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Effects of dietary energy concentration, NSP concentration and particle sizes of NSP on digesta passage rate and gut development in laying hens

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Abstract
MRT in the foregut seemed to be linearly related to the level of dietary NSP intake. The increase of MRT was more pronounced in hens fed coarsely compared to finely ground NSP. These findings seems to be indicators of a higher level of satiety in laying hens, which may contribute to a lower feather pecking pressure.

Keywords: laying hen, mean retention time, NSP, energy content

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Samenvatting
Er is een lineair verband gevonden tussen de hoeveelheid NSP die een leghen dagelijks opneemt en de verblijfstijd van het voer in de krop en klier-/spiermaag. Dit verband was meer uitgesproken bij grove in plaats van bij fijne vezels. Hierdoor behoudt een leghen tussen de maaltijden door langer een verzadigd gevoel. Een hogere mate van verzadiging kan bijdragen aan een afnemende behoefte tot verenpikken.

Trefwoorden: leghen, passagesnelheid, NSP, energiegehalte

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Effects of dietary energy concentration, NSP concentration and particle sizes of NSP on digesta passage rate and gut development in laying hens

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Samenvatting

Deze studie is uitgevoerd in opdracht van het Productschap Diervoeder en het Productschap Pluimvee en Eieren.

Inleiding en doel

In de literatuur wordt verondersteld dat verenpikken van leghennen een vorm is van omgericht voedselzoekgedrag. Verenpikgedrag kan zich ontwikkelen als hennen onvoldoende geprikkeld worden tot foerageergedrag. De relatie tussen voergericht gedrag en verenpikgedrag wordt onderzocht in een meerjarig project, waarvan deze studie onderdeel uitmaakt. In dit project wordt de hypothese getoetst of verenpikgedrag bij leghennen voorkomen of verminderd kan worden door:

- het verlengen van de tijd die hennen aan voergericht gedrag besteden;
- het bevorderen van de mate van verzadiging, waardoor de behoefte tot foerageergedrag vermindert.

Deze studie is uitgevoerd in opdracht van het Productschap Diervoeder en het Productschap Pluimvee en Eieren, en richt zich specifiek op het effect van voersamenstelling op de mate van verzadiging. Het doel van deze studie was het vaststellen van de afzonderlijke effecten van energieniveau, NSP- (Non Starch Polysaccharides) concentratie en deeltjesgrootte van NSP op gemiddelde verblijftijd van het voer per darmsegment en op ontwikkeling van het maag-darmkanaal. Vooraf veronderstelden we dat verstrekking van voer met een laag energiegehalte en/of een hoog gehalte aan grofgemalen niet-wateroplosbare NSP zou resulteren in verlaging van de totale gemiddelde verblijftijd, waardoor hennen weer eerder behoefte hebben aan de volgende maaltijd.

Materiaal

Voor het experiment is gebruik gemaakt van twee identieke afdelingen (9,0 x 9,0 m) met grondhokken (afmetingen 0,9 x 1,50 m). In elk hok stond een voertrog van 1,0 x 0,2 m, zodat er 1,15 m^2 netto leefoppervlak overbleef. Voor elke hok was een legnest geplaatst. Tijdens dit experiment was het niet gewenst dat hennen de beschikking hadden over vezelrijk strooisel. Daarom is gekozen voor zand als strooiselmateriaal. De hennen hadden onbeperkt water en voer ter beschikking. Het voer werd verstrekt in een voertrog en het water in drinknippels. De bodem van het hok was volledig ingericht als scharrelruimte. In elk hok waren twee zitstokken aangebracht die voldoende ruimte boden voor alle hennen.

Op een leeftijd van 16 weken werden 504 niet-gekapte Isa Brown hennen ingezet, verdeeld over 42 grondkooien (zes behandelingen met zeven herhalingen per behandeling). De hennen waren als eendagskuiken aangevoerd en op de proefaccommodatie opgefokt met een bezettingsdichtheid van 13 kuikens/m^2. Ze kregen een gangbaar entschema en lichtschema en water en voer was onbeperkt beschikbaar. Vanaf 8 weken leeftijd kregen de hennen een laagenergetisch opfokvoer (OE_Kuiken = 2500 KCal). Al tijdens de opfokperiode (vanaf 5 weken leeftijd) namen we pikgedrag in de koppel waar.

Het eigenlijke experiment startte toen de hennen 18 weken oud waren en duurde 22 weken. Er werden 12 dieren per hok gehuisvest op 1,15 m^2, wat een duidelijk hogere bezettingsgraad is dan wat gangbaar is voor scharrelkippen (negen hennen/m^2); dit om de kans op verenpikken te verhogen. De hennen werden random over de hokken verdeeld, waarbij gestreefd is naar een gemiddeld gelijk opzetgewicht per hen. Elke hen kreeg een vleugelmerk met daarop een uniek nummer en een unieke combinatie van pootringen, zodat ze individueel herkenbaar waren. Ter beheersing van de lichtintensiteit waren de ramen in de afdelingen geblindeerd en werd er alleen gebruik gemaakt van kunstlicht. Vanaf het indelen van de hennen (week 16) kregen de dieren 10 uur licht per dag met een lichtsterkte van 10 lux (acht peertjes van 25 Watt per afdeling). Wekelijks werd dit met 1 uur verhoogd tot een maximum van 16 uur licht per dag (week 23). Om het verenpikken op te wekken werd de lichtintensiteit geleidelijk opgevoerd. In week 18 verhoogden we de lichtintensiteit naar 20 lux (60 Watt peertjes), terwijl we deze in week 20 verder opvoerden naar 30 lux (100 Watt peertjes). Na een uitbraak van kannibalisme in week 21 is de lichtintensiteit teruggebracht naar 20 lux en vanaf dat moment niet meer gewijzigd.

