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Report 146

Effect of nutrient dilution and Nonstarch Polysaccharide concentration in rearing and laying diets on eating behavior and feather damage of rearing and laying hens

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Abstract
Providing diluted rearing diets increased feed intake from the first wks of life onwards. It was hypothesized that pullets were increasingly 'imprinted' on feed as pecking substrate if dilution level increased. This may decrease feather pecking and this could also explain the improved feather condition of the hens at 49 wk of age that were fed 15% diluted rearing diet.

Keywords: NSP, nutrient dilution, rearing hen, laying hen, feather damage

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Samenvatting
Verstrekking van verdunde opfokvoeders verhoogde de voeropname van de kuikens vanaf de eerste levensweek. Het lijkt erop dat de kuikens het pikgedrag meer op het voer richten als het verdunningsniveau van het opfokvoer toeneemt. Dit kan leiden tot minder verenpikgedrag. In elk geval was bij leghennen op een leeftijd van 49 weken duidelijk minder schade aan het verenkleed als ze tijdens de opfokperiode 15% verdund voer kregen.

Trefwoorden: NSP, voerverdunning, opfokhennen, leghennen, verenschade

Report 146

Effect of nutrient dilution and Nonstarch Polysaccharide concentration in rearing and laying diets on eating behavior and feather damage of rearing and laying hens

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Samenvatting

De studie die in dit rapport is beschreven, is uitgevoerd in opdracht van het Productschap Diervoeder en het Productschap Pluimvee en Eieren.

Aanleiding

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 (Wouw, 1995). Op dit moment wordt snavelkappen gezien als de belangrijkste maatregel om verenpikken en kannibalisme te voorkomen, maar vanaf 2011 zal in Nederland vermoedelijk 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.

Voedingsfactoren kunnen bijdragen aan het reduceren van de mate van verenpikken en kannibalisme (Van Krimpen et al., 2005), met name als deze factoren resulteren in meer voergericht gedrag. Verenpikken kan namelijk het gevolg zijn van het te weinig tijd besteden aan voeropname en foerageren. Deze gedachte wordt ondersteund door (Hoffmeyer, 1969), die grote overeenstemming vond in verenpikgedrag en het normale voedselzoekgedrag (hennen behandelen veren op dezelfde manier als bladeren en andere vegetatie uit hun natuurlijke omgeving), en (Blokhuis and Van der Haar, 1992), die verenpikken beschouwen als een vorm van omgericht bodemzoekgedrag, zoals grondpikken en bodemkrabben. Voeropname en foerageren zijn vormen van natuurlijk gedrag, waaraan hennen in een natuurlijke omgeving een groot deel van de dag (tot wel 60% van de beschikbare tijd) besteden. Voergericht gedrag kan men stimuleren door verstrekking van energiearme voeders (Van der Lee et al., 2001), of van voeders die rijk zijn aan niet-oplosbare NSP (Non Starch Polysaccharides) (Bearse et al., 1940; Hetland et al., 2004a). Ook de maalfijnheid van de NSP-fractie lijkt van invloed te zijn op de mate van verenpikken. Er zijn aanwijzingen zijn dat een grof gemalen NSP-fractie resulteert in minder verenpikken (Hetland et al., 2004a). Ook zijn positieve resultaten bereikt met ruwvoer (Steenfeldt *et al.*, 2001).

Uit een eerste experiment, uitgevoerd door Van Krimpen et al. (2007), met ISA Brown hennen in het traject van 17 tot 24 weken leeftijd, is gebleken dat jonge hennen goed in staat zijn om de voeropname van met zand of NSP-verdunde voeders (10% verdunning t.o.v. een standaard voer) te compenseren. De hennen die voeders kregen die verdund waren met zand of niet-oplosbare NSP's (haverdoppen, stro) presteerden vergelijkbaar of gunstiger dan de hennen die een controlevoer kregen met een gangbaar energie- en een laag NSP-gehalte.

Vervolgens is een tweede experiment uitgevoerd met ISA Brown hennen in het traject van 17 tot 52 weken leeftijd (Van Krimpen et al., 2008). Deze hennen ontwikkelden al verenpikgedrag tijdens de opfokperiode. Uit dit experiment bleek dat het verstrekken van een voer met een laag energiegehalte en een hoog gehalte aan grof gemalen NSP's in vergelijking met een controlevoer leidde tot een vertraging van 10 weken voordat daadwerkelijk verenschade ontstond. Uiteindelijk was de mate van verenschade bij deze behandeling vergelijkbaar met die van de controlegroep. Hieruit kunnen we vaststellen dat het effect van verdunde voeders op voorkomen van veerschade beperkt is op het moment dat dit gedrag al in een eerder stadium is aangeleerd.

Diverse onderzoekers hebben aangetoond dat de aanleg voor verenpikken zich al op jonge leeftijd ontwikkelt (Gunnarsson et al., 1999; Keppler et al., 1999; Huber Eicher and Sebo, 2001; Rodenburg et al., 2004).

Dit wordt bevestigd door een experiment van Johnsen (1998) die opfokhennen tijdens de eerste vier levensweken huisvestte op een bodem van zand, stro of draadgaas, waarna alle hennen in week 5 werden overgeplaatst naar een bodembedekking met zand en stro. De hennen die hun eerste vier levensweken op gaasbodem waren gehouden, vertoonden tijdens de legperiode meer verenpikkerij, hadden een slechter verenkleed en een hoger uitvalspercentage als gevolg van kannibalisme dan hennen op stro of zand. Het gedurende de opfokperiode verrijken van het strooisel met ingestrooid graan resulteerde in een duidelijke vermindering van het verenpikken tijdens de legperiode (Blokhuis, 1989). Daarnaast geldt ook dat een goed ontwikkeld maag-darmkanaal aan het begin van de legperiode het optreden van verenpikken en kannibalisme vermindert (Hadorn and Wiedmer, 2001). Recente praktijkbevindingen tonen aan dat als koppels tijdens de opfokperiode niet pikken, 85% van de koppels dat ook niet doet tijdens de legperiode. Komt tijdens de opfokperiode wel verenpikken voor, dan doet 70% van die koppels dat ook tijdens de legperiode (Bestman, 2006). De verwachting is dat het verstrekken van verdunde voeders vanaf de eerste levensdag bijdraagt aan het ontwikkelen van zowel het maag-darmkanaal als van voergericht gedrag van de opfokhennen, en als gevolg daarvan aan het beperken van verenpikken tijdens zowel de opfok- als de legperiode. Op dit moment is echter niet bekend welk niveau van verdunning en welke wijze van verdunning (zandtoevoeging, NSP-verhoging) van het voer effectief zal zijn. De voerverdunning mag bovendien geen negatief effect hebben op de lichaamsontwikkeling van de opfokhennen.

Uit de tot nu toe uitgevoerde experimenten is gebleken dat een verdunningsniveau van het voer tot 10% geen effect had op de legprestaties van (jonge) leghennen. Vermoedelijk zijn leghennen in staat om te compenseren

voor nog sterkere verdunningsniveaus van het voer. In elk geval had toevoeging van zand aan het voer tot een niveau van 30% geen negatief effect of de legprestaties van leghennen in de leeftijd van 29 tot 36 weken (Van der Meulen et al., 2006). Wel nam de groei van de hennen af bij een toenemend aandeel zand in het voer.
Het doel van het huidige experiment was om na te gaan wat het carry-over effect is van het verstrekken van voeders met een lage nutriëntendichtheid en/of een hoog aandeel niet-water oplosbare NSP's van grove structuur tijdens de opfokperiode op het verminderen van de incidentie van verenpikken tijdens zowel de opfok- als legperiode. Door het verstrekken van verdunde voeders in de opfokperiode, worden het voergericht gedrag mogelijk al van jongs af gestimuleerd. Door het toepassen van hogere verdunningspercentages van de voeders gaan de hennen vermoedelijk nog meer tijd besteden aan voeropname.

