



## Report 09

# Effect of nutrient density, NSP source, coarseness of NSP and feed form on performance and behaviour of hens at early lay



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## **Abstract**

An experiment was conducted to measure the effect of energy dilution and feed structure on performance and behaviour of hens at the beginning of the laying period.

From this experiment we can conclude that feeding low-NSP or high-NSP diets resulted in equal or even improved egg performance of hens at early lay compared with hens that were fed standard diet. Feeding coarse ground meal negatively affects egg production, egg weight, egg mass and body weight, whereas feed form did not affect egg performance. Hens that were fed NSP-high diets spend more time on feed intake during some observations, and had heavier relative gizzard weight and content.

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Effect of nutrient density, NSP source, coarseness of NSP and feed form on performance and behaviour

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# Effect of nutrient density, NSP source, coarseness of NSP and feed form on performance and behaviour of hens at early lay

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# Samenvatting

## Inleiding

De traditionele batterijkooi zal binnen de Europese Unie uiterlijk vanaf 2012 volledig verboden zijn. Een ernstig probleem van alternatieve huisvestingssystemen in vergelijking met batterijkooien is echter een hogere incidentie van verenpikken en kannibalisme (Morgenstern, 1995). In de biologische legpluimveehouderij komen zelfs sterftepercentages van 30% voor als gevolg van kannibalisme (Van der Wouw, 1995). Op dit moment wordt snavelkappen gezien als de belangrijkste maatregel om verenpikken en kannibalisme te voorkomen, maar vanaf 2011 zal er in Nederland een algemeen verbod op snavelkappen van kracht zijn. Juist de combinatie van alternatieve huisvestingssystemen en de afwezigheid van snavelkappen vormt een groot risico voor verenpikken en kannibalisme. De problematiek van verenpikkerij en kannibalisme is voor de legpluimveehouders die hun leghennen nog huisvesten in batterijkooien op dit moment een belangrijke beperking voor omschakeling naar alternatieve huisvestingssystemen.

Het probleem van verenpikkerij en kannibalisme is multifactorieel. Als belangrijke oorzaken hiervoor gelden diereigen factoren, zoals erfelijke aanleg, hormonale status (onvolwassen versus volwassen dieren), mate van angst en sociale factoren, maar ook omgevingsfactoren zoals huisvestingsomstandigheden en voedingsfactoren (Blokhuys, 1989). Het bestrijden van verenpikken vraagt dan ook om een geïntegreerde benadering waarbij rekening wordt gehouden met diverse factoren. Uit een onlangs gepubliceerd literatuuronderzoek [Krimpen, 2005 #332] blijkt dat voedingsfactoren bij kunnen dragen aan het reduceren van de mate van verenpikken en kannibalisme. Een perspectievolle benadering lijkt het stimuleren van de tijd die leghennen besteden aan voeropname gerelateerd gedrag, zoals foerageren (het zoeken naar mogelijke voedselbronnen) en voeropname, en het stimuleren van de mate van verzadiging van leghennen. Beide zaken kunnen gestimuleerd worden door verstrekking van energiearme voeders (Lee *et al.*, 2001), of van voeders die rijk zijn aan niet-oplosbare NSP (Non Starch Polysaccharides) (Bears *et al.*, 1940; Hetland *et al.*, 2002, 2003). Ook de maalfijnheid van de NSP-fractie lijkt van invloed te zijn op de mate van verenpikken. Er zijn aanwijzingen dat een grof gemalen NSP-fractie resulteert in minder verenpikken (Hetland *et al.*, 2002, 2003). Daarnaast zijn positieve resultaten bereikt met het verstrekken van ruwvoer (Steenfeldt *et al.*, 2001). Op dit moment is echter onduidelijk wat de ranking is van bovengenoemde voedingsfactoren of combinatie van factoren in relatie tot het reduceren van verenpikken en wat het werkingsmechanisme ervan is.

We veronderstellen dat hennen die voer met een lagere nutriëntendichtheid krijgen, meer voer gaan opnemen en uiteindelijk een gelijke energieopname realiseren als hennen die een controlevoer met een gangbare nutriëntendichtheid krijgen. Het is echter de vraag of de opnamecapaciteit van de hennen aan het begin van de legperiode voldoende groot is om het verschil in nutriëntendichtheid in het voer te kunnen compenseren, zeker als dit voer ook nog eens een hoog aandeel grof gemalen niet-water oplosbare NSP bevat. Daarnaast is op dit moment nog onvoldoende bekend of de mate van compensatie grondstofafhankelijk is. Onvoldoende compensatie leidt tot een te lage nutriëntenopname, waardoor de dierprestaties afnemen. Bovendien besteden de hennen dan minder tijd aan voeropname, waardoor de positieve effecten op gedrag niet tot uiting komen. Om hierin meer inzicht te krijgen is in opdracht van het Productschap Diervoeder en het Productschap Pluimvee en Eieren een experiment uitgevoerd met hennen aan het begin van de legperiode.