Methode

In het experiment zijn de volgende proeffactoren onderzocht:

- Gangbaar energieniveau (11,8 MJ/kg) versus laag energieniveau (10,6 MJ/kg; 10% verdunning van het voer door toevoeging van zand).
- Gangbaar (133 g/kg) versus hoog (195 g/kg) inert niet-water oplosbaar NSP-gehalte door toevoeging van 10% haverdoppen. Verhoging van het NSP-gehalte verlaagde echter ook de nutriëntendichtheid. Dit werd bij het gangbare energieniveau gecompenseerd door toevoeging van extra vet (behandeling 2 en 3).
- Fijn versus grof malen van de niet-water oplosbare NSP-fractie. Bij fijne maling werden de haverdoppen vooraf fijn gemalen op een 1 mm zeef en daarna buiten de hamermolen om aan de menger toegevoegd.

Bij de grove maling werden de haverdoppen ongemalen buiten de hamermolen om aan de menger toegevoegd. De gemiddelde deeltjesgrootte van de fijn gemalen voeders was 0,87 mm ± 0,02 tegenover 1,05 ± 0,04 voor de fijngemalen voeders.

Deze opzet resulteerde dus in zes behandelingen, zoals aangegeven in onderstaande tabel.
De voeders werden zo samengesteld dat energieniveau en NSP-gehalte onafhankelijk van elkaar varieerden. De voeders 2 en 3 hadden een identieke samenstelling. Deze voeders verschilden alleen in maalfijnheid van de NSP-fractie. Dit gold ook voor resp. voeders 5 en 6.

Behandeling	1	2	3	4	5	6
Energieniveau	Gangbaar	Gangbaar	Gangbaar	Laag	Laag	Laag
Niet-oplosbaar NSP-gehalte	Gangbaar	Hoog	Hoog	Gangbaar	Hoog	Hoog
Maalfijnheid NSP	Geen NSP	Fijn	Grof	Geen NSP	Fijn	Grof

Waarnemingen
Aan het einde van het experiment (40 weken leeftijd) werden drie van de zeven hokken per behandeling geselecteerd. In de geselecteerde hokken kregen vijf aselect gekozen hennen drie capsules met titaniumoxide gevoerd (t=0). Hiermee kreeg elke hen 450 mg titaniumoxide verstrekt. Vervolgens werd op vijf opeenvolgende tijdstippen (t = 30, 90, 180, 270 en 360 minuten na moment van titaniumverstrekking) een van de vijf hennen geëuthanaseerd. Na sectie werd de inhoud van het maag-darmkanaal, opgedeeld in vijf segmenten (krop, klier- en spiermaag, dunne darm, dikke darm en blinde darmen) bepaald door elk segment met en zonder inhoud te wegen. Het titaniumgehalte per darmsegment is een maat voor de passagesnelheid van het voer. De gemiddelde verblijftijd is vervolgens gerelateerd aan het voeropnamegedrag van de hennen in de laatste week voorafgaand aan de sectie.

Modellering en statistische verwerking
Het verloop van het titaniumgehalte per segment in de tijd, als een indicator voor gemiddelde verblijftijd van het voer, is gemodelleerd door gebruik te maken van een multicompartimenten model. Dit model berekende de snelheid (f1…f5) van vullen en ledigen van elk segment. De gemiddelde verblijftijd in de krop werd berekend als 1/f1, in de klier- en spiermaag als 1/f1+ 1/f2 enz.
De gemiddelde verblijftijd per segment, de totale verblijftijd in het maag-darmkanaal, de voeropnamekarakteristieken, het gewicht en inhoud van de darmsegmenten zijn statistisch getoetst met een REML-procedure.
Het experiment was opgezet als een 2 x 3 factoriële proef. De factor op twee niveaus was energie (laag en normaal) en de factor op drie niveaus was een combinatie van de factoren NSP en Grofheid (hoog NSP-fijn, hoog NSP-grof, en laag NSP). De twee vrijheidsgraden voor dit hoofdeffect zijn opgesplitst in een contrast NSP (gemiddelde van fijn en grof hoog NSP t.o.v. laag NSP) en een contrast grofheid (grof hoog NSP t.o.v. fijn hoog NSP). De twee vrijheidsgraden van de interactie zijn gesplitst in een contrast energie x NSP en een contrast energie x grofheid. Het statistische model, waarbij het hok de experimentele eenheid was, zag er als volgt uit:

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

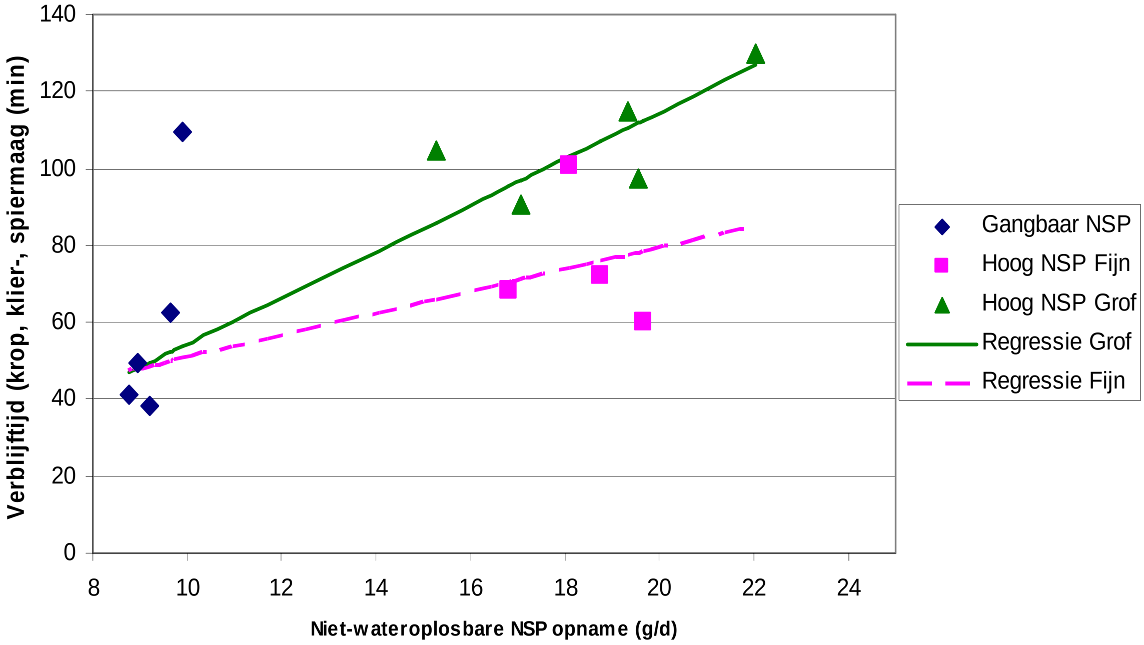
Onder de tabellen met behandelingsgemiddelden zijn overschrijdingskansen (P-waarden) weergegeven van het toetsen van de effecten: energie, NSP, grofheid, energie x NSP en energie x grofheid.

Resultaten

Verblijftijd
Het verstrekken van voer met een hoog NSP-gehalte verdubbelde de gemiddelde verblijftijd van het voer in de krop (68,4 versus 33,7 min), terwijl ook de gemiddelde verblijftijd in de krop + klier-/spiermaag aanzienlijk toenam (90,6 versus 56,8 min) vergeleken met het verstrekken van voer met een gangbaar NSP-gehalte.
Het energiegehalte en de maalfijnheid van de NSP-fractie hadden geen effect op de verblijftijd van het voer aan het begin van het maag-darmkanaal. Het verstrekken van voer met een laag energiegehalte resulteerde wel in een langere verblijftijd in de dikke darm (26,0 versus 6,7 min.), blinde darmen (3,9 versus 1,8 min) en dikke darm + blinde darmen (30,3 versus 8,6 min.) vergeleken met voer met een gangbaar energiegehalte.
Het grof malen van NSP verlaagde in vergelijking met fijn gemalen NSP de gemiddelde verblijftijd in de blinde darmen (1,8 versus 4,6 min.). In tegenstelling tot onze hypothese hadden de behandelingen geen effect op de verblijftijd van het voer in het totale maag-darmkanaal. Dit wijst erop dat de toegenomen verblijftijd van het NSP-rijke voer in de krop + klier-/spiermaag is gecompenseerd door een (niet-significante) afname van de verblijftijd in de rest van het maag-darmkanaal.

Ontwikkeling van darmsegmenten
Door verstrekking van NSP-rijk voer nam het relatieve lege gewicht van de klier-/spiermaag toe met 30% (25,2 versus 19,4 g/kg hen) in vergelijking met verstrekking van voer met een gangbaar NSP-gehalte, terwijl de inhoud van de klier-/spiermaag met 18% toenam (15,4 versus 13,0 g/kg hen). Het relatieve lege gewicht van de spiermaag was tevens afhankelijk van de maalfijnheid van de NSP-fractie. Grofgemalen NSP zorgde voor een 30% zwaardere spiermaag dan bij verstrekking van fijngemalen NSP (28,5 versus 21,9 g/kg hen). De dunne darm van hennen die voer met het gangbare NSP-gehalte kregen, bevatte op drogestofbasis 52% meer inhoud als voer met een laag energieniveauvoer in plaats van een gangbaar energieniveau (4,4 versus 2,9 g/kg hen); terwijl bij verstrekking van voer met het hoge NSP-gehalte geen effect was van het energiegehalte van het voer op de hoeveelheid droge stof in de dunne darm.

Relatie NSP-opname en gemiddelde verblijftijd
Er bleek een lineair verband te zijn tussen de hoeveelheid niet-wateroplosbare NSP die dagelijks via het voer wordt opgenomen en de verblijftijd in de krop + klier-/spiermaag (zie onderstaande figuur). Dit verband was bovendien meer uitgesproken als de NSP's een grove in plaats van fijne structuur hadden.
Opmerking bij figuur: punt (10, 110) is beschouwd als uitbijter en daarom niet meegenomen in de regressie.



