; Release 3. Clarendon Press, Oxford, UK.

Goelema, J. O., A. Smits, L. M. Vaessen and A. Wemmers. 1999. 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. Techn.,* 78:109-126.

Hartini, S., M. Choct, G. Hinch and J. V. Nolan. 2003. Effect of diet composition, gut microbial status and fibre forms on cannibalism in layers. *Australian Egg Corporation Limited.* AECL Publication No 03/03:1-111.

Hetland, H., B. Svihus and A. Krogdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *British Poultry Science,* 44:275-282.

Hetland, H., M. Choct and B. Svihus. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poult. Sci. J.,* 60:415-422.

Hoffmeyer, I. 1969. Feather pecking in pheasants - an ethological approach to the problem. *Danish review of game biology,* 6:1-36.

Hughes, B. O. and C. C. Whitehead. 1979. Behavioural changes associated with the feeding of low-sodium diets to laying hens. *Appl. Anim. Ethology,* 5:255-266.

Jansen, A. I. 1977. BINAS Informatieboek vwo-havo voor het onderwijs in de natuurwetenschappen. Groningen.

Lindstrom, M. J. and D. M. Bates. 1990. Nonlinear mixed effects models for repeated measures data. *Biometrics,* 46:673-786.

Meulen, J. v. d., C. Kwakernaak and C. A. Kan. Submitted. Sand intake by laying hens and its effect on egg production parameters.

Mollenhorst, E. 2005. How to house a hen; assessing sustainable development of egg production systems, *Animal Production Systems*, Wageningen, Thesis Wageningen University,

Morgenstern, R. 1995. Alternative Haltung in der Schweiz: Woran erkranken die Legehennen? *DGS Magazin,* 4-2-1995:11-14.

Noldus. 1993. The Observer®: Base package for Dos. Reference Manual, version 3.0.

NRC. 1994. Nutrient requirements of poultry. *Ninth Revised Edtion.* Washington DC.

Savory, C. J. 1980. Meal occurrence in Japanese quail in relation to particle size and nutrient density. *Anim. Behav.,* 28:160-171.

Schaible, P. J., J. A. Davidson and S. L. Bandemer. 1947. Cannibalism and feather pecking in chicks as influenced by certain changes in a specific ration. *Poultry Science,* 26:651-656.

Steenfeldt, S., R. M. Engberg and J. B. Kjaer. 2001. Feeding roughage ro laying hens affects egg production, gastro-intestinal parameters and mortality. *Proceedings of the 13th European symposium on poultry nutrition.*, Blankenbergen,

Tanaka, T., T. Yoshimoto and K. Mimura. 1983. Feeding behaviour of laying hens fed pellets, crumbles or mash feed. *Japanese Journal of Zootechnical Science,* 54:433-438.

Van der Lee, A. G., G. Hemke and R. P. Kwakkel. 2001. Low density diets improve plumage condition in non-debeaked layers. *Proc.13th Eur. Symp. poult. nutr.*, Blankenbergen, 244-245.

Van Krimpen, M. M., R. P. Kwakkel, B. F. J. Reuvekamp, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2005. Impact of feeding management on feather pecking in laying hens. *World's Poult. Sci. J.,* 61:665-687.

Chapter 4

LOW DIETARY ENERGY CONCENTRATION, HIGH NONSTARCH POLYSACCHARIDE CONCENTRATION, AND COARSE PARTICLE SIZES OF NONSTARCH POLYSACCHARIDES AFFECT THE BEHAVIOR OF FEATHER-PECKING-PRONE LAYING HENS

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LOW DIETARY ENERGY CONCENTRATION, HIGH NON STARCH POLYSACCHARIDES CONCENTRATION, AND COARSE PARTICLE SIZES OF NSP AFFECT BEHAVIOR OF FEATHER PECKING PRONE LAYING HENS

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ABSTRACT

An experiment was conducted with 504 non-cage housed ISA Brown laying hens from 18 to 40 weeks of age to investigate the separate effects of dietary energy concentration, Non Starch Polysaccharides (NSP) concentration, and particle sizes of added NSP source on eating behavior, feather pecking behavior and hen performance of laying hens. Hens were allotted to 1 of 6 dietary treatments according to a 2 x 3 factorial arrangement, with 7 replicates per treatment. The factors were control and low energy concentration (2825 versus 2540 kcal/kg), control and high NSP concentration (133 versus 195 g/kg), and fine versus coarse particle size of the added NSP source in the NSP high diets. We hypothesized that eating time will be increased by feeding low energy diets, and/or coarsely ground NSP high diets, resulting in reduced feather pecking behavior, without negatively affecting hen performance.

Energy reduction, NSP addition and coarse grinding of NSP increased eating time by 14.2% (*P=0.001.*), 17.2% ($P<0.001$) and 7.9% ($P=0.075$), respectively, compared with the control level of these factors. NSP addition decreased eating rate (g/min) by 21.0% ($P=0.010$). Layers performed already gentle feather pecking behavior during the 5th week of the rearing period. Dietary treatments did not affect maximal level of feather condition scores, but arise of feather damage was delayed by 10 weeks in hens fed low energy, coarsely ground NSP rich diets compared to hens fed control diets. Hens fed control NSP diets showed reduced culling rates, due to less cannibalistic pecking, if energy concentration was decreased (44.1% versus 13.1%), whereas in high NSP diets culling rate slightly decreased when hens were fed low energy diets (31.6% versus 28.6%) ($P=0.071$). Hens that were fed low energy diets compensated for 10% reduction in energy concentration by 9.3% higher maximal feed intake (143.0 versus 130.8 g/d). Hen performance and body gain of the hens were not affected by dietary treatments.

It is concluded that hens that were fed low energy or high (coarsely ground) NSP diets spend more time on feed intake, compared with hens that were fed control diets. As a result, some treatments showed less feather pecking behavior.

(Key words: feather pecking, laying hen, energy dilution, NSP, eating behavior)

INTRODUCTION

Feather pecking in layers that is often seen in modern alternative housing systems, is a multi factorial problem that can be caused by environmental, genetic or nutritional factors. Some reports hypothesized that feather pecking behavior is a substitute for normal ground pecking or feeding behavior in the absence of adequate foraging incentives (Hoffmeyer, 1969; Blokhuis and Van der Haar, 1989). In a recent study (Newberry et al., 2006), however, this hypothesis could not be confirmed. In that study, based on behavior of individual birds, a positive association was found between foraging behavior in young hens and severe feather pecking behavior during later ages. These conflicting results supports the need for a thorough analysis of the impact of nutritional factors on feather pecking behavior in layers.

Nutritional factors may positively or negatively affect feather pecking behavior in laying hens (Van Krimpen et al., 2005). Dietary deficiencies, resulting in a marginal supply of nutrients, such as protein (Ambrosen and Petersen, 1997), amino acids (Al Bustany and Elwinger, 1987), or minerals (Hughes and Whitehead, 1979), may increase feather pecking behavior and cannibalism. In contrast, the occurrence of feather pecking behavior seems to be reduced when laying hens are fed mash diets in stead of crumbles or pellets (Aerni et al., 2000). Reduced feather pecking behavior was also observed in hens fed low energy diets, diets with high (in-)soluble Non Starch Polysaccharides (NSP) or roughages (Van Krimpen et al., 2005). Laying hens that are fed low nutrient density diets, will compensate for the lower nutrient concentration by increased feed intake (Savory, 1980; Van der Lee et al., 2001). Therefore, hen performance will be maintained, even in early lay (Van Krimpen et al., 2007). Diets high in insoluble NSP content decreased eating rate and increased the rate of digesta passage (Hartini et al., 2003). Both an increased feeding and foraging time and/or an increased digesta passage rate may reduce feather pecking behavior (Hartini et al., 2003). It can be hypothesized that chickens prefer not just fiber, but coarse fiber (Hetland et al., 2004a). Feather eating and pecking behavior may be partly related to particle size of NSP that play a major role in the volume of gizzard contents (Hetland et al., 2003b).

Although energy and NSP concentrations and particle size of the added NSP source seem to reduce feather pecking behavior in laying hens, these nutritional factors were often confounded in experimental diets. Consequently, it's not clear which factor is most effective in causing these positive effects. Therefore, an experiment was conducted to investigate the separate effects of energy concentration, NSP concentration and particle size of added NSP source on eating behavior, feather pecking behavior and hen performance of laying hens. We hypothesize that eating time will be increased by feeding diets with low energy levels and/or high contents of coarsely ground insoluble NSP's, resulting in reduced feather pecking behavior, without negatively affecting hen performance.

MATERIALS AND METHODS

Housing, Birds and Management

A total of 504 non beak trimmed 16 wk old layers (Isa Brown strain) were housed in two climate controlled rooms. In each room, 21 floor pens (0.90 x 1.50 m) were used for this experiment. A laying nest was placed outside each pen. The pens were built of wire and hens could see their flock mates in other pens. Each pen contained perches, a feeding trough (length of 100 cm), nipple drinkers, while sand was used as litter. Hens were housed with twelve birds per pen (10.4 hens/m^2). To stimulate feather pecking behavior, stocking density was higher than usual in practice (9.0 hens/m^2). At the start of the experiment (18 weeks of age) average body weight was 1713 g (±48.0). The first 8 weeks of age hens received a standard commercial diet (ME = 2610 kcal/kg). To stimulate feed intake capacity, birds were fed a low energy rearing diet (ME = 2515 kcal/kg) from 9 to 17 weeks of age. During the rearing period, gentle feather pecking behavior was shown from 5 weeks of age onwards. Mortality rate during rearing period amounted 1.8% and was not affected by pecking behavior. From 18 to 40 weeks of age, hens received the experimental diets. Hens were fed the experimental diets *ad libitum.* All birds had free access to water. Room temperature was set at 20°C and health status of the hens was monitored daily. At 16 weeks of age, light schedule was set at 10L : 14D (10 Lux) and was gradually extended by one hour per week to 16L: 8D light schedule at the age of 22 weeks. Photoperiod lasted from 1:00 - 17:00 hrs. To stimulate feather pecking behavior, light intensity was increased to 20 Lux (week 18) and 30 Lux (week 20). Due to of an outbreak of cannibalism in week 21, light intensity was reduced to 20 Lux and maintained until the end of the experiment. Throughout the experiment, litter quality was maintained by adding new sand monthly.

Experimental Design

At 18 weeks of age, hens were allotted to 1 of 6 dietary treatments according to a 2 x 3 factorial arrangement, with 7 replicates per treatment (Table 1a and 1b). The factors were control and low energy concentration (2825 versus 2540 kcal/kg), control and high NSP concentration (133 versus 195 g/kg), and fine versus coarse particle size of the added NSP source in the NSP high diets. Sand was used as dilution material to reduce energy concentration in control NSP diets. Oat hulls were used to increase the NSP concentration. Oat hulls were finely ground in diets with fine particle sizes of NSP, whereas whole oat hulls were added in diets with coarse particle sizes of NSP. All diets were in mash form. Energy to protein ratio was similar for all diets.

Diet 1 (control energy and control NSP concentration) met the NRC requirements of laying hens (NRC, 1994). Energy concentration in low energy diets was reduced by 10% (2825 versus 2540 kcal/kg), whereas NSP concentration in high NSP diets was increased by 47% (133 versus 195 g/kg). To maintain energy concentration on the control level, extra fat was added in the high NSP diets of treatment 2 and 3. Addition of 100 g/kg sand to the control diet (treatment 4) increased ash content from 123 to about 225 g/kg, while the other chemical components were diluted up to 10%. Addition of 100 g/kg high-NSP raw materials to the control diet (treatment 5 and 6) decreased the contents of ash, protein and starch up to 10%, whereas the contents of crude fiber, NSP, (hemi-) cellulose and lignin increased.

Measurements

Analytical Procedures. Feed was analyzed for DM, crude ash, crude fat, crude fiber, nitrogen, starch, sugars (mono- and disaccharides as glucose units), calcium, phosphorus, sodium, potassium, NDF, ADF and ADL. All samples were analyzed in duplicate. For determination of the DM content, feed was freeze-dried according to ISO 6496 (1998b). Following freeze-drying, feed was ground to pass a 1 mm screen and kept for analysis. Air-dry feed was dried in a forced air oven at 103^{0}C to an constant weight according to ISO 6496 (1998b). Kjeldahl nitrogen content was measured according to ISO 5983 (1997) in fresh feed. Crude protein content was calculated as nitrogen * 6.25. Crude fat content was determined after acid hydrolysis according to ISO 6492 (1999). For determining crude ash content, samples were incinerated at 550^{0}C in a muffle furnace according to ISO 5984 (2002). The starch content was analyzed enzymatically as described by Brunt (1993). Reducing sugars were extracted from the feed samples, using 40% ethanol, and determined as described by Suárez *et* *al.* (2006). Contents of calcium, phosphorus, sodium and potassium were analyzed by using ICP-AES (ISO, 1998a). Analysis of NDF, ADF and ADL contents were based on a modified method of Van Soest et *al.* (1973), as described by Suárez *et al.* (2006).

Particle Size Distribution. Oat hulls were hammer milled, along with the other raw materials (fine) or ungrounded added to the diet (coarse). Particle size distribution of the diets was analyzed by use of the dry sieve method (Goelema et al., 1999). Seven particle size fractions were separated by using six sieves with diameters of 0.25, 0.50, 1.25, 2.50, 3.15 and 5.0 mm respectively. Average particle size of the diets was calculated as (Fraction < 0.25mm * 0.125) + (Fraction 0.25 – 0.50mm * 0.375) + (Fraction 0.50 – 1.25mm * 0.875) + (Fraction 1.25 – 2.50mm * 1.875) + (Fraction 2.50 – 3.15mm * 2.830) + (Fraction 3.15 – 5.00mm * 4.07) + (Fraction > 5.00mm * 6.50)/100. Average particle size of the finely ground diets was 0.87 ±0.02 mm versus 1.05 ±0.04 mm for the coarsely ground diets.

Observations

Feed Intake, Body Weight, and Hen performance. Feed consumption and hen performance per pen were recorded weekly. All hens were weighed per pen between 18 and 40 weeks of age in a 4-week interval. Egg weight per pen was based on the amount of 'standard' egg mass, i.e. all clean and dirty (blood- or fecal-stained), standard graded eggs. The remaining 'abnormal' egg mass consisted of broken, cracked or shell-less eggs. For the trait 'total egg mass' the entire egg mass production was calculated, assuming shell-less and cracked eggs to weigh the mean 'normal' egg weight of that specific pen and week.

Eating Time. Between 19 and 39 weeks of age, video observations were recorded in a 4-week interval to calculate eating time of hens in a pen. Eating time was defined as percentage of time birds spend on feed intake during the observation period. The day was divided in three blocks, i.e. from 9.00 - 11.30 hrs, 11.30 - 14.00 hrs and from 14.00 until 16.30 hrs. An observation lasted one hour, but to avoid possible disturbances of the cameraman, only the middle 30 observation minutes were analyzed. The number of eating hens (between 0 and 12), was recorded continuously by using Observer 4.1/5.0 software (Noldus, 1993). Eating rate was calculated as feed intake (g/d) divided by number of eating minutes per day. Eating minutes per day were not determined, but calculated as the number of hours with light on (16 h) multiplied by the percentage of observed eating time. Eating time and eating rate were averaged per pen for statistical analysis.

Table 1a. Dietary ingredients of the diets (g/kg, as-fed basis)

Treatment nr.	**1**	**2**	**3**	**4**	**5**	**6**
Energy concentration	Control	Control	Control	Low	Low	Low
NSP concentration	Control	High	High	Control	High	High
Coarseness of NSP	No NSP	Fine	Coarse	No NSP	Fine	Coarse
Ingredients						
Maize (CP=82 g/kg)	383.4	383.4		345.1	345.0	
Wheat (CP= 111 g/kg)	204.8	40.0		184.2	184.3	
Soybean meal, extracted (CP=458 g/kg)	137.9	108.9		124.1	124.1	
Peas (CP=211 g/kg)	84.6	91.9		76.1	76.1	
Oyster shells	72.4	72.0		65.2	65.2	
Rapeseed, extracted (CP=335 g/kg)	30.0			27.0	27.0	
Soybean meal, heat treated (CP=351 g/kg)	25.0	116.1		22.5	22.5	
Soybean oil	23.3	25.0		21.0	21.0	
Limestone	20.0	20.0		18.0	18.0	
Monocalcium phosphate	8.1	9.0		7.2	7.2	
Premix laying hens[1]	5.0	5.0		4.5	4.5	
NaCl	3.7	3.7		3.3	3.3	
DL-Methionine	1.6	2.0		1.4	1.4	
L-Lysine	0.4	-		0.4	0.4	
Palm oil	-	23.2		-	-	
Sand	-	-		100.0	-	
Oat hulls	-	100.0		-	100.0	

[1] Provided the following nutrients per kg of premix: vitamin A, 2,400,000 IU; vitamin D3, 480,000 IU; vitamin E, 8,000 mg; vitamin B1, 960 mg; vitamin B2, 2,400 mg; d-panthothenic acid, 3,200 mg; niacinamide, 9,600 mg; vitamin B6, 1,120 mg; folic acid, 360 mg; vitamin B12, 5,000 µg; vitamin C, 20,000 mg; biotin, 20 mg; vitamin K3, 960 mg; choline chloride 60,000 mg; 20,000 mg; copper, 1,600 mg (as $CuSO_4.5H_2O$), iron, 13,000 mg (as $FeSO_4.7H_2O$); manganese 13,000 mg (as MnO_2); zinc, 10,000 mg (as $ZnSO_4$); cobalt, 80 mg (as $CoSO_4.7H_2O$); iodine, 200 mg (asvKI); selenium, 80 mg (as $Na_2SeO_3.5H_2O$).

Table 1b. Analyzed and calculated nutrients of the diets (g/kg, as-fed basis)

Treatment nr.	**1**	**2**	**3**	**4**	**5**	**6**
Energy concentration	Control	Control	Control	Low	Low	Low
NSP concentration	Control	High	High	Control	High	High
Coarseness of NSP	No NSP	Fine	Coarse	No NSP	Fine	Coarse
Analyzed content[1]						
Dry matter	911.0	920.5	926.9	929.9	925.0	916.1
Ash	123.3	124.3	124.8	223.0	115.9	114.0
Fat	41.7	76.0	86.3	43.7	44.3	39.5
Crude Fiber	26.6	57.9	55.0	22.7	62.1	60.4
Crude Protein	168.1	155.7	154.5	150.2	150.9	151.5
Starch	411.8	338.2	343.4	378.4	388.1	391.5
Reducing Sugars[2]	33.6	29.8	29.0	29.3	30.1	28.6
Calcium	38.9	38.6	41.1	36.0	35.6	35.4
Phosphorus	5.4	4.9	5.0	4.9	4.9	4.8
Sodium	1.4	1.5	1.5	1.5	1.4	1.3
Potassium	7.0	7.1	7.1	6.2	6.8	6.6
NSP[3]	**132.6**	**201.9**	**193.7**	**105.2**	**195.7**	**190.9**
NDF	67.7	127.9	129.9	63.0	140.0	138.7
ADF	26.6	61.7	60.8	29.8	68.0	64.1
ADL (lignin)	6.6	14.1	11.4	6.8	14.1	13.2
Cellulose[4]	20.0	47.6	49.4	23.0	53.9	50.8
Hemi cellulose[4]	41.1	66.2	69.1	33.2	72.0	74.7
Calculated content						
ME (kcal/kg)	**2825**	**2825**	**2825**	**2540**	**2540**	**2540**
LYS	8.09	8.30	8.30	7.33	7.63	7.63
Dig. LYS	6.70	6.70	6.70	6.08	6.20	6.20
Dig. M+C	5.80	5.80	5.80	5.22	5.27	5.27
Dig. THR	4.60	4.52	4.52	4.14	4.20	4.20
Dig. TRP	1.47	1.41	1.41	1.32	1.34	1.34

[1] Based on 1 analysis in duplicate per diet.

[2] Mono- and disaccharides as glucose units.

[3] Non-starch polysaccharide (NSP) content was calculated by subtracting the crude protein, fat, starch, reducing sugars and ash content from the dry matter content.

[4] Cellulose = ADF minus ADL; hemi cellulose = NDF minus ADF.

Feather Condition Scores and Culling Rate. In a 2-week interval, plumage and skin condition per individual hen were scored by using the method described by Bilcik and Keeling (1999). Scores, varying from 0 (intact feathers, no injuries or scratches) to 5 (completely denuded area) were given for each of five body parts (neck, back, rump, tail and belly). The average of these five scores was also used for analysis. Culling rate of birds was recorded on a weekly basis. In all cases, wounded hens were culled from the experiment as a consequence of cannibalistic pecking behavior.

Behavioral Recordings. For scoring the behavioral recordings, the ethogram as described by Van Hierden (2002) was used. Recordings of gentle feather pecking (without removal of feathers) and severe pecking (leading to feather loss), aggressive pecking, vent pecking and cage pecking were made in week 4, 10, 18 and 21 of the experiment. Each pen was observed for 10 min, counting each peck. Results were presented as number of pecks per observed hen per 10 min. Duration of behavior elements was scored during week 11 and 19 of the experiment by using scan sampling technique. Behaviors were classed in four groups: feeding related behavior (pecking at feed or litter, ground scratching), drinking, walking and resting (sitting or standing inactive, preening). For each pen, an observer scored the number of hens per behavior class at 1-min intervals over a 15 min observation period. Based on these 15 observations, average number of hens per behavior class were determined and recalculated to percentages of time spent on the different behaviors.

Curve-fitting procedure and Statistical Analysis. Feather condition scores and performance data from each experimental unit were generated over time at regular intervals as longitudinal data. These data normally show a nonlinear pattern that can be described by logistic and exponential functions. An appropriate method to process such data is the use of general, nonlinear mixed effects models for repeated measures data (Lindstrom and Bates, 1990). An exponential function (1) was used to model feed intake, rate of lay, egg weight, egg mass and body weight of the hens:

$$Y = A + B\left(1 - e^{-\alpha t}\right) \tag{1}$$

where Y is the expected value of the performance parameter; A is performance value at $t = -\infty$; B is the increase of performance value over time; t is point in time (week number-1); α is rate of increase of the performance parameter. Egg weight was corrected for number of weighed eggs, because average weight of the first eggs per pen varied highly as a result of low number of eggs. A residual term was added to account for records with no eggs.

Feather condition scores increased over a number of weeks to an asymptotic value, following a S-shape pattern. Therefore, a logistic curve (2) was used to model feather condition scores:

$$Y = A + \frac{B}{1 + e^{-\alpha(t-\mu)}} \qquad (2)$$

where *Y, A, B, t* and α are as in curve (1); μ is point of inflection.

Feather condition scores of 3 pens (belonging to treatment 1, 2 and 3, respectively) did not fit to the logistic curves and were excluded from the analysis. Due to an outbreak of cannibalism, feather condition scores increased during week 3, but decreased during week 4 and 5 as a result of light dimming and wound treatments. Therefore, only the feather condition scores of week 6 onwards were used for the curve fitting procedure.

A REML procedure in (Genstat 8 Committee, 2002) was used to estimate curve parameters per pen. The nonlinear parameters were estimated by using a two-step iterative procedure, starting from a first order Taylor approach (Lindstrom and Bates, 1990; Engel et al., 2003). Following curve-fitting, the REML variance component analysis procedure tested the effect of the nutritional factors on the determined traits, using the model (3):

$$Y_{ij} = \mu + \text{Energy}_i + \text{NSP}_j + (\text{Energy x NSP}) + e_{ij} \qquad (3)$$

where Y_{ij} = dependent variable; μ = overall mean; energy_i = fixed effect of energy concentration i (i = 2; control and low); NSP_j = fixed effect of NSP concentration j (j = 3; a combination of NSP and coarseness); the contrast of NSP represents control NSP versus the average of high NSP fine and high NSP coarse; the contrast of coarseness represents high NSP fine versus high NSP coarse; the interaction energy x NSP represents the contrast energy x NSP and the contrast energy x coarseness. Below the tables with treatment means, the p-values of energy, NSP, coarseness, energy x NSP and energy x coarseness will be presented. Model (3) was also used to test effects of eating time, eating rate, feather condition score, average culling rate, and behavior traits.

RESULTS

Feed intake on the first day of the experiment (initial feed intake) of the low energy diets was on average 3.3 g/d (± 1.4) higher than the control energy diets (*P*=0.06) (Table 2). Initial feed intake was not affected by NSP concentration and coarseness of NSP.

Table 2. Parameter estimates (A, B and α) of feed intake (g/hen/d) as described by an exponential curve and average feed intake per treatment in ISA Brown laying hens over 18 to 40 weeks of age.

Treatment[1]	Initial feed intake (g/hen/d)[2] (A)	Rate of Increase[2] (α)	Increase in feed intake (g/hen/d)[2] (B)	Asymptotic feed intake (g/hen/d)[2] (A+B)	Average feed intake (g/hen/d)
Control Energy					
Control NSP	93.4	0.27	39.7	133.1	125.8
High NSP-Fine	95.1	0.34	34.6	129.7	124.5
High NSP-Coarse	98.0	0.39	31.6	129.7	125.4
Low Energy					
Control NSP	99.6	0.36	40.4	140.0	134.2
High NSP-Fine	96.4	0.48	37.0	133.3	129.2
High NSP-Coarse	100.3	0.28	55.5	155.8	146.1
Standard error	1.408	0.034	3.180		
***P*-Value**					
Energy	0.060	0.394	**0.021**		
NSP	0.594	0.201	0.928		
Energy*NSP	0.225	0.439	0.140		
Coarseness	0.108	0.138	0.106		
Energy*Coarseness	0.807	**0.011**	**0.025**		

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

[2] Data were fitted by use of the exponential curve: $Y = A + B\left(1 - e^{-\alpha t}\right)$, where *Y* is the expected value of feed intake; *A* is initial feed intake (at $t = -\infty$); *B* is the increase of feed intake over time; *t* is point in time (wk); α is rate of increase of feed intake. Parameter estimates are based on 161 observations per treatment (23 weeks x 7 replicates).

Rate of increase in feed intake over week 18 to 40 was not affected by coarseness of control energy diets, but in low energy diets rate of increase of finely ground high NSP diet was 0.20 (± 0.034) higher compared with coarsely ground high NSP diet (*P*=0.011). Coarseness of NSP in diets with control energy concentration had no effect on increase in feed intake, whereas increase in feed intake in coarsely ground low energy diets was higher (55.5 versus 37.7 g/hen/d) compared with finely ground low energy diets (*P*=0.025).

Hens that were fed low energy diets spent 2.5% more time on feed intake than hens that were fed control energy diets (*P*=0.001), corresponding with a relative increase in eating time of 14.2% (Table 3). Hens that were fed high-NSP diets spent on average 2.9% more time on feed intake than hens that were fed control NSP diets (*P*<0.001), corresponding with a relative increase in eating time of 17.2%.

Table 3. Average eating time (% of observed period) and eating rate (g feed intake/min) in ISA Brown laying hens over 18 to 40 weeks of age.

Treatment[1]	Eating time (%)	Eating rate (g/min)
Control Energy		
Control NSP	15.1	1.04
High NSP-Fine	18.4	0.80
High NSP-Coarse	19.3	0.77
Low Energy		
Control NSP	18.6	0.86
High NSP-Fine	19.7	0.69
High NSP-Coarse	21.9	0.75
Standard error	2.84	0.122
P-Value		
Energy	**0.001**	0.146
NSP	**< 0.001**	**0.010**
Energy*NSP	0.316	0.444
Coarseness	0.075	0.582
Energy*Coarseness	0.475	0.883

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

Coarse grinding of the NSP source slightly (+1.5%; *P*=0.075) increased eating time over week 1–22 (relative increase = 7.9%) compared with feeding finely ground NSP-high diets. Eating rate of hens that were fed high-NSP diets was on average 0.20 g/min lower compared with control energy fed hens (*P*=0.01), which corresponded with a relative decrease in eating rate of 21.0%. Eating rate was not affected by energy concentration and coarseness of NSP.

Table 4. Parameter estimates (α, μ and B) of mean feather condition score (FC) as described by a logistic curve and average FC per treatment in ISA Brown laying hens over 18 to 40 weeks of age.

Treatment[1,2]	Rate of Increase[3] (α)	Point of Inflection (wk)[3] (μ)	Increase in FC[3] (B)	Average FC
Control Energy				
Control NSP	0.52	15.6	1.74	0.55
High NSP-Fine	0.35	13.7	1.32	0.53
High NSP-Coarse	0.33	12.4	1.14	0.53
Low Energy				
Control NSP	0.49	15.7	1.39	0.43
High NSP-Fine	0.07	15.1	1.18	0.52
High NSP-Coarse	0.48	25.4	1.40	0.09
Standard error	0.234	4.84	0.502	
***P*-Value**				
Energy	0.678	0.075	0.793	
NSP	0.158	0.664	0.345	
Energy*NSP	0.913	0.223	0.498	
Coarseness	0.197	0.143	0.925	
Energy*Coarseness	0.186	0.084	0.566	

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse). [2] Initial feather condition score (A) was 0 for all treatments.

[3] Data were fitted by use of the logistic curve: $Y = A + \frac{B}{1+e^{-\alpha(t-\mu)}}$, where *Y* is the expected value of FC; *A* is initial FC (at $t = -\infty$); *B* is the increase of FC over time; *t* is point in time (wk); α is rate of increase of FC, and μ is point of inflection (wk). Parameter estimates are based on 91 observations per treatment (13 weeks x 7 replicates).

Rate of increase of feather condition (FC) varied between 0.07 in birds fed Low Energy/High NSP/Fine diets and 0.52 in birds fed Control Energy/Control NSP diets (Table 4). Due to high variation in rate of increase (se=0.234) dietary treatments did not significantly affect FC. Maximum FC varied between 1.14 in birds fed Control Energy/High NSP/Coarse diets and 1.74 in birds fed Control Energy/Control NSP diets. Due to high variation in increase of FC (se=0.502) dietary treatments did not significantly affect FC. In Control energy diets, inflection point of the logistic curve was not affected by coarseness of NSP, whereas in Low energy diets, inflection point of the curve was increased when the birds were fed coarsely ground high NSP diets compared with finely ground high NSP diets (15.1 versus 25.4; *P*=0.084), indicating that the arise of feather damage was delayed by 10 wk..

Table 5. Average culling rate due to cannibalism per treatment in ISA Brown laying hens over 18 to 40 weeks of age.

Treatment[1]	Average culling rate (%)
Control Energy	
Control NSP	44.1
High NSP-Fine	23.8
High NSP-Coarse	39.3
Low Energy	
Control NSP	13.1
High NSP-Fine	33.3
High NSP-Coarse	23.8
Standard error	3.07
***P*-Value**	
Energy	0.126
NSP	0.731
Energy*NSP	0.071
Coarseness	0.835
Energy*Coarseness	0.206

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

In all cases, wounded hens were culled from the experiment as a consequence of cannibalistic pecking behavior (Table 5). Hens fed control NSP diets showed reduced culling rates if energy concentration was decreased (44.1% versus 13.1%), whereas in high NSP diets culling rate slightly (non significant) decreased when hens were fed low energy diets (31.6% versus 28.6%) (*P*=0.071). Feeding low energy diets numerically (*P*=0.126) reduced culling rate compared with control energy diets (35.7 versus 23.4%). Although non significant (P=0.206), it was shown that coarse grinding of NSP seems to increase culling rate in control energy diets (23.8 versus 39.3%), whereas coarse grinding of NSP seems to decrease culling rate in low energy diets (33.3 versus 23.8%).

Table 6. Mean number of gentle and severe feather pecks and the total number of pecking interactions (nr/observed hen/10 min) per treatment in ISA Brown laying hens over 18 to 40 weeks of age.

Treatment[1]	Gentle FP	Severe FP	Total pecking interactions
Control Energy			
Control NSP	0.098	0.134	0.420
High NSP-Fine	0.118	0.133	0.322
High NSP-Coarse	0.162	0.091	0.426
Low Energy			
Control NSP	0.108	0.153	0.348
High NSP-Fine	0.112	0.165	0.375
High NSP-Coarse	0.114	0.131	0.346
Standard error	0.0147	0.0223	0.0517
***P*-Value**			
Energy	0.414	0.268	0.610
NSP	0.221	0.642	0.805
Energy*NSP	0.336	0.749	0.667
Coarseness	0.307	0.260	0.634
Energy*Coarseness	0.328	0.905	0.393

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

Number of gentle and severe feather pecks and the number of total pecking interactions were not affected by dietary treatments (Table 6).

Percentage of time hens spent on feeding related behavior varied between 49.9 and 59.3% (Table 7). Feeding related behavior was not significantly affected by dietary treatments, although hens fed coarsely ground NSP spent numerically (*P*=0.113) more time on this behavior, compared with hens fed finely ground NSP (52.2 versus 58.7%). Resting behavior was slightly reduced in birds fed coarsely ground NSP, compared with hens fed finely ground NSP (36.6 versus 29.6%; *P*=0.082). In control energy diets, drinking behavior was increased if diets with a control NSP concentration were fed, whereas in low energy diets drinking behavior was not affected by NSP concentration (*P*=0.029).

Table 7. Behavior traits of hens (% of time) per treatment, observed by using scan sampling technique in ISA Brown laying hens over 18 to 40 weeks of age.

Treatment[1]	Feeding related behavior (%)	Resting (%)	Drinking (%)	Walking (%)
Control Energy				
Control NSP	49.9	35.2	11.3	3.6
High NSP-Fine	50.2	38.3	8.0	3.5
High NSP-Coarse	58.9	30.3	6.0	4.7
Low Energy				
Control NSP	59.3	31.6	7.7	1.4
High NSP-Fine	54.1	34.8	7.3	3.9
High NSP-Coarse	58.5	28.8	9.4	3.2
Standard error	5.88	5.66	1.82	2.06
***P*-Value**				
Energy	0.206	0.377	0.782	0.347
NSP	0.817	0.929	0.102	0.302
Energy*NSP	0.283	0.862	**0.029**	0.515
Coarseness	0.113	0.082	0.953	0.830
Energy*Coarseness	0.611	0.805	0.110	0.520

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

Rate of lay parameters were not affected by NSP concentration and coarseness of NSP (Table 8). Initial rate of lay of hens that were fed low energy diets was 4.4% (± 1.80) lower (*P*=0.044), whereas increase in rate of lay was slightly (4.2% ± 2.06) higher (*P*=0.090) comparedwith hens that were fed control energy diets. Therefore, asymptotic rate of lay level was not affected by dietary energy concentration.

Table 8. Parameter estimates (A, B and α) of rate of lay (%) as described by an exponential curve and average rate of lay (%) per treatment in ISA Brown laying hens over 18 to 40 weeks of age.

Treatment[1]	Initial rate of lay (%)[2] (A)	Rate of increase[2] (α)	Increase in rate of lay (%)[2] (B)	Asymptotic rate of lay (%)[2] (A + B)	Average rate of lay (%)
Control Energy					
Control NSP	10.6	0.93	85.5	96.1	90.0
High NSP-Fine	13.7	0.98	82.2	96.0	90.2
High NSP-Coarse	10.0	0.92	86.2	96.2	90.0
Low Energy					
Control NSP	8.4	0.90	91.4	99.8	93.1
High NSP-Fine	9.4	1.00	86.1	95.5	89.6
High NSP-Coarse	5.6	0.85	89.3	94.9	88.2
Standard error	1.797	0.055	2.059		
***P*-Value**					
Energy	**0.044**	0.682	0.090		
NSP	0.565	0.761	0.353		
Energy*NSP	0.965	0.971	0.654		
Coarseness	0.166	0.225	0.251		
Energy*Coarseness	0.998	0.598	0.891		

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

[2] Data were fitted by use of the exponential curve: $Y = A + B\left(1 - e^{-\alpha t}\right)$, where *Y* is the expected value of rate of lay; *A* is initial rate of lay (at $t = -\infty$); *B* is the increase of rate of lay over time; *t* is point in time (wk); α is rate of increase of rate of lay. Parameter estimates are based on 161 observations per treatment (23 weeks x 7 replicates).

Egg weight and body weight parameters were not affected by energy and NSP concentration of the diet or by coarseness of NSP (Table 9 and 10).

Table 9. Parameter estimates (A, B and α) of egg weight (g) as described by an exponential curve and average egg weight (g) per treatment in ISA Brown laying hens over 18 to 40 weeks of age.

Treatment[1]	Initial egg weight (g)[2] (A)	Rate of increase[2] (α)	Increase in egg weight (g)[2] (B)	Asymptotic egg weight level (g)[2] (A + B)	Average egg weight (g)
Control Energy					
Control NSP	48.8	0.25	16.9	65.7	62.4
High NSP-Fine	49.7	0.23	16.8	66.5	63.0
High NSP-Coarse	49.6	0.21	17.0	66.5	62.7
Low Energy					
Control NSP	48.5	0.24	17.4	65.9	62.4
High NSP-Fine	48.2	0.26	17.8	66.0	62.6
High NSP-Coarse	49.4	0.24	17.6	67.0	63.7
Standard error	0.733	0.016	0.650		
***P*-Value**					
Energy	0.379	0.339	0.207		
NSP	0.398	0.466	0.649		
Energy*NSP	0.946	0.522	0.684		
Coarseness	0.656	0.401	0.989		
Energy*Coarseness	0.555	0.966	0.868		

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

[2] Data were fitted by use of the exponential curve: $Y = A + B\left(1 - e^{-\alpha t}\right)$, where *Y* is the expected value of egg weight; *A* is initial egg weight (at $t = -\infty$); *B* is the increase of egg weight over time; *t* is point in time (wk); α is rate of increase of egg weight. Parameter estimates are based on 161 observations per treatment (23 weeks x 7 replicates).

Table 10. Parameter estimates (A, B and α) of body weight (g) as described by an exponential curve and average body weight (g) per treatment in ISA Brown laying hens over 18 to 40 weeks of age.

Treatment[1]	Initial body weight (kg)[2] (A)	Rate of increase[2] (α)	Increase in body weight (g)[2] (B)	Asymptotic body weight level (g)[2] (A + B)	Average body weight (g)
Control Energy					
Control NSP	1727	0.39	249	1976	1943
High NSP-Fine	1718	0.31	281	1999	1953
High NSP-Coarse	1721	0.36	284	2005	1964
Low Energy					
Control NSP	1710	0.40	284	1994	1957
High NSP-Fine	1735	0.26	272	2007	1956
High NSP-Coarse	1704	0.37	278	1982	1964
Standard error	11.7	0.082	14.7		
***P*-Value**					
Energy	0.528	0.806	0.710		
NSP	0.674	0.168	0.538		
Energy*NSP	0.350	0.813	0.266		
Coarseness	0.443	0.174	0.832		
Energy*Coarseness	0.334	0.591	0.943		

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

[2] Data were fitted by use of the exponential curve: $Y = A + B\left(1 - e^{-\alpha t}\right)$, where *Y* is the expected value of body weight; *A* is initial body weight (at $t = -\infty$); *B* is the increase of body weight over time; *t* is point in time (wk); α is rate of increase of body weight. Parameter estimates are based on 49 observations per treatment (7 4-week periods x 7 replicates).

DISCUSSION

BEHAVIOR OF BIRDS PRIOR TO THE EXPERIMENTAL PERIOD

In this experiment, the effects of energy dilution, NSP addition and coarseness of NSP on feather pecking behavior, eating behavior and performance of laying hens were investigated. The reduction in dietary energy is often confounded with changes in the concentration of other nutrients, like amino acid and NSP levels, and until now, the separate effects of energy dilution and NSP supplementation on feather pecking behavior and performance of laying hens are unknown.

The layers in the current experiment showed already feather pecking behavior during the 5th week of the rearing period. The reason for the development of feather pecking behavior during the rearing period in the current experiment is not known. Good litter, consisting of wood shavings, was available during the whole rearing period. Probably, light intensity was too high (over 60 Lux) during the first weeks of the rearing period. After coloring the TL tubs with red paint to reduce light intensity, pecking behavior reduced.

Early rearing conditions can affect feather pecking behavior of hens later in life. Increased feather pecking has been observed in pre-pubertal birds with denied litter availability in their first 2 weeks of life (Huber Eicher and Sebo, 2001) or with denied access to an exploratory-rich environment in their first 5 weeks of life (Chow and Hogan, 2005). Huber Eicher and Sebo (2001) made observations on commercial farms and found that 40% of the flocks developed feather pecking when they were 5 weeks of age and this frequency increased to 77.3% when the same flocks were 14 weeks old. The results in our experiment should be discussed in view of the above stated.

EFFECT OF DIETARY ENERGY DILUTION

Feed intake of hens that were fed diluted diets increased on average by 9.3%, resulting in an almost similar energy intake compared to control energy diets. It seemed that hens need a certain amount of energy per day and that they will eat till their energy demand is fulfilled. Feeding these low energy diets significantly prolonged eating time by 14%, whereas eating rate was slightly reduced. These results are in line with those of Van Krimpen et al. (2007) and Savory (1980), who found proportional increases in eating time due to energy dilution of 10 and 40%, respectively. An increase in feed intake and eating time might compensate for redirected foraging behavior, resulting in less feather pecking behavior (Van Krimpen et al., 2005).

However, feather pecking behavior and feather condition scores in the current experiment were not significantly improved by energy reduction, although culling rate due to cannibalism was lower in the low energy treatments (23.4 versus 35.7%; P>0.05).