Materiaal en methode
Voor het experiment is gebruik gemaakt van twee identieke afdelingen (9,0 x 9,0 m), die elk 24 grondkooien bevatten met afmetingen van 0,9 x 1,50 m. In elk hok was een voertrog geplaatst van 1,0 x 0,2 m, zodat er 1,15 m^2 netto leefoppervlak overbleef. De hokafscheidingen waren 2,20 m hoog en de hokken waren aan de bovenkant afgedekt met gaas. Voor elke grondkooi was een legnest geplaatst. Tijdens dit experiment was het niet gewenst dat hennen de beschikking zouden hebben over vezelrijk strooisel. Daarom is gekozen voor zand als strooiselmateriaal. De hennen kregen onbeperkt water en voer ter beschikking. Het voer is verstrekt in een voertrog (lengte 100 cm) en het water in drinknippels. De bodem van het hok was volledig ingericht als scharrelruimte. In het hok waren aanvankelijk vier zitstokken aangebracht; tijdens de legperiode is dit aantal teruggebracht naar twee. Met twee zitstokken was er nog steeds voldoende ruimte om elke hen een zitplek te geven. De indeling van de stal is weergegeven in bijlage 1.

Op dag 1 van het experiment zijn 16 ISA Brown eendagskuikens per hok (768 kuikens totaal) opgezet, zodat de bezetting 14 kuikens/m^2 bedroeg. Elk kuiken kreeg een vleugelmerk met daarop een uniek nummer. De verdeling van de behandelingen over de hokken per afdelingen is weergegeven in bijlage 2. Het gehanteerde entschema is weergegeven in bijlage 3. De snavels van deze kuikens zijn niet behandeld. Gedurende de eerste 6 weken van de opfokperiode kregen ze voer dat was afgestemd op de nutriënteneisen van opfokmeel 1, waarna ze in week 7 zijn overgeschakeld op voer dat is afgestemd op de nutriënteneisen van opfokmeel 2. De hennen kregen een gangbaar lichtschema wat is overgenomen uit de managementgids van ISA Brown (bijlage 4).
Water en voer was onbeperkt beschikbaar. In week 16 zijn de hennen overgeschakeld op de legvoeders. De legperiode startte toen de hennen 17 weken oud zijn. Vanaf dat moment zijn de hennen nog 35 weken gevolgd. Het experiment eindigde toen de hennen 52 weken oud waren. Aan het eind van dit experiment is expliciet geprobeerd om verenpikgedrag op te wekken. Daartoe kregen alle hennen in week 50 zoutarm voer verstrekt, waarna zij in week 51 en 52 weer de oorspronkelijke proefvoeders kregen. Dit rapport beperkt zich tot het beschrijven van de resultaten van de hennen vanaf 17 tot en met 49 weken leeftijd.

In week 16 zijn de hennen gewogen, waarna het aantal hennen per hok op basis van het gemiddelde gewicht gestandaardiseerd is op twaalf. Mogelijke gewichtsverschillen die tijdens de opfokperiode zijn ontstaan, werkten dus door in de legperiode. Bij aanvang van de legperiode waren er dus 576 hennen (48 hokken x 12 hennen/hok) in het experiment. De bezettinggraad was 10,4 hennen/m^2, wat duidelijk hoger is dan de bezettingsnorm voor scharrelkippen (9 hennen/m^2), dit om de kans op verenpikken te verhogen.

De externe omstandigheden dienden zodanig te zijn dat er een redelijke kans was op het optreden van verenpikken vanaf de 20-ste levensweek. Daarom is gebruik gemaakt van hennen met onbehandelde snavels. Gekozen is voor het merk ISA Brown. Lichtintensiteit is van groot belang bij het ontstaan van verenpikken. Om de lichtintensiteit in de afdelingen zo uniform mogelijk te houden, waren de ramen in de afdelingen geblindeerd, waardoor geen daglicht naar binnen kon dringen. Vanaf het indelen van de hennen (week 16) kregen de dieren 10 uur licht per dag met een lichtsterkte van 10 lux. Wekelijks werd de lichtperiode met 1 uur verlengd tot een maximum van 16 uur licht per dag (week 23). Om het verenpikgedrag te stimuleren is de lichtintensiteit geleidelijk opgevoerd van 20 lux in week 18, naar 30 lux in week 20 en naar 65 lux in week 22.

In het experiment zijn de volgende proeffactoren onderzocht:
1) Verdunning opfokvoer: 0%, 10% of 15%
2) NSP niveau opfokvoer: standaard of hoog
3) Verdunning legvoer: 0%, 10%, 15% of 20%
4) NSP niveau legvoer: standaard of hoog

Deze opzet resulteerde dus in zes behandelingen tijdens de opfokperiode en acht behandelingen tijdens de legperiode.
De voeders waren zo samengesteld dat nutriëntendichtheid en NSP-gehalte onafhankelijk van elkaar varieerden. Het verdunnen van de voeders met een standaard NSP-gehalte gebeurde door het toevoegen van 10%, 15% of 20% zand aan het controlevoer (behandeling 1). Het verhogen van het NSP-gehalte gebeurde door het toevoegen van 10% ongemalen haverdoppen. Alle voeders werden in meelvorm verstrekt.

Resultaten

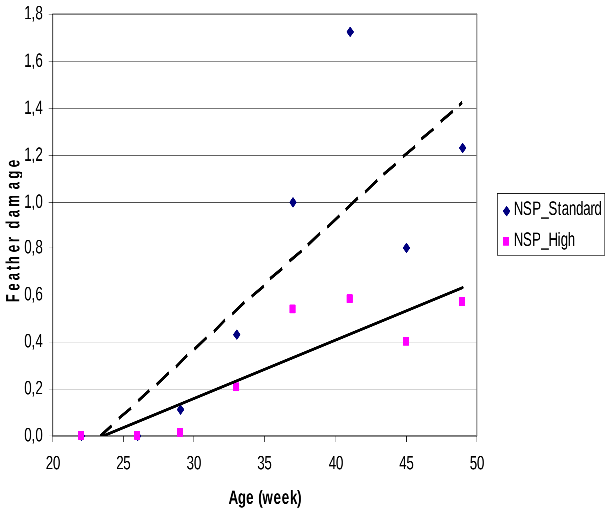
Opfokperiode (week 1 t/m 17)
Verdunning van de nutriënten van het opfokvoer door toevoeging van 10% en 15% zand resulteerde in een verhoging van de voeropname met respectievelijk 13 en 19% (P<0,001). Verdunningsniveaus van 10% en 15% door toevoeging van haverdoppen (en zand bij 15% verdunning) resulteerde in een verhoging van de voeropname met respectievelijk 5% en 7% (P<0,001). De energieopname verschilde niet aantoonbaar tussen de behandelingen. Bij de standaard NSP-voeders was geen effect van verdunning op de eettijd, terwijl bij de NSP-rijke voeders de eettijd bij het 0% verdunde voer lager was dan bij de 10% en 15% verdunde voeders (P < 0,001). Bij de standaard NSP-voeders nam de eetsnelheid toe naarmate het verdunningsniveau van het voer toenam, terwijl bij de NSP-rijke voeders de eetsnelheid juist afnam met een toename van het verdunningsniveau van het voer (P=0,032). Hennen die standaard NSP-voer kregen, hadden een iets hoger gemiddeld lichaamsgewicht in vergelijking met hennen die NSP-rijk voer kregen (635 versus 623 g; P<0,001). Hennen die 0% verdund NSP-rijk opfokvoer kregen hadden een ongunstigere energieconversie in vergelijking met alle andere proefgroepen (P=0,025). Er was geen duidelijk verband tussen de proefbehandelingen en het gedrag van de hennen, gebaseerd op timesampling. Tijdens de opfokperiode is geen verenpikgedrag waargenomen.