Bij elke extra gram grove niet-wateroplosbare NSP die dagelijks werd opgenomen, steeg de gemiddelde verblijftijd in de krop + klier-/spiermaag met 6 min., terwijl deze toename 2,9 minuten bedroeg bij niet-wateroplosbare NSP met fijne structuur. De formule voor de regressielijnen staat in onderstaande tabel.

Regressie	R^2	Significantie
MRT =:		
6,02 x niet-wateroplosbare grove NSP-opname – 6,0	0,89	*P*<0,001
2,86 x niet-wateroplosbare fijne NSP-opname + 22,3	0,42	*P*=0,048

Op basis hiervan mogen we dus veronderstellen dat de hoeveelheid opgenomen NSP positief gecorreleerd is aan de mate van verzadiging van een leghen.

Conclusies

- Verstrekking van voer met een hoog NSP-gehalte resulteert in een aantoonbare verlenging van de verblijftijd van het voer in het begin van het maag-darmkanaal (krop en klier- en spiermaag). Het verband tussen NSP-opname en deze verblijftijd blijkt in de huidige dataset rechtlijnig te zijn en meer uitgesproken bij grove dan bij fijngemalen NSP.
- Verstrekking van voer met een laag energiegehalte verlengt de verblijftijd van het voer in het einde van het maag-darmkanaal (dikke darm en blinde darmen).
- NSP-rijk voer verhoogt zowel het relatieve gewicht van de klier-/spiermaag zelf als van de inhoud van dit darmsegment. Het gewicht van de klier-/spiermaag neemt verder toe als de NSP-fractie grofgemalen is.

Verstrekking van NSP-rijk voer zorgt er dus voor dat dit voer langer aanwezig blijft in de krop en de klier-/spiermaag. Hierdoor houdt een leghen tussen de maaltijden door dus langer een verzadigd gevoel. Een hogere mate van verzadiging kan bijdragen aan een afnemende behoefte tot verenpikken.

Summary

An experiment was conducted with 504 ISA Brown layers from 18 to 40 weeks of age to investigate the effects of energy concentration, non-starch polysaccharides (NSP) concentration and particle size of added NSP source on mean retention time (MRT) of digesta and gut development. Hens were allotted to 6 dietary treatments according to a 2 x 3 factorial arrangement, with 3 replicates per treatment. Experimental factors were energy concentration (11.8 versus 10.6 MJ/kg), 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 MRT will be decreased by feeding diets with low energy levels and/or high contents of coarsely ground insoluble NSP's.
Increasing the dietary NSP concentration, however, significantly 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, conversely, resulted in a longer MRT in the colon (26.0 vs. 6.7 min), caeca (3.9 vs. 1.8 min), and as a result also in the total hind gut (30.3 vs. 8.6 min) 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). In contrast to our hypothesis, total MRT was not affected by dietary treatments, possibly indicating that the increased MRT of NSP high diets in the foregut was counterbalanced by a decrease in the hind gut.
Feeding high NSP diets increased relative empty proventriculus/gizzard weight of hens by even 30% (25.2 versus 19.4 g/kg) and the digesta weight in the proventriculus/gizzard by 18% (15.4 versus 13.0 g/kg) compared with hens fed 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 versus 21.9 g/kg).
In control NSP fed hens, dry content of small intestine was 52% higher in low energy diets compared to control NSP diets (2.9 versus 4.4 g/kg hen), whereas dry content of small intestine was not affected by energy concentration in high NSP diets. Fresh caeca content was 12.5% higher in hens fed low energy diet compared with control energy diet (2.4 versus 2.7 g/kg hen), whereas dry caeca content was similar for all treatments.
It was concluded that addition of NSP to the diet may increase the weights of the gizzard and the gizzard content, and may extend MRT in the foregut. MRT in the foregut seemed to be linearly related to the level of dietary NSP intake. The increase of MRT was more pronounced in hens fed coarsely compared to finely ground NSP. These findings seems to be indicators of a higher level of satiety in laying hens, which may contribute to a lower feather pecking pressure.

Contents

Samenvatting

Summary

1 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 suggested 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). Hens in modern housing systems often spend considerable less time on feeding related behavior compared to hens in a natural environment (Dawkins, 1989). Therefore, we hypothesized that nutritional factors might reduce feather pecking behavior if these factors increase the time laying hens spent on feeding related behavior (Van Krimpen et al., 2005). Indeed, eating time of laying hens can be extended by supplementing diluted diets (Van Krimpen et al., Accepted for publication). Dietary dilution by adding 10% sand to a control diet resulted in a 10% higher feed intake, and a proportional increase of eating time of laying hens, compared to the control diet fed birds. Dietary dilution by addition of 10% non-starch polysaccharides (NSP) to a control diet, however, prolonged eating time by 20% and consequently reduced eating rate (g/min). (Van Krimpen et al., 2007). Thus, dietary dilution is not the only factor that determines eating behavior of laying hens. Eating behavior might also be affected by the level of satiety, as reflected by differences in mean retention time (MRT) of the digesta in the different parts of the GIT.