## Doel

Het doel van dit experiment was om het effect te meten van nutriëntendichtheid, NSP-gehalte, maalfijnheid van de NSP-fractie en voervorm op de voeropname en de mate van voergericht gedrag van leghennen tijdens de eerste 8 weken van de legperiode.

## Materiaal

Er werd gebruik gemaakt van twee identieke afdelingen die elk 24 grondkooien bevatten met afmetingen van 0,90 x 1,50 m. Na aftrek van de oppervlakte van de voertrog (1,0 x 0,2 m) bleef er 1,15 m<sup>2</sup> netto leefoppervlak over. Voor elke grondkooi was een legnest geplaatst. De bodem was ingestrooid met zand. De hennen hadden onbeperkt water en voer ter beschikking. In totaal werden 480 licht getoucheerde ISA Brown hennen ingezet, verdeeld over 48 grondkooien (twaalf behandelingen met vier herhalingen per behandeling). De hennen waren gehuisvest volgens de gangbare oppervlaktenorm voor scharrelkippen (negen hennen/m<sup>2</sup>). Ter beheersing van de lichtintensiteit waren de ramen in de afdelingen geblinderd, zodat geen buitenlicht kon binnendringen. Bij aankomst van de hennen (week 17) kregen de dieren 10 uur licht per dag, oplopend tot 16 uur licht per dag (week 23). De lichtintensiteit werd geleidelijk opgevoerd van 20 lux in week 18 tot 50 lux in week 21.

## Behandelingen

In het experiment zijn de volgende 12 behandelingen onderzocht.

Nr.	Behandeling	Verdunning (%)	NSP Klasse	Niveau van oplosbaar NSP	Deeltjesgrootte NSP-fractie	Voervorm
1	Negatieve Controle – M.	0	Gangbaar	Laag	Fijn	Meel
2	Negatieve Controle – Kr.	0	Gangbaar	Laag	Fijn	Kruimel
3	Zand – M.	10	Laag	Laag	Fijn	Meel
4	Zand – Kr.	10	Laag	Laag	Fijn	Kruimel
5	Grit	10	Laag	Laag	Grof	Meel
6	Haverdoppen fijn	10	Hoog	Laag	Fijn	Meel
7	Haverdoppen grof	10	Hoog	Laag	Grof	Meel
8	Bietenpulp	10	Hoog	Hoog	Grof	Meel
9	Arbocell	10	Hoog	Hoog	Grof	Meel
10	Sojahullen	10	Hoog	Hoog	Grof	Meel
11	Stro	10	Hoog	Hoog	Grof	Meel
12	Positieve controle	5	Hoog	Hoog	Fijn	Meel

In dit onderzoek werden verdunde voeders vergeleken met een onverdund controlevoer dat voldeed aan de normen van een gangbaar legvoer (11,8 MJ/kg, 6,7 g dvllysine/kg). De voeders 3 tot en met 11 waren allemaal 10% verdund. Bij de voeders 3 - 5 werd gebruik gemaakt van verdunningsmateriaal dat geen NSP bevatte (zand, maagkiesel), terwijl de voeders 6 - 11 verdund werden met NSP-rijke grondstoffen. Van enkele behandelingen is zowel een meelvorm als een kruimelvorm meegenomen. Meel is de gangbare praktijk, maar het doel van de kruimels was o.a. om na te gaan wat het effect is van voervorm op voeropnametijd. Anderzijds werd hierdoor voorkomen dat er ongewenste voerselektie optrad, waarbij de hennen het zand niet of onvoldoende opnamen, waardoor het effect van nutriëntverdunding verloren gaat.

De gekozen NSP-bronnen varieerden in samenstelling. Haverdoppen bevatten een hoog aandeel lignine. Bietenpulp bevat veel pectine; sojahullen en arbocell veel cellulose. Op basis van de uitgevoerde chemische analyses bleek dat van de NSP-rijke voeders alleen die met haverdoppen een vergelijkbaar gehalte aan water-oplosbare NSP bevatten als de voeders met een laag NSP-niveau. Verwacht werd dat dit ook voor de voeders met arbocell, sojahullen en stro zou gelden, maar dit was niet het geval.