The limited effect of energy reduction on feather pecking behavior was not in line with earlier publications, probably because we used feather pecking prone layers in this experiment. In an experiment of Elwinger (1981) plumage condition significantly improved as energy concentration of the diet decreased (from 2920 to 12560 kcal/kg). Van der Lee et al. *(2001)* also reported a better plumage condition in hens that were fed diets reduced in energy concentration (2765 versus 2645 kcal/kg).

Feeding low energy diets to hens resulted in similar hen performance parameters compared with hens that were fed control energy diet. These results are in accordance with other experiments. Feeding laying hens a 5% nutrient diluted diet did not affect hen performance compared to hens that were fed a control diet (Van der Lee et al., 2001). Also in a recent trial with laying hens (34-37 weeks of age), a reduced dietary energy concentration (by adding 10, 20, 25 or 30% sand) did not affect hen performance of the hens (Van der Meulen et al., 2006). The hens fully compensated for the effect of added sand in the diet by increasing their daily feed intake. In contrast with these findings, Elwinger (1981) showed that a decreased energy concentration of the diet resulted in a reduced hen performance. However, this decreased energy concentration was confounded with a reduced dietary energy-protein ratio, whereas energy-protein ratio in our experiment was constant for each energy concentration. In the current experiment, the compensation for reduced energy concentration by higher feed intake, however, differed per treatment (Table 11).

Compared to the control energy treatments, in the low energy treatments a 10% higher feed intake was expected, but the observed increase varied between 2% (low energy, control NSP) and 16% (low energy, high NSP, coarse). Due to this variation, protein intake was not similar for all treatments. As a consequence, relative intake level of Methionine + Cysteine, which is an important nutrient for maintaining plumage condition, varied in low energy treatments between 92% and 104% related to control energy treatments. The relatively low intake of Methionine + Cysteine of hens fed diet 5 (low energy, high finely ground NSP diet), however, did not result in reduced hen performance or increased feather condition scores.

We can conclude that feeding low energy diets increased eating time and decreased incidence of cannibalism in laying hens. However, dietary energy reduction did not significantly reduce feather condition scores and pecking behavior.

Table 11. Relative feed intake (%) and Methionine + Cysteine intake (%) per treatment, whereby intake level of treatment 1 (control energy and control NSP) was set at 100%.

Treatment[1]	Feed intake (%)	Methionine + Cystine intake (%)
Control Energy		
Control NSP	100	100
High NSP-Fine	99	99
High NSP-Coarse	100	100
Low Energy		
Control NSP	107	96
High NSP-Fine	102	92
High NSP-Coarse	116	104

[1] The tested factors were energy concentration (2825 versus 2540 kcal/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

EFFECT OF DIETARY NSP CONCENTRATION

Feed intake parameters were not affected by NSP concentration of the diet. Hens fed control NSP diets showed reduced culling rates if energy concentration was decreased (44.1% versus 13.1%), whereas in high NSP diets culling rate slightly (non significant) decreased when hens were fed low energy diets (31.6% versus 28.6%). Birds fed high NSP diets spent more time eating in our experiment, in accordance with the results of (Hetland et al., 2004a). Feeding high-NSP diets prolonged eating time by 17.2% by, whereas eating rate was decreased by 21.0%. These findings are in line with earlier results of Van Krimpen et al. *(2007)*, where eating time and eating rate were affected by 21% and 12% respectively. Increase of dietary NSP concentration had a larger effect on eating rate than energy reduction had, possibly due to differences in specific gravity of the raw materials. For instance, sand has a specific gravity of 1600 kg/m^3, against 780 kg/m^3 for oak wood (Jansen, 1977). Therefore, the same amount of feed intake results in less volume in the GIT when the hens are supplemented with sand-rich diets, compared to NSP-rich diets, resulting in a lower impact on eating rate. Savory (1980) also suggested that differences in meal length were related to dietary bulk.

NSP concentration had no effect on hen performance traits. This was confirmed by Hartini et al. (2003) who found no detrimental effects on performance after substituting wheat by (in)soluble high NSP sources like millrun, barley, rice hulls or oats on an isocaloric and isonitrogenous basis. In some experiments, in which insoluble rich NSP diets were supplemented, nutrient digestibility even increased, possibly due to a better gizzard development and more reflux activity in the fore-gut, resulting in improved hen performance, as reported by (Hetland et al., 2004a).

As a possible effect of extended eating time and decreased eating rate due to NSP addition, culling rate was slightly decreased and feather condition score slightly improved. However this was mainly the case in the low energy groups. Feather pecking behavior was not affected by NSP addition. On the contrary, in literature, insoluble NSP-rich raw materials have been found to decrease feather pecking behavior in laying hens. Hartini et al. (2002) showed that addition of insoluble fiber to the diet might prevent cannibalism mortality in pre lay period (13.2 versus 3.9%) and early lay period (28.9 versus 14.3%). Providing insoluble NSP-rich raw materials also have been found to decrease feather pecking among layers, especially when pellets were fed (Aerni et al., 2000; El Lethey et al., 2000). Hetland et al. *(2004a)* concluded that access to fiber structure from feed and environment may interact with feather pecking behavior. Probably, the absence of clear effects of NSP concentration on feather pecking might be the result of the use of feather pecking prone layers in this experiment.

Based on the results of the current experiment, we can conclude that supplementing NSP high diets increased the amount of feeding related behavior as a results of extended eating time and decreased eating rate. However, incidence of cannibalism and feather condition scores were only slightly reduced in this experiment.

EFFECT OF COARSENESS OF NSP FRACTION

Coarse grinding of the NSP fraction increased eating time by 7.9% as compared to fine grinding of NSP, whereas the development of feather damage was slightly delayed in low energy coarsely ground NSP rich diets. Feather pecking behavior and eating rate, however, were not affected by differences of particle sizes of NSP. In line with these findings, Hetland et al. *(2004b)* found no effect of increased particle sizes, as a result of including whole versus ground oats into the diet, on plumage condition. However, the control diet with ground oats in that experiment contained already considerable amounts of structural components in the form of oat hulls. The authors were suggesting that after a certain level of structural components addition of extra coarse particles will not further improve plumage condition. On the other hand, the lack of a clear positive effect of a coarser feed structure on plumage condition in the current experiment is in contrast to a previous experiment with wheat-based diets (Hetland et al., 2003a), showing improved plumage condition when replacing ground wheat with whole wheat.

In the current experiment, hen performance traits were not affected by particle sizes of NSP. In an earlier experiment we found that feeding coarsely ground diets negatively affected egg weight and body weight gain of hens in early lay (Van Krimpen et al., 2007). In literature, however, numerous positive effects of coarsely ground NSP sources were mentioned. It has been shown that coarse particles accumulate in the gizzard, stimulating gizzard weight and activity, like an increased reflux of bile acids, resulting in an improved starch digestibility and an enhanced emulsification of liberated lipids (Hetland et al., 2003a). The fact that insoluble fiber accumulates in the gizzard may indicate a slower feed passage rate on gizzard level when the coarse fiber content of the diet increased. It is thought that accumulation of insoluble fiber in the gizzard triggers a temporary satiety, but once passed the gizzard, it passes through the gut quickly. This could make the bird feel more satisfied between feeding bouts, but more hungry after gizzard emptying (Hetland et al., 2004a).

In conclusion, feeding coarsely ground NSP did not affect performance, but increased eating time. Moreover feather condition scores were slightly improved by larger feed particles of NSP in the low energy group. However, incidence of cannibalism and pecking behavior were not affected by particle sizes of NSP.

IMPACT OF REARING CONDITIONS

Probably, the fact that the hens started feather pecking before the dietary treatments were applied, may explain the minor effects of the investigated nutritional factors during laying period. Results from a longitudinal study, with birds followed during both rearing and laying period, showed that stereotyped gentle feather pecking in young birds predicted this behavior of these birds in adult stage (Newberry et al., 2006). Once developed, stereotyped behavior can be persistent and hard to extinguish (Garner and Mason, 2002). This is in line with some authors who concluded that more attention should be given to the development of feather pecking during the rearing of laying hen chicks (Huber Eicher and Sebo, 2001). It is also suggested that minimizing differences between the rearing and laying environment via a seamless transition is likely to contribute to making a flock less prone to injurious feather pecking (Van de Weerd and Elson, 2006). Our hypothesis, namely that an extended eating time may reduce feather pecking behavior, has not fully been proofed in the current experiment. This hypothesis seems not to be valid in birds that developed already feather pecking behavior before dietary treatments were started.

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REFERENCES

Aerni, V., H. El Lethey and B. Wechsler. 2000. Effect of foraging material and food form on feather pecking in laying hens. *Br. Poult. Sci.,* 41:16-21.

Al Bustany, Z. and K. Elwinger. 1987. Response of laying hens to different dietary lysine intakes. A comparison of some commercial hybrids with strains selected on a low protein diet. *Acta Agric. Scand.,* 37:27-40.

Ambrosen, T. and V. E. Petersen. 1997. The influence of protein level in the diet on cannibalism and quality of plumage of layers. *Poult. Sci.,* 76:559-563.

Bilcik, B. and L. J. Keeling. 1999. Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. *Br. Poult. Sci.,* 40:444-451.

Blokhuis, H. J. and J. W. Van der Haar. 1989. Effects of floor type during rearing and of beak trimming on ground pecking and feather pecking in laying hens. *Appl. Anim. Behav. Sci.,* 22:359-369.

Brunt, K. 1993. Enzymatic determination of starch content in feed and feedstuffs, Memo 93-302 (in Dutch). *Stichting Nederlands Instituut voor Koolhydraat Onderzoek, Groningen. The Netherlands*.

Chow, A. and J. A. Hogan. 2005. The development of feather pecking in Burmese red junglefowl: the influence of early experience with exploratory-rich environments. *Appl. Anim. Behav. Sci.,* 93:283-294.

El Lethey, H., V. Aerni, T. W. Jungi and B. Wechsler. 2000. Stress and feather pecking in laying hens in relation to housing conditions. *Br. Poult. Sci.,* 41:22-28.

Elwinger, K. 1981. Different energy levels and restricted feeding to three strains of SCWL hybrids. 1. Effects on egg production. *Swedish J. Agric. Res.,* 11:149-157.

Engel, B., K. v. Reenen and W. Buist. 2003. Analysis of correlated series of repeated measurements: application to challenge data. *Biometrical Journal,* 45:866-886.

Garner, J. P. and G. J. Mason. 2002. Evidence for a relationship between cage stereotypes and behavioural disinhibition in laboratory rodents. *Behav. Brain Res.,* 136:83-92.

Genstat 8 Committee. 2002. Genstat 8 Reference Manual; Release 3. Clarendon Press, Oxford, UK.

Goelema, J. O., A. Smits, L. M. Vaessen and A. Wemmers. 1999. 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. Techn.,* 78:109-126.

Hartini, S., M. Choct, G. Hinch and J. V. Nolan. 2003. Effect of diet composition, gut microbial status and fibre forms on cannibalism in layers. *Australian Egg Corporation Limited.* AECL Publication No 03/03:1-111.

Hartini, S., M. Choct, G. Hinch, A. Kocher and J. V. Nolan. 2002. Effects of light intensity during rearing and beak trimming and dietary fiber sources on mortality, egg production, and performance of ISA brown laying hens. *J. Appl. Poult. Res.,* 11:104-110.

Hetland, H., B. Svihus and A. Krogdahl. 2003a. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *Br. Poult. Sci.,* 44:275-282.

Hetland, H., M. Choct and B. Svihus. 2004a. Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poult. Sci. J.,* 60:415-422.

Hetland, H., B. Svihus, S. Lervik and R. Moe. 2003b. Effect of feed structure on performance and welfare in laying hens housed in conventional and furnished cages. *Acta Agric. Scand., Section A, Animal Science,* 53:92-100.

Hetland, H., R. O. Moe, R. Tauson, S. Lervik and B. Svihus. 2004b. Effect of including whole oats into pellets on performance and plumage condition in laying hens housed in conventional and furnished cages. *Acta Agric. Scand., Section A, Animal Science,* 54:206-212.

Hoffmeyer, I. 1969. Feather pecking in pheasants - an ethological approach to the problem. *Danish review of game biology,* 6:1-36.

Huber Eicher, B. and F. Sebo. 2001. The prevalence of feather pecking and development in commercial flocks of laying hens. *Appl. Anim. Behav. Sci.,* 74:223-231.

Hughes, B. O. and C. C. Whitehead. 1979. Behavioural changes associated with the feeding of low-sodium diets to laying hens. *Appl. Anim. Ethology,* 5:255-266.

ISO. 1997. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. Kjeldahl method. ISO 5983. International Organisation for Standardization.

ISO. 1998a. Water quality. Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy. ISO 11885. International Organization for Standardization.

ISO. 1998b. Animal feeding stuffs. Determination of moisture and other volatile matter content. ISO 6496. International Organisation for Standardization.

ISO. 1999. Animal feeding stuffs. Determination of fat content. ISO 6492. International Organisation for Standardization.

ISO. 2002. Animal feeding stuffs. Determination of crude ash. ISO 5984. International Organisation for Standardization.

Jansen, A. I. 1977. BINAS Informatieboek vwo-havo voor het onderwijs in de natuurwetenschappen. Groningen.

Lindstrom, M. J. and D. M. Bates. 1990. Nonlinear mixed effects models for repeated measures data. *Biometrics,* 46:673-786.

Newberry, R. C., L. J. Keeling, I. Estevez and B. Bilcik. 2006. Behaviour when young as a predictor of severe feather pecking in adult laying hens: The redirected foraging hypothesis revisited. *Appl. Anim. Behav. Sci.*: doi:10.1016/j.applanim.2006.10.010.

Noldus. 1993. The Observer®: Base package for Dos. Reference Manual, version 3.0.

NRC, N. R. C. 1994. Nutrient requirements of poultry. *Ninth Revised Edtion.* Washington DC.

Savory, C. J. 1980. Meal occurrence in Japanese quail in relation to particle size and nutrient density. *Anim. Behav.,* 28:160-171.

Suárez, B. J., C. G. Van Reenen, G. Beldman, J. van Delen, J. Dijkstra and W. J. J. Gerrits. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: I. Animal performance and rumen fermentation characteristics. *Journal of Dairy Science,* 89:4365-4375.

Van de Weerd, H. A. and H. A. Elson. 2006. Rearing factors that influence the propensity for injurious feather pecking in laying hens. *World's Poult. Sci. J.,* 62:654-664.

Van der Lee, A. G., G. Hemke and R. P. Kwakkel. 2001. Low density diets improve plumage condition in non-debeaked layers. *Proc.13th Eur. Symp. poult. nutr.*, Blankenbergen, 244-245.

Van der Meulen, J., C. Kwakernaak and C. A. Kan. 2006. Sand intake by laying hens and its effect on egg production parameters. *J. Anim. Nutr. Phys.,* DOI:_10.1111/j.1439-0396.2007.00732.x.

Van Hierden, Y. M., S. M. Korte, E. W. Ruesink, C. G. v. Reenen, B. Engel, J. M. Koolhaas and H. J. Blokhuis. 2002. The development of feather pecking behaviour and targeting of pecking in chicks from a high and low feather pecking line of laying hens. *Appl. Anim. Behav. Sci.,* 77:183-196.

Van Krimpen, M. M., R. P. Kwakkel, B. F. J. Reuvekamp, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2005. Impact of feeding management on feather pecking in laying hens. *World's Poult. Sci. J.,* 61:665-687.

Van Krimpen, M. M., R. P. Kwakkel, G. André, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2007. Effect of nutrient dilution on feed intake, eating time and performance of hens in early lay. *Br. Poult. Sci.,* 48:389-398.

Van Soest, P. J. and R. W. McQueen. 1973. The chemistry and estimation of fiber. *Proc. Nutr. Soc.,* 32:123-130.

Chapter 5

EFFECT OF DIETARY ENERGY CONCENTRATION, NONSTARCH POLYSACCHARIDE CONCENTRATION, AND PARTICLE SIZES OF NONSTARCH POLYSACCHARIDES ON DIGESTA PASSAGE RATE AND GUT DEVELOPMENT IN LAYING HENS

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ABSTRACT

An experiment was conducted with 504 ISA Brown layers from 18 to 40 wk of age to investigate the effects of energy concentration, nonstarch polysaccharide (NSP) concentration and particle sizes of added NSP on digesta mean retention time (MRT) and gut development. Hens were allotted to 6 dietary treatments with 3 replicates per treatment. Experimental factors were energy concentration (11.8 vs. 10.6 MJ/kg), NSP concentration (133 vs. 195 g/kg), and fine vs. coarse particle sizes of NSP in the NSP high diets. Increasing the dietary NSP concentration extended MRT in the crop (68 vs. 34 min.) and total foregut (56.8 vs. 90.6 min) compared to control NSP diets. Feeding low energy diets resulted in a longer MRT in the colon (26.0 vs. 6.7 min), caeca (3.9 vs. 1.8 min), and total hind gut (30.3 vs. 8.6 min) compared to control energy diets. Coarse grinding decreased MRT in the caeca compared to fine grinding (4.6 vs. 1.8 min). Overall MRT was not affected by dietary treatments. Feeding high NSP diets increased relative weights of the empty proventriculus/gizzard and it's contents by 30% (25.2 vs. 19.4 g/kg) and 18% (15.4 vs. 13.0 g/kg), respectively, compared to control NSP diets. In addition, relative empty proventriculus/gizzard weight of hens fed coarsely ground NSP was 30% higher compared to hens fed finely ground NSP (28.5 vs. 21.9 g/kg). It was concluded that addition of NSP to the diet may increase the weights of the gizzard and it's contents, and may extend MRT in the foregut. MRT in the foregut was linearly related to the daily insoluble NSP intake. The increase of MRT was more pronounced in hens fed coarsely compared to finely ground NSP. These findings seem to be indicators of higher levels of satiety in laying hens, which may contribute to a lower feather pecking pressure.

Key words: energy concentration, GIT development, mean retention time, laying hens, NSP concentration, particle sizes

INTRODUCTION

Hens in modern housing systems often spend considerable less time on feeding related behavior, but more time on feather pecking behavior, compared to hens in a natural environment (Dawkins, 1989). Feather pecking is supposed to be a substitute for normal feeding behavior in the absence of adequate foraging incentives (Hoffmeyer, 1969; Blokhuis and Van der Haar, 1989). Therefore, we expect that nutritional factors might reduce feather pecking behavior if these factors increase the level of feeding related behavior (Van Krimpen et al., 2005). Eating time of laying hens can be extended by supplementing diluted diets (Van Krimpen et al., 2008). Dietary dilution by adding 10% sand to a control diet extended eating time by 23%. Addition of 10% coarsely ground non-starch polysaccharides (NSP) to a control diet, however, prolonged eating time by 45%. Thus, dietary dilution is not the only factor that determines eating time. Eating time in relation to diet formulation might also be affected by the level of satiety, as reflected by differences in mean retention time (MRT) of the digesta.

Insoluble NSP seems to decrease overall MRT in the GIT (Roberfroid, 1993). Coarse particles of NSP, however, seem to accumulate in the gizzard, thereby increasing MRT in this part of the GIT (Hetland et al., 2005). Coarse feed particles need to be ground to a critical size before they can leave the gizzard. It is thought that accumulation of insoluble fiber in the gizzard triggers a temporary satiety. But once passed the gizzard, it passes through the gut rather quickly (Hetland and Choct, 2003), finally resulting in an overall shorter MRT. An experiment was conducted to investigate the independent effects of energy concentration, NSP concentration, and particle size of NSP on MRT and gut development. We hypothesized that eating time would be increased and overall MRT prolonged by feeding diets with low energy levels and/or high contents of coarsely ground insoluble NSP's.

MATERIALS AND METHODS

Housing, Birds and Management

A total of 504 non beak trimmed 16 wk old layers (Isa Brown strain) were housed in two climate controlled rooms. One room had 24 and the other 25 floor pens (0.90 x 1.50 m). A laying nest was placed outside each pen. The pens were built of wire and hens could see their flock mates in other pens. Each pen contained perches, a feeding trough (length of 100 cm), nipple drinkers, while sand was used as litter. Hens were housed with twelve birds per pen (10.4

hens/m^2). To stimulate feather pecking behavior, stocking density was higher than usual in practice. At the start of the experiment (18 wk of age) average BW was 1713 g (±48.0). The first 8 wk of age hens received a standard commercial diet (ME = 10.9 MJ/kg). To stimulate feed intake, birds were fed a low energy rearing diet (ME = 10.5 MJ/kg) from 9 to 17 wk of age. During the rearing period, feather pecking behavior was shown from 5 wk of age onwards. From 18 to 40 wk of age, hens received the experimental diets. Hens were fed the experimental diets *ad libitum.* All birds had free access to water. Room temperature was set at 20°C and health status of the hens was monitored daily. At 16 wk of age, light schedule was set at 10L : 14D (10 Lux) and was gradually extended by one hour per wk to 16L: 8D light schedule at the age of 22 wk. Photoperiod lasted from 1:00 - 17:00 hrs. To stimulate feather pecking behavior, light intensity was increased to 20 Lux (wk 18) and 30 Lux (wk 20). Because of an outbreak of cannibalism in wk 21, light intensity was reduced to 20 Lux and maintained until the end of the experiment. Throughout the experiment, litter quality was maintained by adding new sand monthly.

Experimental Design

At 18 wk of age, hens were allotted to 6 dietary treatments (Table 1a and 1b) according to a 2 x 3 factorial arrangement, with 3 replicates per treatment. The factors were energy concentration (11.8 vs. 10.6 MJ/kg), NSP concentration (133 vs. 195 g/kg), and fine vs. coarse particle size of the added NSP source in the NSP high diets. Sand was used as dilution material to reduce energy concentration in control NSP diets. Oat hulls were used to increase the NSP concentration. Oat hulls were finely ground in diets with fine particle sizes of NSP, whereas whole oat hulls were added in diets with coarse particle sizes of NSP. All diets were in mash form.

Diet 1 (control energy and control NSP concentration) met the NRC requirements of laying hens (NRC, 1994). Energy concentration in low energy diets was reduced by 10% (11.8 vs. 10.6 MJ/kg), whereas NSP concentration in high NSP diets was increased by 47% (133 vs. 195 g/kg). To maintain energy concentration on the control level, extra fat was added in the high NSP diets of treatment 2 and 3. Addition of 100 g/kg sand to the control diet (treatment 4) increased ash content from 123 to about 225 g/kg, while the other chemical components were diluted up to 10%. Addition of 100 g/kg high-NSP raw materials to the control diet (treatment 5 and 6) decreased the contents of ash, protein and starch up to 10%, whereas the contents of crude fiber, NSP, (hemi-) cellulose and lignin increased.

Measurements

Analytical Procedures. Feed was analyzed for DM, crude ash, crude fat, crude fiber, nitrogen, starch, sugars (mono- and disaccharides as glucose units), calcium, phosphorus, sodium, potassium, NDF, ADF and ADL. All samples were analyzed in duplicate. For determination of the DM content, feed was freeze-dried according to ISO 6496 (International Organization for Standardization, 1998a). Following freeze-drying, feed was ground to pass a 1 mm screen and kept for analysis. Air-dry feed was dried in a forced air oven at 103^0C to an constant weight according to ISO 6496 (International Organization for Standardization, 1998a). Kjeldahl nitrogen content was measured according to ISO 5983 (International Organization for Standardization, 1997) in fresh feed. Crude protein content was calculated as nitrogen * 6.25. Crude fat content was determined after acid hydrolysis according to ISO 6492 (International Organization for Standardization, 1999). For determining crude ash content, samples were incinerated at 550^0C in a muffle furnace according to ISO 5984 (International Organization for Standardization, 2002). The starch content was analyzed enzymatically as described by Brunt (1993). Reducing sugars were extracted from the feed samples, using 40% ethanol, and determined as described by Suárez *et al.* (2006). Contents of calcium, phosphorus, sodium and potassium were analyzed by using ICP-AES (1998b). Analysis of NDF, ADF and ADL contents were based on a modified method of Van Soest et *al.* (1973), as described by Suárez *et al.* (2006).

Particle Size Distribution. Oat hulls were hammer milled, along with the other raw materials (fine) or ungrounded added to the diet (coarse). Particle size distribution of the diets was analyzed by use of the dry sieve method (Goelema et al., 1999). Seven particle size fractions were separated by using six sieves with diameters of 0.25, 0.50, 1.25, 2.50, 3.15 and 5.0 mm respectively. Average particle size of the diets was calculated as (Fraction < 0.25mm * 0.125) + (Fraction 0.25 – 0.50mm * 0.375) + (Fraction 0.50 – 1.25mm * 0.875) + (Fraction 1.25 – 2.50mm * 1.875) + (Fraction 2.50 – 3.15mm * 2.830) + (Fraction 3.15 – 5.00mm * 4.07) + (Fraction > 5.00mm * 6.50)/100. Average particle size of the finely ground diets was 0.87 ±0.02 mm vs. 1.05 ±0.04 mm for the coarsely ground diets.

Observations

Performance and behavioral recordings. Measurements of performance parameters (feed Intake, BW, and egg production) and behavior parameters (feather condition scores, behavioral recordings, eating time and eating rate) are described in an earlier paper (Van Krimpen et al., 2008).

Feed passage rate determination. Feed passage rate was determined in 5 birds per pen at 40 wks of age, thereby using 3 out of 7 pens per treatment. Titanium dioxide (TiO_2; Catalog No. 10080, Merck KG, Darmstadt, Germany) was used as a marker. TiO_2 is insoluble in water and hydrochloric acid, whereas method of analyses is accurate and simple (Sales and Janssens, 2003). The marker was supplemented by gelatin capsules, containing 150 mg of TiO_2 (corresponding with 90 mg of pure Ti) each, according to the method described by Harlander-Matauschek et al. (2006). Initially, (t = 0) 3 gelatin capsules were manually given to each of the 5 hens per pen. Birds were dissected at 5 successive times (t = 30, 90, 180, 270 and 360 min. after moment of titanium supplementation). After dissection, gut was removed from the body and subdivided in 5 different segments (Crop, proventriculus/gizzard, small intestine, caeca and colon). Digesta was collected from the gut segments by gentle squeezing. Each segment was weighed before and after removing of the digesta from the segment. Titanium concentration was analyzed in the 450 gut samples (5 segments/bird x 5 birds/pen x 6 treatments x 3 pens/treatment).

Titanium determination. The method we used to determine TiO_2 in poultry digesta was developed by Short *et al*. (1996) and further refined by Myers *et al.* (2004). This method is based on digestion of the sample in sulphuric acid and addition of hydrogen peroxide to produce an intense orange/yellow color that is read colorimetrically at 408 nm. Fresh digesta samples were weighed and freeze dried (Edwards, Germany). After drying, samples were reweighed. The difference in weight between fresh and dry samples corresponds to the water content of the fresh sample. Samples were ground to powder form (1 mm) and put in labeled plastic container and closed for titanium determination experiment. Prior to the grinding process, stones were removed from proventriculus/gizzard samples by sieving the material with a 2 mm sieve mesh to minimize stone contamination of sample. Weight of fresh and dried digesta was diminished for stone weight. Titanium content of the gut content was analyzed after drying and digestion. A calibration curve was prepared by pipetting 0.0, 10.0, 20.0, 30.0, 40.0 and 50.0 ml of standard solution $(NH_4)_2TiF_6$ in H_2O. (Merck KG, Darmstad, Germany) into a plastic test tube and

Table 1a. Dietary ingredients of the diets (g/kg, as-fed basis)

<table>
<tr><th>Treatment nr.</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th></tr>
<tr><td>Energy concentration</td><td>Control</td><td>Control</td><td>Control</td><td>Low</td><td>Low</td><td>Low</td></tr>
<tr><td>NSP concentration</td><td>Control</td><td>High</td><td>High</td><td>Control</td><td>High</td><td>High</td></tr>
<tr><td>Coarseness of NSP</td><td>No NSP</td><td>Fine</td><td>Coarse</td><td>No NSP</td><td>Fine</td><td>Coarse</td></tr>
<tr><td>Ingredients</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Maize (CP=82 g/kg)</td><td>383.4</td><td colspan="2">383.4</td><td>345.1</td><td colspan="2">345.0</td></tr>
<tr><td>Wheat (CP= 111 g/kg)</td><td>204.8</td><td colspan="2">40.0</td><td>184.2</td><td colspan="2">184.3</td></tr>
<tr><td>Soybean meal, extracted (CP=458 g/kg)</td><td>137.9</td><td colspan="2">108.9</td><td>124.1</td><td colspan="2">124.1</td></tr>
<tr><td>Peas (CP=211 g/kg)</td><td>84.6</td><td colspan="2">91.9</td><td>76.1</td><td colspan="2">76.1</td></tr>
<tr><td>Oyster shells</td><td>72.4</td><td colspan="2">72.0</td><td>65.2</td><td colspan="2">65.2</td></tr>
<tr><td>Rapeseed, extracted (CP=335 g/kg)</td><td>30.0</td><td colspan="2"></td><td>27.0</td><td colspan="2">27.0</td></tr>
<tr><td>Soybean meal, heat treated (CP=351 g/kg)</td><td>25.0</td><td colspan="2">116.1</td><td>22.5</td><td colspan="2">22.5</td></tr>
<tr><td>Soybean oil</td><td>23.3</td><td colspan="2">25.0</td><td>21.0</td><td colspan="2">21.0</td></tr>
<tr><td>Limestone</td><td>20.0</td><td colspan="2">20.0</td><td>18.0</td><td colspan="2">18.0</td></tr>
<tr><td>Monocalcium phosphate</td><td>8.1</td><td colspan="2">9.0</td><td>7.2</td><td colspan="2">7.2</td></tr>
<tr><td>Premix laying hens[1]</td><td>5.0</td><td colspan="2">5.0</td><td>4.5</td><td colspan="2">4.5</td></tr>
<tr><td>NaCl</td><td>3.7</td><td colspan="2">3.7</td><td>3.3</td><td colspan="2">3.3</td></tr>
<tr><td>DL-Methionine</td><td>1.6</td><td colspan="2">2.0</td><td>1.4</td><td colspan="2">1.4</td></tr>
<tr><td>L-Lysine</td><td>0.4</td><td colspan="2">-</td><td>0.4</td><td colspan="2">0.4</td></tr>
<tr><td>Palm oil</td><td>-</td><td colspan="2">23.2</td><td>-</td><td colspan="2">-</td></tr>
<tr><td>Sand</td><td>-</td><td colspan="2">-</td><td>100.0</td><td colspan="2">-</td></tr>
<tr><td>Oat hulls</td><td>-</td><td colspan="2">100.0</td><td>-</td><td colspan="2">100.0</td></tr>
</table>

[1] Provided the following nutrients per kg of premix: vitamin A, 2,400,000 IU; vitamin D3, 480,000 IU; vitamin E, 8,000 mg; vitamin B1, 960 mg; vitamin B2, 2,400 mg; d-panthothenic acid, 3,200 mg; niacinamide, 9,600 mg; vitamin B6, 1,120 mg; folic acid, 360 mg; vitamin B12, 5,000 µg; vitamin C, 20,000 mg; biotin, 20 mg; vitamin K3, 960 mg; choline chloride 60,000 mg; 20,000 mg; copper, 1,600 mg (as $CuSO_4.5H_2O$), iron, 13,000 mg (as $FeSO_4.7H_2O$); manganese 13,000 mg (as MnO_2); zinc, 10,000 mg (as $ZnSO_4$); cobalt, 80 mg (as $CoSO_4.7H_2O$); iodine, 200 mg (asvKI); selenium, 80 mg (as $Na_2SeO_3.5H_2O$).

Table 1b. Analyzed and calculated nutrients of the diets (g/kg, as-fed basis)

Treatment nr.	**1**	**2**	**3**	**4**	**5**	**6**
Energy concentration	Control	Control	Control	Low	Low	Low
NSP concentration	Control	High	High	Control	High	High
Coarseness of NSP	No NSP	Fine	Coarse	No NSP	Fine	Coarse
Analyzed content[1]						
Dry matter	911.0	920.5	926.9	929.9	925.0	916.1
Ash	123.3	124.3	124.8	223.0	115.9	114.0
Fat	41.7	76.0	86.3	43.7	44.3	39.5
Crude Fiber	26.6	57.9	55.0	22.7	62.1	60.4
Crude Protein	168.1	155.7	154.5	150.2	150.9	151.5
Starch	411.8	338.2	343.4	378.4	388.1	391.5
Reducing Sugars[2]	33.6	29.8	29.0	29.3	30.1	28.6
Calcium	38.9	38.6	41.1	36.0	35.6	35.4
Phosphorus	5.4	4.9	5.0	4.9	4.9	4.8
Sodium	1.4	1.5	1.5	1.5	1.4	1.3
Potassium	7.0	7.1	7.1	6.2	6.8	6.6
NSP[3]	**132.6**	**201.9**	**193.7**	**105.2**	**195.7**	**190.9**
NDF	67.7	127.9	129.9	63.0	140.0	138.7
ADF	26.6	61.7	60.8	29.8	68.0	64.1
ADL (lignin)	6.6	14.1	11.4	6.8	14.1	13.2
Cellulose[4]	20.0	47.6	49.4	23.0	53.9	50.8
Hemi cellulose[4]	41.1	66.2	69.1	33.2	72.0	74.7
Calculated content						
ME (kcal/kg)	**2825**	**2825**	**2825**	**2540**	**2540**	**2540**
LYS	8.09	8.30	8.30	7.33	7.63	7.63
Dig. LYS	6.70	6.70	6.70	6.08	6.20	6.20
Dig. M+C	5.80	5.80	5.80	5.22	5.27	5.27
Dig. THR	4.60	4.52	4.52	4.14	4.20	4.20
Dig. TRP	1.47	1.41	1.41	1.32	1.34	1.34

[1] Based on 1 analysis in duplicate per diet.

[2] Mono- and disaccharides as glucose units.

[3] Non-starch polysaccharide (NSP) content was calculated by subtracting the crude protein, fat, starch, reducing sugars and ash content from the dry matter content.

[4] Cellulose = ADF minus ADL; hemi cellulose = NDF minus ADF.

diluted with water to achieve 0.0 (5.0 ml of water), 20.0 (0.1 ml of TiO_2 + 4.9 ml of water), 40.0 (0.2 ml of TiO_2 + 4.8 ml of water), 60.0 (0.3 ml of TiO_2 + 4.7 ml of water), 80.0 (0.4 ml of TiO_2 + 4.6 ml of water) and 100.0 mg/l (0.5 ml of TiO_2 + 4.5 ml of water) respectively. Thereafter, 0.2 ml of 30 % hydrogen peroxide (Merck KG, Darmstad, Germany) was added to each plastic test tube containing different concentrations of titanium and mixed thoroughly. These solutions were analyzed using a UV-visible spectrophotometer (Varian, CARY 50 probe) and absorbance was measured at 408 nm. The standard containing 0.0 mg of titanium was used to set to zero the instrument. A linear standard curve was produced with a regression equation:

$$Y = 0.006330 * X + 0.005821 \ (R^2 = 0.999) \qquad \text{(eq. 1)}$$

Where Y = the absorbance, measured by the spectrophotometer, and X = the titanium concentration.

From each digesta sample, 0.5 ($\pm$ 0.05) g was weighed with analytical balance (Mettler, AE 240, Tiel Netherlands) into a 300 ml destructive tube (macro Kjeldahl digestion tube). Two tablets of copper (II) tetraoxosulphate (IV) ($CuSO_4$) {10g K_2SO_4 + 0.70g $CuSO_4$}, serving as reagent catalyst and 25 ml of concentrated sulphuric acid (H_2SO_4) were added to this weighed sample. The content was then brought to a heat destruction apparatus (Kjeldatherm; Gerhardt, Germany) to be digested at 406 ^oC for 1h and 45 minutes (appearance of a clear green coloration indicates completion of digestion). After little cooling, 50 ml of demineralized water was added to the sample. Than, the solution was mixed, while a layer of foam was formed. Thereafter, the content of the tube was emptied into a 100 ml volumetric flask. Demineralized water was used to rinse the remaining content of the tube into volumetric flask and made up to the mark (100 ml). After mixing again, sample was cooled down completely (approximately 2 hours) and refilled with demineralized water up to the mark. Thereafter, 5.0 ml of the sample solution was pipetted into two plastic test tubes- one labeled and unlabeled plastic test tube. Than, 0.2 ml of 30% hydrogen peroxide was added into the labeled tube. In both tubes, absorbance was measured at 408 nm, using UV- visible spectrophotometer (Varian, CARY 50 probe). The unlabeled test tube serves as a blank sample for background correction. Absorbance level of the labeled sample was reduced with that of the unlabeled sample.

Curve-fitting procedure and Statistical Analysis. To calculate titanium content per segment, titanium concentration of the digesta (mg/g dm) was multiplied with the weight of the gut segment (g dm). Titanium recovery (%) in the segments was expressed titanium content in segment divided by total supplemented titanium amount times 100. For birds that were dissected at t = 180, 270 and 360 min., total supplemented titanium amount was set at a fixed value (270 mg). We assumed that until 90 min. after supplementation, no titanium was excreted. Total titanium recovery of these birds, however, showed a high variation (236 mg ± 55). Therefore, total supplemented titanium for birds that were dissected at t = 30 and 90 min. was calculated as the sum of the total titanium recovery in the five segments.

The course of titanium recovery per segment over time, as an indicator of feed passage rate through the GIT, is modeled by use of a multi compartmental model (Dhanoa et al., 1985). The alteration in recovery (*dR*) per gut segment at a certain moment (t) can be calculated by use of the following equations (eq. 2 to eq. 7):

$$dR_{Crop}(t)/dt = -f1R_{Crop}(t) \tag{eq. 2}$$

$$dR_{\Pr ov/gizzard}(t)/dt = f1R_{Crop}(t) - f2R_{\Pr ov/gizzard}(t) \tag{eq. 3}$$

$$dR_{Small\_\text{int}estine}(t)/dt = f2R_{\Pr ov/gizzard}(t) - f3R_{Small\_\text{int}estine}(t) \tag{eq. 4}$$

$$dR_{Colon}(t)/dt = f3R_{Small\_\text{int}estine}(t) - f4R_{Colon}(t) \tag{eq. 5}$$

$$dR_{Caeca}(t)/dt = f4R_{Colon}(t) - f5R_{Caeca}(t) \tag{eq. 6}$$

whereas the factors $f1$, $f2$, $f3$, $f4$, and $f5$ are the rate of emptying and filling of the different gut segments, respectively (Crop, Proventriculus/gizzard, small intestine, colon, caeca). These rates are expressed as increase or decrease of segment content (%) at a certain moment (t in min). Curve parameters that fit the course of titanium recovery per pen (eq. 7 to eq. 11) were estimated by solving 5 linear first-order differential equations (eq. 2 to eq. 6) with 5 unknowns by using a iterative procedure (Lindstrom and Bates, 1990; Engel et al., 2003).

$$R_{Crop}(t) = 100e^{-f1t} \tag{eq. 7}$$

$$R_{\Pr ov/gizzard}(t) = 100f1/(f2-f1)e^{-f1t} - e^{-f2t} \tag{eq. 8}$$

$$\begin{aligned} &R_{Small\_\text{int}estine}(t) = \\ &100f1*f2/(f2-f1)e^{-f1t}/(f3-f1) - e^{-f2t}/(f3-f2) + (f2-f1)e^{-f3t}/ \\ &[(f3-f2)(f3-f1)] \end{aligned} \tag{eq. 9}$$

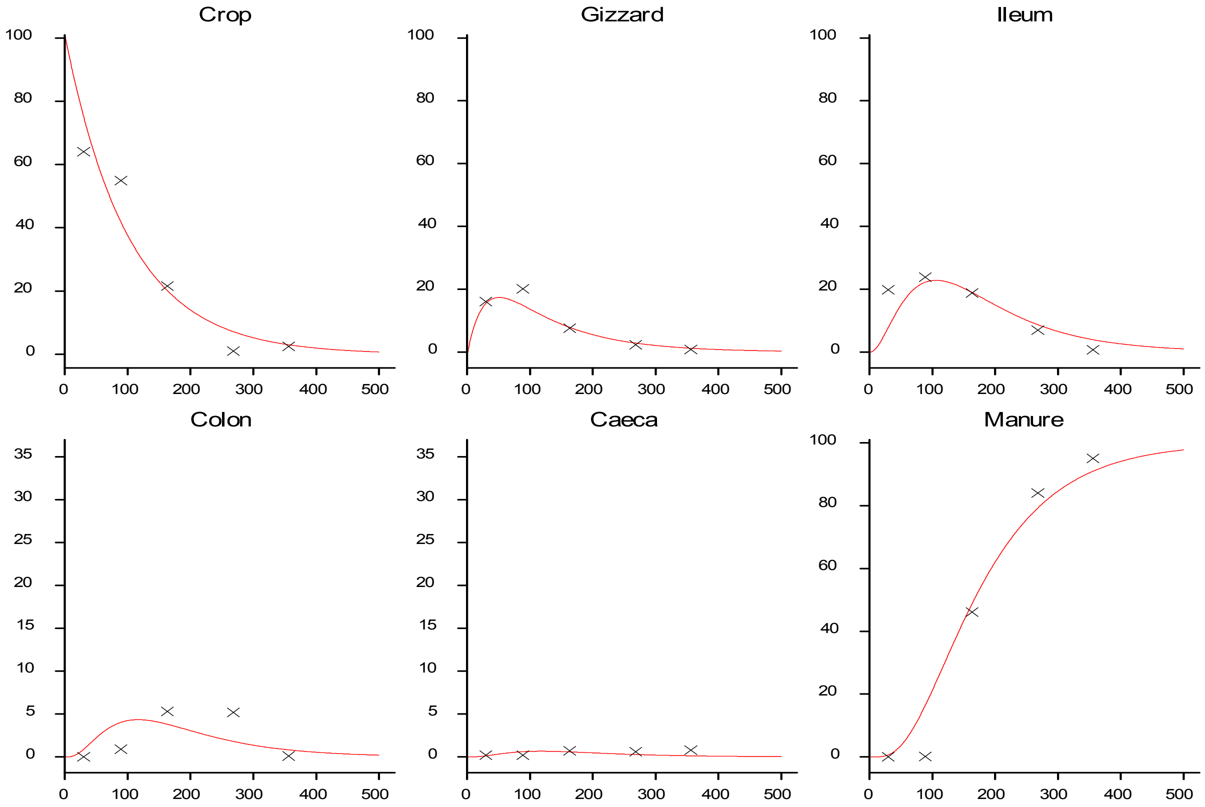
$$\begin{aligned} &R_{Colon}(t) = \\ &100f1*f2*f3/(f2-f1)e^{-f1t}/[(f3-f1)(f4-f1)] - e^{-f2t}/[(f3-f2)(f4-f2)] \\ &+(f2-f1)e^{-f3t}/[(f3-f2)(f3-f1)(f4-f3)] - (f2-f1)e^{-f4t}/ \\ &[(f4-f3)(f4-f2)(f4-f1)] \end{aligned} \tag{eq. 10}$$

$$R_{Caeca}(t)=$$
$$100 f1 * f2 * f3 * f4/(f2-f1)\left(e^{-f1t}-e^{-f5t}\right)/[(f5-f1)(f4-f1)(f3-f1)]-\left(e^{-f2t}-e^{-f5t}\right)/$$
$$[(f5-f2)(f4-f2)(f3-f2)]+(f2-f1)\left(e^{-f3t}-e^{-f5t}\right)/[(f3-f1)(f3-f2)(f4-f3)(f5-f3)]$$
$$-(f2-f1)\left(e^{-f4t}-e^{-f5t}\right)/[(f4-f1)(f4-f2)(f4-f3)(f5-f4)]$$

(eq. 11)

Thus, at t = 0, titanium recovery in the crop = 100%, after which emptying starts based on the value of f1. Recovery in the proventriculus/gizzard is determined by the filling rate from the crop minus the emptying rate towards the small intestine. An example of titanium recovery per gut segment over time is given in Figure 1.