The effect of dietary dilution on MRT in different segments of the GIT depends among others on the solubility and particle sizes of the dilution source. Insoluble NSP seems to shorten overall MRT in the GIT (Roberfroid, 1993). Dietary dilution, by adding 40% cellulose powder, increased crop emptying and overall feed passage rate (Savory, 1980). Coarse particles of NSP seem to accumulate in the gizzard, thereby increasing MRT in this part of the GIT and volume of gizzard contents (Hetland et al., 2004a; Hetland et al., 2005). Coarse feed particles need to be ground to a critical size before they can leave the gizzard. So it can be hypothesized that the gizzard will not retain feed if it has no need for grinding (Hetland et al., 2005). 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).

From our previous studies, it could be deducted that increased eating times and reduced eating rates could delay feather pecking behavior (Van Krimpen et al., Accepted for publication). Thus, feather pecking behavior seems to be related to eating behavior, which could be partly affected by the level of satiety. The effects of nutritional factors on feed passage rate is not similar for all gut segments. However, little information concerning these effects on feed passage rate in different segments of the gut of laying hens, is available. Therefore, an experiment was conducted to investigate the independent effects of energy concentration, NSP concentration, and particle size of added NSP source on gut development and MRT. We hypothesize that total MRT will be decreased and eating time increased by feeding diets with low energy levels and/or high contents of coarsely ground insoluble NSP's.

2 Materials and Methods

2.1 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 weeks of age) average body weight was 1713 g (±48.0). The first 8 weeks 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 weeks of age. During the rearing period, feather pecking behavior was shown from 5 weeks of age onwards. 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 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.

2.2 Experimental Design

At 18 weeks of age, hens were allotted to six dietary treatments according to a 2 x 3 factorial arrangement, with three replicates per treatment. The factors were energy concentration (11.8 versus 10.6 MJ/kg), 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.

Table 1 Dietary ingredients, and 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
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	
Soybeans, 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	

Treatment nr. Energy concentration NSP concentration Coarseness of NSP	1 Control Control No NSP	2 Control High Fine	3 Control High Coarse	4 Low Control No NSP	5 Low High Fine	6 Low High Coarse
Analyzed content						
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[3]	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[4]	**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[5]	20.0	47.6	49.4	23.0	53.9	50.8
Hemi cellulose[5]	41.1	66.2	69.1	33.2	72.0	74.7
Calculated content						
ME (MJ/kg)	**11.8**	**11.8**	**11.8**	**10.6**	**10.6**	**10.6**
ME corrected (MJ/kg)[6]	11.5	11.5	11.8	10.2	10.7	10.4
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] 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 $Na_2SeO_3.5H_2O$).

[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

[6] Based on equation: ME = 18.03 x dig. Protein + 44.65 x dig. Fat + 17.32 x dig. Other carbohydrates; assuming that all analysed nutrients were digestible

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 versus 10.6 MJ/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.

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 < 0.25 mm x 0.125) + (fraction 0.25 – 0.50 mm x 0.375) + (fraction 0.50 – 1.25 mm x 0.875) + (fraction 1.25 – 2.50 mm x 1.875) + (fraction 2.50 – 3.15 mm x 2.830) + (fraction 3.15 – 5.00 mm x 4.07) + (fraction > 5.00 mm x 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.

2.3 Observations

Performance and behavioral recordings

Measurements of performance parameters (feed Intake, body weight, 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., accepted for publication).

Feed passage rate determination

Feed passage rate was determined in 5 birds per pen at 40 wks of age, thereby using three out of seven 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. (2006a). Initially, (t = 0) 3 gelatin capsules were manually given to each of the five hens per pen. Birds were dissected at five 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 five 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 (five segments/bird x five birds/pen x six treatments x three 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 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:

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

Where Y = the absorbance, measured by the spectrophotometer, and X = the titanium concentration.
From each digesta sample, 0.5 (± 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.

2.4 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 equations that are used to model it are presented in Appendix 1.

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

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

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 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 gut segment weights and dry matter content of gut segments.

3 Results

3.1 Feed, energy and NSP intake

Table 2 Feed intake (g/h/d), energy intake (J/h/d), NSP intake (g/h/d), volume intake (ml/h/d) and rate of feed intake (g/ min) during the week prior to week of dissection per treatment

Treatment[1]	Feed intake (g/h/d)	Energy intake (J/h/d)	NSP intake (g/h/d)	Rate of feed intake (g/min)	Rate of energy intake (J/min)	Rate of NSP intake (g/min)
Control Energy						
- Control NSP	138.0	1626	18.3	0.88	10.4	0.12
- High NSP-Fine	136.5	1611	27.6	0.90	10.4	0.17
- High NSP-Coarse	124.5	1469	24.1	1.11	13.0	0.20
Low Energy						
- Control NSP	141.8	1502	14.9	0.91	9.6	0.10
- High NSP-Fine	136.5	1446	26.8	0.75	8.0	0.15
- High NSP-Coarse	146.9	1559	28.0	0.76	8.0	0.14
Standard error	5.26	58.4	0.96	0.233	2.70	0.041
P-Value						
Energy	**0.027**	0.159	**0.004**	0.418	0.216	0.363
NSP	0.366	0.339	**<0.001**	0.992	0.992	0.092
Energy*NSP	0.525	0.525	**0.002**	0.484	0.526	0.801
Coarseness	0.895	0.938	0.368	0.671	0.677	0.792
Energy*Coarseness	**0.038**	**0.034**	**0.018**	0.655	0.648	0.651

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

[2] Estimation of standard error not available

Feed intake over week 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 versus 146.9 g/h/d). Energy intake over week 21, however, was similar for the two high NSP-coarse treatments, whereas in low energy treatments the energy intake was lower in control NSP (1626 versus 1502 J/h/d) and high NSP-fine treatments (1611 versus 1446 J/h/d) compared to control energy treatments. As expected, NSP intake over week 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 versus 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 versus 28.0 g/h/d). Volume intake over week 21 was similar for the two control NSP treatments and for the two high NSP-fine treatments, whereas in high NSP-coarse treatments volume intake was higher in hens fed low energy compared to control energy diet (176.2 versus 219.5 ml/h/d).