Legperiode (week 18 t/m 49)
Verdunning van de nutriënten van het opfokvoer met 10%, 15% en 20% resulteerde in een verhoging van de voeropname met respectievelijk 9,3%, 18,5% en 22,0% (P<0,001), zodat alle behandelingen een vergelijkbare energieopname realiseerden. De voeropname tijdens de legperiode werd niet beïnvloed door de toegepaste behandeling tijdens de opfokperiode. In tegenstelling tot eerdere experimenten nam de eettijd niet evenredig toe met de toename van het verdunningsniveau van het voer. Verstrekking van NSP-rijke voeders resulteerde in toename van het spiermaaggewicht met 71%, terwijl het gewicht van de kliermaaginhoud daalde met 55%. Er was geen effect van de proefbehandelingen op de legprestaties van de hennen. Het percentage vochtig oppervlak was duidelijk lager als hennen NSP-rijk i.p.v. standaard NSP legvoer kregen (64,5 versus 32,0%, P=0,001).
Hennen die 20% verdund voer kregen, besteedden numeriek wel 14% meer tijd aan voeropname dan hennen met 0% verdund voer. De eettijd werd niet beïnvloed door de behandelingen tijdens de opfokperiode of het NSP-niveau tijdens de legperiode. Er was geen effect van de behandelingen op de eetsnelheid. Hennen die tijdens de legperiode NSP-rijk voer kregen, besteedden meer tijd aan lopen (P=0,028) en bodempikken (P=0,111), maar minder tijd aan verzorging van het verenkleed (P=0,009). Het niveau van verenpikken tijdens de legperiode was erg laag. De behandelingen hadden geen aantoonbare effecten op de mate van mild en hard verenpikken.
Door verstrekking van 15% in plaats van 0% of 10% verdund voer tijdens de opfokperiode was er aantoonbaar minder schade aan het verenkleed aan het einde van het experiment (week 49) (0,85 vs. 0,56; P=0,047). Onafhankelijk van het verdunningsniveau van het opfokvoer, was er tijdens de legperiode minder schade aan het verenkleed als NSP-rijk legvoer werd verstrekt (gemiddeld 0.58 vs. 0.30; P<0,001). Het verloop van de verenschade tijdens de legperiode in relatie tot het verdunningsniveau in de opfokperiode is grafisch weergegeven in figuur 1 tot en met 3.

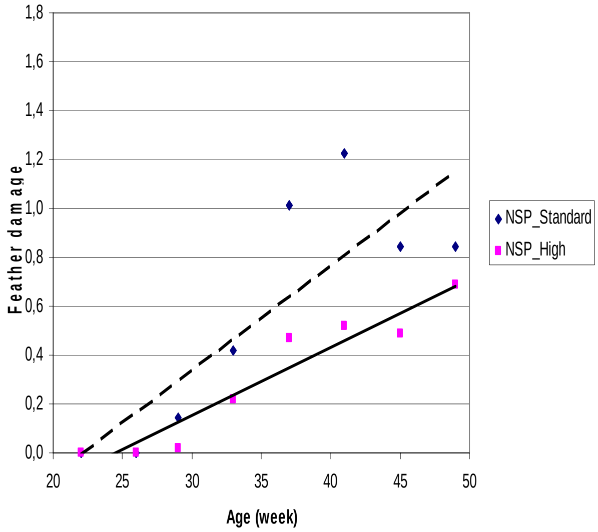
Conclusies

De belangrijkste conclusies uit dit experiment zijn:
- Zowel opfok- als leghennen stemmen de voeropname in sterke mate af op hun energiebehoefte. Verdunningen tot 15% van het opfokvoer en tot 20% van het legvoer (door toevoeging van zand aan het controlevoer) worden volledig gecompenseerd door een evenredig hogere voeropname.
- Opfokhennen zijn niet in staat om de energieopname volledig te compenseren als haverdoppen worden toegevoegd aan het 10% en 15% verdunde voer.
- Het verstrekken van een 15% verdund opfokvoer, waaraan zand of haverdoppen is toegevoegd, resulteert onafhankelijk van het NSP-gehalte van het legvoer in minder schade aan het verenkleed tijdens de legperiode.

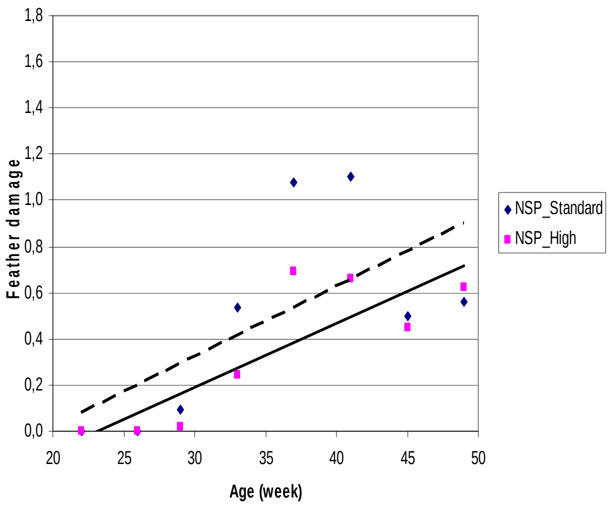
Figuur 1 Effect van NSP concentratie van het legvoer op de ontwikkeling van verenschade tijdens de legperiode bij leghennen die **0%** verdund voer tijdens de opfokperiode kregen.



Figuur 2 Effect van NSP concentratie van het legvoer op de ontwikkeling van verenschade tijdens de legperiode bij leghennen die **10%** verdund voer tijdens de opfokperiode kregen.



Figuur 3 Effect van NSP concentratie van het legvoer op de ontwikkeling van verenschade tijdens de legperiode bij leghennen die **15%** verdund voer tijdens de opfokperiode kregen.



Summary

An experiment was conducted with 768 non-cage housed ISA Brown pullets during the rearing period, of which 576 hens were followed during the laying period, to investigate the separate effects of dietary dilution and Nonstarch Polysaccharides (NSP) concentration of rearing and laying diets on eating behavior, feather damage and performance. Day-old pullets were allotted to one of 6 dietary treatments according to a 3 × 2 factorial (3 dilution levels and 2 NSP concentrations), with 8 replicates (pens) per treatment. At 17 wks of age, pens with hens were allotted to 1 of 8 dietary treatments according to a 4 x 2 factorial arrangement (4 dilution levels and 2 NSP concentrations), with 6 replicates per treatment.
Compared to 0% dilution level, feed intake of laying hens of 10%, 15% and 20% dilution level increased by 8.4% (9.5 g/hen/d), 16.5% (18.1 g/hen/d) and 20.9% (23.6 g/hen/d), respectively. ME intake was similar for all dilution levels. Hens fed low NSP laying diets had similar insoluble NSP intake for all dilution levels (9.3 g/hen/d). Insoluble NSP intake of hens fed high NSP laying diets increased from 15.6 g/hen/d (0% dilution) to 18.9 g/hen/d (20% dilution). Providing high vs. standard NSP layer diet decreased proventriculus content (1.1 vs. 0.3 g/kg BW) and increased empty gizzard weight (14.3 vs. 24.4 g/kg BW). Hens that were fed low NSP diets during laying had more feather damage compared to hens fed standard NSP diets (0.58 vs. 0.30). Increasing the insoluble NSP intake resulted in decreased proventricular weight and increased gizzard weight and it's contents, thereby linearly reducing feather damage. Providing diluted rearing diets increased feed intake from the first wks of life onwards. It was hypothesized that pullets were increasingly 'imprinted' on feed as pecking substrate if dilution level increased. This may decrease feather pecking and this could also explain the improved feather condition of the hens at 49 wk of age that were fed 15% diluted rearing diet.