De voeders zijn geoptimaliseerd volgens de behoefte van jonge leghennen en verstrekt aan de hennen in de leeftijd van 18 – 25 weken. T.o.v. de negatieve controle had de positieve controle een 5% lager energieniveau en een ruim 20% hoger NSP-gehalte. In dit voer waren zonnebloemzaadschilfers de belangrijkste NSP-bron. Uiteindelijk konden de twaalf behandelingen geclusterd worden tot zes hoofdfactoren: 1) controleniveau (meelvoer, gangbaar energiegehalte, laag NSP, fijngemalen voer), 2) effect van laag NSP-niveau, 3) effect van hoog NSP-niveau, 4) effect van kruimel, 5) effect van grof malen en 6) effect van oplosbaarheid van NSP.

## Waarnemingen

De voeders zijn chemisch geanalyseerd. Ook is de deeltjesgrootteverdeling bepaald. Wekelijks werden het voerverbruik en de eiproductiegegevens verzameld. De gewichten van de hennen, de kwaliteit van het verenkleed en de voeropnametijd zijn bepaald in week 1, 4 en 8.

Aan het eind van het experiment is bij vier hennen per hok het gewicht en de inhoud van de spiermaag bepaald.

## Statistische verwerking

Wekelijks verzamelden we de technische resultaten van de hennen, zodat er sprake was van herhaalde waarnemingen (longitudinale data). Deze resultaten bleken ofwel een exponentieel of een logistisch (S-curve) verloop te hebben. Dit verloop is gemodelleerd met behulp van een REML-procedure en een non-lineaire parameterschatting. Vervolgens is de Base-Level methode gebruikt om na te gaan of de geschatte modelparameters van de verschillende hoofdfactoren (NSP-laag, NSP-hoog, kruimel, grof malen, oplosbaarheid van NSP) afweken van die van de controlegroep.

## Resultaten

De belangrijkste resultaten van dit experiment zijn:

- Leghennen zijn aan het begin van de legperiode goed in staat te compenseren voor voeders, die 10% verdund zijn met NSP-vrije of NSP-rijke grondstoffen, door respectievelijk een 10,5% en 8,0% hogere voeropname (zie figuur 2). De vorm (meel versus kruimel) en de maalfijnheid van het voer hebben geen invloed op de voeropname.

- De hennen die NSP-laag of NSP-hoog voer kregen bereikten eerder hun maximale eiproductie dan de hennen die controlevoer kregen, zodat ze tijdens de proefperiode meer eieren produceerden. Het grof malen van het voer tendeeft naar een lager legpercentage, terwijl de vorm van het voer geen invloed heeft op het legpercentage.
- Het startgewicht van de eieren van de hennen die grof gemalen voer kregen was lager dan die van de controlegroep. De toename in eigewicht was lager bij de behandelingen met NSP-hoog en grof gemalen voer.
- Hennen die NSP-laag en NSP-hoog voer kregen produceerden meer eimassa, terwijl hennen die grof gemalen meel kregen juist minder eimassa produceerden dan de controlegroep. De hoeveelheid eimassa werd niet beïnvloed door de vorm van het voer.
- Hennen die NSP-hoog voer kregen hadden een hoger lichaamsgewicht dan de controlegroep, terwijl hennen die grof gemalen voer kregen juist een lager lichaamsgewicht hadden.
- Het verstrekken van voer met veel niet-wateroplosbare NSP's verhoogt het gewicht van zowel de volle als lege spiermaag en van de inhoud van de spiermaag. Het verstrekken van grof gemalen voer verhoogt eveneens het gewicht van de volle en lege spiermaag.
- De tijd die hennen op het controlevoer aan voeropname besteedden nam toe in de loop van de proefperiode van 16,4% in week 4 tot 24,6% in week 9. Het verstrekken van NSP-hoog voer verhoogde de eettijd met 22%, terwijl de andere factoren (NSP-laag, voervorm, maalfijnheid) geen effect hadden op de voeropnametijd.

### **Toepassing voor de Praktijkonderzoek**

Op basis van de resultaten van dit kortlopende onderzoek kunnen we vaststellen dat het verstrekken van NSP-laag of NSP-hoog voer aan hennen in het begin van de legperiode resulteert in vergelijkbare of zelfs verbeterde dierprestaties tijdens de eerste 8 weken van de legperiode in vergelijking met de controlegroep. Het grof malen van het voer vermindert de technische resultaten, terwijl de voervorm hierop geen effect heeft. Hoewel verenpikken zich niet voordeed in dit experiment zijn er toch indicaties dat voer met een hoog gehalte aan niet-wateroplosbare NSP's gunstig kan werken tegen verenpikken. Dieren die dergelijk voer kregen besteedden meer tijd aan voeropname en hadden een hoger spiermaaggewicht (vol en leeg) en spiermaaginhoud. Verhoging van de voeropnametijd en gewicht van de spiermaag zijn beide indicatoren voor meer voeropnamegericht gedrag en een hoger verzadigingsniveau van de hennen. Deze factoren zijn weer gunstig voor het voorkomen van verenpikgedrag. Uit een uit te voeren tweede studie, met een langere looptijd, moet blijken of deze effecten herhaalbaar zijn.