Rate of feed intake, rate of energy intake and rate of volume intake were not affected by dietary treatments. Rate of NSP intake showed a trend to be higher in high NSP diets compared to control NSP diets (0.11 versus 0.17 g/min; *P*=0.092).

3.2 Mean retention time

Table 3 Mean retention time (min) per segment

Treatment[1]	Crop	Proventr./ gizzard	Total Foregut[2]	Small intestine	Colon	Caeca	Total Hindgut[3]	Total 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
Standard error	37.55	14.60	31.55	39.76	13.11	1.45	10.05	33.92
***P*-Value**								
Energy	0.524	0.278	0.796	0.821	**0.029**	**0.030**	**0.025**	0.337
NSP	**0.100**	0.711	**0.074**	0.236	0.095	0.419	0.138	0.737
Energy*NSP	0.974	**0.027**	0.325	0.820	0.330	0.800	0.410	0.777
Coarseness	0.172	0.606	0.165	0.259	0.290	**0.005**	0.173	0.576
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 versus 10.6 MJ/kg), NSP concentration (133 versus 195 g/kg) and particle sizes of the added NSP source (fine versus coarse).

[2] Foregut = sum of gut segments crop and proventriculus/gizzard.

[3] Hindgut = sum of gut segments colon and caeca.

MRT of feed in the crop, proventriculus/gizzard, ileum, 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. 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 versus 9.9 min.; *P*=0.027). NSP addition to the diet resulted in a higher (*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 versus 6.7 min; *P*=0.029), in the caeca (3.9 versus 1.8 min.; *P*=0.030), and as a result also in the total hind gut (30.3 versus 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). Total MRT was not affected by dietary treatments.

3.3 Relative weight of GIT segments and contents

Relative empty weight and content of crop, proventriculus/gizzard, small intestine, colon and caeca, as expressed in g/kg bodyweight of hen are shown in table 4.

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

Treatment[1]	Crop	Proventriculus /gizzard	Small intestine	Colon	Caeca
Control Energy					
- Control NSP	5.0	19.2	29.4	2.8	5.2
- High NSP-Fine	4.5	21.9	29.0	2.7	5.2
- High NSP-Coarse	5.0	29.3	29.6	2.7	4.9
Low Energy					
- Control NSP	4.9	19.5	29.9	2.6	5.1
- High NSP-Fine	4.9	21.8	30.3	2.6	4.6
- High NSP-Coarse	4.8	27.6	30.4	2.8	5.2
Standard error	1.05	0.68	0.65	0.15	1.04
P-Value					
Energy	0.995	0.194	0.056	0.688	0.466
NSP	0.809	**<0.001**	0.728	0.884	0.088
Energy*NSP	0.420	0.286	0.611	0.245	0.555
Coarseness	0.149	**<0.001**	0.464	0.430	0.449
Energy*Coarseness	0.087	0.272	0.675	0.315	**0.004**

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

Dietary treatments had no effect on relative weight of empty crop, small intestine and colon. Feeding high NSP diets, however, increased relative empty proventriculus/gizzard weight of hens by 30% (25.2 versus 19.4 g/kg ± 0.68) compared with hens fed control NSP diets. Relative empty proventriculus/gizzard weight was also affected by coarseness of NSP. Hens fed coarsely ground NSP had 30% higher relative empty proventriculus/gizzard weight compared with hens fed finely ground NSP (28.5 versus 21.9 g/kg ± 0.68). Feeding low energy diets numerically (*P*=0.056) increased relative empty small intestine weight of the hens compared with feeding control energy diets (30.2 versus 29.3 g/kg hen). In control energy fed hens relative empty caeca weight was not affected by coarseness of NSP, whereas in low energy fed hens relative empty caeca weight was lower in hens fed finely ground NSP compared with coarsely ground NSP (*P*=0.004).

Relative fresh and dry digesta weight in GIT contents are shown in table 5.