Contents

1 Introduction

Feather pecking in layers is a very clear welfare problem in non-cage housing systems with a prevalence of between 40-80% (Blokhuis et al., 2007). Some reports hypothesized that feather pecking behavior is a substitute for normal ground pecking or feeding behavior in the absence of adequate foraging incentives (Hoffmeyer, 1969; Blokhuis, 1986).
Thus, nutritional factors which increase duration of feeding behavior may positively affect feather pecking behavior in laying hens (Van Krimpen et al., 2005). In literature, feather pecking behavior was found to reduce in laying hens fed nutrient diluted, high (in-)soluble Nonstarch polysaccharides (NSP) containing diets, or roughages (Van der Lee et al., 2001; Hartini et al., 2003; Hetland et al., 2004b; Steenfeldt et al., 2007). Laying hens that are fed low nutrient density diets, do compensate for this dilution by increased feed intake, resulting in a prolonged eating time (Savory, 1980; Van Krimpen et al., 2008). Hen performance can be maintained, even in early lay (Van Krimpen et al., 2007). Diets high in insoluble NSP content decreased eating rate and the rate of digesta passage in the foregut, suggesting an increased satiety level of the layers (Van Krimpen et al., Submitted).
Although diets with low nutrient and high insoluble NSP contents reduced feather pecking behavior, the beneficial effects were small when feather pecking was already developed before diets were provided (Van Krimpen et al., 2008). Rearing conditions during the first 4 weeks of life have a major influence on the subsequent development of feather pecking in laying hens (Johnsen et al., 1998). Therefore, more measures are recommended to satisfy the needs of pullets in food searching and ingestion to prevent feather pecking in adult birds (Keppler et al., 1999). To validate these recommendations, an experiment was performed to investigate the effects of nutrient dilution and NSP concentration in rearing and laying diets on eating behavior and feather damage of laying hens.

2 Materials and methods

2.1 Housing, birds and management

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

2.2 Experimental Design

At day 0, pullets were allotted to one of six dietary treatments according to a 3 × 2 factorial design. Hens were submitted to a standard vaccination scheme (appendix 3). The factors were dietary dilution (0, 10, and 15% dilution) and insoluble NSP concentration (124 g/kg, (control) vs. 184 g/kg (high). These NSP contents were the average for both rearing phases. Each treatment had 8 replicates. Rearing diets in phase 1 (wk 1 to 7) and phase 2 (wk 8 to 16) (Table 1a, 1b, 1c) had similar energy concentrations, with 2630, 2370 and 2250 kcal/kg for the 0, 10 and 15% diluted diets, respectively.
At the start of the laying period, pens with hens were allotted to one of 8 dietary treatments according to a 4 x 2 factorial design. The factors were dietary dilution (0, 10, 15 and 20% dilution) and insoluble NSP concentration (72 g/kg, (control) vs. 115 g/kg (high); on average for the laying diets), with 6 replicates per treatment. Energy concentrations were 2830, 2540, 2390 and 2250 kcal/kg for the 0, 10, 15 and 20% diluted laying diets, respectively (table 2a, 2b). The experiment comprised of 48 treatment combinations (six treatments in the rearing period x eight treatments in the laying period) and each treatment combination was tested in one pen (Appendix 2). Dietary dilution in the standard NSP diets was realized by adding 10, 15 or 20% sand to the control feed (0% dilution, control NSP). The high insoluble NSP diet was obtained by adding 10% whole oat hulls to the control diet at the expense of all other ingredients. Whole oat hulls were directly added in the mixer, without passing the hammer mill. To maintain the energy concentration in the 0% diluted, high NSP diet, extra fat was added. All feeds were fed in mash form.
Ratio of ME to all other nutrients ratio was similar for all diets, except for ash and NSP. The non-diluted rearing and laying diets had NSP concentrations according to NRC requirements for rearing and laying hens (NRC, 1994).

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

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

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

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

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

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

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

[2] Based on 1 analysis in duplicate per diet

[3] Mono- and disaccharides as glucose units

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

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

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

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

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

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

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

2.3 Measurements

Analytical Procedures

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

Particle Size Distribution

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

Bulk density

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

2.4 Observations

Feed Intake, Body Weight, and Hen performance

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

Eating Time

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

Feather Condition Scores and Culling Rate

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

Behavioral Recordings

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

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

Y_{ijkl} = μ + Dilution $Rearing_i$ + Dilution $Laying_j$ + NSP $Rearing_k$ + NSP $Laying_l$ + Dilution Rearing x NSP Rearing + Dilution Laying x NSP Laying + Dilution Rearing x NSP Laying + Dilution laying x NSP Rearing + Dilution Rearing x Dilution Laying + NSP Rearing x NSP Laying + e_{ijkl}

where Y_{ijkl} = dependent variable; μ = overall mean; Dilution $Rearing_i$ = fixed effect of dilution level of the rearing diet i (i = 3; 0, 10, and 15%); NSP $Rearing_j$ = fixed effect of NSP concentration of the rearing diet j (j = 2; control and high); Dilution $Laying_k$ = fixed effect of dilution level of the laying diet k (k = 4; 0, 10, 15, and 20%); NSP $Laying_j$ = fixed effect of NSP concentration of the rearing diet l (l= 2; control and high); e_{ijkl} = the error term. Model (2) was also used to test effects of eating time, eating rate, feather condition score, behavior traits and gut development parameters.
P-values for Dilution Rearing, NSP Rearing and Dilution Rearing x NSP Rearing are presented for all parameters that were determined in the rearing period. Similarly, *P*-values for Dilution Laying, NSP Laying and Dilution Laying x NSP Laying are presented for all parameters that were determined during the laying period. Besides, only significant carry-over effects of the nutritional factors during rearing on parameters during laying were presented.

3 Results

In general, it can be concluded that the birds had a good health status during the experimental period. An outbreak of coccidiosis occurred during at 14 wks of age, thereby negatively affecting feed intake during that week. Birds were treated against it and recovered quickly. In some weeks with high environmental temperatures, a low pressure of red mite was observed. It was not necessary to treat the birds against it. Overall mortality rate in this experiment was 4.7%, which can be considered as relatively low.

3.1 Results during the rearing period

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

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

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

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

3.2 Results during the laying period

At the start of lay, feather pecking behavior was not observed in any of the pens, irrespective of the dietary treatment during rearing. To encourage the hens to start feather pecking:
(1) light intensity was increased from 10 Lux to 20 Lux (wk 18), 30 Lux (wk 20) and 65 Lux (wk 22);
(2) perch length was reduced from 3.6 m to 2.7 m (wk 24) and to 1.8 m (wk 26);
(3) large part of the sand was removed leaving about 2.0 liter of sand per cage (from wk 28 onwards), and
(4) feeding troughs were blocked for 3 h/d, where blocking period per pen varied during the week (wk 32 to 40).
Average feed intake during the laying period was 126 g/hen/d (table 4). Feed intake was affected by dilution level of the laying diets (P<0.001) and increased proportionally with increased dilution level. Compared to 0% dilution level, feed intake of 10%, 15% and 20% dilution level was increased by 8.3%, 16.5% and 20.9%, respectively. Thus, ME intake was nearly similar for the different dilution levels.