## Summary

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 stresses 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. 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. From the literature it has been shown that nutritional factors may positively or negatively affect feather pecking behaviour in laying hens. Nutritional factors seem to reduce feather pecking behaviour in laying hens if these factors increase the time spent on feeding behaviour, by affecting foraging and feed intake. Laying hens may spend more time on these feeding behaviours when they are fed 1) mash diets in stead of crumbles or pellets, 2) low energy diets, 3) high (in-)soluble fibre diets or 4) roughages. However, such feeding strategies may not reduce egg performance of the hens, which can be the case when hens are not able to fully compensate for the dilution. Especially hens at early lay can have problems with consuming sufficient feed for maintenance and egg production.

Therefore, by order of the Product Board Animal Feed and the Product Board Poultry & Eggs an experiment was conducted to measure the effect of energy dilution and feed structure on performance and behaviour of hens at the beginning of the laying period.

In this experiment, 12 experimental diets varying in level of dilution, NSP content, solubility of NSP, particle size of NSP, and feed form, were tested.

### Overview of the different treatments and their characteristics

	Additive	Dilution (%)	NSP Class	Level of soluble NSP	Particle size of NSP-fraction	Feed form
1	Negative Control – Mash	0	Intermediate	Low	Fine	Mash
2	Negative Control – Crumble	0	Intermediate	Low	Fine	Crumble
3	Sand – Mash	10	Low	Low	Fine	Mash
4	Sand – Crumble	10	Low	Low	Fine	Crumble
5	Grit	10	Low	Low	Coarse	Mash
6	Oat hulls (fine)	10	High	Low	Fine	Mash
7	Oat hulls (coarse)	10	High	Low	Coarse	Mash
8	Beet pulp	10	High	High	Coarse	Mash
9	Arbocell	10	High	High	Coarse	Mash
10	Soya hulls	10	High	High	Coarse	Mash
11	Straw	10	High	High	Coarse	Mash
12	Positive Control	5	High	High	Fine	Mash

Hens were housed in ground pens (10 hens per pen) and pen was the experimental unit. In total 480 hens, divided over 48 pens (4 replicates per diet, were involved in the experiment.

The most important conclusions of this experiment are:

- Laying hens at early lay that were fed NSP-low or NSP-high diets were able to compensate for 10% dietary dilution by a 10.5 and 8.0% higher feed intake, respectively. Feed intake of the soluble NSP diluted diets increased with 6.4%. Feeding crumble or coarsely ground mash did not affect feed intake.
- As a result of the higher rate of increase of hen-day egg production, hens that were fed low-NSP or high-NSP diets on average produced more eggs during this experiment, whereas coarse grinding of the diets tends to a lower egg production. Feed form has no effect on hen-day egg production.
- Coarse grinding of the diets negatively affects initial egg weight, whereas the rate of increase of egg weight decreases when the hens are fed NSP-high or coarse ground diets.
- Egg mass enhances by feeding NSP-low, and both soluble and insoluble NSP-high diets, and decreases by feeding coarse ground meal. Egg mass was not affected by feed form.
- Mean bodyweight of hens that were fed (in-) soluble NSP-high diets is higher than the control, whereas feeding of coarse ground meal reduces mean bodyweight.

- Feeding insoluble NSP-high diets increases full and empty gizzard weight and gizzard content. Coarsely ground diets increases full and empty gizzard weight.
- Eating time of the hens fed the undiluted diets increased over the experimental period from 16.4 to 24.6%, but was not affected by sand or grit addition, particle size distribution or feed form. Feeding NSP-high diets increased eating time with 22%, although eating time of the hens that were fed soluble NSP-high diets over week 7 and 9 was comparable with the undiluted diets.

### **Practical implications**

Based on the literature, feeding diets diluted with NSP-free or NSP-high raw materials were expected to reduce feather pecking behaviour in laying hens. The hens should compensate for dietary dilution by higher feed intake, resulting in a higher proportion of time spend on feed intake, by which less time will remain for feather pecking. However, such feeding strategies may not reduce egg performance of the hens, which can be the case when hens are not able to fully compensate for the dilution.

From this experiment we can conclude that feeding low-NSP or high-NSP diets resulted in equal or even improved egg performance of hens at early lay compared with hens that were fed a standard diet. Feeding coarsely ground meal negatively affects egg production, egg weight, egg mass and body weight, whereas feed form did not affect egg performance. Although feather pecking behaviour in this experiment not occurred, some results of this study are indicating that insoluble NSP-rich diets can have anti feather pecking properties. Hens that were fed these diets spent more time on feed intake and had heavier relative gizzard weight and content. Increased eating time and gizzard weight are both indicators for more feed related behaviour and/or a higher satiety level of the hens, which can prevent feather pecking behaviour.