Table 5 Relative fresh an dry digesta weight in GIT segments (g/kg bodyweight of hen)

Treatment[1]	Crop content fresh	Crop content dry	Prov./ gizzard content fresh	Prov./ gizzard content dry	Small intestine content fresh	Small intestine content dry	Colon content fresh	Colon content dry	Caeca content fresh	Caeca content dry
Control Energy										
- Control NSP	7.9	3.2	12.8	4.7	15.1	2.9	1.6	0.4	2.4	0.7
- High NSP-Fine	11.4	4.5	14.9	5.9	14.8	3.7	2.1	0.6	2.3	0.6
- High NSP-Coarse	12.7	4.9	15.9	5.9	14.3	2.9	1.8	0.4	2.4	0.6
Low Energy										
- Control NSP	8.6	3.2	13.2	5.9	14.0	4.4	1.7	0.5	2.7	0.7
- High NSP-Fine	6.1	2.3	14.9	5.8	15.0	3.4	1.8	0.5	2.6	0.6
- High NSP-Coarse	10.7	4.3	15.8	5.7	14.4	3.0	1.8	0.5	2.7	0.6
Standard error	2.57	1.06	1.35	0.58	0.97	0.31	0.22	0.07	1.09	0.09
P-Value										
Energy	0.190	0.226	0.954	0.503	0.686	0.254	0.572	0.395	**0.036**	0.926
NSP	0.269	0.299	**<0.001**	0.122	0.908	0.809	0.148	0.185	0.885	0.191
Energy*NSP	0.354	0.447	0.739	0.232	0.359	**0.003**	0.416	0.476	0.180	0.933
Coarseness	0.159	0.173	0.174	0.960	0.538	**0.026**	0.446	0.134	0.547	0.930
Energy*Coarse-ness	0.466	0.391	0.996	0.861	0.936	0.503	0.288	0.277	0.159	0.918

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

Fresh and dry weight of digesta in crop and colon were not affected by dietary treatments. Feeding high NSP diets increased fresh gizzard content by 18% (15.4 versus 13.0 g/kg hen ± 1.35) compared with hens fed control NSP diets. Dry gizzard contents did not differ between treatments. Fresh content of small intestine was similar for all treatments. In control NSP fed hens, dry content of small intestine was 52% higher in low energy diets compared to control energy diets (2.9 versus 4.4 g/kg; *P*=0.003), whereas dry content of small intestine was not affected by energy concentration in high NSP diets. Fresh caeca content was 12.5% higher in hens fed low energy diet compared with control energy diet (2.4 versus 2.7 g/kg hen, *P*=0.036), whereas dry caeca content was similar for all treatments.

4 Discussion

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

4.1 Effect of energy dilution

In contrast to our hypothesis, energy dilution of the diet increased MRT of the hindgut, but did not affect MRT of the digesta in the foregut, small intestine nor total MRT. These findings were not in line with earlier reports, showing that feeding 40% diluted mash to Japanese quail increased passage rate through the gut compared to undiluted mash (Savory, 1980). This author also mentioned that diluted mash passed the crop quicker compared to undiluted mash. Furthermore, Savory and Gentle (1976) reported extensions of the GIT by 10-15%, due to feeding diluted diets to Japanese quail. An extension 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. Based on these findings, possible side effects of energy dilution on gut length, and as a result on MRT, can be neglected.

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 specific gravity (Jansen, 1977), which may explain the higher dry digesta weight in the small intestine in this treatment. Caeca content and MRT in the caeca of hens fed low energy diets were both increased compared to hens fed control energy diets. It was assumed that, as a consequence of feeding low energy diets, caeca activity increased to improve energy utilization. Likewise, caeca activity was increased in birds fed protein-deficient diets to increase nitrogen utilization, while caeca activity was not affected in protein-adequate diets (Karasawa and Maeda, 1994).