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

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

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

The amount of consumed insoluble NSP of hens fed low NSP laying diets was similar for all dilution levels (on average 9.3 g/hen/d; table 4), Insoluble NSP intake of hens fed high NSP laying diets increased from 62% (at 0% dilution) to 110% (at 20% dilution) compared to the 0% diluted NSP low diet (P<0.001). Insoluble NSP eaten with diets during the laying phase was not affected by dietary treatments during the rearing phase.
Bulk density of the high NSP diets was substantially lower compared to the low NSP diets. Hens fed high NSP diet consumed more volume of feed (ml/hen/d) for similar weight of feed intake. Volume intake increased by increased dilution levels of the diet. This effect was most pronounced in the high NSP treatments. Volume intake during the laying phase was not affected by dietary treatments during the rearing phase.
In hens fed 0% diluted laying diet, BW was less in standard compared to high NSP laying diet. Hens fed 15% diluted laying diet had less W in the high NSP diet. BW in hens fed 15% diluted laying diet was higher after eating standard NSP rearing diet (1992 vs. 1945 g; table 8). Hens fed 0% diluted rearing diet had lower BW after feeding high NSP laying diet (1941 vs. 1973 g; table 9).
In hens fed high NSP laying diet, BW was not affected by NSP concentration of the rearing diet, whereas in standard NSP laying diet BW was significantly reduced by feeding high vs. standard NSP rearing diet (1980 vs. 1929; table 10). Egg mass was not significantly effected by different rearing and/or laying diet combinations.
Average eating time and eating rate were not affected by dietary treatments during the laying period (table 5). Moreover, eating time during laying was not affected by dietary treatments during rearing. A carry-over effect of NSP concentration of the rearing diet and dilution level of the laying diet on eating rate during laying was observed (table 8). In the 10% diluted laying diets, eating rate slightly increased if hens were previously fed a high

compared to a standard NSP rearing diets (0.67 vs. 0.86 g/min.). In the 15% diluted laying diets, eating rate slightly increased if hens previously were fed a standard NSP compared to a high NSP rearing diets (0.96 vs. 0.74 g/min.).

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

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

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

In this experiment, feather pecking frequency was very low (1.2 pecks/10 min./pen). Gentle and severe feather pecking behaviors during laying were not affected by any of the tested dietary factors.
Over the period 0 to 25 wk of age, feather condition of all the hens was very good. From wk. 29 onwards, feather condition linearly decreased over time.
Feather damage of hens that were fed low NSP diets during laying period was more severe compared to hens fed standard NSP diets (0.58 vs. 0.30; *P*<0.001; table 5).
The worst feather condition (0.73) was found in hens fed the standard feeding regime both during rearing and laying (0% diluted rearing diet and later 0% diluted laying diet). Pair wise comparisons showed that the feather condition of all the other treatment combinations was numerically better compared to the standard regime.
Relative empty crop weight and it's content were on average 5.0 and 5.2 g/kg BW, respectively (table 6). Crop weights and it's contents were not affected by the tested dietary treatments. Relative proventriculus content decreased by 73% after supplementing high compared to low NSP laying diet. (1.1 vs. 0.3 g/kg BW; *P*<0.001; table 6). In hens fed 10% diluted laying diet, relative weight of proventriculus content was increased when previously standard NSP rearing diet was fed (table 8).
In hens fed high NSP laying diets, no effect of NSP concentration on proventriculus content of the rearing diet was observed. In hens fed low NSP laying diets relative weight of proventriculus content increased when previously low vs. high NSP rearing diet was fed (1.5 vs. 0.7; *P*=0.048; table 10). Empty gizzard weight was increased by 71% by feeding high vs. standard NSP laying diet. Relative weight of the gizzard content was not significantly affected by the different rearing and/or laying diet combinations.
Time budgets for eating, dust bathing, resting and feeding related behavior, as observed by a scan sampling technique, were not affected by the dietary factors during laying (table 7). NSP content of the layer diet effected preening, ground searching and walking behavior.

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

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

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

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

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

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

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

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

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

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSP concentration laying period (72 vs. 115 g/kg)

[2] Results are based on the average value of 33 wks. x six replicates per treatment

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

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

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSP concentration laying period (72 vs. 115 g/kg)

[2] Results are based on the average value of 33 wks. x six replicates per treatment

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

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

[1] The tested factors were dilution level rearing period (2630, 2370 and 2540 kcal/kg), dilution level laying period (2830, 2540, 2390 and 2250 kcal/kg), insoluble NSP concentration rearing period (124 vs. 184 g/kg) and insoluble NSP concentration laying period (72 vs. 115 g/kg)

[2] Results are based on the average value of 33 wks. x six replicates per treatment

4 Discussion

4.1 Effect of Nutrient Dilution

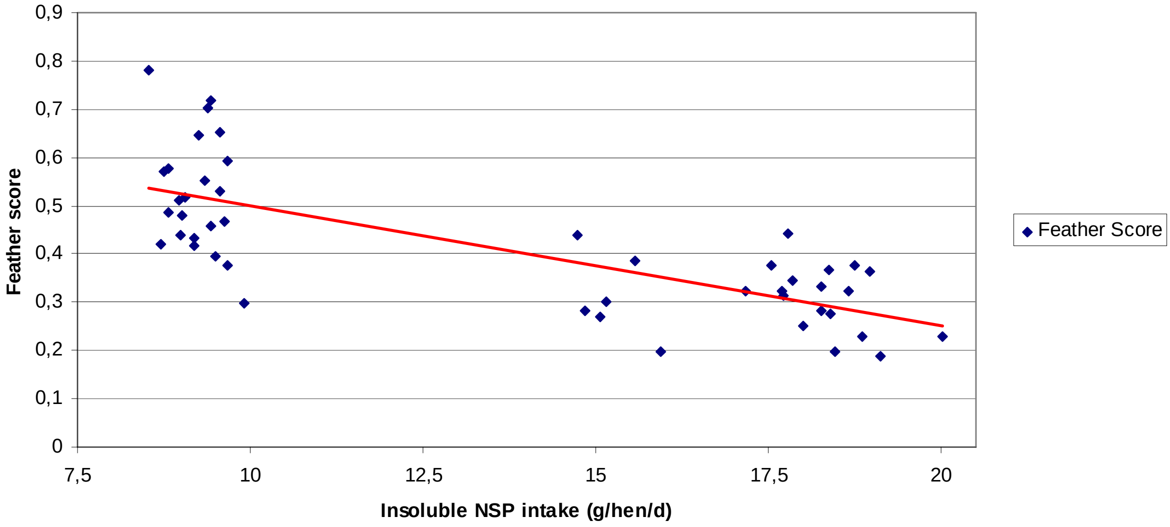
The present experiment was designed to study the impact of diets during rearing on traits during rearing, and on combinations of rearing and laying diets on development and production and on eating traits during laying. Also effects of these treatments on development of proventriculus, gizzard and crop were studied. The aim is to evaluate whether intake and digestion processes are related to the development of feather damage. During rearing, average feed intake of pullets that were fed 10% and 15% diluted diets increased by 8.3 and 13.1%, respectively, resulting in similar ME energy intakes compared to pullets fed control energy diets. This means that rearing hens have a wide range of adaptation to density of their diets. This phenomenon was already known in laying hens (Leeson et al., 2001; Van der Meulen et al., 2006), but not from studies with nutrient diluted diets in rearing hens.
During laying, average energy intake differed only slightly between dilution levels. Relative energy intake was 100%, 97%, 99%, and 97% for the 0%, 10%, 15% and 20% diluted diets, respectively. Contrary to those findings, in the past often overconsumption of feed was observed in hens that were fed diets with increased energy concentrations (Morris, 1968). In the current experiment, energy intake during the laying period was independent of dietary treatments during the rearing period. Van Krimpen et al. (2008) and Van der Meulen et al. (2006) found proportional increases in feed intake with nutrient dilution levels up to 30%. It can be hypothesized that pullets and laying hens need a certain amount of nutrients per day and they will continue to eat until their nutrient demands are fulfilled. It was found that adult layers compensate for dietary dilutions up to 20%, even if whole oat hulls are used as dilution source. Adaptation possibilities are more limited in young pullets because high NSP content could not be fully compensated.
Nutrient dilution of the rearing diet only slightly extended eating time and slightly increased eating rate during the rearing period. Similarly, also during the laying period no significant effects of nutrient dilution on eating time and eating rate were observed. In contrast with those results, earlier findings showed that eating time of laying hens prolonged gradually because of feeding diluted diets (Van Krimpen et al., 2007; Van Krimpen et al., 2008). These differences were not caused by the eating behavior of the hens fed the diluted diets. Eating time and eating rate levels of the 10% diluted diet were comparable with the levels found in an earlier experiment with similar diets (Van Krimpen et al., 2008), Eating time of hens fed the 0% diluted diet in the current experiment, however, was much longer than in the previous experiment (20.4 vs. 15.1%). Probably, eating behavior of hens fed diluted diets in adjacent pens could, encouraged the hens that were fed the undiluted diets to peck more at feed. Keeling and Hurnik (1996) showed that a satiated bird might direct its attention to feed in response to social facilitation effect of a stimulus bird, but that is eats relatively little. Hens might also perform inappropriate feeding pecks in response to a specific deficit in their environment (Savory, 1999). Thus, increased eating behavior of hens fed the undiluted diet because of social facilitation and inappropriate feeding pecks might explain the absence of contrasts in eating time between dilution levels in the current experiment.
Eating time of hens fed the 0% diluted laying diets in the current experiment was in line with that of pullets fed the 0% diluted rearing diets (20.0% vs. 20.3%). Eating time of the birds during the rearing period in the experiment of Van Krimpen et al. (2008) was not determined, but the present results show that hens will spend similar times on eating behavior during both rearing and laying if diets with comparable nutrient densities are supplied .
In the current experiment, feather damage was not affected by nutrient density of the diet. Contrary to those findings, less feather damage was observed if layers were fed a nutrient diluted diet (from 2920 to 2560 kcal/kg; (Elwinger, 1981). Similarly, Van der Lee et al. (2001) reported a better plumage condition in laying hens that were fed diets reduced in energy concentration (2765 versus 2645 kcal/kg). It was hypothesized earlier that an increase in eating time and feeding related behavior because of dietary dilution might compensate for redirected foraging behavior, resulting in less feather pecking behavior (Van Krimpen et al., 2005). In the current experiment, decreasing the nutrient density did not prolong eating time. This might explain the absence of an effect on feather damage. Furthermore, the results of this experiment shows that eating behavior, and subsequent feather pecking behavior, could vary enormously between flocks, even if the same facility, strain, and diet are used. This stresses that feather pecking is a multi factorial problem, which is difficult to control (Leonard et al., 1995; Nicol et al., 2001; Kjaer and Hocking, 2004).
Feeding low nutrient diets to hens resulted in similar hen performance parameters (BW/egg mass) compared with hens that were fed undiluted diet. Similarly, feeding laying hens a 5% nutrient diluted diet did not affect hen performance compared to a control diet (Van der Lee et al., 2001). In a trial with laying hens (34-37 wk of age), dietary dilution (by adding 10, 20, 25 or 30% sand) did also not affect hen performance of the hens (Van der Meulen et al., 2006). The hens fully compensated for the effect of added sand in the diet by increasing their daily feed intake.