# Content

## Samenvatting

### Summary

<b>1</b>	<b>Introduction</b> .....	<b>1</b>
<b>2</b>	<b>Materials and methods</b> .....	<b>2</b>
2.1	Definitions .....	2
2.2	Birds and management.....	2
2.3	Experimental design.....	3
2.4	Observations .....	3
2.5	Statistical analysis .....	5
2.6	General course of the experiment.....	7
<b>3</b>	<b>Results</b> .....	<b>8</b>
3.1	Chemical analyses of the diets .....	8
3.2	Particle size distribution of the diets .....	9
3.3	Hen performance .....	11
3.3.1	Feed intake .....	11
3.3.2	Hen-day egg production.....	13
3.3.3	Egg weight.....	15
3.3.4	Egg mass .....	17
3.3.5	Feed and Energy conversion Ratio.....	18
3.3.6	Bodyweight development.....	20
3.4	Gizzard parameters.....	22
3.5	Eating time and eating rate .....	23
3.6	Feather condition scores .....	25
<b>4</b>	<b>Discussion</b> .....	<b>26</b>
<b>5</b>	<b>Conclusions</b> .....	<b>29</b>
	<b>Literature</b> .....	<b>30</b>
	Appendix 1 Design of the experimental room.....	33
	Appendix 2 Division of experimental treatments.....	34
	Appendix 3 Diet composition .....	35
	Appendix 5 Modelled and realised feed intake (g/h/d) per week per pen.....	38
	Appendix 6 Uncorrected hen-day egg production data.....	39
	Appendix 7 Modelled and realised hen-day egg prod. (%) per week per pen.....	40
	Appendix 8 Uncorrected egg-weight data .....	41
	Appendix 9 Modelled and realised egg-weight (g) per week per pen.....	42
	Appendix 10 Uncorrected egg-mass data.....	43
	Appendix 11 Uncorrected bodyweight data .....	44
	Appendix 12 Modelled and realised egg-weight (g) per week per pen.....	45
	Appendix 13 Effect of individual diet on egg performance per period.....	46
	Appendix 14 Uncorrected gizzard data .....	47
	Appendix 15 Uncorrected eating time data.....	48

## 1 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 fulfil the birds' need to express their natural behaviour. This stresses 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, Mollenhorst, 2005). Feather pecking, especially the severe type, negatively affects the welfare of laying hens (Blokhuys 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). In deep litter systems and organic farming, mortalities of even up to 30%, as a result of feather pecking and cannibalism, have been reported (Morgenstern, 1995, Mollenhorst, 2005). 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 (Blokhuys, 1989). From the literature it has been shown that nutritional factors may positively or negatively affect feather pecking behaviour in laying hens (Krimpen *et al.*, 2005). Some investigations, indeed, show that feather pecking behaviour is a substitute for normal feeding behaviour (Hoffmeyer, 1969, Blokhuys, 1989). 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 (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 behaviour and cannibalism. Nutritional factors seem to reduce feather pecking behaviour in laying hens if these factors increase the time spent on feeding behaviour, by affecting foraging and feed intake. Laying hens may spend more time on these feeding behaviours when they are fed 1) mash diets in stead of crumbles or pellets, 2) low energy diets, 3) high (in-)soluble Non Starch Polysaccharides (NSP) diets or 4) roughages (Krimpen *et al.*, 2005). The particle size of NSP-high raw materials also seems to affect feather pecking behaviour. In the current experiment we will focus on low energy and NSP-high diets, also called low-nutrient density diets.

Nutrient density can be decreased by addition of NSP-low raw materials, like sand and grit, or by NSP-high raw materials, like oat hulls, soya hulls, beet pulp and straw. NSP-high 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). However, the combined effects of nutrient density, and water solubility and particle size of the NSP fraction, on feed intake behaviour is unknown. Laying hens, which were fed low nutrient density diets, normally will compensate for the lower nutrients by increased feed intake (Savory, 1980; Lee *et al.*, 2001). However, feed intake capacity of modern laying strains at early lay, even when fed undiluted diets, often seems not to be sufficient to meet their requirements. Therefore, a reduction in nutrient density could result in too low nutrient intake at early lay, and as a consequence in reduced layer performance. By order of the Product Board Animal Feed and the Product Board Poultry & Eggs an experiment was conducted to measure the effect of different nutritional factors (nutrient density, NSP-content, solubility of NSP-fraction, particle size of NSP-fraction, feed form) on feed intake behaviour and performance of laying hens at early lay. Sand and grit were added as NSP-free dilution materials, whereas oat hulls, beet pulp, arabocell, soya hulls and straw were added as NSP-high dilution materials. Most of the diets were offered in meal form. To exclude the possibility of selective feed intake, however, the sand-rich diets were provided both in meal and crumble form. These treatments were compared with control diets in meal and crumble form (diet 1-2). To measure the effect of particle size of the NSP fraction, both coarsely and finely ground oat hulls were added. The low-nutrient density diets (diet 3-11) were diluted by 10%, adding 10% diluents to 900 grams of control diet. Diet 12 was an intermediate diet, only diluted with 5%, by addition of 10% extracted sunflower seed as dilution material. From this experiment the nutritional factors, which seemed to have the most anti feather pecking properties, have been selected for further research.