Figure 1. Example of curve fitting of titanium recovery (% of original dose) over time per gut segment



Mean retention time (MRT) of the digesta in the crop was calculated as $1/f1$, of the proventriculus/gizzard as $1/f1 + 1/f2$, etc. (Dhanoa et al., 1985). Thus, if $f1 = 0.02$, it means that every minute 2% of the remaining crop content leaves the crop, corresponding with a MRT of 50 min.

Following curve-fitting, the REML variance component analysis procedure tested the effect of the nutritional factors on the determined traits, using the model (1):

$$Y_{ij} = \mu + \text{Energy}_i + \text{NSP}_j + (\text{Energy x NSP}) + e_{ij} \qquad (1)$$

where Y_{ij} = dependent variable; μ = overall mean; energy$_i$ = fixed effect of energy concentration i (i = 2; control and low); NSP$_j$ = fixed effect of NSP concentration j (j = 3; a combination of NSP and coarseness); the contrast of NSP represents control NSP vs. the average of high NSP fine and high NSP coarse; the contrast of coarseness represents high NSP fine vs. high NSP coarse; the interaction energy x NSP represents the contrast energy x NSP and the contrast energy x coarseness. Below the tables with treatment means, the p-values of energy, NSP, coarseness, energy x NSP and energy x coarseness will be presented. Model (1) was also used to test effects of gut segment weights and DM content of gut segments.

RESULTS

Feed intake over wk 21 was similar in the two control NSP and high NSP-fine treatments, whereas in high NSP-course diets feed intake was lower in control energy diets compared to low energy diets (124.5 vs. 146.9 g/h/d; Table 2). Energy intake over wk 21, however, was not statistically different for the two high NSP-coarse treatments. In control NSP treatments, energy intake was lower in the low energy diet compared to control energy diet (1626 vs. 1502 J/h/d). Also in high NSP-fine treatments energy intake was lower in the low energy diet compared to control energy diet (1611 vs. 1446 J/h/d). As expected, NSP intake over wk 21 was higher in the four NSP-high treatments than in the two control NSP treatments. NSP intake was similar in the two high NSP-fine diets. NSP intake of hens fed control NSP diets was lower in the low energy treatment compared to the control energy treatment (18.3 vs. 14.9 g/h/d), whereas NSP intake of hens fed high NSP-coarse diets was higher in the low energy treatment compared to the control energy treatment (24.1 vs. 28.0 g/h/d). Eating time and eating rate were not significantly affected by dietary treatments.

Table 2. Feed intake (g/h/d), energy intake (J/h/d), NSP intake (g/h/d), eating time (% of observation period) and eating rate (g/ min) during the wk prior to wk of dissection per treatment

Treatment[1]	Feed intake (g/h/d)	Energy intake (J/h/d)	NSP intake (g/h/d)	Eating time (%)	Eating rate (g/min)
Control Energy					
Control NSP	138.0	1626	18.3	17.9	0.88
High NSP-Fine	136.5	1611	27.6	16.2	0.90
High NSP-Coarse	124.5	1469	24.1	15.3	1.11
Low Energy					
Control NSP	141.8	1502	14.9	16.5	0.91
High NSP-Fine	136.5	1446	26.8	19.1	0.75
High NSP-Coarse	146.9	1559	28.0	20.6	0.76
SE	5.26	58.4	0.96	4.52	0.233
P-Value of contrasts					
Energy (control vs. low)	**0.027**	0.159	**0.004**	0.483	0.418
NSP (control vs. high)	0.366	0.339	**<0.001**	0.871	0.992
Interaction Energy*NSP	0.525	0.525	**0.002**	0.416	0.484
Contrasts within NSP					
Coarseness (fine vs. coarse)	0.895	0.938	0.368	0.923	0.671
Interaction Energy*Coarseness	**0.038**	**0.034**	**0.018**	0.773	0.655

[1] The tested factors were energy concentration (11.8 vs. 10.6 MJ/kg), NSP concentration (133 vs. 195 g/kg) and particle sizes of the added NSP source (fine vs. coarse).

MRT of feed in the crop, proventriculus/gizzard, small intestine, colon and caeca is on average 56.8, 22.4, 91.3, 16.4 and 2.9 min., corresponding with 29.9, 11.8, 48,1, 8.6 and 1.5% of the total retention time, respectively (Table 3). In the crop, MRT of the four high NSP treatments was as twice as high (*P*=0.10) compared to both control NSP treatments (68 vs. 34 min.). In the two control NSP treatments MRT in the gizzard was not affected by energy concentration, whereas in the high NSP treatments MRT was higher in hens fed low energy diets compared to hens fed standard energy diets (34.3 vs. 9.9 min.; *P*=0.027). NSP addition to the diet resulted in an increased (*P*=0.074) MRT of the digesta in the total foregut (56.8 vs. 90.6

min) compared to the control NSP treatments. Ileal MRT was not significantly affected by dietary treatments, although numerically large differences were observed between treatments. Feeding low energy diets resulted in a longer MRT in the colon (26.0 vs. 6.7 min; *P*=0.029), in the caeca (3.9 vs. 1.8 min.; *P*=0.030), and as a result also in the total hind gut (30.3 vs. 8.6 min; *P*=0.025) compared to control energy diets. Coarse grinding of NSP decreased MRT in the caeca compared to fine grinding of NSP (4.6 vs. 1.8 min; *P*=0.005). Overall MRT was not affected by dietary treatments.

Table 3. Mean retention time (min) per segment.

Treatment[1]	Crop	Proventr./ gizzard	Total Foregut[2]	Small intestine	Colon	Caeca	Total Hindgut[3]	Overall GIT
Control Energy								
Control NSP	42.8	31.2	74.0	102.0	10.9	1.5	12.4	188.3
High NSP-Fine	73.9	10.7	84.7	105.5	5.1	2.5	7.6	197.7
High NSP-Coarse	88.4	9.1	97.5	51.1	4.1	1.5	5.7	154.2
Low Energy								
Control NSP	24.5	15.1	39.6	118.5	39.4	2.9	42.5	200.4
High NSP-Fine	27.4	38.6	66.0	92.5	28.2	6.7	36.1	193.4
High NSP-Coarse	84.0	29.9	114.0	77.9	10.4	2.0	12.2	204.3
SE	37.55	14.60	31.55	39.76	13.11	1.45	10.05	33.92
P-Value of contrasts								
Energy (control vs. low)	0.524	0.278	0.796	0.821	**0.029**	**0.030**	**0.025**	0.337
NSP (control vs. high)	**0.100**	0.711	**0.074**	0.236	0.095	0.419	0.138	0.737
Interaction Energy*NSP	0.974	**0.027**	0.325	0.820	0.330	0.800	0.410	0.777
Contrasts within NSP								
Coarseness (fine vs. coarse)	0.172	0.606	0.165	0.259	0.290	**0.005**	0.173	0.576
Interaction Energy*Coarseness	0.443	0.741	0.447	0.495	0.383	0.077	0.284	0.274

[1] The tested factors were energy concentration (11.8 vs. 10.6 MJ/kg), NSP concentration (133 vs. 195 g/kg) and particle sizes of the added NSP source (fine vs. coarse). [2] Foregut = sum of gut segments crop and proventriculus/gizzard. [3] Hindgut = sum of gut segments colon and caeca.

Table 4. Relative empty weight and content of GIT segments (g/kg bodyweight of hen).

Treatment[1]	Crop empty	Crop content	Gizzard empty	Gizzard content	Small intestine empty	Small intestine content	Colon empty	Colon Content	Caeca empty	Caeca content
Control Energy										
Control NSP	5.0	5.8	19.2	12.8	29.4	15.1	2.8	1.6	5.2	2.4
High NSP-Fine	4.5	4.8	21.9	14.9	29.0	14.8	2.7	2.1	5.2	2.3
High NSP-Coarse	5.0	7.1	29.3	15.9	29.6	14.3	2.7	1.8	4.9	2.4
Low Energy										
Control NSP	4.9	5.4	19.5	13.2	29.9	14.0	2.6	1.7	5.1	2.7
High NSP-Fine	4.9	4.5	21.8	14.9	30.3	15.0	2.6	1.8	4.6	2.6
High NSP-Coarse	4.8	6.6	27.6	15.8	30.4	14.4	2.8	1.8	5.2	2.7
SE	1.05	1.32	0.68	1.35	0.65	0.97	0.15	0.22	1.04	1.09
P-Value of contrasts										
Energy (control vs. low)	0.995	0.759	0.194	0.954	0.056	0.686	0.688	0.572	0.466	**0.036**
NSP (control vs. high)	0.809	0.837	**<0.001**	**<0.001**	0.728	0.908	0.884	0.148	0.088	0.885
Interaction Energy*NSP	0.420	0.829	0.286	0.739	0.611	0.359	0.245	0.416	0.555	0.180
Contrasts within NSP										
Coarseness (fine vs. coarse)	0.149	0.093	**<0.001**	0.174	0.464	0.538	0.430	0.446	0.449	0.547
Interaction Energy*Coarseness	0.087	0.509	0.272	0.996	0.675	0.936	0.315	0.288	0.004	0.159

[1] The tested factors were energy concentration (11.8 vs. 10.6 MJ/kg), NSP concentration (133 vs. 195 g/kg) and particle sizes of the added NSP source (fine vs. coarse).

Relative empty weight and content of crop, gizzard, small intestine, colon and caeca, as expressed in g/kg bodyweight of hen, are shown in Table 4. Dietary treatments had no effect on weight of empty crop and on crop content. Feeding high NSP diets increased empty gizzard weight of hens by 30% (25.2 vs. 19.4 g/kg ± 0.68) compared with hens fed control NSP diets. Empty gizzard weight was also affected by coarseness of NSP. Hens fed coarsely ground NSP had 30% higher empty gizzard weight compared with hens fed finely ground NSP (28.5 vs. 21.9 g/kg ± 0.68). Feeding high NSP diets also increased gizzard content by 18% (15.4 vs. 13.0 ± 1.35) compared with hens fed control NSP diets, whereas no effect of coarseness of NSP on gizzard content was observed. Feeding low energy diets numerically (P=0.056) increased empty small intestine weight of the hens compared with feeding control energy diets (30.2 vs. 29.3 g/kg hen). Small intestine and colon content and empty colon weight were not affected by dietary treatments. In control energy fed hens empty caeca weight was not affected by coarseness of NSP, whereas in low energy fed hens empty caeca weight was lower in hens fed finely ground NSP compared with coarsely ground NSP (P=0.004). Caeca content was 12.5% higher in hens fed low energy diet compared with control energy diet (2.7 vs. 2.4 g/kg, P=0.036).

Dry matter (DM) content (g/kg) of GIT segments are shown in Table 5. DM content of the crop, which was on average 359 g/kg (± 20.3), was not affected by dietary treatments. DM content of the gizzard was 36 g higher compared to the crop (395 g/kg ± 22.9), and also not affected by dietary treatments. Average DM content of the small intestine was much lower compared to crop and gizzard (222 g/kg ± 12.1). Ileal DM content was affected ($P < 0.001$) by Energy * NSP interaction. In control energy diets no effect of NSP on ileal DM content was observed, whereas in low energy diets ileal DM content was 27% higher in standard NSP diets compared to low NSP diets (272 vs. 215 g/kg). Similarly, in control energy diets no effect of NSP on DM content of the colon was observed, whereas in low energy diets DM content of the colon was 20% higher in standard NSP diets compared to low NSP diets (294 vs. 244 g/kg). Caecal DM content was affected ($P = 0.004$) by Energy * Coarseness interaction. In low energy diets caecal DM content was not affected by coarseness of NSP, whereas in control energy diets DM content of caeca in finely ground NSP treatment was 26% higher compared to the coarsely ground NSP treatment (278 vs. 221 g/kg).

Table 5. DM content (g/kg) of digesta per GIT segment

Treatment[1]	Crop/ Proventriculus[2]	Gizzard[2]	Small intestine	Colon	Caeca
Control Energy					
Control NSP	353	402	204	228	247
High NSP-Fine	361	400	224	252	278
High NSP-Coarse	345	375	204	232	221
Low Energy					
Control NSP	373	426	272	294	238
High NSP-Fine	351	394	224	247	214
High NSP-Coarse	372	370	205	241	217
SE	20.3	22.9	12.1	12.0	10.9
P-Value of contrasts					
Energy (control vs. low)	0.453	0.813	**0.009**	**0.015**	**0.002**
NSP (control vs. high)	0.749	0.147	**0.013**	0.079	0.254
Interaction Energy*NSP	0.752	0.471	**< 0.001**	**0.002**	0.173
Contrasts within NSP					
Coarseness (fine vs. coarse)	0.915	0.295	0.072	0.288	**0.009**
Interaction Energy*Coarseness	0.375	0.980	0.984	0.548	**0.004**

[1] The tested factors were energy concentration (11.8 vs. 10.6 MJ/kg), NSP concentration (133 vs. 195 g/kg) and particle sizes of the added NSP source (fine vs. coarse).

[2] Stones were removed before determining DM content of digesta.

DISCUSSION

This experiment was conducted to investigate the independent effects of energy concentration, NSP concentration, and particle sizes of added NSP source on gut development and MRT. We hypothesized that eating time would be increased and overall MRT prolonged by feeding diets with low energy levels or high contents of coarsely ground insoluble NSP's or both.

EFFECT OF ENERGY DILUTION

In contrast to our hypothesis, energy dilution of the diet did not affect MRT in the entire gut but only increased MRT in the hindgut. Savory (1980) showed that MRT in the entire gut of hens fed a 40% diluted mash diet (diluted with cellulose) to Japanese quail decreased compared to undiluted mash. Furthermore, he showed that supplementing the diluted diet decreased MRT in the crop, which is, although non-statistically significant, in line with our results. In contrast to our experiment, Savory (1980) did not separate the effect of (energy)-dilution and the effect of extra NSP addition, which may partly explain the differences between both experiments.

Furthermore, Savory and Gentle (1976) reported increases in length of the GIT by 10-15%, because of feeding diluted diets to Japanese quail. A length increase of the GIT may have consequences for the MRT. Own unpublished data, however, showed that supplementing 10%, 15% or 20% sand to diets of laying hens did not extend the length of any gut segment.

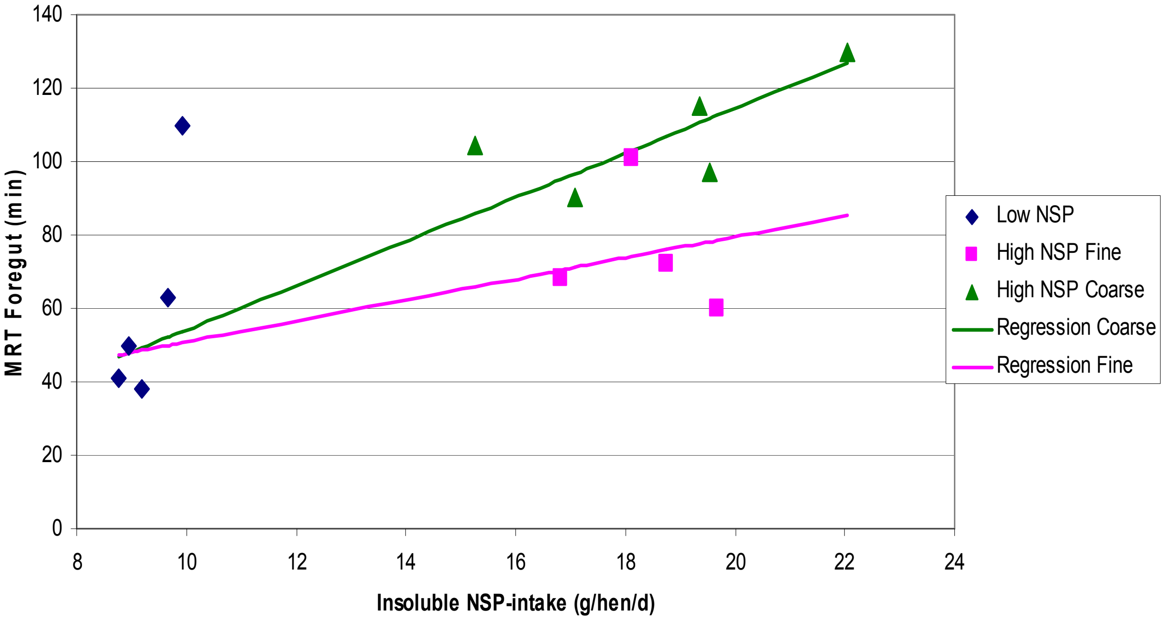
Dry digesta weight in the small intestine was higher if birds were fed low energy/control NSP diet compared to the other treatments. Only in this diet, sand was added as dilution material. Sand has a high bulk density (Jansen, 1977) and sand addition to the diet increases the bulk density of both the complete diet and the digesta. We assumed that the gut segments are able to contain more digesta per volume unit with a high bulk density compared to digesta with a low bulk density. This may explain the higher DM content in the small intestine and colon of hens fed the low energy/control NSP diet. Hens fed low energy diets showed an increased MRT in the caeca compared to hens fed control energy diets. It was assumed that, as a consequence of feeding low density diets for all nutrients, caeca activity could be enhanced to improve nutrient utilization. Karasawa and Maeda (1994) reported that caeca activity was increased in birds fed protein-deficient diets to increase nitrogen (+ energy) utilization, while caeca activity was not affected in protein-adequate diets (Karasawa and Maeda, 1994).

EFFECT OF NSP CONCENTRATION

Because of feeding high NSP diet, relative weight of proventriculus/gizzard and it's contents were increased by 30% and 18%, respectively. Accordingly, supplementing insoluble NSP-rich roughages to layers also increased the relative weights of the gizzard and the gizzard content (Steenfeldt et al., 2007). Increased relative weights of gizzard (+ 26%) and it's contents (+ 55%) were also found in laying hens that had access to wood shavings from the litter (Hetland et al., 2005). In our study, sand was used as litter substrate, so no wood shavings could have been eaten. The effect of wood shavings on gizzard development was evident in hens fed wheat (low insoluble NSP) based diet, but lacking in hens fed oat (high insoluble NSP) based diet. This may indicate that no additional effect of consumption of insoluble NSP's from litter was found when a insoluble NSP source (oat) was already added to the diet.

Insoluble NSP sources seem to accumulate in the gizzard, because insoluble NSP concentration of the gizzard contents was found to be about twice as high as that of the feed (Hetland et al., 2003; Hetland et al., 2005). In line with these findings, gizzard content in the current study was higher in hens fed high NSP diets compared to hens fed control NSP diets. Moreover, feeding a high NSP diet increased MRT in the foregut, especially in the low energy diet. Interestingly, regression analysis revealed that daily insoluble NSP intake as well as the coarseness of NSP clearly affected MRT in the foregut (Figure 2). MRT in the foregut increased by 6.0 min. for each extra gram of *coarsely* ground insoluble NSP that was consumed (MRT = 6.02 x insoluble NSP intake – 6.0; $P<0.001$, $R^2=88.8$). On the other hand, MRT in the foregut increased by only 2.86 min. for each extra gram of *finely* ground insoluble NSP that was consumed (MRT = 2.86 x insoluble NSP intake + 22.3; $P=0.048$, $R^2=42.3$), as shown in Figure 2.

Figure 2. Relation between insoluble NSP intake and MRT in the foregut (crop, proventriculus and gizzard)



Overall MRT, however, was not affected by any of the dietary treatments, indicating that MRT of the digesta in hens fed high NSP diets was decreased after passing the foregut. Accordingly, Hartini et al. (2003) found no effect of high vs. low fiber mash on overall MRT. Also in broiler breeders, an increase of the dietary fiber content was suggested to improve satiety and welfare, as appears from less spot pecking, damaging pecking and cannibalism (Hocking et al., 2004). The increased sense of satiety seemed to be related to the high water-holding capacity of the added fiber sources (sugar beet pulp, sunflower meal and oat hulls) in this study. Dry digesta weights in the broiler breeders were relatively low in the gizzard and ileum, especially for the sugar beet pulp and sunflower ratios. Feeding roughages, that are characterized by low DM contents, resulted in reduced DM contents in the gizzard and ileum of layers (Steenfeldt et al., 2007). In line with earlier findings in broiler breeders and layers, feeding high NSP-diets in the current experiment also resulted in an increased relative weight of fresh digesta in the gizzard. This increase, however, was not related to a lower DM content of digesta. Therefore, it could be hypothesized that a higher level of satiety is partly related to more fresh contents in the foregut, relatively independent of the DM percentage of the digesta in these segments.

EFFECT OF PARTICLE SIZES OF NSP

Feeding coarsely ground NSP increased relative weights of empty proventriculus/gizzard by 30% compared to finely ground NSP. This increase could be explained by the enhanced grinding activity of the gizzard. Coarse feed particles need to be ground to a certain critical size before they can leave the gizzard (Moore, 1999). The gizzard grinds all organic feed ingredients to a very consistent particle size range, regardless of the original particle size of the feed (Hetland et al., 2002). Mean particle sizes of the duodenal contents of birds fed high concentrations of whole wheat, whole oats and whole barley were very similar i.e., 151, 143 and 117 µm, respectively. Thus, coarsely ground insoluble NSP particles accumulate in the gizzard until the particles have the sizes to leave this segment. This may explain the increased volume of the gizzard contents.

Accumulation of coarsely ground NSP in the gizzard, should result in a slower passage out of the gizzard (Hetland et al., 2004a). In the current experiment, MRT in the foregut only non-significantly increased because of coarser NSP. Regression analysis, however, revealed that daily insoluble NSP intake as well as the coarseness of NSP clearly affected MRT in the foregut (Figure 2). These findings accord with that of Hetland et al., (2005) who showed that approximately only 50% of ingested oat hulls from a coarse diet and even 90% of the ingested oat hulls from a fine diet had passed the gizzard after 2h. Surprisingly, MRT in the caeca was higher in hens fed finely ground compared to coarsely ground high NSP diets. The explanation for this is not clear.

Thus, the gizzard will reduce coarse feed particles and letting pass nutrients for digestion. Furthermore, the gizzard plays a major role for gastro-duodenal reflux of digesta (Duke, 1992). To perform well, the gizzard seems to have a demand for structural components (Hetland et al., 2004a). Hens are sometimes motivated to eat feathers and wood shavings (Harlander-Matauschek et al., 2007), probably because they need structural components. Interestingly, high feather pecking hens had a stronger preference for feathers than low feather pecking hens. Comparable to insoluble NSP sources, consumed feathers accelerate feed passage rate (Harlander-Matauschek et al., 2006).

In conclusion, dietary energy dilution increased feed intake, and prolonged MRT in the hindgut. NSP addition to the diet resulted in a higher NSP intake, increased relative weights of the gizzard and it's contents, and in a prolonged MRT in the foregut. The effects of NSP intake on MRT in the foregut were more pronounced in coarsely vs. finely ground NSP. A full gizzard is likely to make the birds feel more satiated, resulting in birds appearing more calm. This may contribute to a lower feather pecking pressure (Hetland et al., 2004b).

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REFERENCES

Blokhuis, H. J. and J. W. Van der Haar. 1989. Effects of floor type during rearing and of beak trimming on ground pecking and feather pecking in laying hens. *Appl. Anim. Behav. Sci.,* 22:359-369.

Brunt, K. 1993. Enzymatic determination of starch content in feed and feedstuffs, Memo 93-302 (in Dutch). *Stichting Nederlands Instituut voor Koolhydraat Onderzoek, Groningen. The Netherlands*.

Dawkins, M. S. 1989. Time Budgets in Red Junglefowl as a Baseline for the Assessment of Welfare in Domestic-Fowl. *Appl. Anim. Behav. Sci.,* 24:77-80.

Dhanoa, M. S., R. C. Siddons, J. France and D. L. Gale. 1985. A Multicompartmental Model to Describe Marker Excretion Patterns in Ruminant Feces. *Br. J. Nutr.,* 53:663-671.

Duke, G. E. 1992. Recent Studies on Regulation of Gastric-Motility in Turkeys. *Poult. Sci.,* 71:1-8.

Engel, B., K. v. Reenen and W. Buist. 2003. Analysis of correlated series of repeated measurements: application to challenge data. *Biometrical Journal,* 45:866-886.

Goelema, J. O., A. Smits, L. M. Vaessen and A. Wemmers. 1999. 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. Techn.,* 78:109-126.

Harlander-Matauschek, A., H. P. Piepho and W. Bessei. 2006. The effect of feather eating on feed passage in laying hens. *Poult. Sci.,* 85:21-25.

Harlander-Matauschek, A., I. Benda, C. Lavetti, M. Djukic and W. Bessei. 2007. The relative preferences for wood shavings or feathers in high and low feather pecking birds. *Appl. Anim. Behav. Sci.,* 107:78-87.

Hartini, S., M. Choct, G. Hinch and J. V. Nolan. 2003. Effect of diet composition, gut microbial status and fibre forms on cannibalism in layers. *Australian Egg Corporation Limited.* AECL Publication No 03/03:1-111.

Hetland, H. and M. Choct. 2003. Role of insoluble non-starch polysaccharides in poultry nutrition. *Proceedings of the 14 th European Symposium of Poultry Nutrition*, Lillehammer, Norway, 64-69.

Hetland, H., B. Svihus and V. Olaisen. 2002. Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *Br. Poult. Sci.,* 43:416-423.

Hetland, H., B. Svihus and A. Krogdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *Br. Poult. Sci.,* 44:275-282.

Hetland, H., M. Choct and B. Svihus. 2004a. Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poult. Sci. J.,* 60:415-422.

Hetland, H., B. Svihus and M. Choct. 2005. Role of insoluble fiber on gizzard activity in layers. *J Appl. Poult. Res.,* 14:38-46.

Hetland, H., R. O. Moe, R. Tauson, S. Lervik and B. Svihus. 2004b. Effect of including whole oats into pellets on performance and plumage condition in laying hens housed in conventional and furnished cages. *Acta Agric. Scand., Section A, Anim. Sci.,* 54:206-212.

Hocking, P. M., V. Zaczek, E. K. M. Jones and M. G. Macleod. 2004. Different concentrations and sources of dietary fibre may improve the welfare of female broiler breeders. *Br. Poult. Sci.,* 45:9-19.

Hoffmeyer, I. 1969. Feather pecking in pheasants - an ethological approach to the problem. *Danish review of game biology,* 6:1-36.

International Organization for Standardization. 1997. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. Kjeldahl method. ISO 5983. Int. Organ. Standardization, Geneva, Switzerland.

International Organization for Standardization. 1998a. Animal feeding stuffs. Determination of moisture and other volatile matter content. ISO 6496. Int. Organ. Standardization, Geneva, Switzerland.

International Organization for Standardization. 1998b. Water quality. Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy. ISO 11885. Int. Organ. Standardization, Geneva, Switzerland.

International Organization for Standardization. 1999. Animal feeding stuffs. Determination of fat content. ISO 6492. Int. Organ. Standardization, Geneva, Switzerland.

International Organization for Standardization. 2002. Animal feeding stuffs. Determination of crude ash. ISO 5984. Int. Organ. Standardization, Geneva, Switzerland.

Jansen, A. I. 1977. BINAS Informatieboek vwo-havo voor het onderwijs in de natuurwetenschappen. Groningen.

Karasawa, Y. and M. Maeda. 1994. Role of caeca in the nitrogen nutrition of the chicken fed on a moderate protein diet or a low protein diet plus urea. *Brit. Poult. Sci.,* 35:383-391.

Lindstrom, M. J. and D. M. Bates. 1990. Nonlinear mixed effects models for repeated measures data. *Biometrics,* 46:673-786.

Moore, S. J. 1999. Food breakdown in an avian herbivore: who needs teeth? *Austr. J. Zoology,* 47:625-632.

Myers, W. D., P. A. Ludden, V. Nayigihugu and B. W. Hess. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.,* 82:179-183.

NRC. 1994. Nutrient requirements of poultry. *Ninth Revised Edtion.* Washington DC.

Roberfroid, M. 1993. Dietary Fiber, Inulin, and Oligofructose - a Review Comparing Their Physiological-Effects. *Critical Reviews in Food Sci. and Nutr.,* 33:103-148.

Sales, J. and G. P. J. Janssens. 2003. The use of markers to determine energy metabolizability and nutrient digestibility in avian species. *World' s Poult. Sci. J.,* 59:314-327.

Savory, C. J. 1980. Meal occurrence in Japanese quail in relation to particle size and nutrient density. *Anim. Behav.,* 28:160-171.

Savory, C. J. and M. J. Gentle. 1976. Effects of Dietary Dilution with Fiber on Food-Intake and Gut Dimensions of Japanese Quail. *Brit. Poult. Sci.,* 17:561-570.

Short, F. J., P. Gorton, J. Wiseman and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim. Feed Sci. Technol.,* 59:215-221.

Steenfeldt, S., J. Kjaer and R. M. Engberg. 2007. Effect of feeding silages or carrots as supplements to laying hens on production performance, nutrient digestibility, gut structure, gut microflora and feather pecking behaviour. *Br. Poult. Sci.,* 48:454-468.

Suárez, B. J., C. G. Van Reenen, G. Beldman, J. van Delen, J. Dijkstra and W. J. J. Gerrits. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: I. Animal performance and rumen fermentation characteristics. *J. Dairy Sci.,* 89:4365-4375.

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. v. d. Peet-Schwering, L. A. d. Hartog and M. W. A. Verstegen. 2008. Low Dietary Energy Concentration, High Nonstarch Polysaccharide Concentration and Coarse Particle Sizes of Nonstarch Polysaccharides Affect the Behavior of Feather-Pecking-Prone Laying Hens. *Poult. Sci.,* 87:485-496.

Van Krimpen, M. M., R. P. Kwakkel, B. F. J. Reuvekamp, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2005. Impact of feeding management on feather pecking in laying hens. *World's Poult. Sci. J.,* 61:665-687.

Van Soest, P. J. and R. W. McQueen. 1973. The chemistry and estimation of fiber. *Proc. Nutr. Soc.,* 32:123-130.

Chapter 6

EFFECTS OF NUTRIENT DILUTION AND NONSTARCH POLYSACCHARIDE CONCENTRATION IN REARING AND LAYING DIETS ON EATING BEHAVIOR AND FEATHER DAMAGE OF REARING AND LAYING HENS

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ABSTRACT

An experiment was conducted with 768 non-cage housed ISA Brown pullets during the rearing period, of which 576 hens were followed during the laying period, to investigate the separate effects of dietary dilution and Nonstarch Polysaccharides (NSP) concentration of rearing and laying diets on eating behavior, feather damage and performance. Day-old pullets were allotted to one of 6 dietary treatments according to a 3 × 2 factorial (3 dilution levels and 2 NSP concentrations), with 8 replicates (pens) per treatment. At 17 wks of age, pens with hens were allotted to 1 of 8 dietary treatments according to a 4 x 2 factorial arrangement (4 dilution levels and 2 NSP concentrations), with 6 replicates per treatment.

Compared to 0% dilution level, feed intake of laying hens of 10%, 15% and 20% dilution level increased by 8.4% (9.5 g/hen/d), 16.5% (18.1 g/hen/d) and 20.9% (23.6 g/hen/d), respectively. ME intake was similar for all dilution levels. Hens fed standard NSP laying diets had a similar insoluble NSP intake for all dilution levels (9.3 g/hen/d). Insoluble NSP intake of hens fed high NSP laying diets increased from 15.6 g/hen/d (0% dilution) to 18.9 g/hen/d (20% dilution). Providing high vs. standard NSP layer diet decreased proventriculus content (1.1 vs. 0.3 g/kg BW) and increased empty gizzard weight (14.3 vs. 24.4 g/kg BW). Hens that were fed standard NSP diets during laying had more feather damage compared to hens fed high NSP diets (0.58 vs. 0.30). Increasing the insoluble NSP intake resulted in decreased proventricular weight and increased gizzard weight and it's contents, thereby linearly reducing feather damage. Providing diluted rearing diets increased feed intake from the first wks of life onwards. It was hypothesized that pullets were increasingly 'imprinted' on feed as pecking substrate if dilution level increased. This may decrease feather pecking and this could also explain the improved feather condition of the hens at 49 wk of age that were fed 15% diluted rearing diet.

Key words: feather damage, pullet, laying hen, dietary dilution, NSP

INTRODUCTION

Feather pecking in layers is a very clear welfare problem in non-cage housing systems with a prevalence of between 40-80% (Blokhuis et al., 2007). Some reports hypothesized that feather pecking behavior is a substitute for normal ground pecking or feeding behavior in the absence of adequate foraging incentives (Hoffmeyer, 1969; Blokhuis, 1986).

Thus, nutritional factors which increase duration of feeding behavior may positively affect feather pecking behavior in laying hens (Van Krimpen et al., 2005). In literature, feather pecking behavior was found to reduce in laying hens fed nutrient diluted, high (in-)soluble Nonstarch polysaccharides (NSP) containing diets, or roughages (Van der Lee et al., 2001; Hartini et al., 2003; Hetland et al., 2004b; Steenfeldt et al., 2007). Laying hens that are fed low nutrient density diets, do compensate for this dilution by increased feed intake, resulting in a prolonged eating time (Savory, 1980; Van Krimpen et al., 2008). Hen performance can be maintained, even in early lay (Van Krimpen et al., 2007). Diets high in insoluble NSP content decreased eating rate and the rate of digesta passage in the foregut, suggesting an increased satiety level of the layers (Van Krimpen et al., Submitted).

Although diets with low nutrient and high insoluble NSP contents reduced feather pecking behavior, the beneficial effects were small when feather pecking was already developed before diets were provided (Van Krimpen et al., 2008). Rearing conditions during the first 4 weeks of life have a major influence on the subsequent development of feather pecking in laying hens (Johnsen et al., 1998). Therefore, more measures are recommended to satisfy the needs of pullets in food searching and ingestion to prevent feather pecking in adult birds (Keppler et al., 1999). To validate these recommendations, an experiment was performed to investigate the effects of nutrient dilution and NSP concentration in rearing and laying diets on eating behavior and feather damage of laying hens.

MATERIALS AND METHODS

Housing, Birds and Management

A total of 768 non beak trimmed day-old layers (Isa Brown strain) were housed in two climate controlled rooms, Within each room there are 24 floor pens (0.90 x 1.50 m). The pens were built of wire and hens could see their flock mates in other pens. Each pen contained 4 perches, a feeding trough (length 100 cm), and 3 nipple drinkers. Sand was used as litter on the floor. A laying nest was placed outside each pen. Throughout the experiment, litter quality was maintained by monthly adding new sand. During rearing from 0 to 16 wks of age and laying from 17 to 49 wks of age the number of birds per pen was 16 and 12, respectively. At the start of the laying period, pen weights were standardized by removing 4 birds which deviated most from the mean. Mean weight of remaining birds is 1475 g (sd 37). To stimulate feather pecking behavior, stocking density was higher (10.4 hens/m^2) than usual in practice (9.0 hens/m^2). Hens were fed *ad libitum* and had free access to water. Temperature was decreased each wk by 2.5 ^{0}C from 33 ^{0}C in wk 1 to a constant value of 21 ^{0}C from wk 5 onwards. At the onset of the experiment the following light scheme for ISA Brown pullets was provided. Light was on during 22 h per d for the first 3 days, followed by a gradual reduction to 10 h per d in wk 7, and this pattern was maintained until week 16; this was also the recommended scheme for these birds. At 17 wk of age, light schedule was gradually extended by one hour per wk to a 16L: 8D light schedule at the age of 22 wk. This photoperiod was maintained until wk 49 and lasted from 1:00 - 17:00 hrs. Health status of the hens was monitored daily.

Experimental Design

At day 0, pullets were allotted to one of 6 dietary treatments according to a 3 × 2 factorial design. The factors were dietary dilution (0, 10, and 15% dilution) and insoluble NSP concentration (124 g/kg, (control) vs. 184 g/kg (high). These NSP contents were the average for both rearing phases. Each treatment had 8 replicates. Rearing diets in phase 1 (wk 1 to 7) and phase 2 (wk 8 to 16) (Table 1a, 1b, and 1c) had similar energy concentrations, with 2630, 2370 and 2250 kcal/kg for the 0, 10 and 15% diluted diets, respectively.

At the start of the laying period, pens with hens were allotted to one of 8 dietary treatments according to a 4 x 2 factorial design. The factors were dietary dilution (0, 10, 15 and 20% dilution) and insoluble NSP concentration (72 g/kg, (control) vs. 115 g/kg (high); on average for the laying diets)., with 6 replicates per treatment. Energy concentrations were 2830, 2540, 2390 and 2250 kcal/kg for the 0, 10, 15 and 20% diluted laying diets, respectively (Table 2a and 2b). The experiment comprised of 48 treatment combinations (6 treatments in the rearing period x 8 treatments in the laying period) and each treatment combination was tested in one pen. Dietary dilution in the standard NSP diets was realized by adding 10, 15 or 20% sand to the control feed (0% dilution, control NSP). The high insoluble NSP diet was obtained by adding 10% whole oat hulls to the control diet at the expense of all other ingredients. Whole oat hulls were directly added in the mixer, without passing the hammer mill.

Table 1a. Dietary ingredients of the phase 1 and 2 rearing diets (g/kg, as-fed basis).

	Phase 1 Rearing diet						Phase 2 Rearing diet					
Treatment nr.	1	2	3	4	5	6	1	2	3	4	5	6
Dilution level	0%	0%	10%	10%	15%	15%	0%	0%	10%	10%	15%	15%
NSP concentration	Control	High	Control	High	Control	High	Control	High	Control	High	Control	High
Ingredients												
Wheat	400.0	313.7	360.0	360.0	340.0	340.0	220.1	86.2	198.1	198.1	187.1	187.1
Barley	100.0	100.0	90.0	90.0	85.0	85.0	100.0	100.0	90.0	90.0	85.0	85.0
Soya bean meal extr.	100.0	100.0	90.0	90.0	85.0	85.0	90.0	90.0	81.0	81.0	76.5	76.5
Wheat Middlings	80.0	100.0	72.0	72.0	68.0	68.0	100.0	100.0	90.0	90.0	85.0	85.0
Maize	67.0	40.0	60.3	60.3	57.0	57.0	252.0	245.1	226.8	226.8	214.2	214.2
Peas	66.6	67.4	60.0	60.0	56.6	56.6	80.9	100.0	72.8	72.8	68.9	68.9
Rape seed extracted	50.0	50.0	45.0	45.0	42.5	42.5	50.0	50.0	45.0	45.0	42.5	42.5
Soya bean heat treated	50.0	50.0	45.0	45.0	42.5	42.5	26.2	37.0	23.6	23.6	22.2	22.2
Lin seed expeller	44.6	0.0	40.2	40.2	37.9	37.9	46.0	21.3	41.4	41.4	39.1	39.1
Chalk	16.0	15.6	14.4	14.4	13.6	13.6	17.8	17.2	16.0	16.0	15.1	15.1
Monocalcium phosphate	12.1	12.9	10.9	10.9	10.3	10.3	8.3	9.1	7.5	7.5	7.1	7.1
Soya oil	0.9	20.0	0.8	0.8	0.7	0.7	–	20.0	–	–	–	–
Palm oil	–	14.2	–	–	–	–	–	14.8	–	–	–	–
Potato Protein	–	2.1	–	–	–	–	–	–	–	–	–	–
Premix[1]	5.0	5.0	4.5	4.5	4.3	4.3	5.0	–	4.5	4.	4.3	4.3
Salt	3.6	3.6	3.2	3.2	3.0	3.0	3.6	3.6	3.2	3.2	3.1	3.1
L-Lysine	1.8	2.1	1.6	1.6	1.5	1.5	–	–	–	–	–	–
DL-Methionine	1.3	1.8	1.1	1.1	1.1	1.1	0.1	0.6	0.09	0.09	0.09	0.09
L-Threonine	1.1	1.5	1.0	1.0	0.9	0.9	–	0.2	–	–	–	–
L-Tryptofaan	0.1	0.3	0.1	0.1	0.1	0.1	–	–	–	–	–	–
Oat hulls	–	**100**	–	**100**	–	**100**	–	**100**	–	**100**	–	**100**
Sand	–	–	**100**	–	**150**	**50**	–	–	**100**	–	**150**	**50**

Table 1b. Analyzed and calculated nutrients of the phase 1 and 2 rearing diets (g/kg, as-fed basis).