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

4.2 Effect of Dietary NSP Concentration

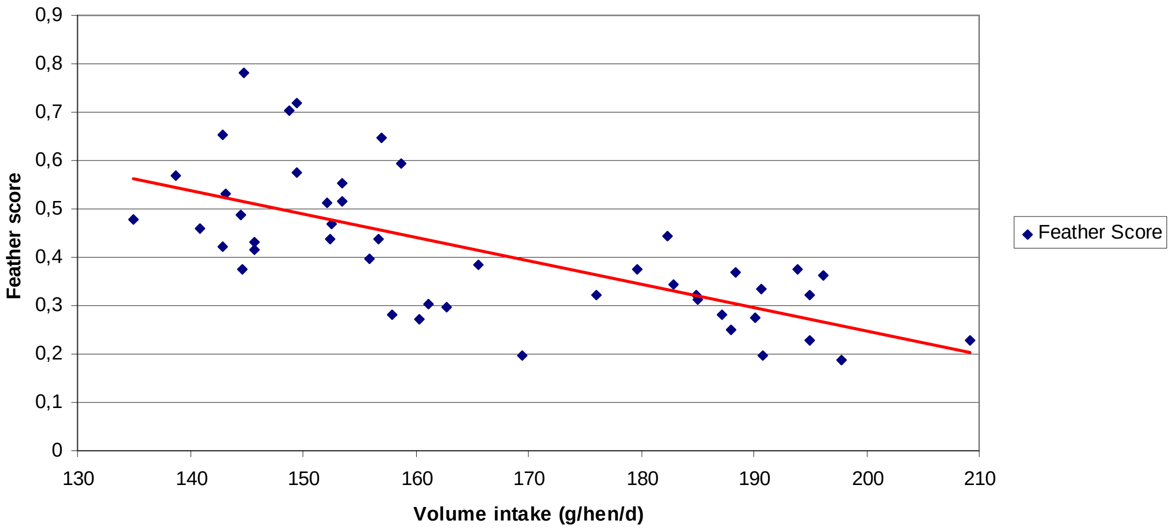
In the current experiment, NSP concentration of the diets was increased by adding 10% whole oat hulls. As a result, NSP concentration of the rearing diet increased from 124 to 184 g/kg. Insoluble NSP level of the laying diets ranged only from 72 (low NSP) to 115 (high NSP) g/kg. Thus, insoluble NSP concentrations of the rearing diets were considerable higher compared to the laying diets.
BW development of pullets during the rearing period that were fed the 10% and 15% diluted high NSP diets was retarded because of reduced energy intake, compared to the 0% diluted high NSP diet. NSP concentration had no effect on hen performance traits. BW of hens that were fed high NSP laying diets was even slightly increased compared to hens fed standard NSP diets. This was confirmed by Hartini et al. (2003) who found no detrimental effects on performance after substituting wheat by (in)soluble high NSP sources like millrun, barley, rice hulls or oats on an isocaloric and isonitrogenous basis. In some experiments, in which insoluble rich NSP diets were supplemented, nutrient digestibility even increased, possibly due to a better gizzard development and more reflux activity in the fore-gut, resulting in improved hen performance, as reported by (Hetland et al., 2004a).
Contrary to our expectations, NSP addition during rearing did not extend eating time and feeding related behavior. Rearing hens fed the high NSP diet simply ate this feed more quickly. Dietary dilution of the high NSP rearing diets, however, resulted in a retarded feed intake and a decreased eating rate, while eating time was only numerically increased. Pullets seem to maintain their eating time in a fixed time budget. In conclusion, no improvement of coarsely ground NSP addition on performance and behavior of the laying period was observed.
Similarly with earlier findings (Van Krimpen et al., 2007; Van Krimpen et al., 2008) feed intake of the layers was not affected by NSP concentration of the laying diet. Oat hulls have a relative low bulk density. Adding 10% whole oat hulls to the control diet decreased the bulk density by 15% (783 vs. 669 g/l), indicating that the hens have to consume more volume for realizing a similar nutrient intake compared to hens that were fed undiluted feed. Hens that were fed the control diet daily consumed 143 ml feed, whereas hens that were fed the 10% diluted high NSP diet consumed 183 ml feed. In a study of Vilarino et al. (1996), volume intake of laying hens even increased from 157 to 279 ml/hen/d by feeding a mash diet diluted by 450 g/kg wheat bran. As a result, eating time increased from 32.5 to 41.3% in that experiment. Hens, however, were not able to completely adjust their feed intake to compensate for the dietary dilution.
Contrary to earlier work, however, no contrasts in eating time and eating rate were found between standard and high NSP laying diets. The eating time and eating rate levels of the high NSP diets in the current experiment were comparable with the levels found in the earlier experiment, but in the current experiment eating time of hens fed the standard NSP laying diets was much higher (21.10 vs. 16.9%), and eating rate much lower (0.80 vs. 0.95 g/min).
As explained in the previous section, increased eating behavior of hens fed the control diet could be the result of social facilitation and inappropriate feeding pecks. Eating rate during laying was affected by a carry-over effect of NSP content of the rearing diet and dilution level of the laying diet (Table 8). In hens fed 10% diluted laying diet eating rate was reduced in standard compared to high NSP rearing diet, whereas the opposite was observed in hens fed 15% diluted laying diet. An explanation for this phenomenon could not be found.
Feather condition of hens fed high NSP laying diets was evidently improved compared to hens fed standard NSP diets. This effect could not be explained by an extended eating time, a decreased eating rate, more feeding related behavior or less feather pecking behavior.
Hens that were fed high NSP laying diets, however, spend more time ground searching and walking, and less time preening. Moreover, hens that were fed high NSP diets consumed more insoluble NSP and more volume of feed per day. Surprisingly, daily insoluble NSP intake was found to be linearly related to feather condition score (FCS = 0.75 – 0.025 * Insoluble NSP intake; $P<0.001$; $R^2 = 0.55$; Figure 1). Likewise, daily volume intake was found to be linearly related to feather condition score (FCS = 1.22 – 0.0049 * volume intake; $P<0.001$; $R^2 = 0.46$; Figure 2). Earlier findings showed that insoluble NSP intake was linearly related to the mean retention time of digesta in the foregut (Van Krimpen et al., Submitted). An increased insoluble NSP intake resulted in a decreased mean retention time in the foregut, which was associated with a higher level of satiety. A higher level of satiety may contribute to a lower feather pecking pressure (Hetland et al., 2004a).