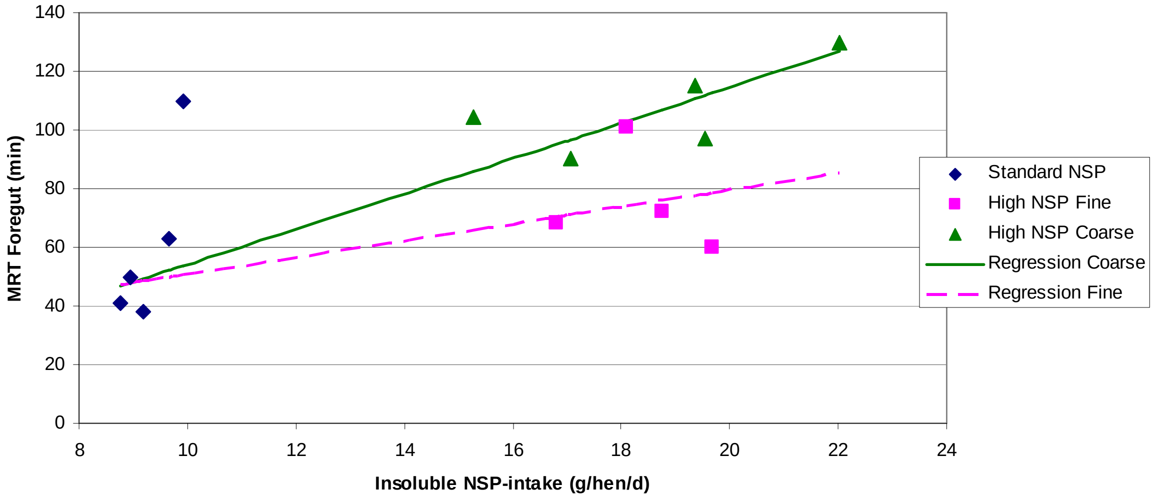
4.2 Effect of NSP concentration

As a result of feeding high NSP diet, relative empty proventriculus/gizzard weight and relative weight of proventriculus/gizzard contents were increased by 30% and 18%, respectively. Accordingly, supplementing insoluble NSP-rich roughages to laying hens were also shown to increase the relative weights of the gizzard and the gizzard content (Steenfeldt et al., 2007). Increased relative weights of empty gizzard (+ 26%) and gizzard content (+ 55%) were also found in laying hens that had access to wood shavings from the litter (Hetland et al., 2005). The effect of wood shavings was apparent in hens fed wheat based diet, but not in hens fed oat based diet. This may indicate that no additional effect of consumption of insoluble NSP's from litter was found when a 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, NSP addition to the diet increased MRT of the foregut. The effect of NSP addition was more pronounced in low energy diets, compared to control energy diets. Birds that were fed the low energy/high NSP diets consumed more feed, and thereby more, NSP compared to birds fed the control energy/high NSP diets. Interestingly, regression analysis revealed that daily insoluble NSP intake as well as the coarseness of NSP clearly affected MRT in the foregut (Figure 1). 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 1.

Figure 1 Relation between dietary NSP intake and Mean Retention Time in the foregut; point (10, 110) was considered as outlier and therefore not included in the regression analysis



Total MRT, however, was not affected by dietary treatments, indicating that MRT in hens fed high NSP diets was decreased after passing the foregut. Accordingly, Hartini et al., (2003) found no effect of high versus low fiber mash on average MRT. These authors suggested that a lower MRT may increase the feeling of hunger more quickly. As a consequence, birds will spend more time feeding and less time pecking (Hartini et al., 2003). 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). 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 dry matter contents, resulted in reduced dry matter 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, went not together with lower dry matter content. Therefore, it could be hypothesized that a higher level of satiety is partly related to increased content weights of the foregut, independent of the dry matter contents of these segments.

4.3 Effect of particle sizes of NSP

Feeding coarsely ground NSP increased relative empty proventriculus/gizzard weight 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). Although the diets in that experiment contained up to 440 g/kg whole wheat, oats or barley, mean particle size of duodenal contents varied only from 112 µm (moderate concentration whole wheat) to 211 µm (moderate concentration ground oats). Mean particle size of the duodenal contents of birds fed high concentrations of whole wheat, whole oats and whole barley were very similar; 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 explains the increased volume of the gizzard contents.
In the current experiment, no effect of coarseness of NSP on MRT in the foregut was observed. These findings did not 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. No oat hulls were found in birds fed the fine diet after 48 h., whereas at that time still more than 30% of the ingested oat hulls were found in the gizzard of hens fed the coarse diet. Accumulation of NSP in the gizzard, and thus its slower passage out of the gizzard, are contrary to the conventional theory that insoluble NSP speeds up feed passage rate. The conventional theory, however, is still valid for fine particles. Feed passage rate of fine NSP particles increased if coarse NSP sources were fed to broilers (Hetland and Svihus, 2001; Svihus and Hetland, 2001). Probably, the absence of a clear effect of particle sizes of NSP on MRT in the proventriculus/gizzard in the current experiment may be explained by the limited contrast in average particle size between the fine and

coarse diets (0.87 versus 1.05 mm). 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 requirement for structural components (Hetland et al., 2004a). Hens are motivated to eat feathers and wood shavings (Harlander-Matauschek et al., 2006b). Probably, this motivation can be explained by the hens need of 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., 2006a).

In conclusion, addition of coarsely ground NSP to the diet resulted in an increased relative gizzard weight, weight of gizzard content, and MRT in the foregut. 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). MRT was not affected by energy level and particle sizes of NSP.

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Appendix 1 Equations for modeling mean retention time (MRT)

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):

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

$$dR_{Prov/gizzard}(t)/dt = f1R_{Crop}(t) - f2R_{Prov/gizzard}(t) \qquad \text{(eq. 3)}$$

$$dR_{Small\_intestine}(t)/dt = f2R_{Prov/gizzard}(t) - f3R_{Small\_intestine}(t) \qquad \text{(eq. 4)}$$

$$dR_{Colon}(t)/dt = f3R_{Small\_intestine}(t) - f4R_{Colon}(t) \qquad \text{(eq. 5)}$$

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

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).

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

$$R_{Prov/gizzard}(t) = 100f1/(f2-f1)e^{-f1t} - e^{-f2t} \qquad \text{(eq. 8)}$$

$$R_{Small\_intestine}(t) =$$
$$100f1*f2/(f2-f1)e^{-f1t}/(f3-f1) - e^{-f2t}/(f3-f2) + (f2-f1)e^{-f3t}/[(f3-f2)(f3-f1)] \qquad \text{(eq. 9)}$$

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

$$R_{Caeca}(t) =$$
$$100f1*f2*f3*f4/(f2-f1)\left(e^{-f1t}-e^{-f5t}\right)/[(f5-f1)(f4-f1)(f3-f1)] - \left(e^{-f2t}-e^{-f5t}\right)/$$
$$[(f5-f2)(f4-f2)(f3-f2)] + (f2-f1)\left(e^{-f3t}-e^{-f5t}\right)/[(f3-f1)(f3-f2)(f4-f3)(f5-f3)]$$
$$-(f2-f1)\left(e^{-f4t}-e^{-f5t}\right)/[(f4-f1)(f4-f2)(f4-f3)(f5-f4)]$$
$$.. \qquad \text{(eq. 11)}$$

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. 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.