## 2 Materials and methods

This chapter describes the design of the experiment. The animal experiment committee of ASG-Lelystad, the Netherlands, approved this experiment.

### 2.1 Definitions

In this report a number of specialist terms is used, which in this chapter are explained.

- NSP = Non starch polysaccharides. It's plant cell wall material, which is calculated as:  
1000 – ash – crude protein – fat – starch – sugar (dry matter base).
- NSP (structural) = the water insoluble plant cell wall material, which is determined by the NDF analysis.
- Water soluble (non structural) NSP can be calculated as: NSP – NDF.
- NSP-low diets are diets, which are diluted with sand or grit.
- NSP-high diets are diets, which are diluted with NSP-rich raw materials like oat hulls, beet pulp, arabocell, soya hulls and straw.
- Grinding structure: diets with an average particle size of 0.82 mm or lower were considered as fine, whereas diets with an average particle size of 0.85 or higher were considered as coarse.

### 2.2 Birds and management

Hens were housed in two rooms, both measuring 9 x 9 metres. Each of the rooms contained 24 floor pens, divided over three rows, and three sliding doors. The room design is shown in Appendix 1. The pens measured 90 x 150 centimetres each. Subtracting the area of the feeding trough – 20 x 100 centimetres – results in 1,15m<sup>2</sup> for 10 hens, that is 9 hens per m<sup>2</sup>, which is the density required for free-range chickens. The pens were built from wires and hens could see the chickens in the other pens.

A laying nest was placed at the outside of the pen. In the back of the pen, 2 perches were present at two different suitable heights. A feeding trough was placed at the long side of the pen, between the laying nest and the perches. The water tube with nipple drinkers was placed at the front side of the pen, between the laying nest and the feeding trough.

Two times a day, a check-up was done to control the (health) status of the hens. Temperature was set to 20°C. The animals used were ISA Brown laying hens. The hens were obtained from a commercial poultry trader. The hens arrived at 16 weeks of age. The hens were not beak trimmed, but the beaks had been touched. Beak length, however, showed a large variety. Initially, the hens were housed with eleven animals per pen. Three days after the arrival, all hens were selected based on their bodyweight. Hens were allowed to weigh between 1130 and 1550 grams. Lighter or heavier hens were removed from the experiment. All selected hens were marked with a wing mark and their numbers were listed. Finally, 10 hens per pen were placed.

At arrival, the hens were fed a commercial diet (OE<sub>Broiler</sub> = 2600 Kcal) for rearing laying hens, ageing over 6 weeks. When the hens were 17.5 weeks old, the start of the experiment was made and all hens received their experimental laying diet. Every pen had an own, marked bucket with cover, containing weighed feed. Weekly, the feeding troughs were emptied in buckets and the leftovers of the feed were weighed. After that, new feed was put in the bucket and weighed again. Twice a week the troughs were filled from the bucket. Hens were fed *ad libitum*. Water was given unrestricted. Each pen had three or four nipple drinkers.

At arrival, on the age of 16 weeks, the hens got 10 hours of light per day. The hens only received artificial light; the windows were covered with black agricultural plastic. Weekly, the light period was extended with one hour, by switching on the light one hour earlier, till they had 16 hours of light per day at the age of 22 weeks. To induce feather pecking behaviour, light intensity was increased three times. Starting light intensity was around 10 lux, using bulbs. The first light increase was done when the hens were 18 weeks old to 20 lux and at the age of 19.5 weeks to 20-30 lux. At 21.5 weeks, the bright lights were used all day, giving a light intensity varying between 40 and 60 lux, depending on the place in the room.

Weekly, together with the fill of the feeding trough, the sand of the pens was turned over to transport dryer sand to the places in the pen where most droppings fell. The moister sand was placed to the dust bathing part, so it could dry. Then, the laying nests were also vacuum cleaned, as was the rest of the room.