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



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

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



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

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

In the current experiment, NSP addition was confounded with particle sizes distribution. Thus, average particle size increased in high NSP diets compared to standard NSP diets. In earlier work we observed a delay in feather damage in low energy – coarsely ground – high NSP diets compared to hens fed a control diet (Van Krimpen et al., 2008). Replacing ground wheat with whole wheat also showed a positive effect on plumage condition of laying hens (Hetland et al., 2003). In literature, numerous positive effects of coarsely ground NSP sources were mentioned. They stimulate gizzard weight and increase reflux of bile acids, resulting in improved starch digestibility and an enhanced emulsification of liberated lipids (Hetland et al., 2003).

Indeed, NSP addition in the current experiment increased gizzard weight and gizzard content. Moreover, NSP addition resulted in a considerable reduced proventriculus content of the hens (0.9 vs. 0.3 g/kg hen). Contents of gut segments of the foregut can be influenced by feedback control from the duodenum, mediated through receptors sensitive to e.g. acidity, particle size and rheological properties of digesta (Bach Knudsen, 2001). Probably, this feedback control affects the proventriculus content. In broilers, increased particle sizes reduce the incidence of proventricular dilatation, stimulate gizzard development and extend mean retention time in the foregut, This was associated with less mortality (Jones and Taylor, 2001; Taylor and Jones, 2004).
In conclusion, adding coarsely ground insoluble NSP to the diet resulted in similar performance and in similar eating behavior, Moreover, it reduced proventricular weight and increased gizzard weight and it's contents All this was associated with better feather condition scores.

4.3 Carry over Effects of Nutritional Factors in the Rearing Diets on Parameters During Laying

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

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

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

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

Thus, supplementing 15% diluted diet during rearing resulted in a good feather condition at the end of the laying period, independent of the NSP concentration of the laying diet. The development of feather damage during the laying period related to dilution levels of the rearing diet are graphically shown in figures 3, 4 and 5. From these figures it can be concluded that the development of feather damage is retarded if hens were fed high instead of standard NSP laying diets. Feather damage, however, was also retarded in hens that were fed 15% diluted diet during rearing (figure 5). Providing 10% diluted rearing diet had no beneficial effect on feather condition during laying (figure 4). Thus, the feeding strategy of 15% diluted diet during rearing followed by standard NSP laying diet is as efficient as 0% diluted rearing diet followed by high NSP laying diet in retarding feather damage.

This result demonstrates the importance of conditions during the rearing period in preventing feather pecking behavior during the laying period, which was confirmed by different authors (Blokhuis, 1989; Vestergaard and Lisborg, 1993; Johnsen et al., 1998; Chow and Hogan, 2005). Layer pullets that do not get the right substrate to peck early in life may feather peck later on (Johnsen et al., 1998). Supplementing extra straw or spreading 10% of the estimated feed intake as whole wheat into the litter of layer pullets markedly reduced feather damage in the layer period (Blokhuis and Van der Haar, 1992). The birds showed more foraging-related behaviours like ground scratching and ground pecking, suggesting that the incentive value of the ground, and the substrate covering it, might be increased with grain during the rearing period. Similarly, Chow and Hogan (2005) concluded that young Burmese red jungle fowl from 1 to 5 wk of age that were deprived of exploratory-rich environments performed significantly more gentle feather pecking, and tended to show more severe feather pecking than the experienced birds. This suggested that chicks deprived of exploratory-rich environments may consider pen mates as appropriate exploratory stimuli and subsequently direct exploratory behavior towards conspecifics. Chow and

Hogan (2005) hypothesized that providing early experience with enriched environments could reduce the likelihood of severe feather pecking developing. It is suggested that pullets become imprinted on pecking substrates very early in life (Vestergaard, 1994), with a sensitive period estimated on day 0-6 (Braastad, 1990), around day 3 (Vestergaard and Baranyiova, 1996), or below day 10 (Huber Eicher and Wechsler, 1997) post hatching.

In the current experiment, diluted diets were provided from day 0 post hatch onwards. Feed intake, and probably also the number of feeding pecks, of pullets that were fed the diluted diets increased from the first week of life onwards. Likely, these pullets were more 'imprinted' on their feed and therefore less oriented towards the feathers of their conspecifics. This could explain the improved feather condition of the hens at 49 wk of age, that were fed the 15% diluted rearing diets.

Figure 3 Effect of NSP concentration of the layer diet on feather damage development in hens that were fed **0%** diluted rearing diet

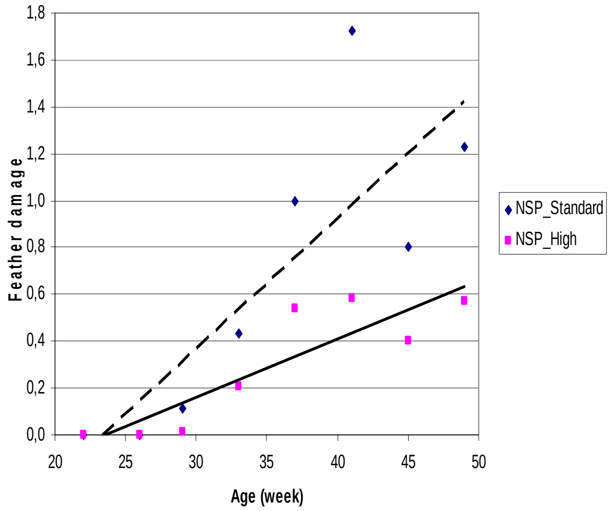


Figure 4 Effect of NSP concentration of the layer diet on feather damage development in hens that were fed **10%** diluted rearing diet

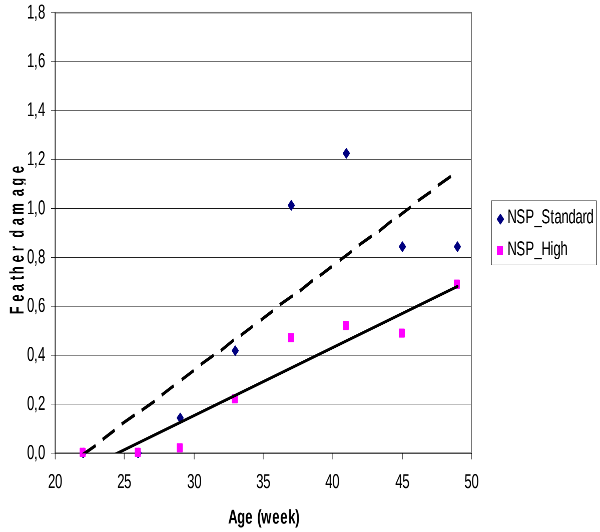
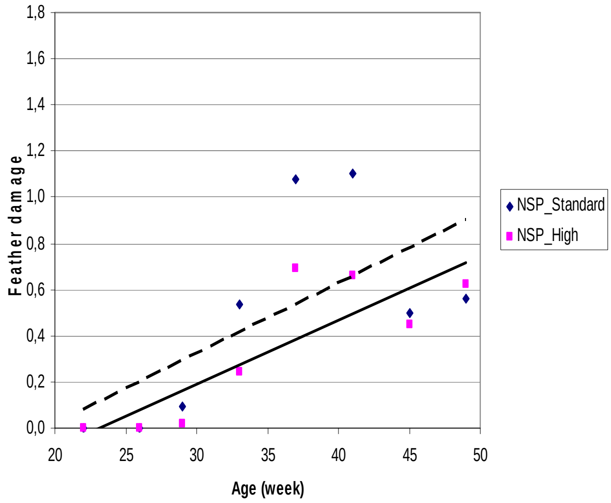