	Phase 1 Rearing diet						Phase 2 Rearing diet					
Treatment nr.	1	2	3	4	5	6	1	2	3	4	5	6
Dilution level	0%	0%	10%	10%	15%	15%	0%	0%	10%	10%	15%	15%
NSP concentration	Control	High	Control	High	Control	High	Control	High	Control	High	Control	High
Analyzed content[2]												
Dry matter	876	885	889	877	896	885	881	888	892	880	899	888
Crude ash	60.6	62	161.5	57.6	210.8	106.7	56.6	58.1	149.6	53.0	202.9	110.1
Crude protein	191	169	166	173	159	165	175	161	155	163	148	156
Crude fat	35.9	64.3	30.3	35.7	29.4	31.9	35.0	63.0	32.9	34.4	31.5	33.3
Crude fiber	43.9	63.5	39.4	56.5	36.9	54.4	40.2	68.9	37.9	52.6	36.6	54.3
Starch	343	289	307	310	297	295	376	304	330	353	314	323
Reducing sugars[3]	40	36	35	37	30	31	34	31	33	35	31	32
Ca	11.7	12.2	10.4	10.1	9.6	9.8	10.6	10.9	8.5	8.9	8.8	9.6
P	7.0	7.0	6.3	7.0	5.9	6.8	6.4	6.4	5.7	6.2	5.7	5.9
K	7.7	7.8	6.8	7.9	6.4	7.2	7.5	7.8	7.0	7.8	6.4	7.2
Na	1.8	1.6	1.5	1.5	1.3	1.2	1.5	1.6	1.4	1.5	1.3	1.6
NDF	134	181	116	178	108	191	141	200	128	195	116	156
ADF	67	87	51	77	48	96	54	90	53	75	47	62
ADL (Lignin)	13	16	12	14	10	14	10	13	9	13	9	13
NSP[4]	206	265	189	264	170	255	204	271	192	242	172	234
Cellulose[5]	54	71	39	63	38	82	44	77	44	62	38	49
Hemi-Cellulose[5]	67	94	65	101	60	95	87	110	75	120	69	94

Table 1c. Calculated nutrients and mean particle sizes (mm) of the phase 1 and 2 rearing diets (g/kg, as-fed basis).

	Phase 1 Rearing diet						Phase 2 Rearing diet					
Treatment nr.	1	2	3	4	5	6	1	2	3	4	5	6
Dilution level	0%	0%	10%	10%	15%	15%	0%	0%	10%	10%	15%	15%
NSP concentration	Control	High	Control	High	Control	High	Control	High	Control	High	Control	High
Calculated contents												
ME (kcal/kg)	2630	2630	2370	2370	2250	2250	2630	2630	2370	2370	2250	2250
Dig. Lysine	8.4	8.4	7.6	7.6	7.1	7.1	6.4	6.4	5.8	5.8	5.4	5.4
Dig. Meth. + Cyst.	6.2	6.2	5.6	5.6	5.3	5.3	4.8	4.8	4.3	4.3	4.1	4.1
Dig. Threonine	6.1	6.1	5.5	5.5	5.2	5.2	4.7	4.7	4.3	4.3	4.0	4.0
Dig. Tryptophan	2.0	2.0	1.8	1.8	1.7	1.7	1.6	1.5	1.5	1.5	1.4	1.4
Dig. Isoleucine	5.8	5.4	5.2	5.2	4.9	4.9	5.4	5.1	4.8	4.8	4.6	4.6
Absorbable Phosphorus	4.0	4.0	3.6	3.6	3.4	3.4	3.2	3.2	2.9	2.9	2.7	2.7
Physical characteristics												
Mean particle size (mm)	0.72	0.82	0.68	0.83	0.66	0.84	0.70	0.95	0.61	0.83	0.61	0.78

[1] Provided the following nutrients per kg of premix: vitamin A, 2,400,000 IU; vitamin D3, 480,000 IU; vitamin E, 8,000 mg; vitamin B1, 960 mg; vitamin B2, 2,400 mg; d-panthothenic acid, 3,200 mg; niacinamide, 9,600 mg; vitamin B6, 1,120 mg; folic acid, 360 mg; vitamin B12, 5,000 µg; vitamin C, 20,000 mg; biotin, 20 mg; vitamin K3, 960 mg; choline chloride 60,000 mg; 20,000 mg; copper, 1,600 mg (as $CuSO_4.5H_2O$), iron, 13,000 mg (as $FeSO_4.7H_2O$); manganese 13,000 mg (as MnO_2); zinc, 10,000 mg (as $ZnSO_4$); cobalt, 80 mg (as $CoSO_4.7H_2O$); iodine, 200 mg (as KI); selenium, 80 mg (as $Na_2SeO_3.5H_2O$). [2] Based on 1 analysis in duplicate per diet. [3] Mono- and disaccharides as glucose units.

[4] Non-starch polysaccharide (NSP) content was calculated by subtracting the crude protein, fat, starch, reducing sugars and ash content from the dry matter content. [5] Cellulose = ADF minus ADL; hemi cellulose = NDF minus ADF.

To maintain the energy concentration in the 0% diluted, high NSP diet, extra fat was added. All feeds were fed in mash form. Ratio of ME to all other nutrients ratio was similar for all diets, except for ash and NSP. The non-diluted rearing and laying diets had NSP concentrations according to NRC requirements for rearing and laying hens (NRC, 1994).

Measurements

Analytical Procedures. Feed was analyzed for DM, crude ash, crude fat, crude fiber, nitrogen, starch, sugars (mono- and disaccharides as glucose units), calcium, phosphorus, sodium, potassium. NDF, ADF and ADL were measured to obtain cellulose and hemicellulose . All samples were analyzed in duplicate. For determination of the DM content, feed was freeze-dried according to ISO 6496 (1998b). Following freeze-drying, feed was ground to pass a 1 mm screen and kept for analysis. Air-dry feed was dried in a forced air oven at 103^0C to an constant weight according to ISO 6496 (1998b). Kjeldahl nitrogen content was measured according to ISO 5983 (1997) in fresh feed. Crude protein content was calculated as nitrogen * 6.25. Crude fat content was determined after acid hydrolysis according to ISO 6492 (1999). For determining crude ash content, samples were incinerated at 550^0C in a muffle furnace according to ISO 5984 (2002). The starch content was analyzed enzymatically as described by Brunt (1993). Reducing sugars were extracted from the feed samples, using 40% ethanol, and determined as described by Suárez *et al.* (2006). Contents of calcium, phosphorus, sodium and potassium were analyzed by using ICP-AES (1998a). Analysis of NDF, ADF and ADL contents were based on a modified method of Van Soest et *al.* (1973), as described by Suárez *et al.* (2006).

Particle Size Distribution. Oat hulls were added as whole to the diet. Particle size distribution of the diets was analyzed by use of the dry sieve method (Goelema et al., 1999). Seven particle size fractions were separated by using six sieves with diameters of 0.09, 0.18, 0.36, 0.71, 1.40, and 2.80 mm, respectively. Average particle size of the diets was calculated as (Fraction < 0.09mm * 0.045) + (Fraction 0.09 – 0.18mm * 0.135) + (Fraction 0.18 – 0.36mm * 0.27) + (Fraction 0.36 – 0.71mm * 0.53) + (Fraction 0.71 – 1.40mm * 1.06) + (Fraction 1.40 – 2.80mm * 2.20) + (Fraction > 2.88mm * 4.20)/100. Average particle size of the finely ground rearing diets was 0.66 ±0.046 mm versus 0.84 ±0.057 mm for the coarsely ground diets. Average particle size of the finely ground laying diets was 0.74 ±0.039 mm versus 1.06 ±0.111 mm for the coarsely ground diets.

Table 2a. Dietary ingredients of the laying diets (g/kg, as-fed basis)

Treatment nr.	1	2	3	4	5	6	7	8
Energy dilution	0%	0%	10%	10%	15%	15%	20%	20%
NSP concentration	Control	High	Control	High	Control	High	Control	High
Ingredients								
Maize	365.6	328.5	329.0	329.0	310.8	310.8	292.5	292.5
Wheat	229.0	80.0	206.1	206.1	194.7	194.7	183.2	183.2
Maize starch	50.0	100.0	45.0	45.0	42.5	42.5	40.0	40.0
Soya been meal CF< 50	155.0	100.0	139.5	139.5	131.8	131.8	124.0	124.0
Maize gluten meal	20.0	91.3	18.0	18.0	17.0	17.0	16.0	16.0
Peas CP < 220	22.0	20.0	19.8	19.8	18.7	18.7	17.6	17.6
Limestone	72.4	72.1	65.2	65.2	61.5	61.5	57.9	57.9
Rape meal extract	30.0	24.3	27.0	27.0	25.5	25.5	24.0	24.0
Palm oil	–	16.6	–	–	–	–	–	–
Soya oil	16.6	25.0	14.9	14.9	14.1	14.1	13.3	13.3
Chalk	20.0	20.0	18.0	18.0	17.0	17.0	16.0	16.0
Monocalcium phosphate	8.4	9.6	7.6	7.6	7.1	7.1	6.7	6.7
Premix[1]	5.0	5.0	4.5	4.5	4.3	4.3	4.0	4.0
Salt	3.8	3.7	3.4	3.4	3.2	3.2	3.0	3.0
DL-Methionine	1.3	1.0	1.2	1.2	1.1	1.1	1.0	1.0
L-Lysine	1.0	2.6	0.9	0.9	0.9	0.9	0.8	0.8
L-Tryptofaan	–	0.3	–	–	–	–	–	–
Oat hulls	–	**100**	–	**100**	–	**100**	–	**100**
Sand	–	–	**100**	–	**150**	**50**	**200**	**100**

[1] Provided the following nutrients per kg of premix: vitamin A, 2,400,000 IU; vitamin D3, 480,000 IU; vitamin E, 8,000 mg; vitamin B1, 960 mg; vitamin B2, 2,400 mg; d-panthothenic acid, 3,200 mg; niacinamide, 9,600 mg; vitamin B6, 1,120 mg; folic acid, 360 mg; vitamin B12, 5,000 µg; vitamin C, 20,000 mg; biotin, 20 mg; vitamin K3, 960 mg; choline chloride 60,000 mg; 20,000 mg; copper, 1,600 mg (as $CuSO_4.5H_2O$), iron, 13,000 mg (as $FeSO_4.7H_2O$); manganese 13,000 mg (as MnO_2); zinc, 10,000 mg (as $ZnSO_4$); cobalt, 80 mg (as $CoSO_4.7H_2O$); iodine, 200 mg (as KI); selenium, 80 mg (as $Na_2SeO_3.5H_2O$).

Table 2b. Analyzed and calculated nutrients, and physical characteristics of the laying diets (g/kg, as-fed basis)

Treatment nr.	1	2	3	4	5	6	7	8
Energy dilution	0%	0%	10%	10%	15%	15%	20%	20%
NSP concentration	Control	High	Control	High	Control	High	Control	High
Analyzed contents[1]								
Dry matter	884	891	896	883	899	891	910	896
Crude ash	127	125	221	123	238	183	322	219
Crude protein	157	160	136	148	139	145	122	130
Crude fat	39.9	69	35.6	37.7	33.3	33.6	31	33.3
Crude fiber	38.5	52	34.4	58.7	36.8	48.1	23.6	49
Starch	391	365	358	357	333	344	322	342
Reducing sugars[2]	34	25	30	31	29	29	24	29
Ca	39.8	38.3	37.6	34.7	33.4	33.9	30.4	31.1
P	5.1	5.3	4.6	4.8	4.4	4.5	4.1	4.2
K	6.3	5	5.5	6.4	5.3	5.7	4.7	5.3
Na	1.6	1.8	1.5	1.7	1.2	1.2	1.4	1.4
NDF	81	104	73	129	71	108	62	118
ADF	32	42	31	53	29	46	26	50
ADL (Lignin)	8	9	6	10	6	8	6	9
NSP[3]	135	147	115	186	127	157	89	143
Cellulose[4]	24	33	25	43	23	38	20	41
Hemi-Cellulose[4]	49	62	42	76	42	62	36	68
Calculated contents								
ME (kcal/kg)	2830	2830	2540	2540	2390	2390	2250	2250
Dig Lysine	6.7	6.7	6.0	6.1	5.7	5.8	5.4	5.5
Dig Meth. + Cyst.	5.8	5.8	5.2	5.3	4.9	5.0	4.6	4.6
Dig. Threonine	4.6	4.6	4.1	4.2	3.9	4.0	3.7	3.7
Dig Tryptophan	1.5	1.5	1.3	1.3	1.2	1.3	1.2	1.2
Dig. Isoleucine	5.2	5.3	4.7	4.7	4.4	4.5	4.1	4.2
Absorbable Phosphorus	2.8	2.8	2.5	2.5	2.4	2.4	2.2	2.2
Physical characteristics								
Mean particle size (mm)	0.78	1.20	0.76	1.00	0.69	1.10	0.73	0.95
Bulk density (g/l)	783	707	832	669	858	679	891	710

[1] Based on 1 analysis in duplicate per diet. [2] Mono- and disaccharides as glucose units. [3] Non-starch polysaccharide (NSP) content was calculated by subtracting the crude protein, fat, starch, reducing sugars and ash content from the dry matter content. [4] Cellulose = ADF minus ADL; hemi cellulose = NDF minus ADF.

Bulk density. To determine bulk density of the laying diets, a filling hopper on top of a cylinder, with a known volume of 1 l, was filled with mash. Hopper and cylinder were separated by a slide with a fall weight on top of it. After removing the slide, the weight felled down, thereby sucking down the mash. Access of feed was removed by placing the slide back in the cylinder. Bulk density was determined by dividing net weight of the mash by the volume of the container (Balandran-Quintana et al., 1998).

Observations

Feed Intake, Body Weight, and Hen performance. Feed consumption and hen performance per pen were recorded weekly. All hens were weighed per pen in a 4-week intervals. For the trait 'total egg mass' the entire egg mass production was calculated, assuming that shell-less and cracked eggs had the same weight as the mean 'normal' egg weight of that specific pen in that week.

Eating Time. Video observations were recorded in a 4-week interval to calculate eating time of birds in a pen. Eating time was defined as percentage of time birds spend on feed intake during the observation period. An observation day was divided in three blocks, i.e. from 9.00 - 11.30 hrs, 11.30 - 14.00 hrs and from 14.00 until 16.30 hrs. An observation lasted one hour, but to avoid possible disturbances of the cameraman at the start and end of the observation period, only the middle 30 observation minutes were analyzed. The number of eating birds (between 0 and 16), was recorded continuously by using Observer 4.1/5.0 software (Noldus, 1993). Eating rate was calculated as feed intake (g/d) divided by number of eating minutes per day. Eating minutes per day were estimated by multiplying the number of minutes with light on (16 h x 60 min) times the percentage of observed eating time. Eating time and eating rate were averaged per pen per day.

Feather Condition Scores and Culling Rate. In a 4-week interval, plumage and skin condition per individual hen were scored by using the method described by Bilcik and Keeling (1999). Scores, varying from 0 (intact feathers, no injuries or scratches) to 5 (completely denuded area) were given for each of five body parts (neck, back, rump, tail and belly). The average of these five scores was also used for analysis. Culling of birds was recorded on a weekly basis.

Behavioral Recordings. For scoring the behavioral recordings, the ethogram as described by Van Hierden (2002) was used. All hens were fitted with colored leg rings to enable individual identification. Recordings of gentle feather pecking (without removal of feathers) and severe pecking (leading to feather loss), aggressive pecking, vent pecking and cage pecking were made in wks 4, 10, 18 and 21 of the experiment. Each pen was observed for 10 min, counting each peck. Results were presented as number of pecks per observed hen per 10 min. Duration of behavior elements was scored during wks 11 and 19 of the experiment by using scan sampling technique. Behaviors were classed in four groups: feeding related behavior (pecking at feed or litter, ground scratching), drinking, walking and resting (sitting or standing inactive, preening). For each pen, an observer scored the number of hens per behavior class at 1-min intervals over a 15 min observation period. Based on these 15 observations, average number of hens per behavior class were determined and recalculated to percentages of time spent on the different behaviors.

Statistical Analysis. The REML variance component analysis procedure tested the effect of the nutritional factors on the determined traits, using the model (1):

$$Y_{ijkl} = \mu + \text{Dilution Rearing}_i + \text{Dilution Laying}_j + \text{NSP Rearing}_k + \text{NSP Laying}_l + \text{Dilution Rearing x NSP Rearing} + \text{Dilution Laying x NSP Laying} + \text{Dilution Rearing x NSP Laying} + \text{Dilution laying x NSP Rearing} + \text{Dilution Rearing x Dilution Laying} + \text{NSP Rearing x NSP Laying} + e_{ijkl}$$

where Y_{ijkl} = dependent variable; μ = overall mean; Dilution Rearing_i = fixed effect of dilution level of the rearing diet i (i = 3; 0, 10, and 15%); NSP Rearing_j = fixed effect of NSP concentration of the rearing diet j (j = 2; control and high); Dilution Laying_k = fixed effect of dilution level of the laying diet k (k = 4; 0, 10, 15, and 20%); NSP Laying_j = fixed effect of NSP concentration of the rearing diet l (l= 2; control and high); e_{ijkl} = the error term. Model (2) was also used to test effects of eating time, eating rate, feather condition score, behavior traits and gut development parameters.

P-values for Dilution Rearing, NSP Rearing and Dilution Rearing * NSP Rearing are presented for all parameters that were determined in the rearing period. Similarly, *P*-values for Dilution Laying, NSP Laying and Dilution Laying * NSP Laying are presented for all parameters that were determined during the laying period. Besides, only significant carry-over effects of the nutritional factors during rearing on parameters during laying were presented.

RESULTS

Results during the rearing period.

Average feed intake during the rearing period was 60.6 g/hen/d (sd = 0.52). In the low NSP rearing diets, feed intake increased by 13% and 19% in the 10% and 15% diluted diets, respectively (*P*<0.001; Table 3). In some high NSP diets, however, feed intake did not increase enough to ensure similar ME intake as in the low NSP diets . Feed intake increased by only 5% and 7% in the 10% and 15% diluted diets, respectively. Despite this, dilution level and NSP concentration did not significantly affect energy intake during the rearing period. In the low NSP rearing diets, eating time was not affected by dilution level, whereas eating time in the high NSP rearing diets was significantly prolonged in the 10% and 15% diluted diet compared to the 0% diluted diet (*P*=0.001; Table 3). In the low NSP rearing diets, eating rate of pullets linearly increased with increasing dilution levels, Eating rate in pullets fed high NSP rearing diets slightly decreased with increasing dilution levels (*P*=0.032; Table 3). In pullets fed the 0% diluted diets, body weight increased with feeding high NSP diet, Bodyweight of pullets fed the 10% and 15% diluted was less after feeding high NSP diet (*P*<0.001; Table 3). Energy conversion ratio decreased in the 10% (11,520 kcal/kg growth) and 15% diluted diets (11,410 kcal/kg growth), compared to the 0% diluted diet (12,050 kcal/kg growth; *P*=0.025). Feeding related behavior was not affected by the tested dietary factors during the rearing period.

Results during the laying period.

At the start of lay, feather pecking behavior was not observed in any of the pens, irrespective of the dietary treatment during rearing. To encourage the hens to start feather pecking,

(1) light intensity was increased from 10 Lux to 20 Lux (wk 18), 30 Lux (wk 20) and 65 Lux (wk 22);

(2) perch length was reduced from 3.6 m to 2.7 m (wk 24) and to 1.8 m (wk 26);

(3) large part of the sand was removed leaving about 2.0 liter of sand per cage (from wk 28 onwards), and

(4) feeding troughs were blocked for 3 h/d, where blocking period per pen varied during the week (wk 32 to 40).

Table 3. Performance traits per treatment during the rearing period in ISA Brown rearing pullets from 1 to 17 wk of age

Treatment[1,2]	Feed Intake (g/hen/d)	Energy Intake (J/hen/d)	Eating time (%)	Eating rate (g/min)	BW (g)	Energy conversion ratio[3]	FRB[4] (%)
Standard NSP Rearing							
0% Dilution Rearing	55.4^{e}	610.1	22.7^{a}	0.38^{c}	628.2^{b}	11.6^{b}	35.0
10% Dilution Rearing	62.4^{ab}	614.9	20.6^{ab}	0.48^{abc}	639.3^{a}	11.6^{b}	30.5
15% Dilution Rearing	66.1^{a}	619.2	20.8^{ab}	0.52^{a}	638.1^{a}	11.5^{b}	32.8
High NSP Rearing							
0% Dilution Rearing	57.7^{d}	633.9	17.9^{b}	0.50^{ab}	637.1^{a}	12.5^{a}	31.3
10% Dilution Rearing	60.1^{c}	595.9	23.3^{a}	0.43^{abc}	618.3^{cd}	11.4^{b}	30.8
15% Dilution Rearing	61.9^{ab}	581.2	23.8^{a}	0.39^{bc}	614.4^{d}	11.3^{b}	37.0
SE	0.69	22.8	1.97	0.057	3.8	0.28	2.32
P-Value							
Dilution Rearing	**<0.001**	0.606	0.225	0.860	0.291	**0.025**	0.299
NSP Rearing	**<0.001**	0.554	0.980	0.858	**<0.001**	0.400	0.745
Dilution Rearing * NSP Rearing	**<0.001**	0.380	**0.001**	**0.032**	**<0.001**	0.060	0.273

[1] The tested factors were dilution level rearing period (2630, 2370 and 2250 kcal/kg) and insoluble NSP concentration rearing period (124 vs. 184 g/kg). [2] Results are based on the average value of 16 wks x 8 replicates. [3] Expressed as kcal x 1000 per kg gain. [4] FRB = Feeding Related Behavior (Eating + Ground searching), based on scan sampling observations.

Average feed intake during the laying period was 126 g/hen/d (Table 4). Feed intake was affected by dilution level of the laying diets ($P<0.001$) and increased proportionally with increased dilution level. Compared to 0% dilution level, feed intake of 10%, 15% and 20% dilution level was increased by 8.3%, 16.5% and 20.9%, respectively. Thus, ME intake was nearly similar for the different dilution levels.

Table 4. Performance traits per treatment in ISA Brown laying hens over 18 to 49 weeks of age

Treatment^{1,2}	**Feed Intake (g/hen/d)**	**ME Intake (kcal/hen/d)**	**Ins. NSP Intake (g/hen/d)**	**Volume Intake (ml/hen/d)**	**BW (g/hen)**	**Egg mass (g/hen/d)**
Standard NSP Laying						
0% Dilution Laying	111.2	314	9.6^{d}	144.1^{f}	1944^{b}	52.5
10% Dilution Laying	122.2	311	9.2^{d}	145.8^{ef}	1944^{b}	52.8
15% Dilution Laying	131.3	315	9.3^{d}	152.9^{ef}	1987^{a}	53.3
20% Dilution Laying	135.0	305	9.0^{d}	153.0^{e}	1943^{b}	52.2
High NSP Laying						
0% Dilution Laying	114.4	323	15.6^{c}	165.4^{d}	1990^{a}	52.6
10% Dilution Laying	122.3	311	17.8^{b}	182.4^{c}	1958^{ab}	51.6
15% Dilution Laying	130.4	313	18.4^{ab}	191.8^{ab}	1950^{b}	54.2
20% Dilution Laying	137.7	311	18.9^{a}	195.6^{a}	1971^{ab}	53.7
SE	1.70	4.30	0.33	4.00	16.30	0.82
***P*-value**						
Dilution Laying	**< 0.001**	0.107	**<0.001**	**<0.001**	0.665	0.308
NSP Laying	0.304	0.283	**<0.001**	**<0.001**	0.278	0.578
Dilution Laying * NSP Laying	0.593	0.556	**<0.001**	**<0.001**	**0.061**	0.397

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSP concentration laying period (72 vs. 115 g/kg). [2] Results are based on the average value of 33 wks. x 6 replicates per treatment.

The amount of consumed insoluble NSP of hens fed low NSP laying diets was similar for all dilution levels (on average 9.3 g/hen/d; Table 4), Insoluble NSP intake of hens fed high NSP laying diets increased from 62% (at 0% dilution) to 110% (at 20% dilution) compared to the 0% diluted NSP low diet (*P*<0.001). Insoluble NSP eaten with diets during the laying phase was not affected by dietary treatments during the rearing phase.

Bulk density of the high NSP diets was substantially lower compared to the low NSP diets. Hens fed high NSP diet consumed more volume of feed (ml/hen/d) for similar weight of feed intake. Volume intake increased by increased dilution levels of the diet. This effect was most pronounced in the high NSP treatments. Volume intake during the laying phase was not affected by dietary treatments during the rearing phase.

In hens fed 0% diluted laying diet, BW was less in standard compared to high NSP laying diet. Hens fed 15% diluted laying diet had less W in the high NSP diet. BW in hens fed 15% diluted laying diet was higher after eating standard NSP rearing diet (1992 vs. 1945 g; Table 8). Hens fed 0% diluted rearing diet had lower BW after feeding high NSP laying diet (1941 vs. 1973 g; Table 9). In hens fed high NSP laying diet, BW was not affected by NSP concentration of the rearing diet, whereas in standard NSP laying diet BW was significantly reduced by feeding high vs. standard NSP rearing diet (1980 vs. 1929; Table 10). Egg mass was not significantly effected by different rearing and/or laying diet combinations.

Average eating time and eating rate were not affected by dietary treatments during the laying period (Table 5). Moreover, eating time during laying was not affected by dietary treatments during rearing. A carry-over effect of NSP concentration of the rearing diet and dilution level of the laying diet on eating rate during laying was observed (Table 8). In the 10% diluted laying diets, eating rate slightly increased if hens were previously fed a high compared to a standard NSP rearing diets (0.67 vs. 0.86 g/min.). In the 15% diluted laying diets, eating rate slightly increased if hens previously were fed a standard NSP compared to a high NSP rearing diets (0.96 vs. 0.74 g/min.).

In this experiment, feather pecking frequency was very low (1.2 pecks/10 min./pen). Gentle and severe feather pecking behaviors during laying were not affected by any of the tested dietary factors.

Over the period 0 to 25 wk of age, feather condition of all the hens was very good. From wk. 29 onwards, feather condition linearly decreased over time. Feather damage of hens that were fed low NSP diets during laying period was more severe compared to hens fed standard NSP diets (0.58 vs. 0.30; $P<0.001$; Table 5). The worst feather condition (0.73) was found in hens fed the standard feeding regime both during rearing and laying (0% diluted rearing diet and later 0% diluted laying diet). Pair wise comparisons showed that the feather condition of all the other treatment combinations was numerically better compared to the standard regime.

Table 5. Eating and pecking characteristics in ISA Brown laying hens from 18 to 49 wk of age

Treatment[1,2]	**Eating time (%)**[3]	**Eating rate (g/min)**	**Gentle feather pecking**[4]	**Severe feather pecking**[4]	**Feather damage score**
Standard NSP laying					
0% Dilution laying	20.4	0.73	1.6	0.4	0.71
10% Dilution laying	21.1	0.72	1.0	0.3	0.55
15% Dilution laying	21.2	0.91	0.9	0.4	0.43
20% Dilution laying	21.6	0.82	1.2	0.4	0.63
High NSP laying					
0% Dilution laying	19.6	0.77	1.4	0.7	0.37
10% Dilution laying	20.2	0.81	1.2	0.5	0.33
15% Dilution laying	20.9	0.79	0.8	0.3	0.24
20% Dilution laying	23.8	0.68	1.2	0.5	0.27
SE	1.51	0.082	0.29	0.18	0.083
***P*-value**					
Dilution Laying	0.338	0.485	0.227	0.577	0.418
NSP Laying	0.930	0.607	0.883	0.383	**<0.001**
Dilution Laying * NSP Laying	0.712	0.424	0.863	0.739	0.666

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSP concentration laying period (72 vs. 115 g/kg). [2] Results are based on the average value of 33 wks. x 6 replicates per treatment. [3] % of the observation period. [4] Nr of pecks per 10 min. per pen.

Relative empty crop weight and it's content were on average 5.0 and 5.2 g/kg BW, respectively (Table 6). Crop weights and it's contents were not affected by the tested dietary treatments. Relative proventriculus content decreased by 73% after supplementing high compared to low NSP laying diet. (1.1 vs. 0.3 g/kg BW; $P<0.001$; Table 6).

Table 6. Relative empty weight and content of crop, proventriculus and gizzard (g/kg bodyweight of hen) in ISA Brown laying hens

Treatment[1,2]	Crop Empty	Crop Content	Proventriculus Empty	Proventriculus Content	Gizzard Empty	Gizzard Content
Standard NSP Laying						
0% Dilution Laying	5.2	6.5	4.4	0.8	13.4	11.0
10% Dilution Laying	5.3	2.7	4.3	1.7	14.3	12.7
15% Dilution Laying	4.9	3.4	4.2	1.0	14.2	9.8
20% Dilution Laying	4.7	5.3	4.7	1.0	15.2	13.3
High NSP Laying						
0% Dilution Laying	4.7	6.8	4.0	0.3	24.6	13.5
10% Dilution Laying	5.5	4.7	4.4	0.3	25.2	13.0
15% Dilution Laying	5.0	9.4	4.3	0.3	25.2	12.7
20% Dilution Laying	5.0	3.1	4.2	0.3	22.7	12.7
SE	0.52	4.25	0.33	0.34	1.54	1.37
***P*-Value**						
Dilution Laying	0.732	0.843	0.838	0.436	0.952	0.652
NSP Laying	0.772	0.791	0.301	**<0.001**	**< 0.001**	**0.098**
Dilution Laying * NSP Laying	0.669	0.440	0.659	0.288	0.138	0.400

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSP concentration laying period (72 vs. 115 g/kg). [2] Results are based on the average value of 33 wks. x 6 replicates per treatment.

In hens fed 10% diluted laying diet, relative weight of proventriculus content was increased when previously standard NSP rearing diet was fed (Table 8). In hens fed high NSP laying diets, no effect of NSP concentration on proventriculus content of the rearing diet was observed. In hens fed low NSP laying diets relative weight of proventriculus content increased when previously low vs. high NSP rearing diet was fed (1.5 vs. 0.7; *P*=0.048; Table 10). Empty gizzard weight was increased by 71% by feeding high vs. standard NSP laying diet. Relative weight of the gizzard content was not significantly affected by the different rearing and/or laying diet combinations.

Time budgets for eating, dust bathing, resting and feeding related behavior, as observed by a scan sampling technique, were not affected by the dietary factors during laying (Table 7). NSP content of the layer diet effected preening, ground searching and walking behavior.

Table 7. Behavior traits (% of time), observed by using a scan sampling technique, in ISA Brown layers from 18 to 49 wk of age

Treatment[1]	Eating (%)	Dust bathing (%)	Preening (%)	Ground Searching (%)	Walking (%)	Resting (%)	FRB[3] (%)
Standard NSP Laying							
0% Dilution Laying	16.1	1.7	10.2	17.3	5.7	49.0	33.4
10% Dilution Laying	19.1	1.5	9.9	20.2	5.5	43.9	39.3
15% Dilution Laying	19.5	1.6	9.6	20.7	6.6	42.1	40.2
20% Dilution Laying	16.4	3.6	8.7	18.6	5.8	47.2	35.0
High NSP Laying							
0% Dilution Laying	16.3	1.7	8.7	22.0	6.0	45.8	38.2
10% Dilution Laying	17.5	1.5	7.0	19.7	7.7	47.0	37.2
15% Dilution Laying	17.8	1.5	8.3	20.5	6.6	45.5	38.4
20% Dilution Laying	18.0	1.7	7.2	22.0	7.8	43.5	40.0
SE	1.37	0.67	0.93	1.63	0.79	2.59	2.31
***P*-Value**							
Dilution Laying	0.329	0.142	0.334	0.954	0.468	0.784	0.633
NSP Laying	0.619	0.233	**0.009**	**0.111**	**0.028**	0.937	0.407
Dilution Laying * NSP Laying	0.547	0.436	0.803	0.298	0.382	0.325	0.209

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSP concentration laying period (72 vs. 115 g/kg). [2] Results are based on the average value of 33 wks. x 6 replicates per treatment. [3] FRB = Feeding Related Behavior = Eating + Ground searching.

Preening behavior was reduced in hens fed high compared to standard NSP laying diet (9.6 vs. 7.8%; *P*=0.009). Ground searching behavior was slightly increased in hens that were fed high compared to standard NSP laying diet (21.1 vs. 19.2%; *P*=0.111; Table 7). Walking time was increased in hens fed high NSP laying diet compared to standard NSP laying diet (7.0 vs. 5.9%; *P*=0.028; Table 7).

Table 8. Carry-over effects of NSP concentration of the rearing diet and dilution level of the laying diet on eating rate and relative proventriculus content of ISA Brown laying hens

Treatment[1,2]	**Eating rate (g/min)**	**Proventriculus content g/kg BW**
Standard NSP Rearing		
0% Dilution Laying	0.83^{abc}	0.3^{b}
10% Dilution Laying	0.67^{c}	1.6^{a}
15% Dilution Laying	0.96^{a}	0.8^{b}
20% Dilution Laying	0.77^{bc}	0.7^{b}
High NSP Rearing		
0% Dilution Laying	0.67^{c}	0.7^{b}
10% Dilution Laying	0.86^{ab}	0.3^{b}
15% Dilution Laying	0.74^{bc}	0.6^{b}
20% Dilution Laying	0.75^{bc}	0.6^{b}
SE	0.082	0.35
***P*-value**		
Dilution Laying	0.413	0.436
NSP Rearing	0.485	**0.038**
Dilution Laying * NSP Rearing	**0.079**	**0.036**

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSP concentration laying period (72 vs. 115 g/kg). [2] Results are based on the average value of 33 wks. x 6 replicates per treatment.

Table 9. Carry-over effect of dilution level in the rearing diet and NSP concentration in the laying diet on average BW of ISA Brown laying hens from 18 to 49 wk of age

Treatment	BW (g)
Standard NSP laying	
0% Dilution Rearing	1973^{ab}
10% Dilution Rearing	1952^{bc}
15% Dilution Rearing	1939^{c}
High NSP laying	
0% Dilution Rearing	1941^{c}
10% Dilution Rearing	1983^{a}
15% Dilution Rearing	1977^{ab}
SE	14.14
***P*-value**	
NSP Laying	0.278
Dilution Rearing	0.726
NSP Laying * Dilution Rearing	0.023

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSP concentration laying period (72 vs. 115 g/kg). [2] Results are based on the average value of 33 wks. x 6 replicates per treatment.

Table 10. Carry-over effect of NSP concentration in the rearing diet and NSP concentration in the laying diets on average BW and relative proventriculus content of ISA Brown laying hens

Treatment[1,2]	BW (g)	Proventriculus content (g/kg BW)
Standard NSP laying		
Standard NSP rearing	1980^{a}	1.5^{a}
High NSP rearing	1929^{b}	0.7^{b}
High NSP laying		
Standard NSP rearing	1968^{a}	0.2^{c}
High NSP rearing	1966^{a}	0.3^{bc}
SE	11.57	0.27
***P*-value**		
NSP Laying	**0.020**	**<0.001**
NSP Rearing	0.278	**0.038**
NSP Laying * NSP Rearing	**0.033**	**0.048**

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSPoncentration laying period (72 vs. 115 g/kg). [2] Results are based on the average value of 33 wks. x 6 replicates per treatment.

DISCUSSION

EFFECT OF NUTRIENT DILUTION

The present experiment was designed to study the impact of diets during rearing on traits during rearing, and on combinations of rearing and laying diets on development and production and on eating traits during laying. Also effects of these treatments on development of proventriculus, gizzard and crop were studied. The aim is to evaluate whether intake and digestion processes are related to the development of feather damage. During rearing, average feed intake of pullets that were fed 10% and 15% diluted diets increased by 8.3 and 13.1%, respectively, resulting in similar ME energy intakes compared to pullets fed control energy diets. This means that rearing hens have a wide range of adaptation to density of their diets. This phenomenon was already known in laying hens (Leeson et al., 2001; Van der Meulen et al., 2006), but not from studies with nutrient diluted diets in rearing hens.

During laying, average energy intake differed only slightly between dilution levels. Relative energy intake was 100%, 97%, 99%, and 97% for the 0%, 10%, 15% and 20% diluted diets, respectively. Contrary to those findings, in the past often overconsumption of feed was observed in hens that were fed diets with increased energy concentrations (Morris, 1968). In the current experiment, energy intake during the laying period was independent of dietary treatments during the rearing period. Van Krimpen et al. (2008) and Van der Meulen et al. (2006) found proportional increases in feed intake with nutrient dilution levels up to 30%. It can be hypothesized that pullets and laying hens need a certain amount of nutrients per day and they will continue to eat until their nutrient demands are fulfilled. It was found that adult layers compensate for dietary dilutions up to 20%, even if whole oat hulls are used as dilution source. Adaptation possibilities are more limited in young pullets because high NSP content could not be fully compensated.

Nutrient dilution of the rearing diet only slightly extended eating time and slightly increased eating rate during the rearing period. Similarly, also during the laying period no significant effects of nutrient dilution on eating time and eating rate were observed. In contrast with those results, earlier findings showed that eating time of laying hens prolonged gradually because of feeding diluted diets (Van Krimpen et al., 2007; Van Krimpen et al., 2008). These differences were not caused by the eating behavior of the hens fed the diluted diets. Eating time and eating rate levels of the 10% diluted diet were comparable with the levels found in an earlier experiment with similar diets (Van Krimpen et al., 2008), Eating time of hens fed the 0% diluted diet in the current experiment, however, was much longer than in the previous experiment (20.4 vs. 15.1%). Probably, eating behavior of hens fed diluted diets in adjacent pens could, encouraged the hens that were fed the undiluted diets to peck more at feed. Keeling and Hurnik (1996) showed that a satiated bird might direct its attention to feed in response to social facilitation effect of a stimulus bird, but that is eats relatively little. Hens might also perform inappropriate feeding pecks in response to a specific deficit in their environment (Savory, 1999). Thus, increased eating behavior of hens fed the undiluted diet because of social facilitation and inappropriate feeding pecks might explain the absence of contrasts in eating time between dilution levels in the current experiment.

Eating time of hens fed the 0% diluted laying diets in the current experiment was in line with that of pullets fed the 0% diluted rearing diets (20.0% vs. 20.3%). Eating time of the birds during the rearing period in the experiment of Van Krimpen et al. (2008) was not determined, but the present results show that hens will spend similar times on eating behavior during both rearing and laying if diets with comparable nutrient densities are supplied .

In the current experiment, feather damage was not affected by nutrient density of the diet. Contrary to those findings, less feather damage was observed if layers were fed a nutrient diluted diet (from 2920 to 2560 kcal/kg; (Elwinger, 1981). Similarly, Van der Lee et al. (2001) reported a better plumage condition in laying hens that were fed diets reduced in energy concentration (2765 versus 2645 kcal/kg). It was hypothesized earlier that an increase in eating time and feeding related behavior because of dietary dilution might compensate for redirected foraging behavior, resulting in less feather pecking behavior (Van Krimpen et al., 2005). In the current experiment, decreasing the nutrient density did not prolong eating time. This might explain the absence of an effect on feather damage. Furthermore, the results of this experiment shows that eating behavior, and subsequent feather pecking behavior, could vary enormously between flocks, even if the same facility, strain, and diet are used. This stresses that feather pecking is a multi factorial problem, which is difficult to control (Leonard et al., 1995; Nicol et al., 2001; Kjaer and Hocking, 2004).

Feeding low nutrient diets to hens resulted in similar hen performance parameters (BW/egg mass) compared with hens that were fed undiluted diet. Similarly, feeding laying hens a 5% nutrient diluted diet did not affect hen performance compared to a control diet (Van der Lee et al., 2001). In a trial with laying hens (34-37 wk of age), dietary dilution (by adding 10, 20, 25 or 30% sand) did also not affect hen performance of the hens (Van der Meulen et al., 2006). The hens fully compensated for the effect of added sand in the diet by increasing their daily feed intake.

Thus, feeding low nutrient diets during rearing and laying resulted in a similar nutrient intake. Nutrient density, however, did not affect feeding related behavior and feather damage.