#### Water delivery problems

The water supply was hampered during two days in department 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 department,

all treatments were equally affected by this. To correct for this effect, a factor was included in the statistical model which predicts feed intake.

## 2.3 Experimental design

In total, 12 treatments with each 4 replications were divided among the rooms. In each of the two rooms, 2 replications were present. Each room was divided in two blocks (see Appendix 1). To correct for differences in light intensity, air movements and other environmental parameters, the first four pens of each row in a room were allocated to one block, as were the last four. In each block one complete replication was present. The division of treatments between the two rooms is shown in Appendix 2.

Two NSP-low dilution materials were tested (diet 3-5). Five different NSP-sources, differing in the content of soluble NSP, were tested (diet 6-11). Some diets were produced as crumble (diet 2 and 4) and some diets were coarsely ground (diet 5, 7-11) to test the effect of feed form (meal vs crumble) and particle size of the NSP-fraction (finely versus coarsely ground meal). The control diet met the needs of the laying hens. Diets 3-11 were 10% diluted, adding 100 grams diluents to 900 grams of control diet. Finally, a positive control diet was tested (treatment 12). The dilution level of this diet was 5% and the diet composition deviated from the other diets; it contained less wheat and soybean meal expeller, mostly compensated by peas. The characteristics of the different treatments are shown in Table 1, while the diet composition and calculated chemical analysis are shown in Appendix 3.

**Table 1** Overview of the different treatments and their characteristics

	Additive	Dilution (%)	NSP Class	Level of soluble NSP	Particle size of NSP-fraction	Feed form
1	Negative Control – Mash	0	Intermediate	Low	Fine	Mash
2	Negative Control – Crumble	0	Intermediate	Low	Fine	Crumble
3	Sand – Mash	10	Low	Low	Fine	Mash
4	Sand – Crumble	10	Low	Low	Fine	Crumble
5	Grit	10	Low	Low	Coarse	Mash
6	Oat hulls (fine)	10	High	Low	Fine	Mash
7	Oat hulls (coarse)	10	High	Low	Coarse	Mash
8	Beet pulp	10	High	High	Coarse	Mash
9	Arbocell	10	High	High	Coarse	Mash
10	Soya hulls	10	High	High	Coarse	Mash
11	Straw	10	High	High	Coarse	Mash
12	Positive Control	5	High	High	Fine	Mash

Diets 1 and 2 were considered as standard diets, diets 3-5 as diets with low NSP-level; diets 6-11 as NSP-high diets, whereas diet 12 was an intermediate diet. The NSP sources were added to the feed after grinding, except for the oat hulls of diet 6, which were hammer milled together with the other raw materials.

## 2.4 Observations

### Chemical analysis of the diets and particle size distribution

The diets were chemically analysed on dry matter, ash, crude protein (nitrogen x 6.25), fat, crude fibre, starch, sugar, NDF, ADF, ADL (= Lignin), potassium, sodium, phosphor, calcium, chloride, copper, zinc and iron. The content of cellulose was calculated as ADF minus ADL, whereas the hemi-cellulose content was calculated as the difference between NDF and ADF. The soluble NSP content on dry matter base was calculated as: 1000 – ash – crude protein – fat – starch – sugar – NDF.

The particle size distribution of the meal diets was analysed according to the dry sieve method. To make the particle size of the meals and crumbles comparable, the particle size distribution of diets 1 – 4 (control and sand diets in both meal and crumble form) were analysed by using the wet sieve method (Zandstra, 2001). For this method 200 g of feed has to be dissolved in 500 ml water, soaked for 45 min, after which this solution has to be flushed by the sieves. The content of each sieve is dried and weighed. Seven particle size fractions were distinguished by using sieves with diameters of 0.25, 0.50, 1.25, 2.50, 3.15 and 5.0 mm respectively. The average particle size was calculated as:

$(\text{Fraction} < 0.25 * 0.125) + (\text{Fraction } 0.25 - 0.50 * 0.375) + (\text{Fraction } 0.50 - 1.25 * 0.875) + (\text{Fraction } 1.25 - 2.50 * 1.875) + (\text{Fraction } 2.50 - 3.15 * 2.830) + (\text{Fraction } 3.15 - 5.00 * 4.07) + (\text{Fraction} > 5.00 * 6.50)/100$ . An average particle size of 0.82 mm or lower was considered as fine, whereas an average particle size of 0.85 mm or higher was considered as coarse. The diets to which the coarsely ground NSP sources were added, and also the grit diet were defined as course; the other diets as fine.

### Start of the experiment

The experiment started on the 26<sup>th</sup> of April, when the hens aged 17.5 weeks, and lasted 8 weeks till the 20<sup>th</sup> of June. In week 9, no performance parameters but only video observations were recorded. For some analyses, three periods were distinguished, as shown in Table 2.