Figure 5 Effect of NSP concentration of the layer diet on feather damage development in hens that were fed **15%** diluted rearing diet



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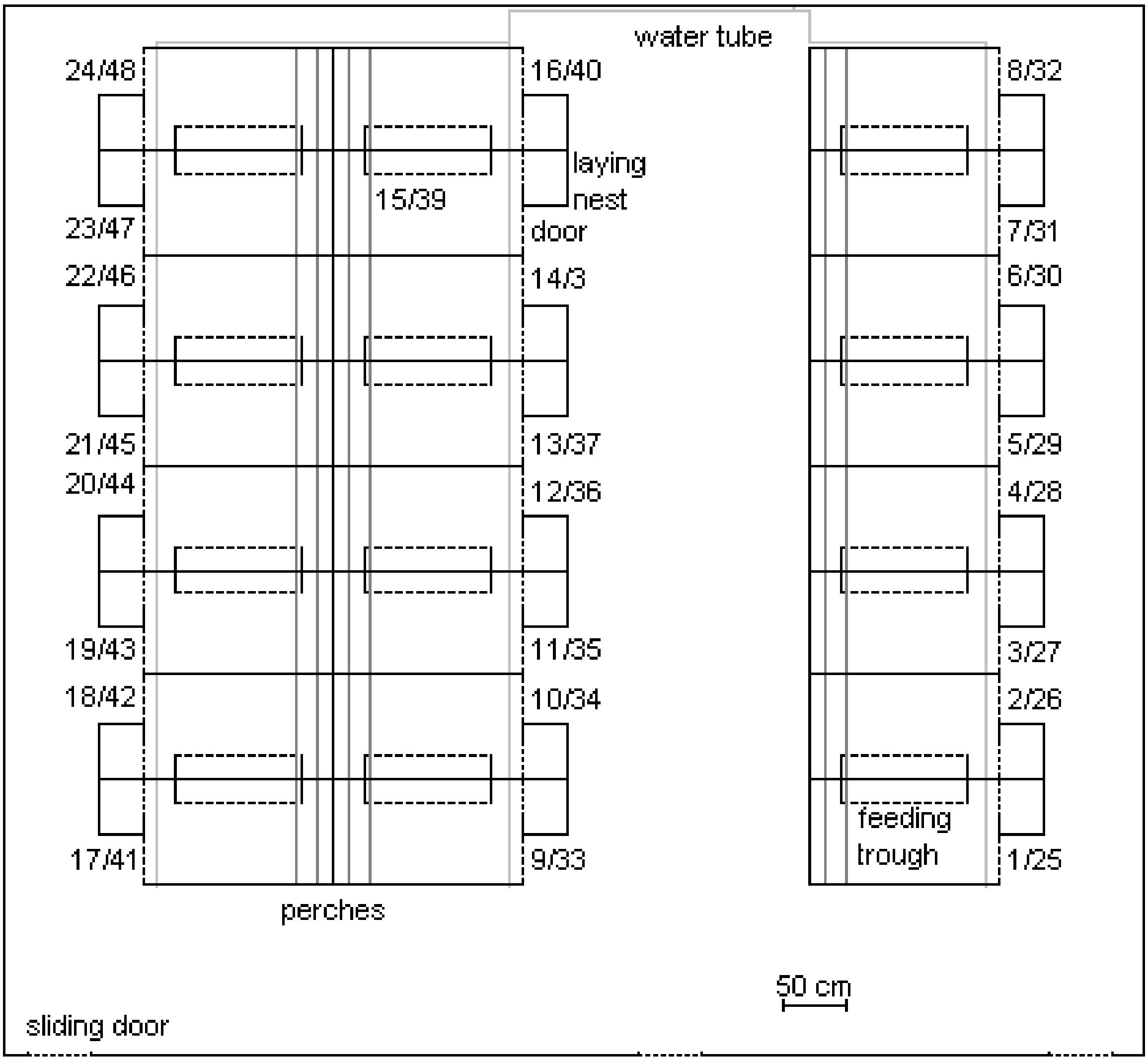
Appendices

Appendix 1 Room overview



Appendix 2 Distribution of treatments over pens

Room	Pen	Code Rearing diet	Code ME Rearing	Code NSP Rearing	Code Laying diet	Code ME Laying	Code NSP Laying
1	1	6	3	2	5	3	1
1	2	2	1	2	1	1	1
1	3	1	1	1	1	1	1
1	4	4	2	2	6	3	2
1	5	5	3	1	2	1	2
1	6	2	1	2	4	2	2
1	7	1	1	1	4	2	2
1	8	6	3	2	6	3	2
1	9	1	1	1	8	4	2
1	10	4	2	2	5	3	1
1	11	4	2	2	8	4	2
1	12	4	2	2	4	2	2
1	13	1	1	1	5	3	1
1	14	5	3	1	6	3	2
1	15	3	2	1	6	3	2
1	16	5	3	1	4	2	2
1	17	2	1	2	6	3	2
1	18	6	3	2	1	1	1
1	19	1	1	1	3	2	1
1	20	5	3	1	3	2	1
1	21	5	3	1	5	3	1
1	22	6	3	2	8	4	2
1	23	6	3	2	3	2	1
1	24	6	3	2	7	4	1
2	25	6	3	2	4	2	2
2	26	2	1	2	7	4	1
2	27	4	2	2	3	2	1
2	28	6	3	2	2	1	2
2	29	3	2	1	8	4	2
2	30	4	2	2	7	4	1
2	31	1	1	1	6	3	2
2	32	3	2	1	1	1	1
2	33	4	2	2	2	1	2
2	34	2	1	2	8	4	2
2	35	5	3	1	1	1	1
2	36	1	1	1	2	1	2
2	37	3	2	1	2	1	2
2	38	3	2	1	3	2	1
2	39	3	2	1	5	3	1
2	40	3	2	1	4	2	2
2	41	5	3	1	7	4	1
2	42	2	1	2	5	3	1
2	43	1	1	1	7	4	1
2	44	3	2	1	7	4	1
2	45	4	2	2	1	1	1
2	46	2	1	2	3	2	1
2	47	2	1	2	2	1	2
2	48	5	3	1	8	4	2

Explanation codes:

ME Rearing:	1 = Dilution 0% 2 = Dilution 10% 3 = Dilution 15%
NSP Rearing:	1 = NSP standard 2 = NSP high
ME Laying:	1 = Dilution 0% 2 = Dilution 10% 3 = Dilution 20% 4 = Dilution 20%
NSP Laying:	1 = NSP standard 2 = NSP high

Appendix 3 Vaccination scheme rearing hens

Day	Vaccination
0	Marek Rispens +HVT / IB MA 5
10	NCD
21	Gumboro
28	NCD
42	PDww
70	IB primer
84	ILT
91	AE drinkwater
98	NCD / ND
112	IB H52

Appendix 4 Light scheme rearing hens

Age	Hours light	Light intensity (Lux)
Day 1-3	22	20-40
Day 4-7	20	15-30
Day 8-14	18	10-20
Day 15-21	16	10
Day 22-28	15	10
Day 29-35	13:30	10
Day 36-42	12	10
Day 43-49	11	10
Day 49 – Week 17	10	10