EFFECT OF DIETARY NSP CONCENTRATION

In the current experiment, NSP concentration of the diets was increased by adding 10% whole oat hulls. As a result, NSP concentration of the rearing diet increased from 124 to 184 g/kg. Insoluble NSP level of the laying diets ranged only from 72 (low NSP) to 115 (high NSP) g/kg. Thus, insoluble NSP concentrations of the rearing diets were considerable higher compared to the laying diets.

BW development of pullets during the rearing period that were fed the 10% and 15% diluted high NSP diets was retarded because of reduced energy intake, compared to the 0% diluted high NSP diet. NSP concentration had no effect on hen performance traits. BW of hens that were fed high NSP laying diets was even slightly increased compared to hens fed standard NSP diets. This was confirmed by Hartini et al. (2003) who found no detrimental effects on performance after substituting wheat by (in)soluble high NSP sources like millrun, barley, rice hulls or oats on an isocaloric and isonitrogenous basis. In some experiments, in which insoluble rich NSP diets were supplemented, nutrient digestibility even increased, possibly due to a better gizzard development and more reflux activity in the fore-gut, resulting in improved hen performance, as reported by (Hetland et al., 2004a).

Contrary to our expectations, NSP addition during rearing did not extend eating time and feeding related behavior. Rearing hens fed the high NSP diet simply ate this feed more quickly. Dietary dilution of the high NSP rearing diets, however, resulted in a retarded feed intake and a decreased eating rate, while eating time was only numerically increased. Pullets seem to maintain their eating time in a fixed time budget. In conclusion, no improvement of coarsely ground NSP addition on performance and behavior of the laying period was observed.

Similarly with earlier findings (Van Krimpen et al., 2007; Van Krimpen et al., 2008) feed intake of the layers was not affected by NSP concentration of the laying diet. Oat hulls have a relative low bulk density. Adding 10% coarse oat hulls to the control diet decreased the bulk density by 15% (783 vs. 669 g/l), indicating that the hens have to consume more volume for realizing a similar nutrient intake compared to hens that were fed undiluted feed. Hens that were fed the control diet daily consumed 143 ml feed, whereas hens that were fed the 10% diluted high NSP diet consumed 183 ml feed. In a study of Vilarino et al. (1996), volume intake of laying hens even increased from 157 to 279 ml/hen/d by feeding a mash diet diluted by 450 g/kg wheat bran. As a result, eating time increased from 32.5 to 41.3% in that experiment. Hens, however, were not able to completely adjust their feed intake to compensate for the dietary dilution.

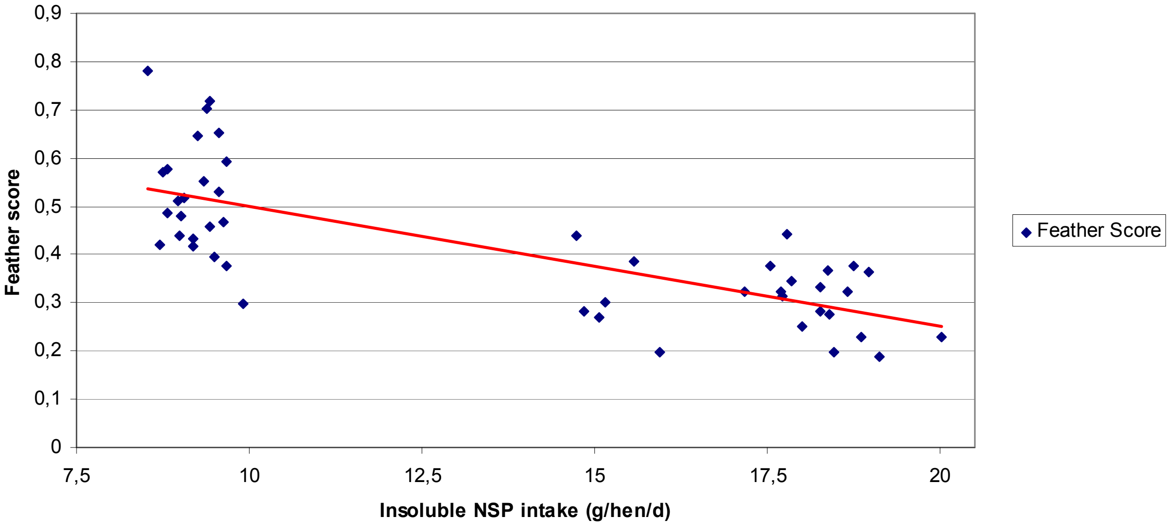
Contrary to earlier work, however, no contrasts in eating time and eating rate were found between standard and high NSP laying diets. The eating time and eating rate levels of the high NSP diets in the current experiment were comparable with the levels found in the earlier experiment, but in the current experiment eating time of hens fed the standard NSP laying diets was much higher (21.10 vs. 16.9%), and eating rate much lower (0.80 vs. 0.95 g/min).

As explained in the previous section, increased eating behavior of hens fed the control diet could be the result of social facilitation and inappropriate feeding pecks. Eating rate during laying was affected by a carry-over effect of NSP content of the rearing diet and dilution level of the laying diet (Table 8). In hens fed 10% diluted laying diet eating rate was reduced in standard compared to high NSP rearing diet, whereas the opposite was observed in hens fed 15% diluted laying diet. An explanation for this phenomenon could not be found.

Feather condition of hens fed high NSP laying diets was evidently improved compared to hens fed standard NSP diets. This effect could not be explained by an extended eating time, a decreased eating rate, more feeding related behavior or less feather pecking behavior.

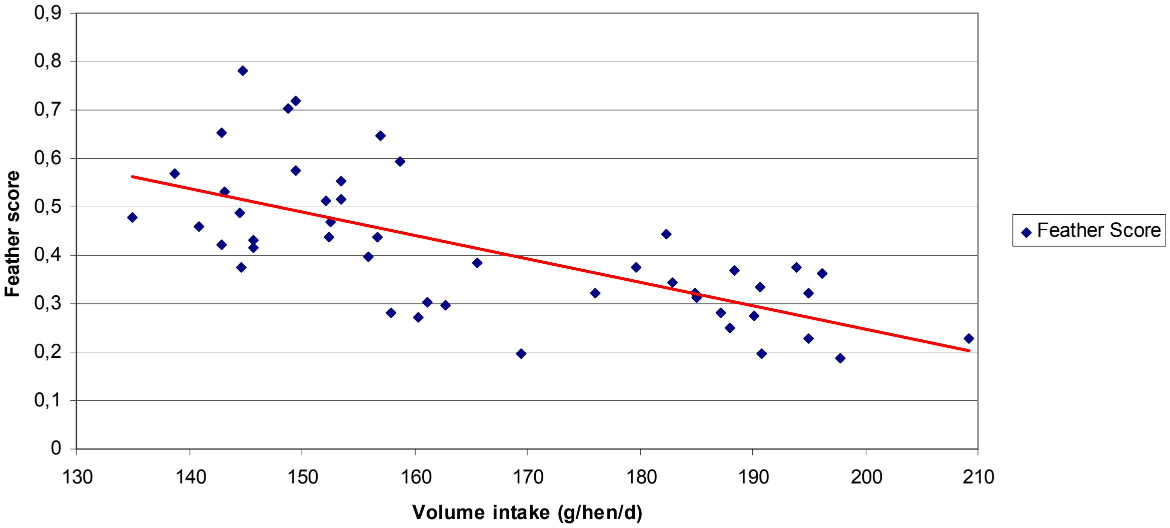
Hens that were fed high NSP laying diets, however, spend more time ground searching and walking, and less time preening. Moreover, hens that were fed high NSP diets consumed more insoluble NSP and more volume of feed per day. Surprisingly, daily insoluble NSP intake was found to be linearly related to feather condition score (FCS = 0.75 – 0.025 * Insoluble NSP intake; $P<0.001$; $R^2 = 0.55$; Figure 1). Likewise, daily volume intake was found to be linearly related to feather condition score (FCS = 1.22 – 0.0049 * volume intake; $P<0.001$; $R^2 = 0.46$; Figure 2). Earlier findings showed that insoluble NSP intake was linearly related to the mean retention time of digesta in the foregut (Van Krimpen et al., Submitted). An increased insoluble NSP intake resulted in a decreased mean retention time in the foregut, which was associated with a higher level of satiety. A higher level of satiety may contribute to a lower feather pecking pressure (Hetland et al., 2004a).

Figure 1. Relation between insoluble NSP intake (g/hen/d) and feather condition score[1]



[1] One pen was excluded from the analysis because of a large standardized residual (9.8 g insoluble NSP intake, 1.77 FCS)

Figure 2. Relation between volume intake (g/hen/d) and feather condition score[1]



[1] One pen was excluded from the analysis because of a large standardized residual (146 g volume intake, 1.77 FCS)

In literature it was shown that adding insoluble NSP-rich raw materials to the diet decreased feather pecking behavior in laying hens. Hartini et al. (2002) showed that addition of insoluble fiber to the diet might prevent cannibalism mortality in pre lay period (13.2 versus 3.9%) and early lay period (28.9 versus 14.3%). Providing insoluble NSP-rich raw materials also have been found to decrease feather pecking among layers, especially when pellets were fed (Aerni et al., 2000; El Lethey et al., 2000). Hetland et al. (2004a) concluded that access to fiber structure from feed and environment may interact with feather pecking behavior.

In the current experiment, NSP addition was confounded with particle sizes distribution. Thus, average particle size increased in high NSP diets compared to standard NSP diets. In earlier work we observed a delay in feather damage in low energy – coarsely ground – high NSP diets compared to hens fed a control diet (Van Krimpen et al., 2008). Replacing ground wheat with whole wheat also showed a positive effect on plumage condition of laying hens (Hetland et al., 2003). In literature, numerous positive effects of coarsely ground NSP sources were mentioned. They stimulate gizzard weight and increase reflux of bile acids, resulting in improved starch digestibility and an enhanced emulsification of liberated lipids (Hetland et al., 2003). Indeed, NSP addition in the current experiment increased gizzard weight and gizzard content. Moreover, NSP addition resulted in a considerable reduced proventriculus content of the hens (0.9 vs. 0.3 g/kg hen). Contents of gut segments of the foregut can be influenced by feedback control from the duodenum, mediated through receptors sensitive to e.g. acidity, particle size and rheological properties of digesta (Bach Knudsen, 2001). Probably, this feedback control affects the proventriculus content. In broilers, increased particle sizes reduce the incidence of proventricular dilatation, stimulate gizzard development and extend mean retention time in the foregut, This was associated with less mortality (Jones and Taylor, 2001; Taylor and Jones, 2004).

In conclusion, adding coarsely ground insoluble NSP to the diet resulted in similar performance and in similar eating behavior, Moreover, it reduced proventricular weight and increased gizzard weight and it's contents All this was associated with better feather condition scores.

CARRY-OVER EFFECTS OF NUTRITIONAL FACTORS IN THE REARING DIETS ON PARAMETERS DURING LAYING

Some authors stated that more attention should be given to the development of feather pecking during the rearing of laying hen chicks (Huber Eicher and Sebo, 2001). It is also suggested that minimizing differences between the rearing and laying environment via a seamless transition may contribute to make a laying flock less prone to injurious feather pecking (Van de Weerd and Elson, 2006). Results from a longitudinal study, with birds followed during both rearing and laying period, showed that stereotyped gentle feather pecking in young birds predicted this behavior of these birds in adult stage (Newberry et al., 2006). Once developed, stereotyped behavior can be persistent and hard to extinguish (Garner and Mason, 2002).

The current experiment was also performed to test the suggestion of Keppler et al. (1999) that stimulating the food-searching behavior of young pullets could prevent feather pecking in adult birds. Although rearing hens that were fed diluted diets consumed more feed, eating time during the rearing period was only slightly increased in the 10% and 15% diluted high NSP diets, whereas feeding related behavior was not affected by dietary dilution level. NSP concentration of the rearing diet had no effect at all on eating behavior during the rearing period. So, it was not striking that significant carry-over effects of the dietary treatments during the rearing period on the average feather condition and feather pecking behavior during the laying period were absent. Nevertheless, feather condition of the hens in the last week of the experiment (wk 49) was positively affected by the dilution level of the rearing diet (Table 11).

Table 11. Carry-over effect of dilution level of the rearing diet and NSP concentration of the laying diet on feather damage score in ISA Brown laying hens of 49 wk of age (SE=0.186; *P*=0.047)

Treatment	Standard NSP Laying	High NSP laying
0% Dilution Rearing	1.23	0.57
10% Dilution Rearing	0.91	0.69
15% Dilution Rearing	**0.52**	0.60

Thus, supplementing 15% diluted diet during rearing resulted in a good feather condition at the end of the laying period, independent of the NSP concentration of the laying diet. This result demonstrates the importance of conditions during the rearing period in preventing feather pecking behavior during the laying period, which was confirmed by different authors (Blokhuis, 1989; Vestergaard and Lisborg, 1993; Johnsen et al., 1998; Chow and Hogan, 2005). Layer pullets that do not get the right substrate to peck early in life may feather peck later on (Johnsen et al., 1998). Supplementing extra straw or spreading 10% of the estimated feed intake as whole wheat into the litter of layer pullets markedly reduced feather damage in the layer period (Blokhuis and Van der Haar, 1992). The birds showed more foraging-related behaviors like ground scratching and ground pecking, suggesting that the incentive value of the ground, and the substrate covering it, might be increased with grain during the rearing period. Similarly, Chow and Hogan (2005) concluded that young Burmese red jungle fowl from 1 to 5 wk of age that were deprived of exploratory-rich environments performed significantly more gentle feather pecking, and tended to show more severe feather pecking than the experienced birds. This suggested that chicks deprived of exploratory-rich environments may consider pen mates as appropriate exploratory stimuli and subsequently direct exploratory behavior towards conspecifics. Chow and Hogan (2005) hypothesized that providing early experience with enriched environments could reduce the likelihood of severe feather pecking developing. It is suggested that pullets become imprinted on pecking substrates very early in life (Vestergaard, 1994), with a sensitive period estimated on day 0-6 (Braastad, 1990), around day 3 (Vestergaard and Baranyiova, 1996), or below day 10 (Huber Eicher and Wechsler, 1997) post hatching. In the current experiment, diluted diets were provided from day 0 post hatch onwards. Feed intake, and probably also the number of feeding pecks, of pullets that were fed the diluted diets increased from the first week of life onwards. Likely, these pullets were more ‘imprinted’ on their feed and therefore less oriented towards the feathers of their conspecifics. This could explain the improved feather condition of the hens at 49 wk of age, that were fed the 15% diluted rearing diets.

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REFERENCES

Aerni, V., H. El Lethey and B. Wechsler. 2000. Effect of foraging material and food form on feather pecking in laying hens. *Br. Poult. Sci.,* 41:16-21.

Bach Knudsen, K. E. 2001. The nutritional significance of "dietary fibre" analysis. *Animal Feed Science and Technology,* 90:3-20.

Balandran-Quintana, R. R., G. V. Barbosa-Canovas, J. J. Zazueta-Morales, A. Anzaldua-Morales and A. Quintero-Ramos. 1998. Functional and nutritional properties of extruded whole pinto bean meal (Phaseolus vulgaris L.). *Journal of Food Science,* 63:113-116.

Bilcik, B. and L. J. Keeling. 1999. Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. *Br. Poult. Sci.,* 40:444-451.

Blokhuis, H. J. 1986. Feather-pecking in poultry: its relation with ground-pecking. *Applied Animal Behaviour Science,* 16:63-67.

Blokhuis, H. J. 1989. The effect of a sudden change in floor type on pecking behaviour in chicks. *Applied Animal Behaviour Science,* 22:65-73.

Blokhuis, H. J. and J. W. Van der Haar. 1992. Effects of pecking incentives during rearing on feather pecking of laying hens. *British Poultry Science,* 33:17-24.

Blokhuis, H. J., T. Fiks-Van Niekerk, W. Bessei, A. Elson, D. Guémené, J. Kjaer, G. A. Maria Levrino, C. J. Nicol, R. Tauson, C. A. Weeks and H. A. Van de Weerd. 2007. The LayWel project: welfare implications of changes in production systems for laying hens. *World's Poult. Sci. J.,* 63:101-114.

Braastad, B. O. 1990. Effects on behaviour and plumage of a key-stimuli floor and a perch in triple cages for laying hens. *Applied Animal Behaviour Science,* 27:127-139.

Brunt, K. 1993. Enzymatic determination of starch content in feed and feedstuffs, Memo 93-302 (in Dutch). *Stichting Nederlands Instituut voor Koolhydraat Onderzoek, Groningen. The Netherlands*.

Chow, A. and J. A. Hogan. 2005. The development of feather pecking in Burmese red junglefowl: the influence of early experience with exploratory-rich environments. *Appl. Anim. Behav. Sci.,* 93:283-294.

El Lethey, H., V. Aerni, T. W. Jungi and B. Wechsler. 2000. Stress and feather pecking in laying hens in relation to housing conditions. *Br. Poult. Sci.,* 41:22-28.

Elwinger, K. 1981. Different energy levels and restricted feeding to three strains of SCWL hybrids. 1. Effects on egg production. *Swedish J. Agric. Res.,* 11:149-157.

Garner, J. P. and G. J. Mason. 2002. Evidence for a relationship between cage stereotypes and behavioural disinhibition in laboratory rodents. *Behav. Brain Res.,* 136:83-92.

Goelema, J. O., A. Smits, L. M. Vaessen and A. Wemmers. 1999. 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. Techn.,* 78:109-126.

Hartini, S., M. Choct, G. Hinch and J. V. Nolan. 2003. Effect of diet composition, gut microbial status and fibre forms on cannibalism in layers. *Australian Egg Corporation Limited.* AECL Publication No 03/03:1-111.

Hartini, S., M. Choct, G. Hinch, A. Kocher and J. V. Nolan. 2002. Effects of light intensity during rearing and beak trimming and dietary fiber sources on mortality, egg production, and performance of ISA brown laying hens. *J. Appl. Poult. Res.,* 11:104-110.

Hetland, H., B. Svihus and A. Krogdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *Br. Poult. Sci.,* 44:275-282.

Hetland, H., M. Choct and B. Svihus. 2004a. Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poult. Sci. J.,* 60:415-422.

Hetland, H., R. O. Moe, R. Tauson, S. Lervik and B. Svihus. 2004b. Effect of including whole oats into pellets on performance and plumage condition in laying hens housed in conventional and furnished cages. *Acta Agric. Scand., Section A, Anim. Sci.,* 54:206-212.

Hoffmeyer, I. 1969. Feather pecking in pheasants - an ethological approach to the problem. *Danish review of game biology,* 6:1-36.

Huber Eicher, B. and B. Wechsler. 1997. Feather pecking in domestic chicks: its relation to dustbathing and foraging. *Animal Behaviour,* 54:757-768.

Huber Eicher, B. and F. Sebo. 2001. The prevalence of feather pecking and development in commercial flocks of laying hens. *Appl. Anim. Behav. Sci.,* 74:223-231.

International Organization for Standardization. 1997. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. Kjeldahl method. ISO 5983. Int. Organ. Standardization, Geneva, Switzerland.

International Organization for Standardization. 1998a. Water quality. Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy. ISO 11885. Int. Organ. Standardization, Geneva, Switzerland.

International Organization for Standardization. 1998b. Animal feeding stuffs. Determination of moisture and other volatile matter content. ISO 6496. Int. Organ. Standardization, Geneva, Switzerland.

International Organization for Standardization. 1999. Animal feeding stuffs. Determination of fat content. ISO 6492. Int. Organ. Standardization, Geneva, Switzerland.

International Organization for Standardization. 2002. Animal feeding stuffs. Determination of crude ash. ISO 5984. Int. Organ. Standardization, Geneva, Switzerland.

Johnsen, P. F., K. S. Vestergaard and G. Norgaard Nielsen. 1998. Influence of early rearing conditions on the development of feather pecking and cannibalism in domestic fowl. *Applied Animal Behaviour Science,* 60:25-41.

Jones, G. P. D. and R. D. Taylor. 2001. The incorporation of whole grain into pelleted broiler chicken diets: production and physiological responses. *British Poultry Science,* 42:477-483.

Keeling, L. J. and J. F. Hurnik. 1996. Social facilitation acts more on the appetitive than the consummatory phase of feeding behaviour in domestic fowl. *Animal Behaviour,* 52:11-15.

Keppler, C., K. Lange and D. W. Folsch. 1999. Development of behaviour of layer chicks and pullets in improved rearing systems. *Fachgruppe Verhaltensforschung vom 12. bis 14. November 1998 in Freiburg Breisgau. KTBL Schrift*:70-80.

Kjaer, J. B. and P. M. Hocking. 2004. The genetics of feather pecking and cannibalism. In: *Welfare of the Laying Hen*. Ed. G. C. Perry. CABI Publishing 109-122.

Leeson, S., J. D. Summers and L. J. Caston. 2001. Response of layers to low nutrient density diets. *Journal of Applied Poultry Research,* 10:46-52.

Leonard, M. L., A. G. Horn and R. W. Fairfull. 1995. Correlates and consequences of allopecking in White Leghorn chickens. *Applied Animal Behaviour Science,* 43:17-26.

Morris, T. R. 1968. Effect of Dietary Energy Level on Voluntary Calorie Intake of Laying Birds. *British Poultry Science,* 9:285-&.

Newberry, R. C., L. J. Keeling, I. Estevez and B. Bilcik. 2006. Behaviour when young as a predictor of severe feather pecking in adult laying hens: The redirected foraging hypothesis revisited. *Appl. Anim. Behav. Sci.*: doi:10.1016/j.applanim.2006.10.010.

Nicol, C. J., A. C. Lindberg, A. J. Phillips, S. J. Pope, L. J. Wilkins and L. E. Green. 2001. Influence of prior exposure to wood shavings on feather pecking, dustbathing and foraging in adult laying hens. *Applied Animal Behaviour Science,* 73:141-155.

Noldus. 1993. The Observer®: Base package for Dos. Reference Manual, version 3.0.

NRC. 1994. Nutrient requirements of poultry. *Ninth Revised Edtion.* Washington DC.

Savory, C. J. 1980. Meal occurrence in Japanese quail in relation to particle size and nutrient density. *Anim. Behav.,* 28:160-171.

Savory, C. J. 1999. Temporal control of feeding behaviour and its association with gastrointestinal function. *Journal of Experimental Zoology,* 283:339-347.

Steenfeldt, S., J. Kjaer and R. M. Engberg. 2007. Effect of feeding silages or carrots as supplements to laying hens on production performance, nutrient digestibility, gut structure, gut microflora and feather pecking behaviour. *Br. Poult. Sci.,* 48:454-468.

Suárez, B. J., C. G. Van Reenen, G. Beldman, J. van Delen, J. Dijkstra and W. J. J. Gerrits. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: I. Animal performance and rumen fermentation characteristics. *J. Dairy Sci.,* 89:4365-4375.

Taylor, R. D. and G. P. D. Jones. 2004. The influence of whole grain inclusion in pelleted broiler diets on proventricular dilatation and ascites mortality. *British Poultry Science,* 45:247-254.

Van de Weerd, H. A. and H. A. Elson. 2006. Rearing factors that influence the propensity for injurious feather pecking in laying hens. *World's Poult. Sci. J.,* 62:654-664.

Van der Lee, A. G., G. Hemke and R. P. Kwakkel. 2001. Low density diets improve plumage condition in non-debeaked layers. *Proc.13th Eur. Symp. poult. nutr.*, Blankenbergen, 244-245.

Van der Meulen, J., C. Kwakernaak and C. A. Kan. 2006. Sand intake by laying hens and its effect on egg production parameters. *J. Anim. Nutr. Phys.,* DOI:_10.1111/j.1439-0396.2007.00732.x.

Van Hierden, Y. M., S. M. Korte, E. W. Ruesink, C. G. v. Reenen, B. Engel, J. M. Koolhaas and H. J. Blokhuis. 2002. The development of feather pecking behaviour and targeting of pecking in chicks from a high and low feather pecking line of laying hens. *Appl. Anim. Behav. Sci.,* 77:183-196.

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. v. d. Peet-Schwering, L. A. d. Hartog and M. W. A. Verstegen. 2008. Low Dietary Energy Concentration, High Nonstarch Polysaccharide Concentration and Coarse Particle Sizes of Nonstarch Polysaccharides Affect the Behavior of Feather-Pecking-Prone Laying Hens. *Poult. Sci.,* 87:485-496.

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. Submitted. Effects of dietary energy concentration, Nonstarch Polysaccharide concentration and particle sizes of Nonstarch Polysaccharides on digesta passage rate and gut development in laying hens. *Poult. Sci.*

Van Krimpen, M. M., R. P. Kwakkel, B. F. J. Reuvekamp, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2005. Impact of feeding management on feather pecking in laying hens. *World's Poult. Sci. J.,* 61:665-687.

Van Krimpen, M. M., R. P. Kwakkel, G. André, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2007. Effect of nutrient dilution on feed intake, eating time and performance of hens in early lay. *Br. Poult. Sci.,* 48:389-398.

Van Soest, P. J. and R. W. McQueen. 1973. The chemistry and estimation of fiber. *Proc. Nutr. Soc.,* 32:123-130.

Vestergaard, K. S. 1994. Dustbathing and its relation to feather pecking in the fowl: motivational and developmental aspects, Denmark, Royal Veterinary and Agricultural University Copenhagen.,

Vestergaard, K. S. and L. Lisborg. 1993. A model of feather pecking development which relates to dustbathing in the fowl. *Behaviour,* 126:291-308.

Vestergaard, K. S. and E. Baranyiova. 1996. Pecking and scratching in the development of dust perception in young chicks. *Acta Veterinaria Brno,* 65:133-142.

Vilarino, M., M. L. Picard, J. P. Melcion and J. M. Faure. 1996. Behavioural adaptation of laying hens to dilution of diets under mash and pellet form. *British Poultry Science,* 37:895-907.

Chapter 7

GENERAL DISCUSSION

GENERAL DISCUSSION

INTRODUCTION

Feather pecking in layers is still a very dominant welfare problem in non-cage housing systems with a prevalence of between 40-80% (Blokhuis et al., 2007). Furthermore, feather pecking, which is painful in itself (Gentle and Hunter, 1990), can cause injury and bleeding, thereby increasing the risk of cannibalism (Allen and Perry, 1975; Hughes, 1982; Blokhuis et al., 2000).The prevalence of cannibalism is lower than feather pecking but up to 20% of flocks were affected in one survey and up to 40% in another. Feather pecking is a multi factorial problem (Hughes and Duncan, 1972; Blokhuis and Van der Haar, 1989; Nørgaard-Nielsen et al., 1993; Leonard et al., 1995; Huber Eicher and Audige, 1999; Jones and Hocking, 1999; Nicol et al., 2001; Kjaer and Hocking, 2004), and nutritional factors may contribute to this problem. From literature, it can be concluded that nutrition could reduce feather pecking behavior (Chapter 2).

The present study was performed to test the hypothesis that nutritional factors may reduce feather pecking behavior if they increase:

3) the time hens spend on feeding related behavior, and/or
4) the (temporary) level of satiety by affecting retention time of digesta in the gut.

Increasing feeding related behavior meets the natural drive of laying hens to spend a lot of time on these type of behaviors (Dawkins, 1989). If the theory of Blokhuis et al. (1986), i.e. that feather pecking is a form of redirected ground or feed pecking behavior is valid, increasing feeding related behavior, will result in less feather pecking behavior. Increasing the level and duration of satiety may result in a temporary reduction of the need to peck to feed, thereby also reducing the risk of performing redirected behavior like feather pecks. This project focused on the parameters: feed characteristics – gut fill/satiety/organ development – feeding related behavior – feather pecking behavior.

IMPORTANCE OF FEEDING RELATED BEHAVIOR

Jungle fowl that are housed in a semi-wild environment have a high motivation for foraging. They spend 60% of their active time on feeding related behaviors, like eating and foraging (Dawkins, 1989). In modern laying hens, this behavior is strongly affected by housing and management conditions. In a study of Aerni et al. (1999), feeding related behavior of laying hens housed in floor pens ranged from 29% in hens that were fed pelletised feed without access to straw to 52% in hens that were fed mash with access to long-cut straw as foraging material.

Feather pecking behavior was dramatically increased in those hens that spent only 29% of their time on feeding related behavior. These results show that the behavior of the domesticated laying hens is very similar to that of their ancestors (Dawkins, 1989). The motivation to perform feeding related behavior will be reduced in the absence of the right stimuli. A significant reduction in feeding related behavior can result in increased feather pecking behavior (Aerni et al., 1999).

Laying hens spend more time on feeding related behavior if they are fed low energy diets (Savory, 1980), or high insoluble fiber diets (Aerni et al., 2000). Moreover, supply of coarse particles (Al Bustany and Elwinger, 1988; Hetland et al., 2004) also reduced feather pecking behavior.

In several studies, reduction in dietary energy concentration, however, is confounded with changes in the concentration of other ingredients and nutrients, like amino acids and Nonstarch Polysaccharides (NSP) (Savory, 1980; Elwinger, 1981; Valkonen et al., 2008). Therefore, the separate effects of nutrient dilution and NSP supplementation on feeding related behavior and feather pecking is still unknown.

In this project, we studied the independent effects of nutrient dilution, NSP supplementation and particle sizes of NSP in rearing and laying diets on feed intake, time budgets of the hens, feed passage rate, gut segment development and feather pecking behavior of rearing and laying hens.

IMPACT OF NUTRIENT DILUTION ON FEEDING RELATED BEHAVIOR

The effects of nutrient dilution on feeding related behavior have been studied by Savory, already in (1980), He fed male Japanese quail 40% diluted (with cellulose powder) and undiluted diets. Those receiving the diluted mash consumed about 40% more feed (14.9 vs. 10.8 g/d), spent a higher proportion of total time (24 h) on feed intake (23.8 vs. 9.1%), had a longer meal length (1.54 vs. 0.87 min), a shorter inter-meal interval (4.98 vs. 8.92 min) and more meals per day (128 vs. 86). Despite meal length being longer with diluted mash, the weight eaten per meal (av. 0.116 g) was equal to the amount with undiluted mash.

In our project, the effect of nutrient dilution alone was studied by adding sand to the diet. The effects of nutrient dilution on feed intake and eating time during the laying period are summarized in Table 1a.

Table 1a. Effects of sand dilution on feed intake and eating time of laying hens

Energy content (MJ/kg)	Sand level (%)	Feed intake (g/hen/d)	Feed intake (Relative)	Eating time (%)	Eating time (Relative)	Eating rate (g/min)	Eating rate (Relative)	Reference
Early lay (18 – 25 wks of age)								
11.8	0	105	100	20.9	100	0.61	100	Chapter 3
10.6	10	115	110	23.2	111	0.58	95	
Laying period (18 – 40 wks of age)								
11.8	0	126	100	15.1	100	1.04	100	Chapter 4
10.6	10	134	106	18.6	123	0.86	83	
Laying period (17 – 49 wks of age)								
11.8	0	111	100	20.4	100	0.73	100	Chapter 6
10.6	10	122	110	21.1	103	0.72	99	
10.0	15	131	118	21.2	104	0.91	125	
9.4	20	135	122	21.6	106	0.82	112	

Adding up to 20% sand to laying diets resulted in a proportional increase of feed intake. Therefore, no adverse effects of nutrient dilution on nutrient intake and egg performance were found. Similarly, feeding a reduced dietary energy concentration (by adding 10, 20, 25 or 30% sand) to laying hens (34-37 weeks of age) did not affect hen performance of the hens (Van der Meulen et al., 2006). For the tested dilution levels, it can be concluded that hens adjust their feed intake to their nutrtional need, independent of the amount of feed that needs to be consumed. In all experiments during the laying period, addition of sand to the diet prolonged eating time during the laying phase (Chapter 3, 4, and 6). In two experiments (Chapter 3 and 4) eating rate of hens fed the diluted decreased. In Chapter 6, however, eating rate increased at higher dilution levels. As a result, eating time in that experiment, only slightly increased with increasing dilution levels. In chapter 6, we included the rearing phase as well. The effects of nutrient dilution on feed intake and eating time during this period are summarized in Table 1b.

Table 1b. Effects of sand dilution on feed intake and eating time of rearing hens

Energy content (MJ/kg)	Sand level (%)	Feed intake (g/hen/d)	Feed intake (Relative)	Eating time (%)	Eating time (Relative)	Eating rate (g/min)	Eating rate (Relative)	Reference
Rearing period (1 – 16 wks of age)								
11.0	0	55	100	22.7	100	0.38	100	Chapter 6
9.9	10	62	113	20.6	91	0.48	126	
9.4	15	66	120	20.8	92	0.52	137	

Adding up to 15% sand to rearing diets resulted in a proportional increase of feed intake. Thus, both in rearing and laying hens, feed intake of sand diluted diets is not limited by physical properties of feed, but rather by chemostatic conditions. Contrary to our expectations, providing sand diluted diets during the rearing phase even reduced time spend on eating in this phase (Chapter 6). Apparently, rearing hens preferred to increase their eating rate, thereby keeping eating time within fixed margins.

Other behavior traits, both during rearing and laying, and weights of gut segments were not affected by dietary dilution level. It was observed, however, that dietary dilution prolonged mean retention time in the hindgut (8.6 vs. 30.3 min) and increased DM content in small intestine, colon and caeca.

CHARACTERISTICS OF DIETARY NSP AND LIGNIN

Dietary fiber is predominantly found in plant cell walls and consist mainly of NSP. The NSP fraction can be separated into two physiochemical groups; the insoluble NSP's, which are mainly composed of cellulose, lignin and some hemicelluloses, and the soluble NSP's, such as pectins, gums, mucilages and other hemicelluloses (Schneeman, 1986; Graham and Aman, 1991). Cellulose is the main structural component of plant cell wall (Southgate, 1995). Although cellulose is water-insoluble, it still has the property of taking up water (4 g water/g cellulose). The hemicelluloses comprises a series of heteroglycans, the largest group consisting of pentosans such as xylans and arabinoxylans; a second group consists of hexose polymers such as galactan. The acidic hemicelluloses containing galacturonic or glucuronic acids, form a third group of hemicelluloses. Lignin is a non-carbohydrate cell wall component, which is resistant against microbial degradation (Bach Knudsen, 2001).

Solubility of the polysaccharide increases with increasing flexibility of the chain between sugar residues. The ratio between insoluble and soluble non-cellulosic polysaccharides ranges from 0.4 in sugar beet pulp to 22.7 in oat hull meal (Bach Knudsen, 2001). Since polysaccharides are hydrophilic molecules, both soluble and insoluble polysaccharides have the ability to hold water. Soluble fiber holds up to 10 times its own weight in water, whereas insoluble fiber 4-6 times its own weight (Hartini et al., 2003).

Almost all water-soluble polysaccharides, such as guar gum and pectins, produce a viscous solution. Viscosity reduced the transit time through the gut (Salih et al., 1990) and consequently give more time for gut micro organisms to proliferate. Insoluble NSP, however, is only slightly fermented. Therefore, insoluble NSP serves almost entirely as bulking agent and shortens overall transit time (Roberfroid, 1993).

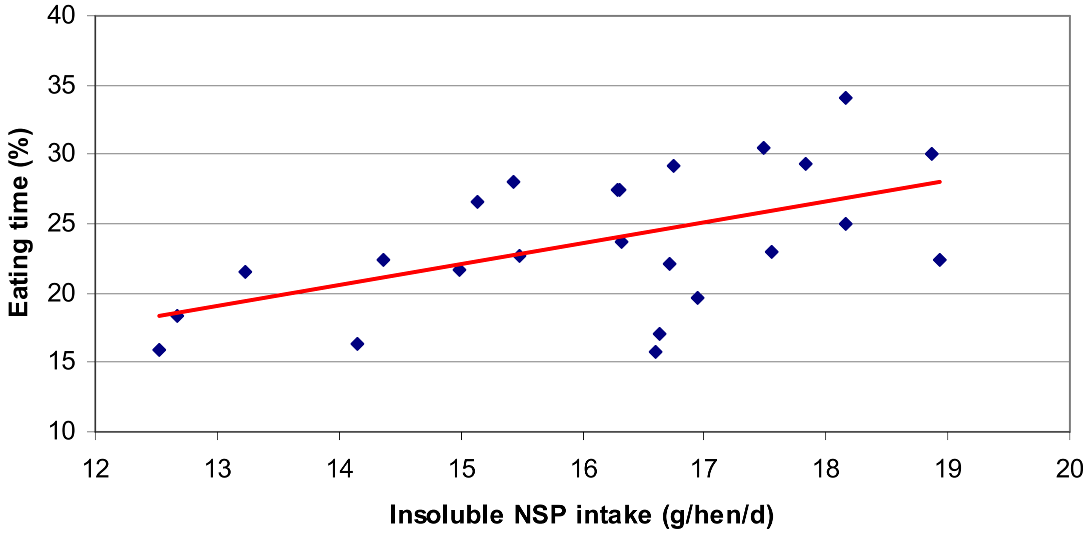
IMPACT OF DIETARY NSP ON FEEDING RELATED BEHAVIOR

Providing NSP sources to layers will affect feeding related behavior (Savory, 1980; Aerni et al., 2000; Hartini et al., 2002). We concluded from our studies that NSP content and solubility of NSP affected eating time. In Chapter 3, it was shown that eating time of hens in early lay that were fed the high NSP diluted diets increased on average by 22% compared to hens fed a standard NSP diet (21.2 vs. 25.8%). The increase in eating time, however, was not similar for all high NSP treatments. This may be related to the water-solubility of the tested NSP sources. Within the high NSP treatments, a positive linear relationship between daily insoluble NSP intake (g/hen/d) and eating time (% of observation period) was found (equation 1; Figure 1).

$$\text{Eating time} = 0.23 \times \text{insoluble NSP intake} - 6.4 \qquad (P{=}0.010;\ R^2{=}0.24) \tag{1}$$

Thus, eating time of hens is prolonged with increased consumption of insoluble NSP's. On the contrary, eating time is reduced with increased consumption of soluble NSP's (Eating time = -1,27 x soluble NSP intake + 36.0; P=0.051; R^2=0.12). Compared to soluble NSP, more time is probably needed for soaking insoluble NSP in the crop and degrading it in the proventriculus/gizzard before entering the small intestine.

Figure 1. Effect of insoluble NSP intake on eating time in high NSP treatments from Chapter 3 (Eating time = 0.23 x insoluble NSP intake – 6.4; P=0.010; R^2=0.24)



From the NSP sources that were tested in Chapter 3, oat hulls had the highest insoluble NSP content. Therefore, to prolong eating time in the following experiments (Chapter 4 to 6), oat hulls were chosen as NSP source. The effects of dietary dilution by use of oat hulls on feed intake and eating time are summarized in Table 2a (laying period) and 2b (rearing period).

Table 2a. Effects of NSP dilution by addition of oat hulls on feed intake and eating time of laying hens

Energy content (MJ/kg)	Oat hulls level (%)	Structure of oat hulls	Sand level (%)	Feed intake (g/hen/d)	Feed intake (Relative)	Eating time (%)	Eating time (Relative)	Eating rate (g/min)	Eating rate relative	Ref.
Early lay (18 – 25 wks of age)										
11.8	0	–	0	105	100	20.9	100	0.61	100	Chapt. 3
10.6	10	Fine	0	114	109	28.0	134	0.47	77	
10.6	10	Coarse	0	111	106	24.7	118	0.56	92	
Laying period (18 – 40 wks of age)										
11.8	0	–	0	126	100	15.1	100	1.04	100	Chapt. 4
10.6	10	Fine	0	129	102	19.7	130	0.69	66	
10.6	10	Coarse	0	146	116	21.9	145	0.75	72	
Laying period (17 – 49 wks of age)										
11.8	0	–	0	111	100	20.4	100	0.73	100	Chapt. 6
10.6	10	Coarse	0	122	110	20.2	99	0.81	111	
10.0	10	Coarse	5	130	117	20.9	102	0.79	108	
9.4	10	Coarse	10	138	124	23.8	117	0.68	93	

It can be concluded that dietary dilution with oat hulls proportionally increased feed intake. Compared to the sand diluted diets we observed more variation in feeding related behavior between the studies. In the experiments, described in Chapter 3 and 4, the effects of dietary dilution with oat hulls on eating time were more than proportional. This was the result of a significant decrease in eating rate. In Chapter 6, the effects of oat hulls addition on eating time were absent or less pronounced. Contrary to the findings in Chapter 3 and 4, hens that were fed 10% and 15% diluted diets in Chapter 6 reacted by increasing their eating rate. Eating time and eating rate levels of the diluted diets were comparable with the levels found in Chapter 4, Compared to the previous experiment, eating time of hens fed the 0% diluted diet in the current experiment, however, was extended (20.4 vs. 15.1%) and eating rate decreased (0.73 vs. 1.04). Probably, eating behavior of hens fed diluted diets in adjacent pens could, encouraged the hens that were fed the undiluted diets to peck more at feed. Keeling and Hurnik (1996) showed that a satiated bird might direct its attention to feed in response to social facilitation effect of a stimulus bird, but that it eats relatively little. Furthermore, hens might also perform inappropriate feeding pecks in response to a specific deficit in their environment (Savory, 1999). Thus, an already high eating time of hens fed the undiluted diet might be explained by social facilitation and inappropriate feeding pecks.

In chapter 6, we included the rearing phase as well. The effects of dietary dilution with oat hulls on feed intake and eating time during this period are summarized in Table 2b.