**Table 2** Time periods in the experiment

Period	Experimental week	Starting Tuesday	Age of the hens (weeks)	Ending Monday
1	1-3	26 April	17.5	16 May
2	4-6	17 May	20.5	6 June
3	7, 8	7 June	23.5	20 June

### Climate

Twice a day, temperature and air humidity of both rooms was recorded, together with the check-up of the hens.

### Egg production

Daily, eggs laid per pen per day were counted, together with the amount of ground eggs, shell-less eggs, and broken eggs. Weekly, eggs per pen were collected and sorted. Egg weight per pen was based on the amount of 'normal' egg mass, i.e. all clean and dirty (blood- or faecal-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.

### Feed intake

Weekly, the weight of the refusals and the bucket at the end of the week was subtracted from the weight of the full bucket at the start of the week, resulting in the amount of feed eaten in that week. Feed intake per hen per day was calculated as the weekly amount of feed intake, divided by the number of hens present in that week, multiplied by 7. Water consumption was not recorded.

### Body weight

All hens have been weighed five times; individually in the pre-experiment period, and per pen in week 1, 4, 7 and 9 of the experiment.

### Video observations

In week 4, 7 and 9, video observations have been made from which eating time per cage could be calculated. The day was divided in three blocks, from 9 am to 11 am, 11.30 am to 1.30 pm and from 2 pm to 4 pm. In each block on every day, eight cages were observed using 4 cameras. Each observation lasted one hour. From these, the number of hens, who were eating (between 0 and 10), was recorded continuously until the end of each observation. A computer, programmed with the Observer 4.1/5.0 software (Noldus, 1993) was used to analyse the observations. Based on the video observations, the total number of eating minutes per cage per observation period was calculated. Then, this number was divided by the number of hens per cage and by the duration of the observation period, resulting in the average percentage of time spent on feed intake per cage. Relative eating rate (eating minutes/g feed intake) on a weight base was calculated as daily eating minutes divided by feed intake (g). 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.

### Feathers

In week 1 and week 9 the feathers of all hens have been scored, using the method of (Bilcik and Keeling, 1999). The scoring values are shown in Table 3. Based on the experience of experts, the scoring could be restricted to four places – neck, back, rump and belly of the hen. Feather scoring was done together with the weighing. The same person has done scoring all the time.

**Table 3** Description of feather scoring system (Bilcik and Keeling, 1999)

Score	Body	Skin injuries
0	Intact feathers	No injuries or scratches
1	Some feathers scruffy, up to 3 missing feathers	<5 pecks or scratches
2	More damaged feathers, >3 feathers missing	5 or more pecks and scratches or 1 wound <1 cm diameter
3	Bald patch <5 cm diameter or <50% of the area	Wound >1 cm in diameter but <2 cm
4	Bald patch >5 cm diameter or >50% of the area	Wound >2 cm in diameter
5	Completely denuded area	-

**Dissection**

On the Friday of week 9, 24 June, two hens per pen were selected at random, based on the number on their wing mark. These hens were killed using an injection containing pentobarbital sodium 200 mg/ml. Dosing used was 1 ml per 2 kg of live weight. Dead hens were weighed, after which the gizzard was removed. The weights of the full and empty gizzard were calculated, related to the weight of the corresponding hen, resulting in grams per kilogram of hen. The gizzard surface was scored, using the values as mentioned in Table 4.

**Table 4** Gizzard erosion scores

Score	Description
0	No erosion
1	Light erosion (roughness of epithelia)
2	Modest erosion (roughness and gaps)
3	Severe erosion (roughness, gaps and ulcer on stomach wall)

**2.5 Statistical analysis****Curve fitting procedure**

During the experimental period performance data from the same experimental units were generated at regular intervals as longitudinal data. Furthermore, performance data of laying hens normally show a nonlinear development. For instance, hen-day egg production starts at 0% and increases to nearly 100%, following an exponential pattern. An appropriate method to model the development of 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 is presumed on the knowledge of the development of the specific performance parameters. A REML procedure in (Genstat\_8\_Committee, 2002) was used to estimate the parameters of the model. The nonlinear parameters are estimated by using a two-step iterative procedure, starting from a first order Taylor approach (Lindstrom and Bates, 1990; Engel *et al.*, 2003). Exponential curves are used for modelling feed intake and bodyweight, while logistic curves are used for modelling hen-day egg production, egg weight and egg mass. These curves can be characterised by the parameters  $Y$ ,  $t$ ,  $A$ ,  $B$ ,  $\alpha$  and  $\mu$ , as shown in Figure 1.

































































