Table 2b. Effects of NSP dilution by addition of oat hulls on feed intake and eating time of rearing hens

Energy content (MJ/kg)	Oat hulls level (%)	Structure of oat hulls	Sand level (%)	Feed intake (g/hen/d)	Feed intake (Relative)	Eating time (%)	Eating time (Relative)	Eating rate (g/min)	Eating rate (relative)	Ref.
Rearing period (1 – 16 wks of age)										
11.0	0	—	0	55	100	22.7	100	0.38	100	Chapt. 6
9.9	10	Coarse	0	60	109	23.3	103	0.43	113	
9.4	10	Coarse	5	62	113	23.8	105	0.39	103	

During rearing, hens also compensated for dietary dilution with oat hulls by a higher feed intake. This higher feed intake resulted in an increased eating time and eating rate. Rearing hens that were eating sand diluted diets seem to have a preference for increasing their eating rate, thereby keeping eating time within fixed margins. Apparently, rearing hens could no fully apply this eating strategy if oat hulls were used as dilution source.

The question is why a dietary dilution with *insoluble NSP sources* additively affected eating behavior of laying hens? We hypothesized that eating time of NSP diluted diets prolonged as a result of increased (1) attractiveness of feed, (2) feed intake, (3) volume intake, and (4) mean retention time (MRT) in the foregut.

1) Hens will compensate for dietary dilution by higher feed intake to maintain their nutrient intake, thereby prolonging eating time in most of the experiments.
2) The high NSP diets, especially the coarsely ground ones, contained structure rich components. Therefore, a high NSP diet in mash form might be an attractive foraging substrate for the hens, in which they will spend more time searching for the NSP particles, compared to low NSP mash diets.
3) Oat hulls have a relative low bulk density. Adding 10% coarse oat hulls on a weight basis to the control diet increased diet volume by 15% (783 vs. 669 g/l; Chapter 6), indicating that the hens have to consume more volume for realizing a similar nutrient intake compared to hens that were fed undiluted feed. Hens that were fed the control diet consumed 143 ml feed, whereas hens that were fed the 10% diluted high NSP diet consumed 183 ml feed (Chapter 6). This increase of 28% in volume intake might also partly explain the prolonged eating time.
4) Feeding high NSP diets resulted in a 100% increase of the MRT in the crop (34 vs. 68 min), whereas MRT of the total foregut increased by 60% (from 57 vs. to 91 min), compared to standard NSP diets. Surprisingly, a linear relationship was found between daily insoluble NSP intake and mean retention time in the foregut (Chapter 5). Apparently, soaking the high insoluble NSP diet in the crop, and degrading this structural diet in the proventriculus/gizzard takes more time compared to diets with a high content of water-soluble nutrients. In line with our findings, results of others showed that insoluble NSP accumulate in the gizzard, indicating a prolonged MRT in this gut segment (Hetland et al., 2003; Hetland et al., 2005). Because of these changes in MRT, eating time and level of satiety increased. In line with these findings, it is reported that supplying cellulose diluted diet increased meal length and shortened inter-meal interval in Japanese quail, thereby increasing overall eating time (Savory, 1980). Savory (1980) suggested that gut-emptying, and particularly filling and emptying of the gizzard or duodenum, could be the main activating mechanism in meal

initiation and termination. Reduction in the gastric emptying rate has been correlated with the feeling of fullness or satisfaction, which is a signal to stop eating (Read, 1992). Zorilla (1998) described satiety as 'those processes that determine the length of time between meals". Slowing gastric emptying will increase the duration of gastric distension which as a consequence extends the feeling of satiety.

IMPACT OF NSP CONCENTRATION AND NSP SOLUBILITY ON GIZZARD DEVELOPMENT

In all experiments within this project, relative empty gizzard weight increased between 30 and 71% because of feeding high vs. standard NSP diet; i.e. from 14.5 to 20.5 g/kg BW in experiment 1, from 19.4 to 25.2 g/kg BW in experiment 2, and from 14.3 to 24.4 g/kg in experiment 3. Within the high NSP treatments, however, relative gizzard weight varied considerable. In the first experiment (Chapter 3), relative empty gizzard weight ranged from 14.0 g/kg BW in the arbocel treatment to 22.2 g/kg BW in the whole oat hulls treatment. Therefore, more insights in the chemical composition of the NSP fraction, as separated by Van Soest and McQueen (1973), is necessary to understand the various effects of the tested NSP sources on gizzard development. Based on the combined data of the three experiments within the current project, the relationships between daily intake of insoluble NSP, cellulose, hemicellulose, and lignin on empty gizzard weight are graphically shown in Figures 2 to 5, respectively.

Figure 2. Relation between daily insoluble NSP intake and gizzard development over the three experiments of this project

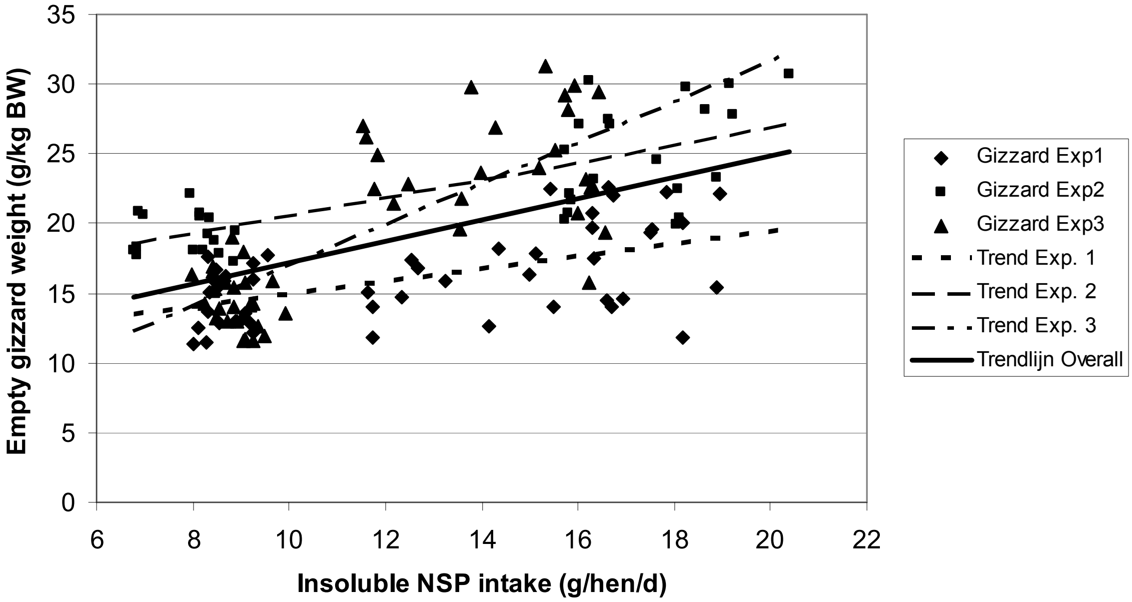


Figure 3. Relation between daily cellulose intake and gizzard development over the three experiments of this project

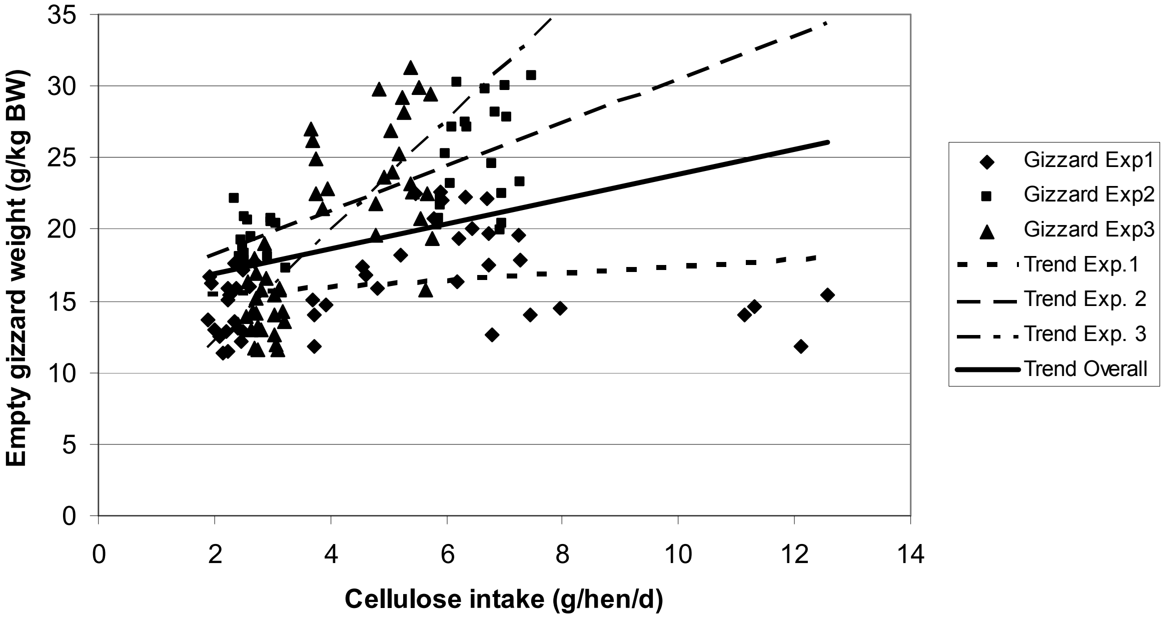


Figure 4. Relation between daily hemicellulose intake and gizzard development over the three experiments of this project

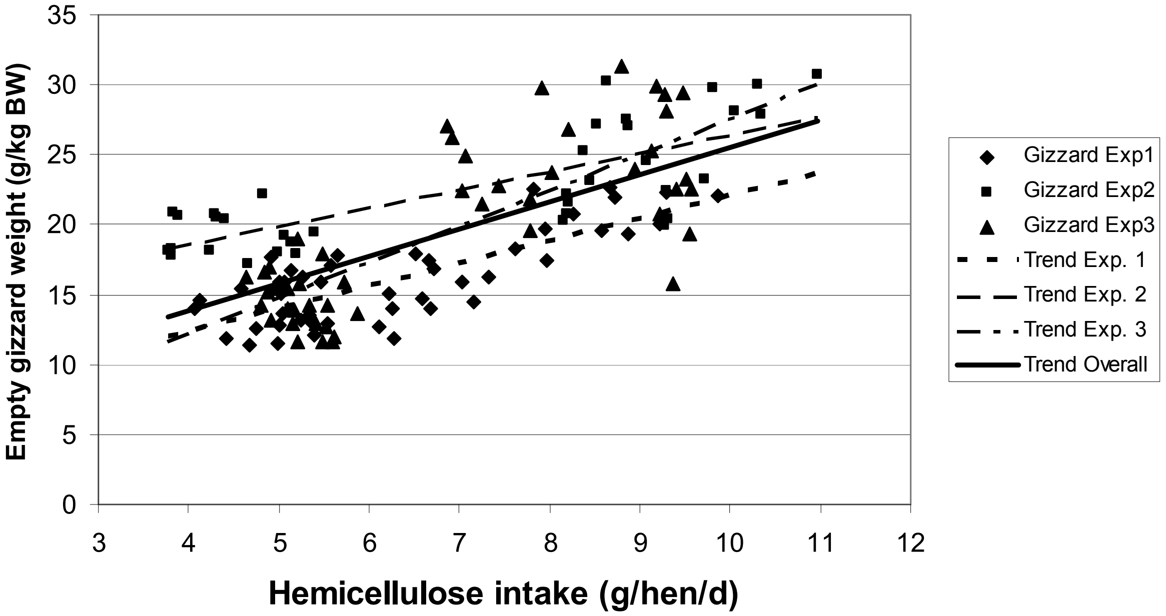
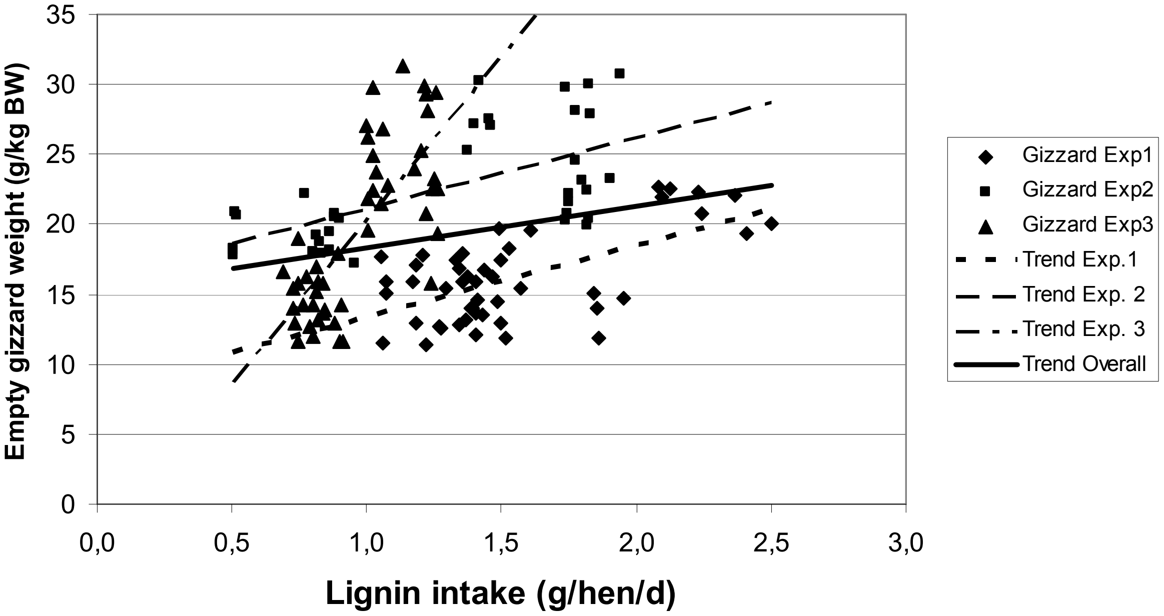


Figure 5. Relation between daily lignin intake and gizzard development over the three experiments of this project



Linear regression was used to find relationships between daily intake of NSP fractions, and empty gizzard weight, based on the combined data of the three experiments of the current project. The linear relation between intake of insoluble NSP, cellulose, hemicellulose and lignin on empty gizzard weight are shown in equations 2 to 5, respectively.

Relative empty gizzard weight = 0.76 x insoluble NSP intake + 9.61 (P<0.001; $R^2 = 0.32$) (2)

Relative empty gizzard weight = 0,86 x cellulose intake + 15.24 (P<0.001; $R^2 = 0.12$) (3)

Relative empty gizzard weight = 1.94 x hemicellulose intake + 6.09 (P<0.001; $R^2 = 0.51$) (4)

Relative empty gizzard weight = 3.00 x lignin intake + 15.31 (P<0.003; $R^2 = 0.06$) (5)

The daily intake of all mentioned NSP fractions are significantly related to empty gizzard weight. The percentage of accounted variance, however, ranged from 6% (lignin) to 51% (hemicellulose). Thus, of the determined fiber fractions, daily hemicellulose intake seems to be the best predictor of gizzard development in laying hens. Gizzard development was only limited explained by the daily cellulose and lignin intake of the hens. Cellulose is the most structural component of plant cell walls (Hartini et al., 2003). These structures have to degraded to a certain critical size before they can leave the gizzard (Moore, 1999). Therefore, a more pronounced effect of cellulose intake on gizzard development was expected. The absence of a linear relation was mainly caused by the arbocel treatment. Arbocel was added to the diet in powder form, and therefore, no further degradation of these particles in the gizzard was necessarily. After exclusion of the arbocel treatment from the dataset, percentage of accounted variance increased to 34%. Relationship between lignin intake and gizzard development largely varied between the experiments (Figure 5). This explains the low overall percentage of accounted variance of lignin intake related to gizzard weight.

IMPACT OF PARTICLE SIZES DISTRIBUTION ON FEEDING RELATED BEHAVIOR

In the study of chapter 3, no effects of particle sizes of NSP on feed intake, eating time, eating rate were observed. Coarsely ground diets, however, negatively affected egg performance of young layers. In chapter 4, eating time and total feeding related behavior increased because of feeding coarsely ground high NSP diets, whereas resting time decreased. In that experiment, however, no effects of particle sizes of NSP on egg performance was observed. Besides, particle sizes of NSP significantly affected relative empty gizzard weight. Compared to the standard NSP treatment (133 g/kg), relative empty gizzard weight increased by 13% after adding 10% finely ground oat hulls to the diet, whereas relative empty gizzard weight increased even by 47% because of adding whole oat hulls to the diet (19.4 vs. 21.9 vs. 28.5 g/kg BW; Chapter 5). Coarseness of NSP did not affect other gut segments. In chapter 6, particle sizes and NSP concentration were confounded, because only whole oat hulls were used as NSP source. It can be concluded that the effects of particle sizes distribution on feeding related behavior are rather limited.

ADDITIVE EFFECTS BETWEEN NUTRIENT DILUTION X NSP (CONTENT/FORM)

In the sections above, the independent effects of nutrient dilution, NSP concentration, and particle sizes of NSP were described. Besides, some additive effects were observed if these nutritional factors were combined (Chapter 4). Providing a low energy - coarsely ground – high NSP diet resulted in additive effects on feed intake, eating time, mean retention time (MRT) in the foregut, and the arise of feather damage (Table 3).

Table 3. Additive effects of nutrient dilution, NSP concentration and particle sizes of NSP on feed intake, eating time, delay in the arise of feather damage and culling rate (Chapter 4 and 5)

Treatment	Feed intake (g/hen/d)	Eating time (%)	MRT Foregut (min)	Moment of maximal increase in feather damage (wk of the laying period)
Control (standard energy/standard NSP)	126	15.1	74	15.6
Low energy	134	18.6	40	15.7
High NSP	131	19.8	91	16.7
Coarse NSP	136	20.6	106	18.9
High NSP + Coarse				
Low energy x high NSP x coarse NSP	146	21.9	114	25.4

Feeding a low energy diet (diluted with 10% sand) proportionally increased feed intake and eating time. Hens that were fed a low energy - high NSP diet (diluted with 10% whole oat hulls) also increased their feed intake. Simultaneously, they also consumed more insoluble NSP and also more coarse particles. This resulted in additive effects on eating time and MRT in the foregut. None of the individual dietary factors (low energy, high NSP, coarse NSP) affected the level of feather damage, but the combination of these factors in one diet resulted in a significant delay (about 10 wk) until the moment of maximal increase of feather damage was reached.

Eating time is a behavior trait that shows a very large variation. Linear regressions between nutritional characteristics and eating time (based on data of Chapter 4) only explain a limited part of this variation (Table 4).

Table 4. Linear regression equations between nutritional characteristics and eating time (Chapter 4)

Variable part of equation	Constant	*P*-value	R^2
Eating time =:			
-2.13 x Energy content (MJ/kg)	- 42.9	0.021	0.10
0.16 x Coarse particles[1] intake (g/hen/d)	+ 15.6	0.029	0.09
0.35 x Insoluble NSP intake (g/hen/d)	+ 14.1	0.006	0.15
-1.73 x Energy content + 0.30 x Insoluble NSP intake	+ 34.1	0.004	0.21
-2.20 x Energy content + 0.17 x coarse particles intake[1]	+ 40.1	0.004	0.21

[1] Calculated as % of feed particles >= 1.25 mm x feed intake (g/hen/d)

Linear regressions with each nutritional characteristics explained a maximum of 15% of the variation of eating time. Additive effects were found by combining energy content and insoluble

NSP intake, or energy content and coarse particles intake, thereby explaining 21% of variation in eating time. Furthermore, the results from our different studies of this experiment shows that eating behavior, and subsequent feather pecking behavior, could vary enormously between flocks, even if the same facility, strain, and diet are used. This stresses that feather pecking is a multi factorial problem, which is difficult to control (Leonard et al., 1995; Nicol et al., 2001; Kjaer and Hocking, 2004).

IMPACT OF NUTRITIONAL FACTORS IN REARING AND LAYING DIETS ON FEATHER DAMAGE DURING LAYING

Nutrient dilution in laying diets

In our studies, no significant effects of nutrient dilution as such on feather pecking behavior and feather damage were observed. Despite the absence of explanatory changes in behavior, culling rate was dramatically reduced in a feather pecking prone laying hens because of adding 10% sand (44.1 vs. 13.1%) to the diet (Chapter 4). In one of the studies (Chapter 6), decreasing the nutrient density had only a limited effect on prolonging eating time. This might explain the absence of an effect on feather damage.

NSP content in laying diets

Increasing the NSP content of the laying diet of feather pecking prone laying hens did not affect feather damage, feather pecking behavior and other behavior traits (Chapter 4). In a study with a low feather pecking pressure (Chapter 6), feather damage was significantly reduced after feeding high compared to standard NSP diet (0.58 vs. 0.30; $P<0.001$). Apparently, NSP addition may only prevent feather damage if pecking behavior was not developed in an earlier stage.

Particle sizes of NSP in laying diets

In our studies, no effects of particle sizes of NSP on feather condition and pecking behavior were observed. In the study of Chapter 6, only whole oat hulls were used as NSP source. Thus, NSP content and particle sizes of NSP were confounded in that experiment.

Nutrient dilution x NSP content x particles sizes of NSP in laying diets

Providing a nutrient diluted – high NSP – coarsely ground diet to laying hens showed additive effects on eating behavior, gizzard development en MRT. As a result, the maximal increase in feather damage occurred 10 wks later compared to hens fed the standard diet (Table 3). This was a striking effect, because those hens showed already feather pecking behavior during the rearing period (Chapter 4). Moreover, it can be concluded from those results that nutritional factors might be more effective in preventing feather damage if these factors are applied before feather pecking behavior has been developed.

Nutrient dilution and NSP addition in rearing diets

It has been suggested that there might be a critical period in early life, in which young hens are sensitive for the development of feather pecking behavior (Klein et al., 2000). Results from a longitudinal study, with birds followed during both rearing and laying period, showed that stereotyped gentle feather pecking in young pullets predicts this behavior when the birds became adult (Newberry et al., 2006). Layer pullets that do no get the right pecking experience early in life may feather peck later on (Johnsen et al., 1998). Once developed, stereotyped behavior can be persistent and hard to extinguish (Garner and Mason, 2002). Thus, stimulating eating and foraging behavior in early life may give perspectives with regard to preventing feather pecking behavior at adult stage.

Chapter 6 focused on the effects of nutritional factors during rearing and laying period on eating behavior and subsequent feather pecking behavior of rearing and laying hens. Relevant carry-over effects were found between dietary dilution during the rearing period and NSP concentration during the laying period on the development of feather damage over time (Figure 7, 8 and 9).

Figure 7. Effect of NSP concentration of the layer diet on feather damage development in hens that were fed **0%** diluted rearing diet

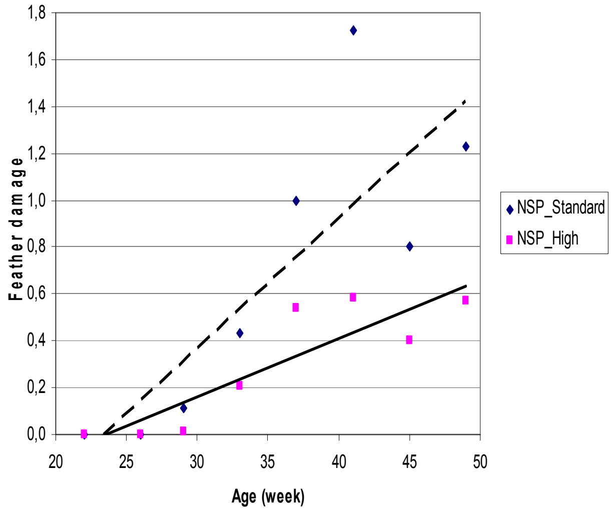


Figure 8. Effect of NSP concentration of the layer diet on feather damage development in hens that were fed **10%** diluted rearing diet

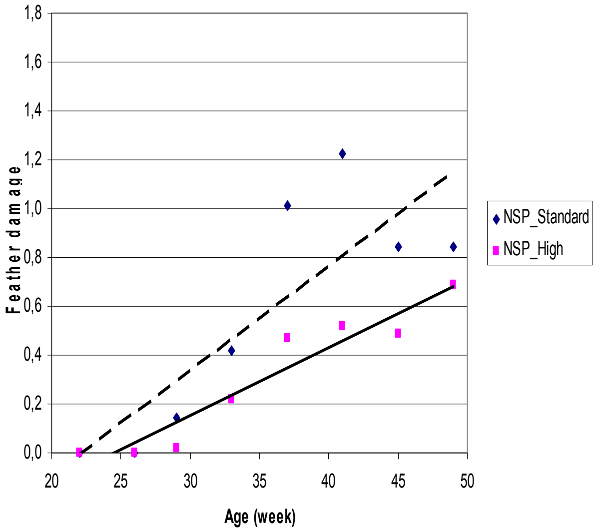
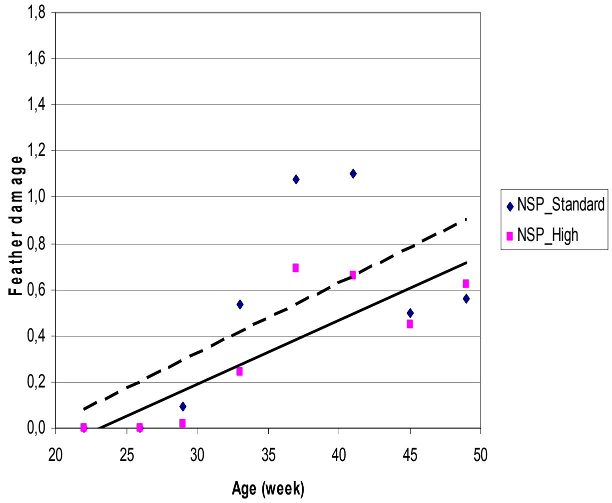


Figure 9. Effect of NSP concentration of the layer diet on feather damage development in hens that were fed **15%** diluted rearing diet



From these figures it can be concluded that the development of feather damage is retarded if hens were fed high instead of standard NSP laying diets. Feather damage, however, was also retarded in hens that were fed 15% diluted diet during rearing (Figure 9). Providing 10% diluted rearing diet had no beneficial effect on feather condition during laying (Figure 8). Thus, the feeding strategy of 15% diluted diet during rearing followed by standard NSP laying diet is as efficient as 0% diluted rearing diet followed by high NSP laying diet in retarding feather damage.

These studies are performed to test the hypothesis that feather pecking behavior will be reduced if hens are spending more on feeding related behavior, or if the hens are more satiated. The rearing hens that were fed the 15% diluted diet consumed 19% more feed (66.1 vs. 55.4 g/hen/d) compared to the hens fed the 0% diluted diet. Dietary dilution, however, did not prolong eating time during the rearing period, whereas other behavior traits remained also unchanged. Therefore, our hypothesis seems not to be valid for rearing hens. Assuming that the feed intake per peck was similar for the diluted and undiluted rearing diets, hens had also to perform 19% more feeding pecks. Laying hens on average will consume 5.2 mg of mash feed per peck (Tanaka et al., 1983). This might indicate that hens fed the 15% diluted diets performed 12,700 pecks/d against 10,650 pecks/d in hens fed the undiluted diet. For this calculation, we assumed that rearing hens have similar feed intake per peck as adult layers. Although eating time of the rearing hens fed the 15% diluted diets was not prolonged, these hens had to peck much often to their feed, even in the first weeks of live, compared to hens fed the undiluted diet. Probably, the number of feeding pecks per day is more important for the imprinting process of young rearing hens than the time spend on this behavior. In that case, our hypothesis needs to be formulated more specific.

IMPACT OF DIETARY NUTRIENTS ON PHYSIOLOGICAL AND NEUROBIOLOGICAL PARAMETERS

The current project focused on the direct impact of nutritional factors on feeding related behavior and feather damage. Nutritional factors, however, will also affect behavior of animals by physical and neurobiological pathways. A stabilized blood glucose level was found to decrease physical activity and to increase satiety level of pregnant sows (De Leeuw et al., 2004). Increased levels of plasma corticosterone and hetrophiles/lymphocytes ratio are associated with higher levels of stress, frustration, fear, anxiety, and stereotypic pecking behavior (Gross and Siegel, 1983; Korte, 2001).

In the second experiment (Chapter 4), also some (unpublished) physiological and neurobiological parameters were determined. Blood plasma parameters (corticosterone and glucose in mg/l) and blood cell counts (heterophiles and lymfocytes in %) per treatment are shown in Table 6. Serotonin and dopamine turnover, and noradrenalin concentration were determined in the forebrains (Table 7). These traits were determined after 5 minutes of manual restraint in 10 hens per treatment.

Table 6. Blood plasma parameters (corticosterone and glucose) and blood cell counts per treatment, determined in the experiment described in Chapter 4.

Treatment	Glucose (mg/dl)	Corticosterone (ng/ml)	Ratio Heterophiles/ Lymphocytes
Standard Energy			
Standard NSP	272.5	12.4	0.66
High NSP-Fine	268.2	8.5	1.32
High NSP-Coarse	259.1	11.0	2.26
Low Energy			
Standard NSP	276.0	6.0	1.52
High NSP-Fine	270.8	15.9	1.64
High NSP-Coarse	255.5	8.5	1.84
Standard error	7.47	2.85	0.49
***P*-Value**			
Energy	0.907	0.960	0.380
NSP	0.057	0.572	**0.043**
Energy*NSP	0.612	**0.037**	0.258
Coarseness	**0.046**	0.302	0.154
Energy*Coarseness	0.612	**0.029**	0.355

Hens fed high NSP diets had slightly lower plasma glucose concentration than hens fed low NSP diets (263.4 versus 274.3 mg/dl). Plasma glucose concentration was lower in hens fed coarsely in stead of finely ground high NSP diets (257.3 versus 269.5 mg/l; *P*=0.046). In restricted fed sows, blood glucose peaks were observed after a meal, following by a decline in glucose content several hours after feeding (De Leeuw et al., 2004). Sugar beet pulp, as a source of fermentable dietary fiber, was found to stabilize blood glucose levels and to reduce physical activity, indicating a prolonged feeling of satiety in these sows. In the current project, hens were fed unrestricted, which enabled the hens to maintain their glucose homeostasis. Nevertheless, blood glucose levels of the hens fed coarsely ground NSP was 4.5% lower compared to hens fed finely ground NSP. Hens that were fed coarsely ground NSP had a prolonged mean retention time in the foregut (Chapter 5), indicating that the supply of nutrients in the small intestine and the blood were spread over a longer period. This could explain the decreased blood glucose level in this treatment. In the current project, blood samples were collected during two days, but only one blood sample per hen was taken. Therefore, these data provides no insight in possible differences in blood glucose profiles during the day of hens fed finely versus coarsely ground high NSP diets. For future experiments, it might be interesting to determine such blood glucose profiles as possible indicators of satiety.

Plasma corticosterone concentrations were not affected by NSP concentrations in normal energy fed hens, whereas hens fed low energy/low NSP diets had lower corticosterone concentrations compared with hen fed low energy/high NSP diets (6.0 versus 12.2 ng/ml). Hens fed low energy/coarsely ground NSP diets had lower corticosterone concentrations compared with hen fed low energy/finely ground NSP diets (8.5 versus 15.9 ng/ml). Heterophiles to lymphocytes ratio was increased in high NSP diets compared to standard NSP diets (1.09 vs. 1.78). Based on the observed effects of dietary manipulation on feather pecking behavior, lowest levels of corticosterone and hetrophiles/lymphocytes ratio were expected in hens fed the low energy coarsely ground high NSP diet. Indeed, corticosterone level was relatively low in this treatment but simultaneously, hetrophiles/lymphocytes ratio was relatively high. Moreover, corticosterone level was unexpected high in the low energy finely ground high NSP diet. Hens fed the standard energy standard NSP diet had high corticosterone levels, whereas hetrophiles/lymphocytes ratio was low.

Levels of serotonin and dopamine turnover were decreased in high feather pecking birds compared to low feather pecking birds (Van Hierden et al., 2002). Moreover, serotonin turnover was negatively associated with the frequency of feather pecking (Van Hierden et al., 2004). Serotonin and dopamine turnover, and noradrenalin concentration in the forebrains are shown in Table 7. Hens fed low energy diets had lower serotonin turnover compared with normal energy fed birds (0.829 versus 1.128 versus 0.829 mg/g; *P*=0.058). Likewise, hens fed low energy diets had lower dopamine turnover compared with normal energy fed birds (1.58 versus 2.77). Feeding coarsely ground NSP resulted in lower dopamine turnover compared with feeding finely ground NSP (1.62 versus 2.64). NSP concentration did not affect brain parameters. Serotonin turnover was reduced in the low energy treatments, in which less feather pecking behavior was observed. In contrast with our findings, Van Hierden et al. (2004) reported that serotonin turnover was negatively associated with the frequency of feather pecking. These conflicting results showed that stress and behavior indicators in blood and brains could be related to nutritional factors. More research, however, is necessarily to understand the mode of action of these indicators.

Table 7. Serotonin and dopamine turnover, and noradrenalin concentration (mg/g) in the forebrains

Treatment	Serotonin turnover	Dopamine turnover	Noradrenalin
Standard Energy			
Standard NSP	1.132	2.873	0.547
High NSP-Fine	1.210	3.035	0.569
High NSP-Coarse	1.042	2.079	0.611
Low Energy			
Standard NSP	0.640	1.328	0.599
High NSP-Fine	1.041	2.244	0.590
High NSP-Coarse	0.805	1.165	0.631
Standard error	1.146	1.197	1.048
***P*-Value**			
Energy	0.058	**0.014**	0.447
NSP	0.376	0.956	0.488
Energy*NSP	0.310	0.508	0.703
Coarseness	0.309	0.052	0.402
Energy*Coarseness	0.794	0.612	0.980

SUGGESTIONS FOR FUTURE RESEARCH

In the current project, the direct effect of feed characteristics on eating time and feed passage rate, and the indirect effect on feather pecking behavior were studied. The mechanisms that determine feed intake regulation, however, have not been investigated. Scientists agree that animals in general aim to have a balance between energy use and energy intake (Schwartz et al., 2000; Berthoud, 2002). This energy balance is controlled by the central neural system. This neural network is sensible for short term and long term changes in energy need. Finally, this central nerves system will control eating behavior (eating time, eating rate, meal size, inter meal interval, meal content). Short term signals affect the level of satiety, eating time, meal size, digestion and absorption processes and thermoregulation.

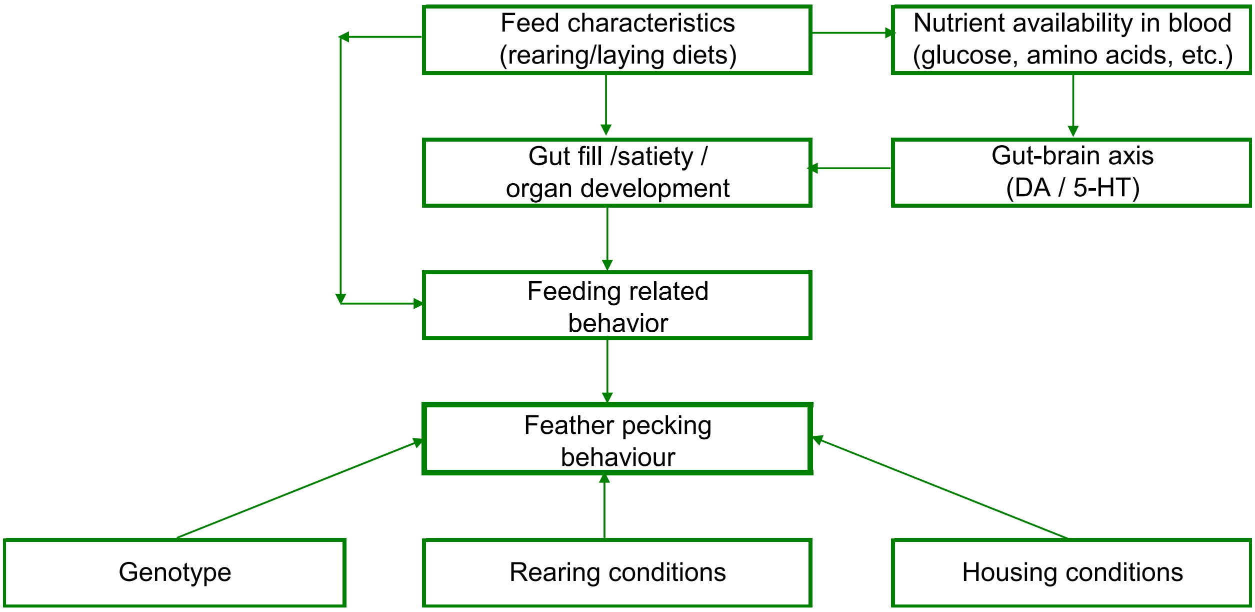
Birds of a high and a low feather pecking line differed in the activity of their Dopamine (DA) systems (van Hierden et al., 2005) and an inverse relationship between the level of DA and feather pecking has been found (Kjaer et al., 2004). Thus, stimulation of both the central 5-HT and DA systems apparently decreases the expression of feather pecking in laying hens.

Apart from 5-HT and DA, several other neurotransmitters and neuro modulators are involved in the regulation of feed intake and feeding behavior, and they simultaneously play a role in behavioral and mood disorders (e.g. obsessive compulsive disorders, depression). Future experiments have to investigate the role of cholecystokinine (CCK) and neuropeptide Y (NPY) both part of the gut-brain axis (Jimerson and Wolfe, 2004), glucose (De Leeuw et al., 2004) and the stress hormone corticosterone in feather pecking. CCK, NPY, glucose (insulin) and corticosterone exert some of their effects on behavior and appetite through interaction with 5-HT and DA pathways, e.g. (Morley and Blundell, 1988).

Thus, alterations in feeding management can affect feather pecking via changes in the 5-HT and DA pathways, blood parameters and gastro intestinal characteristics, as shown in a modified scheme (Figure 5). New experiments might focus on the impact of selected feed characteristics, like energy dilution, (coarsely) ground insoluble Non Starch Polysaccharides (NSP), and tryptophan in the diets of pullets and laying hens on their feather pecking behavior. The mode of action of such nutritional factors on feather pecking will be established by determining the underpinning neuro endocrine (5-HT and/or DA pathways), physiological (corticosterone, glucose, energy metabolism) and behavioral (feeding, foraging) mechanisms. The potential effects of variations in the birds' housing and rearing conditions should also be taken into account. A better understanding of the relationships between these internal and external factors might lead to new solutions to the feather pecking problem, and hence improve poultry welfare.

Feather damage and feather pecking behavior are important parameters in experiments that aimed to reduce the incidence of feather pecking. In our studies, it was decided to keep the hens under practical conditions. Hens had the possibility to perform ground pecking behavior in their pens. Feather pecking behavior was not controlled, and this behavior was or nearly absent (Chapter 3 and 6), or relatively violent (Chapter 4), and both situations were not perfect. In new experiments, it might be better to test nutritional factors under challenged feather pecking conditions. An appropriate model to stimulate gentle and severe feather pecking behavior in young rearing hens, is described by Van Hierden (2003). We advise to combine such a model and our approach in future experiments in the area of nutrition and feather pecking.

Figure 5. Modified scheme of possible pathways between feed characteristics and feeding related behavior and feather pecking



ECONOMIC RETURNS

Feather pecking hens cause damage to the plumage and loss of feathers. This adversely affects the costs of egg production, since loss of feathers results in increased feed intake, feed conversion, and feeding costs (Leeson and Morrisson, 1978; Tullet et al., 1980; Keeling et al., 1988; Herremans et al., 1989; Peguri and Coon, 1993). Furthermore, feather pecking can cause injury and bleeding, thereby increasing the risk of cannibalism (Allen and Perry, 1975; Hughes, 1982; Blokhuis et al., 2000). In this section, the economical impact of different feeding scenario's were estimated. For this, a computer model (BedrijfsWijzer Pluimvee) was used as a tool to simulate the effects of some feeding strategies on economical parameters (Vermeij and Kanis, 2005). Costs and yield are adjust on a free-range farm (25,000 hens). Feed and egg prices, and costs of young laying hens, mortality and manure delivery are based on KWIN-V (Animal Sciences Group, 2007). Five different strategies are compared:

1) *No feather pecking*:

   This strategy describes the economical status of a flock in which no pecking behavior occurs, with a standard mortality level of 9%.

2) *Feather pecking*:

   The effects of an outbreak of feather pecking are simulated. Because of less feather cover, heat loss increased. For maintaining thermoregulation, feed intake was assumed to increase by 10% (Peguri and Coon, 1993). Consequently, manure production also increased by 10%. The impact of feather pecking behavior and subsequent cannibalism on mortality level could range from absent (Huber Eicher and Sebo, 2001) to more than 10% (Johnsen et al., 1998). In this scenario, the level of mortality was set on 14%.

3) *Diluted diet during laying*:
   Hens are fed a 10% diluted high NSP diet. Therefore, feed intake and manure production both increased by 10%. The costs of feed decreased by 5%. This scenario was supposed to prevent feather pecking behavior (Chapter 6) and therefore, feather condition and mortality were similar with strategy 1.
4) *Diluted diet during rearing*:
   From Chapter 6, it can be concluded that providing a diluted diet during rearing resulted in an improved feather condition during laying. In this simulation, rearing hens were fed a 15% diluted diet. The costs per kg of feed reduced by 5%, whereas manure production of the rearing hens increased by 15%.
5) *Diluted diet during both rearing and laying*:
   This simulation combined the measurements of the strategies 4 and 5.

The technical parameters, costs, yields and gross margins per scenario, expressed per 100 purchased 17 wk laying hens, are shown in Table 8. Gross margin of the default (scenario 1) amounted € 410 per 100 purchased hens. An outbreak of feather pecking and cannibalistic behavior might have a tremendous effect on economical performance. Based on the assumptions of scenario 2, gross margin even decreased by 44%. Providing a 10% diluted laying diet (scenario 3) increased costs of feeding and manure delivery. Assuming that this strategy successfully prevent feather pecking and cannibalism, gross margin only decreased by 10% compared to scenario 1. This scenario, however, will not be effective if hens developed already feather pecking behavior during the rearing period (Chapter 4).

Table 8. Technical parameters, costs, yields and gross margins per scenario, expressed per 100 purchased 17-wk laying hens

Scenario	1 No feather pecking	2 Feather pecking	3 10% diluted laying diet	4 15% diluted rearing diet	5 Diluted rearing and laying diet
Technical and economical parameters					
Feed intake (g/h/d)	121	133	133	121	133
Egg production/purchased hen	318	302	318	318	318
Feed price (€/100 kg)	19.30	19.30	18.34	19.30	18.34
Egg price (€/100 eggs)	5.40	5.40	5.40	5.40	5.40
Mortality (%)	9	14	9	9	9
Manure production (kg/hen/year)	18.0	19.8	19.8	18.0	19.8
Yield (€/100 purchased hens)					
Eggs	1711	1625	1711	1711	1711
Slaughtering hens	20	19	20	20	20
Costs (€/100 purchased hens)					
17-wk laying hen (€)	350	350	350	372	372
Feed	940	1029	975	940	975
Manure delivery	32	36	36	32	36
Gross margin (Yield – Costs) (€)	410	229	370	387	348

Therefore, it might be more safe to provide diluted diets to rearing birds from day-old onwards (scenario 4). Birds than become imprinted on feed very early in life, which might prevent feather pecking at adult stage (Braastad, 1990; Nicol et al., 2001). This feeding strategy increased costs of rearing hens by 6%, thereby also decreasing gross margin by 6%. Combining scenario 3 and 4 is probably most effective in diminishing the risk of a cannibalistic outbreak of feather pecking. The premium for this risk reduction amounted 15% of gross margin (€ 348 vs. € 410/100 purchased hens; scenario 5). This gross margin, however, is still considerable higher than that of the feather pecking flock. In scenario 2, it was assumed that feed intake increased by 10% and mortality by 5%, but the effects of feather pecking on these parameters could be more or less pronounced, depending of the severity of the outbreak, Gross margin will be reduced with € 10.20/100 purchased hens for each percent extra feed intake. Gross margin will be reduced with € 15.90/100 purchased hens for each percent extra mortality.

We have to realize that these results are only indicative, based on the price levels of 2007/2008 (Animal Sciences Group, 2007). Alterations in price levels might affect the current conclusions. For instance, an increase of the feed price will enlarge the differences in gross margins of scenario 2 – 5 compared to scenario 1. At changing price levels, feed producers and advisers have to update these calculations to estimate the actual costs that have to be paid for preventing feather pecking behavior.

PRACTICAL IMPLICATIONS OF THIS STUDY

Increasing feeding related behavior and satiety by dietary manipulation are successful strategies in preventing feather pecking behavior, as long as this behavior is not developed in an earlier stage. In laying hens, nutrient dilution and addition of (coarse) insoluble NSP increase feeding related behavior, as expressed by prolonged eating time and decreased eating rate. Providing 15% diluted diets to rearing hens results in less feather damage during the laying period. Although dilution of the rearing diet does not prolong eating time in this stage, this might stimulate imprinting of pecks on feed, rather than on feathers of flock mates. Feeding related behavior and satiety of laying hens are mostly affected by eating diets with a high insoluble NSP content. Additive effects, however, are found if dietary energy content is reduced and the NSP source is coarsely ground. The most perspective full feeding strategy to prevent feather damage is the supply of a 15% diluted diet during the rearing period, followed by a 10% diluted – coarsely ground – high NSP diet during the laying period.

SUMMARIZING CONCLUSIONS

The studies described in this thesis have shown results which allowed the following conclusions:

Feed intake

- Rearing and laying hens strongly adjust feed intake on their energy need. Dilution levels up to 15% in rearing diets and up to 20% in laying diets (by adding sand to a control diet) are fully compensated by a proportional higher feed intake.
- Rearing hens are not able to fully compensate their energy intake if whole oat hulls are used as dilution source in the 10% and 15% diluted diets.
- An adjusted feed intake in laying hens as a result of dilution with sand or NSP sources will not affect egg performance.

Eating time and eating rate

- In the laying period, sand addition to the diet will prolong eating time without a large effect on eating rate.
- In the rearing period, sand addition to the diet increases eating rate, without prolonging eating time.
- Eating time is linearly prolonged as the daily insoluble NSP intake increases.

Empty gizzard weight and mean retention time

- Nutrient dilution does not affect empty gizzard weight and mean retention time in the foregut.
- Coarsely ground insoluble NSP's, in particular the cellulose and hemicellulose fractions, increase empty gizzard weight.
- Mean retention time in the foregut, i.e., the crop, proventriculus and gizzard, is linearly related to the daily consumption of insoluble NSP's. This relation is more pronounced in coarsely compared to finely ground NSP's.
- Overall mean retention time is not affected by the NSP content of the diet, indicating that passage rate of digesta increases in the intestines and hindgut.

Feather damage

- The combined supply of a low energy - coarsely ground - high insoluble NSP layer diet might delay by about 10 wks the increase in feather damage during the layer period.
- The supply of a 15% diluted diet, either by sand or oat hulls, during the rearing period results in less feather damage during the laying period, irrespective of the NSP concentration of the layer diet.

REFERENCES

Aerni, V., H. El Lethey and B. Wechsler. 1999. Feather pecking by laying hens: effects of foraging material and food form. *Fachgruppe Verhaltensforschung vom 12. bis 14. November 1998 in Freiburg Breisgau. KTBL Schrift*:63-69.

Aerni, V., H. El Lethey and B. Wechsler. 2000. Effect of foraging material and food form on feather pecking in laying hens. *Br. Poult. Sci.,* 41:16-21.

Al Bustany, Z. and K. Elwinger. 1988. Whole grains, unprocessed rapeseed and beta-glucanase in diets for laying hens. *Swedish Journal of Agricultural Research,* 18:31-40.

Allen, J. and G. C. Perry. 1975. Feather pecking and cannibalism in a caged layer flock. *British Poultry Science,* 16:441-451.

Animal Sciences Group. 2007. KWIN-V; Kwantitatieve Informatie Veehouderij 2007-2008. *Animal Sciences Group*. Lelystad.

Bach Knudsen, K. E. 2001. The nutritional significance of "dietary fibre" analysis. *Animal Feed Science and Technology,* 90:3-20.

Berthoud, H. R. 2002. Multiple neural systems controlling food intake and body weight. *Neuroscience and Biobehavioral Reviews,* 26:393-428.

Blokhuis, H. J. 1986. Feather-pecking in poultry: its relation with ground-pecking. *Applied Animal Behaviour Science,* 16:63-67.

Blokhuis, H. J. and J. W. Van der Haar. 1989. Effects of floor type during rearing and of beak trimming on ground pecking and feather pecking in laying hens. *Appl. Anim. Behav. Sci.,* 22:359-369.

Blokhuis, H. J., E. D. Ekkel, S. M. Korte, H. Hopster and C. G. v. Reenen. 2000. Farm animal welfare research in interaction with society. *Veterinary Quarterly,* 22:217-222.

Blokhuis, H. J., T. Fiks-Van Niekerk, W. Bessei, A. Elson, D. Guémené, J. Kjaer, G. A. Maria Levrino, C. J. Nicol, R. Tauson, C. A. Weeks and H. A. Van de Weerd. 2007. The LayWel project: welfare implications of changes in production systems for laying hens. *World's Poult. Sci. J.,* 63:101-114.

Braastad, B. O. 1990. Effects on behaviour and plumage of a key-stimuli floor and a perch in triple cages for laying hens. *Applied Animal Behaviour Science,* 27:127-139.

Dawkins, M. S. 1989. Time Budgets in Red Junglefowl as a Baseline for the Assessment of Welfare in Domestic-Fowl. *Appl. Anim. Behav. Sci.,* 24:77-80.

De Leeuw, J. A., A. W. Jongbloed and M. W. A. Verstegenl. 2004. Dietary fiber stabilizes blood glucose and insulin levels and reduces physical activity in sows (Sus scrofa). *Journal of Nutrition,* 134:1481-1486.

Elwinger, K. 1981. Different energy levels and restricted feeding to three strains of SCWL hybrids. 1. Effects on egg production. *Swedish J. Agric. Res.,* 11:149-157.

Garner, J. P. and G. J. Mason. 2002. Evidence for a relationship between cage stereotypes and behavioural disinhibition in laboratory rodents. *Behav. Brain Res.,* 136:83-92.

Gentle, M. J. and L. N. Hunter. 1990. Physiological and behavioural responses associated with feather removal in *Gallus gallus var domesticus*. *Research in Veterinary Science,* 50:95-101.

Graham, H. and P. Aman. 1991. Nutritional Aspects of Dietary-Fibers. *Animal Feed Science and Technology,* 32:143-158.

Gross, W. B. and H. S. Siegel. 1983. Evaluation of the Heterophil Lymphocyte Ratio as a Measure of Stress in Chickens. *Avian Diseases,* 27:972-979.

Hartini, S., M. Choct, G. Hinch and J. V. Nolan. 2003. Effect of diet composition, gut microbial status and fibre forms on cannibalism in layers. *Australian Egg Corporation Limited.* AECL Publication No 03/03:1-111.

Hartini, S., M. Choct, G. Hinch, A. Kocher and J. V. Nolan. 2002. Effects of light intensity during rearing and beak trimming and dietary fiber sources on mortality, egg production, and performance of ISA brown laying hens. *J. Appl. Poult. Res.,* 11:104-110.

Herremans, M., E. Decuypere and O. Siau. 1989. Effects of feather wear and temperature on prediction of food intake and residual food consumption. *British Poultry Science,* 30:15-22.

Hetland, H., B. Svihus and A. Krogdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *Br. Poult. Sci.,* 44:275-282.

Hetland, H., B. Svihus and M. Choct. 2005. Role of insoluble fiber on gizzard activity in layers. *J Appl. Poult. Res.,* 14:38-46.

Hetland, H., R. O. Moe, R. Tauson, S. Lervik and B. Svihus. 2004. Effect of including whole oats into pellets on performance and plumage condition in laying hens housed in conventional and furnished cages. *Acta Agric. Scand., Section A, Anim. Sci.,* 54:206-212.

Huber Eicher, B. and L. Audige. 1999. Analysis of risk factors for the occurrence of feather pecking in laying hen growers. *British Poultry Science,* 40:599-604.

Huber Eicher, B. and F. Sebo. 2001. The prevalence of feather pecking and development in commercial flocks of laying hens. *Appl. Anim. Behav. Sci.,* 74:223-231.

Hughes, B. O. 1982. Feather pecking and cannibalism in domestic fowl. In: *Disturbed Behaviour in Farm Animals*. Ed. W. Bessei. Verlag Eugen Ulmer 121:138-146.

Hughes, B. O. and I. J. H. Duncan. 1972. The influence of strain and environmental factors upon feather pecking and cannibalism in fowls. *British Poultry Science,* 13:525-547.

Jimerson, D. C. and B. E. Wolfe. 2004. Neuropeptides in eating disorders. *CNS Spectrums,* 9:516-522.

Johnsen, P. F., K. S. Vestergaard and G. Norgaard Nielsen. 1998. Influence of early rearing conditions on the development of feather pecking and cannibalism in domestic fowl. *Applied Animal Behaviour Science,* 60:25-41.

Jones, R. B. and P. M. Hocking. 1999. Genetic selection for poultry behaviour: big bad wolf or friend in need? *Animal Welfare,* 8:343-359.

Keeling, L. J. and J. F. Hurnik. 1996. Social facilitation acts more on the appetitive than the consummatory phase of feeding behaviour in domestic fowl. *Animal Behaviour,* 52:11-15.

Keeling, L. J., B. O. Hughes and P. Dun. 1988. Performance of free-range laying hens in a polythene house and their behaviour on range. *Farm Building Progress,* 94:21-28.

Kjaer, J. B. and P. M. Hocking. 2004. The genetics of feather pecking and cannibalism. In: *Welfare of the Laying Hen*. Ed. G. C. Perry. CABI Publishing 109-122.

Kjaer, J. B., B. M. Hjarvard, K. H. Jensen, J. Hansen-Møller and O. Naesbye Larsen. 2004. Effects of haloperidol, a dopamine D2 receptor antagonist, on feather pecking behaviour in laying hens. *Applied Animal Behaviour Science,* Volume 86:77-91.

Klein, T., E. Zeltner and B. Huber Eicher. 2000. Are genetic differences in foraging behaviour of laying hen chicks paralleled by hybrid-specific differences in feather pecking? *Applied Animal Behaviour Science,* 70:143-155.

Korte, S. M. 2001. Corticosteroids in relation to fear, anxiety and psychopathology. *Neuroscience and Biobehavioral Reviews,* 25:117-142.

Leeson, S. and W. D. Morrisson. 1978. Effect of feather cover on feed efficiency in laying birds. *Poultry Science,* 57:1094-1096.

Leonard, M. L., A. G. Horn and R. W. Fairfull. 1995. Correlates and consequences of allopecking in White Leghorn chickens. *Applied Animal Behaviour Science,* 43:17-26.

Moore, S. J. 1999. Food breakdown in an avian herbivore: who needs teeth? *Austr. J. Zoology,* 47:625-632.

Morley, J. E. and J. E. Blundell. 1988. The neurobiological basis of eating disorders: Some formulations. *Biological Psychiatry,* 23:53-78.

Newberry, R. C., L. J. Keeling, I. Estevez and B. Bilcik. 2006. Behaviour when young as a predictor of severe feather pecking in adult laying hens: The redirected foraging hypothesis revisited. *Appl. Anim. Behav. Sci.*: doi:10.1016/j.applanim.2006.10.010.

Nicol, C. J., A. C. Lindberg, A. J. Phillips, S. J. Pope, L. J. Wilkins and L. E. Green. 2001. Influence of prior exposure to wood shavings on feather pecking, dustbathing and foraging in adult laying hens. *Applied Animal Behaviour Science,* 73:141-155.

Nørgaard-Nielsen, G., K. S. Vestergaard and H. B. Simonsen. 1993. Effects of rearing experience and stimulus enrichement on feather damage in laying hens. *Applied Animal Behaviour Science,* 38:345-352.

Peguri, A. and C. Coon. 1993. Effect of feather coverage and temperature on layer performance. *Poultry Science,* 72:1318-1329.

Read, N. W. 1992. Role of Gastrointestinal Factors in Hunger and Satiety in Man. *Proceedings of the Nutrition Society,* 51:7-11.

Savory, C. J. 1980. Meal occurrence in Japanese quail in relation to particle size and nutrient density. *Anim. Behav.,* 28:160-171.

Savory, C. J. 1999. Temporal control of feeding behaviour and its association with gastrointestinal function. *Journal of Experimental Zoology,* 283:339-347.

Schneeman, B. O. 1986. Dietary Fiber - Physical and Chemical-Properties, Methods of Analysis, and Physiological-Effects. *Food Technology,* 40:104-110.

Schwartz, M. W., S. C. Woods, P. Y. Porte, R. J. Seeley and D. G. Bas Kin. 2000. Central nervous system control of food intake. *Nature,* 404:661-671.

Southgate, D. A. T. 1995. The Diet as a Source of Dietary Fiber. *European Journal of Clinical Nutrition,* 49:S22-S26.

Tanaka, T., T. Yoshimoto and K. Mimura. 1983. Feeding behaviour of laying hens fed pellets, crumbles or mash feed. *Japanese Journal of Zootechnical Science,* 54:433-438.

Tullet, S. G., M. G. Macleod and T. R. Jewitt. 1980. The effects of partial defeathering on energy metabolism in the laying fowl. *British Poultry Science,* 21:241-245.

Valkonen, E., E. Venäläinen, L. Rossow and J. Valaja. 2008. Effects of Dietary Energy Content on the Performance of Laying hens in Furnished and Conventional Cages. *Poult. Sci.,* 87:844-852.

Van der Meulen, J., C. Kwakernaak and C. A. Kan. 2006. Sand intake by laying hens and its effect on egg production parameters. *J. Anim. Nutr. Phys.,* DOI:_10.1111/j.1439-0396.2007.00732.x.

Van Hierden, Y. M., S. M. Korte and J. M. Koolhaas. 2003. Chronic increase of dietary L-Tryptophan decreases feather pecking behaviour. *Applied Animal Behaviour Science*:Submitted for publication.

Van Hierden, Y. M., S. F. d. Boer, S. M. Korte and J. M. Koolhaas. 2004. The control of feather pecking by serotonin. *Behavioural Neuroscience,* 118:575-583.

Van Hierden, Y. M., S. M. Korte, E. W. Ruesink, C. G. v. Reenen, B. Engel, G. A. H. Korte Bouws, J. M. Koolhaas and H. J. Blokhuis. 2002. Adrenocortical reactivity and central serotonin and dopamine turnover in young chicks from a high and low feather-pecking line of laying hens. *Physiology and Behavior,* 75:653-659.

van Hierden, Y. M., J. M. Koolhaas, P. Košťál, P. Výboh, M. Sedlacková, M. Rajman, M. Juráni and S. M. Korte. 2005. Chicks from a high and low feather pecking line of laying hens differ in apomorphine sensitivity. *Physiology and Behavior,* 84:471-477.

Van Soest, P. J. and R. W. McQueen. 1973. The chemistry and estimation of fiber. *Proc. Nutr. Soc.,* 32:123-130.

Vermeij, I. and J. Kanis. 2005. BedrijfsWijzer Pluimvee geeft u het antwoord: wijziging zinvol en haalbaar? *De Pluimveehouderij,* 35:25.

Zorrilla, G. 1998. Hunger and satiety: Deceptively simple words for the complex mechanisms that tell us when to eat and when to stop. *Journal of the American Dietetic Association,* 98:1111.

SUMMARY

SUMMARY

Feather pecking remains one of the major problems facing the poultry industry because it is a significant welfare insult for the hens, an economic burden for the farmer, and a pressing societal concern. Hens peck and pull at the feathers of conspecifics, causing damage to the plumage and loss of feathers. This adversely affects the costs of egg production, since loss of feathers results in increased feed intake, feed conversion, and feeding costs. Furthermore, feather pecking, which is painful in itself, can cause injury and bleeding, thereby increasing the risk of cannibalism. Thus, apart from these serious economic losses, there is also an ethical aspect to the problem. Clearly, feather pecking is extremely detrimental to the welfare of the birds.

Beak trimming is a common and effective precautionary measure practiced by poultry farmers to prevent serious feather damage and mortality. However, it has associated welfare problems. Beak trimming can cause acute and chronic pain, and it has therefore already been prohibited in several European countries, such as Norway, Sweden, and Switzerland. In the Netherlands beak trimming is expected to be banned from 2011.

In 2012, changes in EU-legislation with regard to animal welfare and husbandry will be implemented that might increase the risk of feather pecking in layers. These changes include the ban on beak trimming and a ban on the use of traditional battery cages. The latter ban results from a societal debate that led to the conclusion that battery cages could not fulfill the birds' need to express their natural behavior. Clearly, it is imperative that we develop viable alternative housing systems for layers, e.g., free range or aviaries. However, a widespread introduction of such alternatives, specifically designed with the aim to improve poultry welfare, is hampered by the increased likelihood of outbreaks of feather pecking and cannibalism. This may occur because the presence of a few feather peckers in a free housing system has a much greater impact because larger numbers of potential victims are present. Feather pecking in layers is still a very dominant welfare problem in non-cage housing systems.

Jungle fowl that are housed in a semi-wild environment have a high foraging motivation, spending 60% of their active time on feeding related behaviors, like eating and foraging. Feeding related behavior, however, is significantly reduced in laying hens housed in modern housing systems. Feather pecking behavior has been hypothesized to arise from ground pecking

behavior or feeding behavior that is redirected towards feathers in the absence of adequate foraging incentives. The level of feather pecking is inversely related to the time spent feeding and foraging. Furthermore, the ontogeny of feather pecking behavior in mature hens seems to be influenced by early life experiences and rearing conditions. But also the development of the digestive tract during the rearing period, by appropriate nutritional strategies, that results in an appropriate volume and digestive capacity of the gut at the beginning of lay, is thought to be of great importance in determining the expression of feather pecking behavior during the laying period.

The assumption that feather pecking behavior is a substitute for normal ground pecking or feeding behavior in the absence of adequate foraging incentives justifies a nutritional approach of this problem. It is hypothesized here, that nutritional factors may reduce feather pecking behavior if these factors increase:

1) the time hens are spending on feeding related behavior, or
2) the (temporary) level of satiety by affecting retention time of digesta in the gut.

Although energy and Non Starch Polysaccharides (NSP) concentrations and particle size of the added NSP source seem to reduce feather pecking behavior in laying hens, these nutritional factors were often confounded in experimental diets. Consequently, it’s not clear which factor is most effective in causing these positive effects. In this project, the relationships between feed characteristics – satiety/ development of gut segments – feeding related behavior – feather pecking behavior are studied.

The objectives of the present study were:

1. To review the impact of nutritional factors and feeding strategies on feather pecking behavior in laying hens.
2. To determine the independent effects of nutrient density, NSP concentration, and particle sizes of NSP on eating behavior, feather pecking, performance and digesta mean retention time in laying hens between 18 and 40 wk of age.
3. To investigate the carry-over effects of nutrient density and NSP concentration in rearing diets on eating behavior, feather pecking and performance in laying hens.

The overall aim of the project is to provide the basis for the development of a new feeding strategy that meet the requirements of the modern layer breeds and help to solve the feather pecking problem in current husbandry systems.

This thesis provides a literature review and describes the results of three experiments in which different nutritional factors were tested in hens of varying ages. For essential nutrients, like amino acids, minerals and vitamins, nutrients to energy ratio's were similar in all diets within an experiment. All experiments were carried out with ISA Brown hens.

Chapter 2 discusses the relative importance of specific deficiencies in layer diets, as well as the effectiveness and possible modes of action of certain nutritional factors and feeding strategies on feather pecking behavior in laying hens. Nutritional factors can have positive and negative effects on feather pecking behavior in laying hens. Severe feather pecking has been demonstrated in birds that were fed a too low mineral level in the diet, a too low protein level or a too low amino acid level (methionine, arginine). Sometimes somewhat more feather pecking was found when layers were fed diets with mainly vegetable protein sources as compared with diets with protein from animal origin. Also more feather pecking may occur when the diets were fed restrictedly, fed coarsely ground, or fed as pellets. Feeding high-fiber diets, low energy diets, or roughages reduced feather pecking. Providing additional grain or straw in the litter during rearing could result in lower levels of feather pecking behavior in adult stages. Some of these positive effects on feather pecking seem to be related to the time birds spend on feed intake and foraging. From this review study it was decided to focus the following experiments on the effects of nutrient dilution, NSP concentration and particle sizes of NSP.

In Chapter 3, the effects of dietary dilution, particle sizes of NSP, and feed form on feed intake, eating behavior and performance of laying hens at early lay were investigated. An experiment with 480 ISA Brown layer strains was conducted to measure the effect of dietary energy (11.8, 11.2 and 10.6 MJ/kg) and NSP (128, 146 and 207 g/kg) concentration, soluble NSP content (64 and 85 g/kg), particle size distribution of the NSP fraction (fine and coarse) and feed form (mash and crumble) on feed intake, eating time and egg-performance of laying hens in early lay (from 18 to 26 weeks of age). Twelve experimental diets were tested, each replicated four times. Diets were diluted by low NSP (sand and grit), or by high NSP sources (oat hulls, straw, soya hulls, cellulose fiber, beet pulp, and sunflower meal). The high NSP sources differed in water solubility of the NSP fraction, which may affect feed intake, feeding related behavior, viscosity of the digesta and feed passage rate. Control diets and sand diluted diets were provided both in mash and crumble. Differences in particle sizes were created by adding finely ground versus whole oat hulls. Laying hens in early lay that were fed low- or high-NSP diets

were able to compensate for 10% dietary dilution by a 9.5 and 4.9% higher feed intake, respectively. Feeding crumble or coarsely ground mash did not affect feed intake. Eating time of the hens fed the undiluted diets increased over the experimental period from 16.4 to 24.6% of the observation period, but was not affected by sand or grit addition, particle size distribution or feed form. Feeding high-NSP diets increased eating time by 22%. Egg performance and body gain of the hens that were fed low-NSP or high-NSP diets were similar or better compared to the undiluted diets, whereas coarse grinding of the diets showed 7-10% lower egg performance and body gain. Egg performance and body gain were not affected by feed form. It is concluded that hens in early lay, that were fed energy diluted diets, as a result of addition of sand or grit (low-NSP) or NSP-rich raw materials (high-NSP) to the control diet, were able to increase their feed intake, resulting in a comparable energy intake and egg performance as the control group. Supplementing diets with insoluble NSP also decreased eating rate. Prolonged eating time using insoluble NSP could be useful in reducing feather pecking behavior.

Based on the results of Chapter 3, in the further experiments (Chapters 4 to 6) sand was chosen as dilution source in low NSP diets, whereas oat hulls were chosen to increase the NSP concentration. Therefore, Chapter 4 describes an experiment in which the separate effects of energy concentration, NSP concentration and particle size of added NSP source on eating behavior, feather pecking behavior and hen performance of laying hens were investigated. This experiment was conducted with 504 non-cage housed ISA Brown laying hens from 18 to 40 weeks of age. Hens were allotted to 1 of 6 dietary treatments according to a 2 x 3 factorial arrangement, with 7 replicates per treatment. The factors were control and low energy concentration (2825 versus 2540 kcal/kg), control and high NSP concentration (133 versus 195 g/kg), and fine versus coarse particle size of the added NSP source in the NSP high diets. We hypothesized that eating time will be increased by feeding low energy diets, and/or coarsely ground NSP high diets, resulting in reduced feather pecking behavior, without negatively affecting hen performance. Energy reduction, NSP addition and coarse grinding of NSP increased eating time by 14.2% ($P=0.001.$), 17.2% ($P<0.001$) and 7.9% ($P=0.075$), respectively, compared with the control level of these factors. NSP addition decreased eating rate (g/min) by 21.0% ($P=0.010$). Layers performed already gentle feather pecking behavior during the 5th week of the rearing period. Dietary treatments did not affect maximal level of feather condition scores, but arise of feather damage was delayed by 10 weeks in hens fed low energy, coarsely ground NSP rich diets compared to hens fed control diets. Hens fed control

NSP diets showed reduced culling rates, due to less cannibalistic pecking, if energy concentration was decreased (44.1% versus 13.1%), whereas in high NSP diets culling rate slightly decreased when hens were fed low energy diets (31.6% versus 28.6%) (*P*=0.071). Hens that were fed low energy diets compensated for 10% reduction in energy concentration by 9.3% higher maximal feed intake (143.0 versus 130.8 g/d). Hen performance and body gain of the hens were not affected by dietary treatments. It is concluded that hens that were fed low energy or high (coarsely ground) NSP diets spend more time on feed intake, compared with hens that were fed control diets. As a result, some treatments showed less feather pecking behavior.

Chapter 5 provides mean retention time per gut segment (crop, proventriculus/ gizzard, small intestine, colon and caeca) of diets varying in dilution level, NSP concentration and particle sizes of NSP. In this chapter, the weights and contents of the different gut segments are also presented. These parameters were investigated at the end of the experiment, that was described in Chapter 4. Feed passage rate was determined in 5 birds per pen at 40 wks of age, thereby using 3 out of 7 pens per treatment. Titanium dioxide was used as a marker. Initially, (t = 0) 3 gelatin capsules were manually given to each of the 5 hens per pen. Birds were dissected at 5 successive times (t = 30, 90, 180, 270 and 360 min. after moment of titanium supplementation). After dissection, gut was removed from the body and subdivided in 5 different segments (Crop, proventriculus/gizzard, small intestine, caeca and colon). Digesta was collected from the gut segments by gentle squeezing. Each segment was weighed before and after removing of the digesta from the segment. Titanium concentration was analyzed in the 450 gut samples (5 segments/bird x 5 birds/pen x 6 treatments x 3 pens/treatment).

Increasing the dietary NSP concentration extended MRT in the crop (68.0 vs. 34.0 min.) and total foregut (90.6 vs. 56.8 min) compared to control NSP diets. Feeding low energy diets resulted in a longer MRT in the colon (26.0 vs. 6.7 min), caeca (3.9 vs. 1.8 min), and total hind gut (30.3 vs. 8.6 min) compared to control energy diets. Coarse grinding decreased MRT in the caeca compared to fine grinding (4.6 vs. 1.8 min). Overall MRT was not affected by dietary treatments. Feeding high NSP diets increased relative weights of the empty proventriculus/gizzard and it's contents by 30% (25.2 vs. 19.4 g/kg) and 18% (15.4 vs. 13.0 g/kg), respectively, compared to control NSP diets. In addition, relative empty proventriculus/gizzard weight of hens fed coarsely ground NSP was 30% higher compared to hens fed finely ground NSP (28.5 vs. 21.9 g/kg). It was concluded that addition of NSP to the diet may increase the weights of the gizzard and it's contents, and may extend MRT in the foregut. MRT in the foregut was linearly related to the daily insoluble NSP intake. The increase of MRT was more pronounced in hens fed coarsely compared to finely ground NSP. These findings seem to be indicators of higher levels of satiety in laying hens, which may contribute to a lower feather pecking pressure.

To investigate the carry-over effects of nutrient density and NSP concentration in rearing diets on eating behavior, feather pecking and performance in laying hens, an experiment was performed (Chapter 6). In this experiment different levels of nutrient dilution and NSP concentration in rearing and laying diets were applied. Feed intake, eating behavior, feather pecking and development of gut segments in rearing and laying hens were measured. This experiment was conducted with 768 non-cage housed ISA Brown pullets during the rearing period, of which 576 hens were followed during the laying period, to investigate the separate effects of dietary dilution and NSP concentration of rearing and laying diets on eating behavior, feather damage and performance. Day-old pullets were allotted to one of 6 dietary treatments according to a 3 × 2 factorial (3 dilution levels and 2 NSP concentrations), with 8 replicates (pens) per treatment. At 17 wks of age, pens with hens were allotted to 1 of 8 dietary treatments according to a 4 x 2 factorial arrangement (4 dilution levels and 2 NSP concentrations), with 6 replicates per treatment.

Compared to 0% dilution level, feed intake of laying hens of 10%, 15% and 20% dilution level increased by 8.4% (9.5 g/hen/d), 16.5% (18.1 g/hen/d) and 20.9% (23.6 g/hen/d), respectively. ME intake was similar for all dilution levels. Hens fed standard NSP laying diets had a similar insoluble NSP intake for all dilution levels (9.3 g/hen/d). Insoluble NSP intake of hens fed high NSP laying diets increased from 15.6 g/hen/d (0% dilution) to 18.9 g/hen/d (20% dilution). Providing high vs. standard NSP layer diet decreased proventriculus content (1.1 vs. 0.3 g/kg BW) and increased empty gizzard weight (14.3 vs. 24.4 g/kg BW). Hens that were fed standard NSP diets during laying had more feather damage compared to hens fed high NSP diets (0.58 vs. 0.30). Increasing the insoluble NSP intake resulted in decreased proventricular weight and increased gizzard weight and it’s contents, thereby linearly reducing feather damage. Providing diluted rearing diets increased feed intake from the first wks of life onwards. It was hypothesized that pullets were increasingly ‘imprinted’ on feed as pecking substrate if dilution level increased. This may decrease feather pecking and this could also explain the improved feather condition of the hens at 49 wk of age that were fed 15% diluted rearing diet.

In the General Discussion (Chapter 7), the results reported in the Chapters 2-6 are discussed and evaluated with respect to theories on feather pecking behavior and nutrition. Prospects for further research are suggested. Practical implications for feeding strategies of rearing and laying hens, which could prevent feather pecking behavior, are presented.

The studies described in this thesis have shown results which allowed the following conclusions:

Feed intake

- Rearing and laying hens strongly adjust feed intake on their energy need. Dilution levels up to 15% in rearing diets and up to 20% in laying diets (by adding sand to a control diet) are fully compensated by a proportional higher feed intake.
- Rearing hens are not able to fully compensate their energy intake if whole oat hulls are used as dilution source in the 10% and 15% diluted diets.
- An adjusted feed intake in laying hens as a result of dilution with sand or NSP sources will not affect egg performance.

Eating time and eating rate

- In the laying period, sand addition to the diet will prolong eating time without a large effect on eating rate.
- In the rearing period, sand addition to the diet increases eating rate, without prolonging eating time.
- Eating time is linearly prolonged as the daily insoluble NSP intake increases.

Empty gizzard weight and mean retention time

- Nutrient dilution does not affect empty gizzard weight and mean retention time in the foregut.
- Coarsely ground insoluble NSP's, in particular the cellulose and hemicellulose fractions, increase empty gizzard weight.
- Mean retention time in the foregut, i.e., the crop, proventriculus and gizzard, is linearly related to the daily consumption of insoluble NSP's. This relation is more pronounced in coarsely compared to finely ground NSP's.
- Overall mean retention time is not affected by the NSP content of the diet, indicating that passage rate of digesta increases in the intestines and hindgut.

Feather damage

- The combined supply of a low energy - coarsely ground - high insoluble NSP layer diet might delay by about 10 wks the increase in feather damage during the layer period.
- The supply of a 15% diluted diet, either by sand or oat hulls, during the rearing period results in less feather damage during the laying period, irrespective of the NSP concentration of the layer diet.

This study might have some practical implications. Increasing feeding related behavior and satiety by dietary manipulation are successful strategies in preventing feather pecking behavior, as long as this behavior is not developed in an earlier stage. In laying hens, nutrient dilution and addition of (coarse) insoluble NSP increase feeding related behavior, as expressed by prolonged eating time and decreased eating rate. Providing 15% diluted diets to rearing hens results in less feather damage during the laying period. Although dilution of the rearing diet does not prolong eating time in this stage, this might stimulate imprinting of pecks on feed, rather than on feathers of flock mates. Feeding related behavior and satiety of laying hens are mostly affected by eating diets with a high insoluble NSP content. Additive effects, however, are found if dietary energy content is reduced and the NSP source is coarsely ground. The most perspective feeding strategy to prevent feather damage is the supply of a 15% diluted diet during the rearing period, followed by a 10% diluted – coarsely ground – high NSP diet during the laying period.

SAMENVATTING

Verenpikgedrag blijft een van de grote problemen voor de legpluimveehouderij omdat dit gedrag het welzijn van hennen schaadt, economische verliezen voor de pluimveehouder met zich meebrengt en maatschappelijke bezorgdheid opwekt. Hennen kunnen namelijk pikken en trekken aan de veren van hokgenoten, wat resulteert in schade aan het verenkleed en verlies van veren. Dit heeft een negatief effect op de productiekosten van de eieren, omdat verenverlies leidt tot een verhoging van de voeropname, de voederconversie en de voerkosten. Bovendien kan verenpikgedrag, dat op zichzelf al pijnlijk is, verwondingen en bloedingen veroorzaken waardoor ook het risico op kannibalisme toeneemt. Naast de economische consequenties, is er dus tevens een ethisch aspect aan dit probleem. Het is duidelijk dat verenpikgedrag een bijzonder schadelijk effect heeft op het welzijn van de hennen.

Het behandelen van de snavels van hennen is een effectieve voorzorgsmaatregel ter voorkoming van ernstige verenschade en sterfte en deze behandeling wordt algemeen in de pluimveehouderij toegepast. Deze behandeling veroorzaakt tegelijkertijd echter ook weer andere welzijnsproblemen. Snavelbehandelen kan bij hennen acute en chronische pijn veroorzaken en is hierom al verboden in verschillende Europese landen, zoals Noorwegen, Zweden en Zwitserland. In Nederland wordt een verbod op snavelbehandelen in 2011 verwacht.

In 2012 zal nieuwe Europese wetgeving met betrekking tot huisvesting en houden van dieren van kracht worden met als doel het dierenwelzijn te verbeteren. Tegelijkertijd neemt door deze wetgeving het risico op verenpikken bij leghennen juist toe. Deze wijzigingen betreffen het verbod op snavelbehandelen en het verbod op het gebruik van traditionele kooisystemen. Het laatste verbod is het resultaat van een maatschappelijk debat, waarin vastgesteld is dat hennen in batterijkooien niet in staat zijn om hun natuurlijk gedrag uit te oefenen. Scharrel- en volièrestallen zijn voorbeelden van economisch levensvatbare alternatieve huisvestingssystemen voor leghennen. Het massaal overschakelen naar dergelijke systemen, die speciaal ontworpen zijn om het welzijn van pluimvee te verbeteren, wordt echter geremd door de verhoogde kans op uitbraken van verenpikken en kannibalisme. De aanwezigheid van enkele verenpikkers in een scharrelstal kan, vanwege de grote aantallen potentiële slachtoffers, namelijk een zeer groot effect hebben op de kwaliteit van het verenkleed in een koppel. Gebleken is dat verenpikken juist in niet-kooisystemen een groot welzijnsprobleem is.

Boskippen, die in een seminatuurlijke omgeving gehouden werden, bleken een hoge behoefte te hebben aan voergericht gedrag, zoals eten en foerageren. Ze besteedden ongeveer 60% van hun actieve tijd dit gedrag. In de huidige huisvestingssystemen voor leghennen komt voergericht gedrag echter duidelijk minder vaak voor. Ethologen veronderstellen dat verenpikgedrag eigenlijk een vorm is van grondpik- of eetgedrag. Dit gedrag kan bij afwezigheid van geschikt foerageersubstraat omgericht worden naar het verenkleed van hokgenoten. De mate van verenpikken is omgekeerd evenredig met de tijd die besteed wordt aan eten en foerageren. De oorsprong van het verenpikgedrag bij volwassen hennen lijkt samen te hangen met ervaringen in de vroege jeugd en met omstandigheden tijdens de opfokperiode. Ook het toepassen van de juiste voedingsstrategie tijdens de opfokperiode, resulterend in een goede ontwikkeling van het maag-darmkanaal op het moment dat de hennen aan de leg komen, lijkt van wezenlijk belang bij het tot uiting komen van verenpikgedrag tijdens de legperiode.

De veronderstelling dat verenpikken een vervanging is voor normaal grondpik gedrag of eetgedrag rechtvaardigt een voedingskundige benadering van dit probleem. Onze hypothese is dat voedingskundige factoren verenpikgedrag zullen verminderen, als deze factoren zorgen voor:

1) een toename van de tijd die hennen besteden aan voergericht gedrag, of
2) een (tijdelijke) verhoging van de mate van verzadiging, door beïnvloeding van de verblijftijd van de spijsbrij in het maag-darmkanaal.

Uit literatuuronderzoek bleek dat zowel het energiegehalte van het voer, als het plantaardige vezelgehalte (Non Starch Polysacchariden; NSP), als de deeltjesgrootte van de toegevoegde vezels in het voer kunnen bijdragen aan vermindering van het verenpikgedrag bij leghennen. Deze factoren bleken in proefvoeders vaak verstrengeld te zijn, zodat niet duidelijk was welke factor het meest effectief was in het veroorzaken van deze positieve effecten. In dit project is zijn de voedingsfactoren nutriëntendichtheid, NSP gehalte en deeltjesgrootte van NSP onafhankelijk van elkaar onderzocht. Nagegaan is wat het effect van deze factoren was op: 1) de mate van verzadiging en ontwikkeling van darmsegmenten, 2) de hoeveelheid voergericht gedrag en 3) het verenpikgedrag.

De doelstellingen in de project waren als volgt:

1. Het uitvoeren van een literatuurstudie naar het belang van voedingsfactoren en voerstrategieën bij het optreden van verenpikgedrag bij leghennen.

2. Het onafhankelijk van elkaar vaststellen van de effecten van nutriëntendichtheid, NSP-gehalte en deeltjesgrootte van NSP in het voer op eetgedrag, verenpikken, dierprestaties en verblijftijd van spijsbrij in het maag-darmkanaal bij leghennen.
3. Nagaan in hoeverre er sprake was van carry-over effecten van nutriëntendichtheid en NSP-gehalte in het opfokvoer op eetgedrag, verenpikgedrag en dierprestaties van hennen tijdens de legperiode.

Het uiteindelijke doel van dit project was het ontwikkelen van een nieuwe voerstrategie, die bij kan dragen aan het oplossen van de verenpikproblemen in de huidige legpluimveehouderij, zonder dat tekort gedaan wordt aan de voedingskundige behoeften van de moderne leghen.

Dit proefschrift bevat een literatuurstudie en beschrijft de resultaten van een drietal experimenten waarin verschillende voedingsfactoren zijn uitgetest bij hennen met verschillende leeftijden. Voor alle essentiële nutriënten, zoals aminozuren, mineralen en vitaminen, gold dat de energie : nutriënt verhouding gelijk was bij alle voeders binnen een experiment. Alle experimenten zijn uitgevoerd met hennen van het merk ISA Brown.

In hoofdstuk 2 worden de effecten van voedingskundige tekorten in legvoeders, evenals de effectiviteit en mogelijke werkingsmechanismen van bepaalde voedingsfactoren en voerstrategieën op verenpikgedrag van leghennen besproken. Voedingskundige factoren kunnen het verenpikgedrag zowel positief als negatief beïnvloeden. Het optreden van ernstige vormen van verenpikken is aangetoond bij hennen die voer verstrekt kregen met te lage gehalten aan mineralen, eiwit of aminozuren (methionine, arginine). In sommige experimenten nam het verenpikgedrag toe als voer verstrekt werd dat uitsluitend eiwit bevatte van plantaardige herkomst in vergelijking met voer dat ook dierlijk eiwit bevatte. Ook kan het verenpikgedrag toenemen als leghennen beperkt gevoerd worden, als het voer grof gemalen is, of als het voer in gepelleteerde vorm verstrekt wordt. Het verstrekken van voer met een hoog vezelgehalte of een laag energiegehalte, of het bijvoeren van ruwvoer verminderde juist het verenpikgedrag. Het verspreiden van extra graan of stro over het strooisel tijdens de opfokperiode bleek te kunnen leiden tot minder verenpikken tijdens de legperiode. Sommige van deze positieve effecten op het verenpikgedrag lijken samen te hangen met de tijd die hennen besteden aan voeropname- en foerageergedrag. Op basis van de resultaten die in dit hoofdstuk beschreven zijn, is besloten om de hierop volgende experimenten te richten op nutriëntenverdunning, NSP gehalte en deeltjesgrootte van NSP in het voer.

In hoofdstuk 3 is het effect bestudeerd van nutriëntverdunning, deeltjesgrootte van NSP en de vorm van het voer op voeropname, eetgedrag en legprestaties van leghennen aan het begin van de legperiode (18 tot 26 weken leeftijd). Hiertoe is een experiment uitgevoerd met 480 ISA Brown leghennen. In dit experiment werd het effect van drie energieniveaus (11,8, 11,2 en 10,6 MJ/kg), drie NSP niveaus (128, 146 en 207 g/kg), twee niveaus van oplosbaar NSP (64 en 85 g/kg), twee niveaus van deeltjesgrootte van de NSP-fractie (fijn versus grof) en twee voervormen (meel en kruimel) op dierprestaties en gedrag vergeleken. Er waren in totaal twaalf verschillende proefvoeders, die elk vier keer herhaald werden. De voeders werden 10% verdund door toevoeging van NSP-arme (zand of grit) of NSP-rijke grondstoffen (haverdoppen, stro, sojahullen, cellulosevezels, bietenpulp en zonnebloemzaadschilfers). De NSP-rijke grondstoffen varieerden in de mate van oplosbaarheid van de NSP-fractie, hetgeen een effect zou kunnen hebben op de voeropname, het voergerichte gedrag, en de stroperigheid en passagesnelheid van de spijsbrij. Het controlevoer en het met zand verdunde voer werd zowel in meelvorm als in kruimelvorm verstrekt. De verschillen in deeltjesgrootte werden gecreëerd door fijngemalen of ongemalen haverdoppen aan het voer toe te voegen. Uit dit onderzoek bleek dat hennen aan het begin van de legperiode in staat waren om te compenseren voor een verdunningsniveau van 10% door een hogere voeropname. Verstrekking van voer dat verdund was met NSP-arme grondstoffen resulteerde in een 9,5% hogere voeropname, terwijl de voeropname steeg met 4,9% bij verdunning met NSP-rijke grondstoffen. Er was geen effect van voervorm of deeltjesgrootte van de NSP-bron op de voeropname. De eettijd van de hennen die het controlevoer kregen steeg gedurende de proefperiode van 16,4 naar 24,6% van de observatieperiode. De eettijd werd niet beïnvloed door verdunning met zand of grit, de voervorm of de deeltjesgrootte van de NSP-bron. Het verstrekken van voer dat verdund was met NSP-rijke grondstoffen verlengde de eettijd echter met 22%. De legprestaties en het lichaamsgewicht van de hennen die verdund voer kregen was vergelijkbaar of beter in vergelijking met hennen die het onverdunde voer kregen, terwijl deze kenmerken niet beïnvloed werden door de vorm van het voer. Op basis van dit experiment kan geconcludeerd worden dat het verstrekken van voeders met een 10% lagere nutriëntendichtheid, door toevoeging van NSP-arme of NSP-rijke grondstoffen aan een controlevoer, aan het begin van de legperiode resulteert in een vergelijkbare energieopname en vergelijkbare legprestaties in vergelijking met hennen die onverdund voer kregen. Het verstrekken van voer met een hoog gehalte aan niet-wateroplosbare NSP's verlengt bovendien de eettijd en verlaagt de eetsnelheid. Een verlengde eettijd als gevolg van het gebruik van niet-wateroplosbare NSP's kan bijdragen aan vermindering van het verenpikgedrag.

Op basis van de resultaten van hoofdstuk 3 is in de volgende experimenten (hoofdstuk 4 tot en met 6) gekozen voor zand als verdunningsbron in NSP-arme voeders en voor haverdoppen voor het verhogen van het NSP-gehalte van het voer. Hoofdstuk 4 beschrijft de resultaten van een experiment waarin de onafhankelijke effecten van energiegehalte, NSP-gehalte en deeltjesgrootte van NSP op eetgedrag, verenpikgedrag en legprestaties van leghennen is onderzocht. Dit experiment is uitgevoerd met 504 ISA Brown leghennen in de leeftijd van 18 tot 40 weken leeftijd, die gehuisvest waren in grondhokken. De hennen werden verloot over een van de zes verschillende voerbehandelingen. De proef kende een 2 x 3 factorieel ontwerp met 7 herhalingen per behandeling. De factoren waren standaard en laag energie (2825 versus 2540 kcal/kg), standaard en hoog NSP-gehalte (133 versus 195 g/kg) en fijne versus grove structuur van de toegevoegde NSP-bron. Onze hypothese was dat eettijd zou toenemen door verstrekking van voer met een laag energiegehalte, of met een hoog gehalte aan NSP's van grove structuur, resulterend in minder verenpikgedrag, zonder dat deze voeders een negatief effect zouden hebben op de legprestaties. Energieverlaging, NSP-verhoging en grove structuur van de NSP's verlengden de eettijd met respectievelijk 14,2%, 17,2% en 7,9% in vergelijking met de controleniveaus van deze factoren. NSP-verhoging vertraagde de eetsnelheid (gram voer/min.) met 21%. De hennen vertoonden al mild verenpikgedrag vanaf de vijfde week van de opfokperiode. De voerbehandelingen hadden geen effect op het uiteindelijke niveau van verenschade, maar het moment waarop de verenschade zich begon te ontwikkelen werd vertraagd met 10 weken als hennen voer verstrekt kregen met een laag energiegehalte als gevolg van het toevoegen van NSP's met grove structuur. In de behandelingen met het gangbare NSP-gehalte was het percentage hennen dat uit de proef verwijderd moest worden als gevolg van kannibalistisch verenpikgedrag aantoonbaard verminderd als de hennen voer kregen met een laag in plaats van een gangbaar energiegehalte (44,1 versus 13,1%). In de behandelingen met het hoge NSP-gehalte was het percentage hennen dat uit de proef verwijderd moest worden slechts in lichte mate verminderd als de hennen voer kregen met een laag in plaats van een gangbaar energiegehalte (31,6 versus 28,6%). Hennen die voer met een 10% lager energiegehalte kregen compenseerden voor deze verdunning door 9,3% meer voer op te nemen (143,0 versus 130,8 g/d). De legprestaties en het lichaamsgewicht van de hennen waren niet beïnvloed door de voerbehandelingen. Op basis van dit experiment kon vastgesteld worden dat hennen die voer kregen met een laag energiegehalte of een hoog NSP-gehalte van grove structuur meer tijd besteedden aan het opnemen van het voer in vergelijking met hennen die het standaard voer kregen. Als gevolg van deze voerbehandelingen vertoonden sommige behandelingen minder verenpikgedrag.

Hoofdstuk 5 geeft inzicht in de gemiddelde verblijfstijd van de spijsbrij per darmsegment (krop, kliermaag/spiermaag, dunne darm, dikke darm, blinde darm) na het verstrekken van voer dat varieerde in energiegehalte, NSP-gehalte en structuur van NSP. Ook zijn in dit hoofdstuk de gewichten van de lege darmsegmenten en van de inhoud van deze segmenten per voerbehandeling gepresenteerd. Deze parameters zijn onderzocht aan het einde van het experiment dat in hoofdstuk 4 is beschreven. De verblijfstijd van het voer is bepaald bij vijf hennen per hok, en bij drie hokken per behandeling, op het moment dat de hennen 40 weken oud waren. Titaniumdioxide is gebruikt als markerstof. Op het begintijdstip (t=0) werd bij elk van de vijf hennen handmatig drie gelatine capsules gevuld met titanium via de bek ingebracht. Vervolgens werden deze hennen geëuthanaseerd op vijf verschillende tijdstippen (t = 30, 90, 180, 270 en 360 minuten na het moment dat de titaniumcapsules waren verstrekt). Hierna werd de darm uit het lichaam verwijderd en onderverdeeld in vijf segmenten (krop, kliermaag/ spiermaag, dunne darm, dikke darm, blinde darm). De spijsbrij per darmsegment werd verzameld door de darmen licht te strippen. Elk segment werd voor en na verwijdering van de spijsbrij gewogen. In elk van de 450 darmmonsters (5 segmenten x 5 hennen/hok x 6 behandelingen x 3 herhalingen/behandeling) is het titaniumgehalte is geanalyseerd.

Verhoging van het NSP-gehalte in het voer verlengde de gemiddelde verblijfstijd van de spijsbrij in de krop (68,0 versus 34,0 min.) en in het totale voorste deel van het maag-darmkanaal (90,6 versus 56,8 min.) in vergelijking met de voeders met het gangbare NSP-gehalte. Het verstrekken van voer met een laag energiegehalte verlengde de gemiddelde verblijfstijd van het voer in de dikke darm (26,0 versus 6,7 min.), blinde darm (3,9 versus 1,8 min.) en in het totale achterste gedeelte van het maag-darmkanaal (30,3 versus 8,6 min.) in vergelijking met de voeders met het gangbare energiegehalte. Een grove structuur van de NSP-bron verminderde ten opzichte van een fijne structuur de gemiddelde verblijfstijd van de spijsbrij in de blinde darm (4,6 versus 1,8 min). De totale verblijfstijd van de spijsbrij in het maag-darmkanaal werd niet beïnvloed door de voerbehandelingen. Het verstrekken van NSP-rijk voer verhoogde de relatieve gewichten van zowel de lege klier-/spiermaag als van de klier-

/spiermaaginhoud met respectievelijk 30% (25,2 versus 19,4 g/kg) en 18% (15,4 versus 13,0 g/kg) in vergelijking met voer met een gangbaar NSP-gehalte. Het gewicht van de lege klier-/spiermaag was nog eens 30% hoger als het NSP-rijke voer een grove in plaats van een fijne structuur had (28,5 versus 21,9 g/kg). Op basis van deze resultaten kan geconcludeerd worden dat het toevoegen van NSP-rijke grondstoffen aan voer resulteert in een toename van zowel het lege gewicht van de lege klier-/spiermaag en als van de inhoud ervan, terwijl de gemiddelde verblijfstijd van de spijsbrij in het voorste deel van het maag-darmkanaal hierdoor toeneemt. De gemiddelde verblijfstijd in het voorste deel van het maag-darmkanaal blijkt recht evenredig toe te nemen met de dagelijks opgenomen hoeveelheid niet-water oplosbare NSP's. Deze bevindingen kunnen wijzen op een hogere mate van verzadiging van de hennen, wat weer kan samenhangen met vermindering van het verenpikgedrag.

Het laatste experiment was gericht op het onderzoeken van carry-over effecten van nutriëntendichtheid en NSP-gehalte in opfokvoeders op eetgedrag, verenpikgedrag en legprestaties van hennen tijdens de legperiode (hoofdstuk 6). In dit experiment zijn verschillende verdunningsniveaus en NSP-concentraties vergeleken in zowel opfok- als legvoeders. Voeropname, eetgedrag, verenpikgedrag en de ontwikkeling van het maag-darmkanaal zijn bepaald bij opfok- en leghennen. Dit experiment is uitgevoerd met 768 ISA Brown opfokhennen, waarvan er 576 zijn gevolgd tijdens de legperiode. De hennen waren gehuisvest in grondhokken. Eendagskuikens werden toegewezen aan een van de zes voerbehandelingen. Het experiment was factorieel opgezet met 3 verdunningsniveaus x 2 NSP-concentraties, met 8 herhalingen (hokken) per behandeling. Toen de hennen 17 weken oud waren zijn ze toegewezen aan een van de acht voerbehandelingen tijdens de legperiode. Het experiment was in deze fase factorieel opgezet met 4 verdunningsniveaus x 2 NSP concentraties, elk met 6 herhalingen per behandeling.

In vergelijking met hennen die het onverdunde voer kregen steeg de voeropname van de hennen die het 10%, 15% of 20% verdunde voer kregen met respectievelijk 8,4% (9,5 g/hen/d), 16,5% (18,1 g/hen/d) en 20,9% (23,6 g/hen/d). Uiteindelijk was de nutriëntenopname gelijk voor alle verdunningsniveaus. Het verdunningsniveau van de voeders met een gangbaar NSP gehalte had geen effect op de niet-wateroplosbare NSP-opname tussen de verdunde voeders (gemiddeld 9,3 g/hen/d). Bij hennen die het NSP-rijke voer kregen nam de dagelijkse opname van niet-wateroplosbare NSP toe van 15,6 g/hen/d bij het 0% verdunde voer tot 18,9 g/hen/d bij het 20% verdunde voer. Het verstrekken van legvoer met een hoog in plaats van een gangbaar NSP gehalte resulteerde in een geringere inhoud van de kliermaag (1,1 versus 0,3 g/kg lichaamsgewicht), terwijl het gewicht van de lege spiermaag toenam van 14,3 naar 24,4 g/kg lichaamsgewicht. Hennen die tijdens de legperiode voer kregen met een gangbaar NSP-gehalte ontwikkelden meer verenschade dan hennen die NSP-rijk legvoer kregen (0,58 versus 0,30). Verhoging van het niet-wateroplosbare NSP-gehalte van het voer verminderde het gewicht van de lege kliermaag en verhoogde het gewicht van zowel de spiermaag als de spiermaaginhoud. Er was een rechtlijnig verband tussen de dagelijkse opname van niet-wateroplosbare NSP's en de afname van de schade aan het verenkleed. Het verstrekken van verdunde opfokvoeders verhoogde de voeropname. Dit effect was vanaf de eerste week merkbaar. We veronderstellen dat kuikens hun pikgedrag meer gaan richten op het voer, naarmate het verdunningsniveau van het voer toeneemt, waardoor het verenpikgedrag zou kunnen verminderen. Dit zou ook kunnen verklaren waarom de conditie van het verenkleed van de leghennen aan het einde van het experiment beter was als ze tijdens de opfokperiode 15% verdund voer hadden gekregen.

In hoofdstuk 7 (General Discussion) zijn de resultaten, die in de voorgaande hoofdstukken vermeld zijn, bediscussieerd en geëvalueerd in het licht van theorieën over verenpikgedrag en voeding. Er worden voorstellen gedaan voor toekomstig onderzoek. Ook geeft dit hoofdstuk aanwijzingen voor het vertalen van de kennis uit dit project naar praktisch toepasbare voerstrategieën voor opfok- en leghennen ter voorkoming van verenpikken.

Op basis van de studies die in dit proefschrift beschreven worden, kunnen de volgende conclusies getrokken worden:

Voeropname

- Opfok- en leghennen stemmen de voeropname in sterke mate af op hun energiebehoefte. Verdunningsniveaus tot 15% in opfokvoeders en tot 20% in legvoeders (door toevoeging van zand aan een controlevoer) worden volledig gecompenseerd door een evenredig hogere voeropname.
- Opfokhennen zijn niet in staat om volledig te compenseren voor verdunningsniveaus van 10% en 15% als ongemalen haverdoppen zijn gebruikt als verdunningsmateriaal.
- Het verstrekken van verdund voer, door toevoeging van zand of NSP-rijke grondstoffen, heeft geen effect op de legprestaties van leghennen.

Eettijd en eetsnelheid

- Het toevoegen van zand aan het voer verlengt de eettijd van leghennen, zonder dat dit een groot effect heeft op de eetsnelheid.
- Het toevoegen van zand aan het voer verhoogt de eetsnelheid van opfokhennen, zonder dat dit een groot effect heeft op de eettijd.
- De eettijd van leghennen is positief gecorreleerd met de hoeveelheid dagelijks opgenomen niet-wateroplosbare NSP's.

Spiermaaggewicht en verblijfstijd van het voer

- Het verlagen van de nutriëntendichtheid heeft geen effect op het gewicht van de spiermaag en op de verblijfstijd van het voer in het voorste deel van het maag-darmkanaal.
- Grof gemalen niet-wateroplosbare NSP's, en in het bijzonder de cellulose- en hemicellulosefracties, verhogen het gewicht van de spiermaag.
- De gemiddelde verblijfstijd in het voorste deel van het maag-darmkanaal, de krop, klier- en spiermaag, is positief gecorreleerd met de hoeveelheid dagelijks opgenomen niet-wateroplosbare NSP's. Dit verband is sterker bij grofgemalen dan bij fijngemalen NSP's.
- De totale verblijfstijd in het maag-darmkanaal is niet beïnvloed door het NSP-gehalte van het voer, wat aangeeft dat de passagesnelheid van de digesta is toegenomen na passage van de spiermaag.

Verenschade

- Het verstrekken van verdund legvoer met een hoog gehalte aan grof gemalen niet-wateroplosbare NSP's kan resulteren in een vertraging van 10 weken voordat verenschade zich begint te ontwikkelen.
- Het verstrekken van 15% verdund voer – door toevoeging van zand of haverdoppen – tijdens de opfokperiode resulteert onafhankelijk van het NSP-gehalte van het legvoer in minder verenschade tijdens de legperiode.

Uit deze studie blijkt dat het verhogen van de mate van voergericht gedrag en de mate van verzadiging via aanpassingen van het voer succesvolle strategieën zijn voor het voorkomen van verenpikken, zolang dit gedrag niet is aangeleerd in een eerder stadium. Verdunning van de nutriëntendichtheid en verhoging van het gehalte aan grof gemalen niet-wateroplosbare NSP's in het legvoer resulteert bij leghennen in meer voergericht gedrag, wat blijkt uit een verlengde eettijd en een vertraagde eetsnelheid. Het verstrekken van 15% verdunde voeders tijdens de opfokperiode voorkomt schade aan het verenkleed tijdens de legperiode. Hoewel het van jongs af aan verstrekken van verdund opfokvoer niet resulteert in een verlengde eettijd, lijkt het toch te bevorderen dat kuikens hun pikken meer richten op het voer dan op het verenkleed van hokgenoten. Voergericht gedrag en verzadiging van leghennen worden vooral bevorderd door het verstrekken van voer met een hoog gehalte aan niet-wateroplosbare NSP's. Deze effecten worden echter verder versterkt door de nutriëntendichtheid te verlagen en de NSP's in grove vorm aan te bieden. De meest perspectiefvolle voerstrategie ter voorkoming van schade aan het verenkleed is het verstrekken van een 15% verdund voer tijdens de opfokperiode, gevolgd door een 10% verdund, grofgemalen NSP-rijk voer tijdens de legperiode.

DANKWOORD

Het doorlopen van een promotieonderzoek is in mijn ogen te vergelijken met het bouwen van een huis. Hoewel ik geen ervaringsdeskundige ben, stel ik me zo voor dat je zo'n bouwproject begint met het opstellen van de wensen en eisen, waaraan het huis moet voldoen. Deze eisen bepalen vervolgens welk prijskaartje eraan komt te hangen. Als de financiering geregeld en alle andere voorbereidingen getroffen zijn, kan begonnen worden met het leggen van het fundament. Daarna volgen geleidelijk aan de muren, de verdiepingen en het dak. Maar op het moment dat de buitenkant voltooid is, moeten er in het huis nog veel zaken afgewerkt worden. Als het huis eindelijk af is, hebben de omstanders er vaak geen notie van hoeveel moeite het heeft gekost om tot dit resultaat te komen. Aan het einde van dit traject terugkijkend, zie ik veel parallellen tussen het bouwen van een huis en het uitvoeren van een promotieonderzoek.

Al bij mijn aanstelling als wetenschappelijk onderzoeker in 1999 is het doen van promotieonderzoek ter sprake gekomen. Tijdens de promotie van collega Teun Veldkamp in november 2002 kwam ik in gesprek met René Kwakkel. René verzorgde onderwijs en onderzoek met betrekking tot pluimveevoeding bij het Departement Dierwetenschappen in Wageningen. Hij wilde graag meedenken over een geschikt promotieonderwerp. Zijn enthousiasme werkte erg aanstekelijk op mij, zodat ik mij verder ben gaan oriënteren. Uit een brainstormsessie met Martin Verstegen, Leo den Hartog, René Kwakkel, Koos van Middelkoop en Carola van der Peet bleek dat verenpikgedrag bij leghennen een actueel probleem was. Hoewel er al veel onderzoek naar dit thema was uitgevoerd, bleek er tot dan toe nog weinig bekend te zijn over de relatie voeding en verenpikken. Zo zijn de eerste ideeën over het 'nieuwe huis' ontstaan.

Het Productschap Diervoeder was in 2003 bereid een literatuurstudie te financieren die de relaties tussen diervoeding en verenpikken bij leghennen inzichtelijk maakte. Uit deze studie bleek dat vermindering van verenpikken via voeding zeker perspectiefvol was. Deze uitkomsten waren voor het Productschap Diervoeder en het Productschap Pluimvee en Eieren aanleiding om in te stemmen met een meerjarig vervolgproject (2005 – 2007), waarin meerdere dierexperimenten uitgevoerd zouden worden om de effecten van voedingsfactoren op vermindering van het verenpikgedrag bij leghennen te meten. Hiermee was er een financieel fundament en een duidelijk plan van aanpak voor het bouwen van 'het huis'. Bouwprojecten worden echter financieel vaak onderschat. Dit gold ook voor het huidige project. Gelukkig heeft de Animal Sciences Group de afgelopen twee jaren aanzienlijke hoeveelheden eigen middelen beschikbaar gesteld, zodat ik toch twee werkdagen per week aan dit project kon blijven besteden. Ik ben mijn werkgever zeer erkentelijk voor deze financiële ondersteuning en voor de ruimte die ik gekregen heb om dit onderzoek uit te kunnen voeren.

Achteraf kan vastgesteld worden dat deze studie zeer voorspoedig is verlopen. Er zijn gelukkig geen echte tegenslagen geweest. Wel bleek dat we het pikgedrag van de hennen niet in de hand hadden. In het eerste en derde experiment was er geen of weinig pikkerij. In het tweede experiment, daarentegen, pikten de hennen bijzonder heftig, wat resulteerde in grote aantallen pikslachtoffers. Het gepubliceerd krijgen van een artikel is toch elke keer weer een soort bevalling. Toch kwam in de afgelopen jaren langzaam maar zeker het ene na het andere hoofdstuk van dit proefschrift gereed. Het ‘bouwwerk’ kreeg zo geleidelijk aan steeds meer haar definitieve vorm. De laatste maanden is intensief gewerkt aan de afwerking (introduction, general discussion, summary, samenvatting en - niet te vergeten - de layout), een fase die zeker niet onderschat moet worden. Gelukkig is inmiddels ook deze fase voltooid.

Zonder hulp van een groot aantal studenten had dit project werkelijk nooit binnen het huidige tijdsbestek en binnen het beschikbare financiële budget uitgevoerd kunnen worden. Zij verzamelden de eieren, observeerden de hennen, scoorden het verenkleed en besteedden vele uren aan het uitlezen van de videobanden, uitmondend in waardevolle verslagen. Daarom wil ik in chronologische volgorde Suzanne Siegers, Eefke Weesendorp, Marielle Vijfvinkel, Yann Cloarec, Emeka Ubah, Lane Pineda, Bijaya Shresta, Ariyati, Jérémie Renaud en Anne-Marie Frijters bijzonder hartelijk bedanken voor al hun inzet en betrokkenheid. Daarnaast wil ik ook mijn directe collega Gisabeth Binnendijk heel hartelijk bedanken voor alle momenten dat zij de waarnemingen heeft uitgevoerd. Soms was er een studentloze periode, terwijl experimenten ook vaak doorliepen tijdens de zomervakantie, als de studenten naar huis waren. Jij was in die perioden gelukkig altijd bereid om in te springen. De belangrijkste conclusies uit mijn laatste experiment zijn met name gebaseerd op jouw nauwkeurige verenscores aan het einde van de legperiode. Ik ben blij dat je tijdens de promotie de taak van paranimf op je wil nemen. Ik weet zeker dat jij op het laatst nog aan dingen denkt, die ik zou vergeten.

De experimenten zijn uitgevoerd op proefaccommodatie ‘De Haar’ in Wageningen. De samenwerking met het personeel van deze accommodatie heb ik altijd bijzonder gewaardeerd. Ten behoeve van de experimenten zijn mooie hokjes getimmerd. De dierverzorgers stonden steeds weer te trappelen van enthousiasme als er een nieuwe proef van start ging. Wat mij vooral aansprak was jullie flexibele opstelling. Willem, Ben, Andre, Peter, Ries, Marleen en Roel, jullie hebben de proeven altijd consequent begeleid. Bovendien waren jullie altijd bereid om bij te springen op momenten dat er meer gedaan moest worden dan vooraf was afgesproken. Of het nu ging om een extra dierweging, het tellen van de eieren omdat de betreffende student tentamen had, of het controleren van de pootringen, jullie pakten het als vanzelfsprekend op. Graag wil ik ook jullie super bedanken voor alle hulp.

Een verantwoorde proefopzet en een goede statistische onderbouwing van de resultaten is voor een onderzoeker van groot belang. De persoon die hierbij een grote rol heeft gespeeld, is collega en statisticus Geert André. Geert, jij wist alle behandelingseffecten die in het eerste experiment door elkaar heen liepen te groeperen tot een paar overzichtelijke hoofdeffecten. Daarnaast adviseerde je mij om de resultaten van wekelijks gemeten kengetallen niet in eindeloze tabellen te presenteren. In plaats daarvan wist jij, door gebruik te maken van exponentiële en logistische curven, de beschrijving van de resultaten drastisch te comprimeren. Jij was ook de geestelijke vader van het model dat de verblijfstijd van de spijsbrij per darmsegment berekende. Geert, jouw inbreng heeft een fundamentele bijdrage geleverd aan dit proefschrift. Hiervoor ben ik je zeer erkentelijk. Later is het rekenwerk overgenomen door Jac Thissen. Jac, ook jij heel hartelijk bedankt voor al het werk dat je hebt gedaan.

Dit project werd wetenschappelijk gecoacht door mijn begeleidingscommissie, bestaande uit Martin Verstegen (promotor), Leo den Hartog (co-promotor), René Kwakkel (co-promotor) en collega Carola van der Peet. We vergaderden altijd 's ochtends vroeg van 8.00 tot 10.00 uur. Het waren bijzonder gezellige, maar ook zeer constructieve bijeenkomsten. Het eerste kwartier werd steevast besteed aan het uitwisselen van allerlei nieuwtjes. Elke begeleider had zo zijn specifieke kracht. Martin zorgde voor de nieuwe ideeën, Leo bewaakte de grote lijnen, terwijl Carola scherpe vragen stelde en de papers van kritisch commentaar voorzag. Carola, heel fijn dat ook jij de taak van paranimf op je wil nemen. René had zijn eigen stijl van begeleiden. Hij gaf er de voorkeur aan om 's avonds bij hem thuis aan artikelen te werken. Vele avonden hebben we samen boven op de studeerkamer van Emmy gezeten. Deze altijd keurig opgeruimde kamer, voor een groot deel gevuld met theologische boeken, verschafte ons een ideale werkplek. Emmy, hartelijk dank voor het afstaan van jouw kamer, voor de vele kopjes cafeïnevrije koffie met speculaasjes die je boven gebracht hebt en ook voor al die keren dat ik mee mocht eten. René, het was geweldig om met jou samen te werken. Je hebt de gave om direct na een eerste keer lezen suggesties ter verbetering aan te reiken. Dankzij jouw inbreng kregen de papers een duidelijker structuur en verbeterde de kwaliteit van het Engels. Soms kwamen we helemaal niet aan de teksten toe, omdat we bleven steken in het proberen te doorgronden van bepaalde proefuitkomsten. Ook deze discussies heb ik buitengewoon gewaardeerd. Onze werkwijze heeft mij echt gemotiveerd en mede hierdoor ben ik zelfs aan het einde van dit ‘bouwproject’ nog steeds enthousiast. Alle begeleiders wil ik hierbij bedanken voor het meedenken en voor de waardevolle suggesties die ik tijdens deze jaren mocht ontvangen.

Een bijzonder woord van dank wil ik richten aan mijn moeder. Helaas heeft mijn vader het einde van dit promotieonderzoek niet mee mogen maken. Ma, ik ben u, en uiteraard ook pa, heel erg dankbaar dat ik de gelegenheid heb gekregen om te studeren. Voordat ik met dit onderzoek begon, wist u de oplossing voor het verenpikprobleem al: ‘bloedmeel verstrekken’. Dit proefschrift toont aan dat er ook andere voedingsmogelijkheden zijn om verenpikken te voorkomen. Overigens zijn er dit moment velen in de pluimveesector die uw oplossingsrichting ondersteunen. Daarom onderzoeken we op dit moment of het opnemen van dierlijk eiwit in het voer een bakerpraatje is of daadwerkelijk bijdraagt aan vermindering van de problematiek. Ik wens u Gods troost en nabijheid toe.

Het uitvoeren van promotieonderzoek is voor de thuissituatie nu niet direct een sociaal gebeuren, hoewel er gelukkig altijd nog wel tijd was voor gezamenlijk eten en koffiedrinken. Dit project was echter zo intensief dat er, ondanks mijn fysieke aanwezigheid met de laptop op de huiskamertafel, heel veel van het gezinsgebeuren langs mij heen is gegaan. Jessica, Bouke en Tabitha, ik hoop dat jullie er niet teveel last van gehad hebben dat ik aan het promoveren was. Lieve Gerda, je hebt al die jaren intensief meegeleefd. Naarmate de tijd vorderde kreeg je er wel steeds meer moeite mee dat dit traject zoveel tijd en energie opslokte. Dit is overigens goed te begrijpen. Daarnaast hadden we nog de bijzondere gezinssituatie, die de laatste jaren alleen maar intensiever is geworden. Het waren echt tropenjaren voor ons. Ik ben je bijzonder dankbaar voor de offers die jij gebracht hebt en voor de ruimte die je mij hebt gegeven om deze studie tot een goed einde te brengen. Nu mijn ‘bouwwerk’ af is, wil ik er weer voor 100% zijn voor jou en de kinderen.

Tot slot wil ik mijn dank naar God toe uitspreken. Ik zie het als Zijn leiding dat dit promotieonderzoek er gekomen is. Hij heeft mij tijdens deze jaren bovendien de gezondheid en de energie gegeven om door te gaan en dit project tot een goed einde te brengen. Ik ben Hem dankbaar dat Hij, om in bouwkundige termen te spreken, het fundament van mijn leven is. Hij is de grote Bouwmeester, die een ‘woning’ beschikbaar heeft voor iedereen die in Hem gelooft (zie Johannes 14:2).

CURRICULUM VITAE

Marinus Maarten van Krimpen werd geboren op 23 april 1964 te Vlaardingen. Na de basisschool werd het VWO doorlopen op de Reformatorische Scholengemeenschap Guido de Brès te Rotterdam. In 1983 behaalde hij zijn eindexamen. In datzelfde jaar begon hij met de studierichting Zoötechniek aan de toenmalige Landbouwuniversiteit te Wageningen. In 1988 studeerde hij af in de afstudeerrichtingen Veehouderij, Agrarische Bedrijfseconomie en Pedagogiek. Na zijn afstuderen werkte hij gedurende een jaar als beleidsmedewerker bij de Vereniging van Mengvoerfabrikanten (Nimo/VNMF) te Rijswijk. Na een intermezzo van een half jaar als leraar wiskunde op een LTS te Rotterdam, werkte hij in 1990/1991 bij de TAK-organisatie voor de Rundveehouderij (TAURUS) te Lelystad. In deze functie maakte hij rundveehouders in West- en Zuid-Nederland vertrouwd met een elektronisch netwerk (VeeNET). Via dit netwerk konden de veehouders allerlei bedrijfsspecifieke gegevens binnenhalen en integreren met hun managementsysteem. Van 1992 tot 1999 werkte hij als voedingsdeskundige (varkens en legpluimvee) bij CAVO-LATUCO, een middelgroot mengvoerbedrijf te Utrecht. Hier was hij verantwoordelijk voor de samenstellingen van de varkens- en legpluimveevoeders en voor kennisoverdracht naar de buitendienst. Sinds 1999 is hij aangesteld als wetenschappelijk onderzoeker in de varkens- en pluimveevoeding bij het toenmalige Proefstation voor de Varkenshouderij te Rosmalen, dat inmiddels met een aantal andere instituten is gefuseerd tot de Animal Sciences Group van Wageningen UR. Vanaf 2001 is de standplaats gewijzigd van Rosmalen naar Lelystad. Zijn projecten richtten zich op de thema's: voeding – gezondheid (alternatieven voor antimicrobiële groeibevorderaars), voeding – vlees-/eikwaliteit, voeding van biologische varkens en kippen, voeding van vleeseenden en voeding – gedrag van leghennen. Dit laatste thema vormde het onderwerp van zijn promotieonderzoek, waaraan vanaf 2004 circa 50% van de tijd is besteed. In het kader van dit onderzoek werkte hij twee dagen per week bij de leerstoelgroep Diervoeding van Wageningen Universiteit.

Publications

Refereed scientific journals

Van Krimpen, M. M., R. P. Kwakkel, B. F. J. Reuvekamp, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2005. Impact of feeding management on feather pecking in laying hens. *World's Poult. Sci. J.,* 61:665-687.

Van Krimpen, M. M., R. P. Kwakkel, G. André, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2007. Effect of nutrient dilution on feed intake, eating time and performance of hens in early lay. *Br. Poult. Sci.,* 48:389-398.

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. v. d. Peet-Schwering, L. A. d. Hartog and M. W. A. Verstegen. 2008. Low Dietary Energy Concentration, High Nonstarch Polysaccharide Concentration and Coarse Particle Sizes of Nonstarch Polysaccharides Affect the Behavior of Feather-Pecking-Prone Laying Hens. *Poult. Sci.,* 87:485-496.

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. Effects of nutrient dilution and Nonstarch Polysaccharide concentration in rearing and laying diets on eating behaviour and feather damage of laying hens. (accepted).

Conference proceedings

Van Krimpen, M. M., R. P. Kwakkel, B. F. J. Reuvekamp, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2005. Reduction of feather pecking behaviour in laying hens by feeding management - a review. *Proceedings of 7th European Symposium of Poultry Welfare, 15-19 June 2005, Lublin, Poland*, Lublin, Polish Academy of Sciences, Institute of Genetics and Animal Breeding, Jastrzebiec, Polish Academy of Sciences, Jastrzebiec. 23:161-174.

Van Krimpen, M. M., R. P. Kwakkel, G. André, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2007. Impact of nutritional factors on feather pecking behavior of laying hens in non-cage housing sytems. *Proceedings of the XVI European Symposium on Poultry Nutrition, August 26-30, Strasbourg, France*, Strasbourg.

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2008. Effect of dietary energy concentration, NSP concentration and coarse particle sizes of NSP on mean retention time and gut development in laying hens. *Proceedings of the XXIII World's Poultry Congress, 30 June - 4 July 2008, Brisbane, Australia*, Brisbane, Australia.

Reports

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2007. Effect of dietary energy and NSP concentration and particle size of NSP on eating behaviour, feather pecking behaviour and performance of laying hens. *Animal Sciences Group.* PraktijkRapport 46.

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2008. Effects of nutrient dilution and Nonstarch Polysaccharide concentration in rearing and laying diets on eating behavior and feather damage of laying hens. *Animal Sciences Group van Wageningen UR.* PraktijkRapport 146.

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. Verstegen. 2008. Effects of dietary energy concentration, Nonstarch Polysaccharide concentration and particle sizes of Nonstarch Polysaccharides on digesta passage rate and gut development in laying hens. *Animal Sciences Group.* PraktijkRapport 145.

Pubications in popular media

Van Krimpen, M. M. 2007. Voeropname en eettijd goed te sturen. *Pluimveehouderij,* 13 januari 2007:10-11.

Van Krimpen, M. M., R. P. Kwakkel, C. M. C. Van der Peet-Schwering, L. A. Den Hartog and M. W. A. verstegen. 2007a. Aangepast voer verbetert gedrag. *Pluimveehouderij,* 37:7-9.

van Krimpen, M. M., R. P. Kwakkel, C. M. C. Van der Peet-Schwering, L. A. den Hartog and M. W. A. Verstegen. 2007b. Niet-wateroplosbare vezels verminderen verenpikken. *V-Focus,* Augustus 2007:39-41

Training and Supervision Plan	**Graduate School WIAS**
Name:	Marinus van Krimpen
Group:	Animal Nutrition Group
Daily supervisors:	Dr. Ir. R. Kwakkel, Dr. ir. C. van der Peet-Schwering
Supervisors:	Prof. Dr. Ir. M. Verstegen, Prof. Dr. Ir. L. den Hartog

The Basic Package	year	credits *
WIAS Introduction Course (mandatory, 1.5 credits)	2006	1,5
Course on philosophy of science and/or ethics (mandatory, 1.5 credits)	2005	1,5
International conferences		
WPSA Welfare conference, Bristol (18-19 July)	2003	0,5
WPSA Welfare conference, Lublin, Poland (15 - 19 June)	2005	1,2
European Symposium on Poultry Nutrition, Strassbourg, Fr. (27-29 August)	2007	1,0
WPSA Poultry Symposium, June, Brisbane (Australia) (30-06 t/m 4-07)	2008	1,2
Seminars and workshops		
Workshop Voeropnameregulatie 14-06-07	2007	0,2
Workshop Verenpikken 1-02-08	2008	0,2
PHLO cursus Voeropnameregulatie 24-04-08	2008	0,2
Presentations		
Oral Presentation WPSA Welfare conference Lublin (Poland) 15-19 June 2005	2005	1,0
Oral Presentation WIAS Science Day 08-03-07	2007	1,0
Oral Presentation European Symposium on Poultry Nutrition, Strassbourg, Fr. (27-29 August)	2007	1,0
Oral Presentation WPSA Poultry Symposium, June, Brisbane (Australia) 01-07-08	2008	1,0
Oral Presentation Nederlandstalige dag voor voedingsonderzoekers 24-04-08	2008	1,0
Disciplinary and interdisciplinary courses		
PHLO cursus Growth Modelling of the Pig	1995[P]	1,0
PHLO cursus Vruchtbaarheid en voortplanting van het varken	1997[P]	0,8
PHLO cursus Varkensvoeding; nieuwe ontwikkelingen en praktijk	1999[P]	0,8
Cursus Toegepaste Statistiek Centrum voor Biometrie	2001[P]	2,0
Cursus 'Methods of Farm Animal Ethology' 3-8 september Hohenheim (Dld)	2007	2,0
Cursus Nutrition in the Omics area	2008	0,4
Statutory Courses		
Use of Laboratory Animals (mandatory when working with animals)	2005	3,0
Professional Skills Support Courses		
Techniques for Writing and Presenting a Scientific Paper (Michael Grossman)	2001[P]	0,6
Course 'Presentation techniques'	2003	1,0
Course 'Project management'	2003	1,0
Course Techniques for Scientific Writing (Linda McPhee)	2004	1,5
Research Skills Training	year	credits
Preparing own PhD research proposal	2004	4,0
Didactic Skills Training		
Lecture during course 'Principles of Poultry Science' 2005/2006/2007	2005	0,6
Lectures during PHLO Course 'Pluimveevoeding en Management	2006	0,5
Lecture PTC+ (28-08-2006)	2006	0,2
Lecture ForFarmers (26-10-2006)	2006	0,2
Lecture Ned. Ver. Techniek in de Landbouw (13-02-2007)	2007	0,2
Lecture voor buitendienst Verbeek/Rijnvallei (7-02 en 14-02)	2007	0,4
Lecture tijdens Workshop Voeropnameregulatie 14-06-07	2007	0,2
Lecture tijdens Workshop Verenpikken 1-02-08	2008	1,0
Lecture tijdens PHLO cursus Voeropnameregulatie 24-04-08	2008	1,0
Supervising theses		
Suzanne Siegers (MSc Major))	2005	2,0
Emeka Ubah (MSc Major))	2006	2,0
Bijaya Shresta (MSc Major)	2007	2,0
Ariyati Ariyati (MSc Major)	2007	2,0
Anne-Marie Frijters (MSc minor)	2008	1,5
Organisation of seminars and courses		
Workshop Voeropnameregulatie (14-06-2007)	2007	1,0
Workshop Verenpikken (01-02-2008)	2008	1,0
Membership of boards and committees		
Member of the Committee Feed Tabel (CVB) 2004-2007		4,0
Total		50,4

*One ECTS (European Credit Transfer System) credit equals a study load of approximately 28 hours

[P] Previous work before starting this PhD project

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ANIMAL SCIENCES GROUP WAGENINGEN UR

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