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Report 46

Effect of dietary energy and NSP concentration and particle size of NSP on eating behaviour, feather pecking behaviour and performance of laying hens

April 2007

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Effect of dietary energy and NSP concentration and particle size of NSP on eating behaviour, feather pecking behaviour and performance of laying hens

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Abstract

1. An experiment with 588 ISA Brown layer strains was conducted to measure the effect of dietary energy (11.8 versus 10.6 MJ/kg) and NSP (133 versus 195 g/kg) concentration and particle size of the NSP fraction (fine versus coarse) on eating behaviour, feather pecking behaviour and egg-performance of laying hens from 18 to 40 weeks of age. Seven experimental diets were tested, each replicated seven times.
2. Energy reduction, NSP addition and coarse grinding of NSP increased average eating time with 14.2%, 17.2% and 7.9%, respectively. Eating rate was not affected by energy concentration and coarseness of NSP, but NSP addition decreased eating rate with 21.0%.
3. Dietary treatments did not affect feather condition scores convincingly, although energy reduction and NSP addition improved feather condition scores in some individual weeks. Hens fed normal energy diets showed lower mortality rates if high-NSP diets were supplemented (31.6 versus 44.1%), whereas in low energy diets mortality decreased when hens were fed low-NSP diets (13.1 versus 28.6%) (P=0.071).
4. Hens that were fed low energy diets compensated for 10% reduction in energy concentration by 9.3% higher asymptotic feed intake (143.0 versus 130.8 g/d). Egg performance and body gain of the hens were not affected by dietary treatments.
5. It is concluded that hens that were fed low energy, high (coarsely ground) NSP diets spend more time on feed intake, compared with hens that were fed normal energy or low NSP diets. However, these effects were not necessarily reflected by a reduced feather pecking behaviour.

Samenvatting

Inleiding

De traditionele batterijkooi zal binnen de Europese Unie uiterlijk vanaf 2012 volledig verboden zijn. Een ernstig probleem van alternatieve huisvestingssystemen in vergelijking met batterijkooien is echter een hogere incidentie van verenpikken en kannibalisme (Morgenstern, 1995, Mollenhorst, 2005). In de biologische legpluimveehouderij komen zelfs sterftepercentages van 30% voor als gevolg van kannibalisme (Van der Wouw, 1995). Op dit moment wordt snavelkappen gezien als de belangrijkste maatregel om verenpikken en kannibalisme te voorkomen, maar vanaf 2011 zal er in Nederland waarschijnlijk ook een algemeen verbod op snavelkappen van kracht zijn. Juist de combinatie van alternatieve huisvestingssystemen en de afwezigheid van snavelkappen vormt een groot risico voor verenpikken en kannibalisme. De problematiek van verenpikkerij en kannibalisme is voor de legpluimveehouders die hun leghennen nog huisvesten in batterijkooien op dit moment een belangrijke beperking voor omschakeling naar alternatieve huisvestingssystemen.
Het probleem van verenpikkerij en kannibalisme is multifactorieel. Als belangrijke oorzaken hiervoor gelden diereigen factoren, zoals erfelijke aanleg, hormonale status (onvolwassen vs. volwassen dieren), mate van angst en sociale factoren, maar ook omgevingsfactoren, zoals huisvestingsomstandigheden en voedingsfactoren (Blokhuis, 1989). Het bestrijden van verenpikken vraagt dan ook om een geïntegreerde benadering waarbij rekening wordt gehouden met diverse factoren. Uit een onlangs gepubliceerd literatuuronderzoek (Krimpen *et al.*, 2005) blijkt dat voedingsfactoren bij kunnen dragen aan het reduceren van de mate van verenpikken en kannibalisme. Een perspectiefvolle benadering lijkt het stimuleren van de tijd die leghennen besteden aan voeropname gerelateerd gedrag, zoals foerageren (het zoeken naar mogelijke voedselbronnen) en voeropname, en het stimuleren van de mate van verzadiging van leghennen. Beide zaken kunnen gestimuleerd worden via verstrekking van energiearme voeders (Lee *et al.*, 2001), of van voeders die rijk zijn aan niet-oplosbare NSP (Non Starch Polysaccharides) (Bearse *et al.*, 1940; Hetland *et al.*, 2002, 2003). Ook de maalfijnheid van de NSP-fractie lijkt van invloed te zijn op de mate van verenpikken. Er zijn aanwijzingen dat een grof gemalen NSP-fractie resulteert in minder verenpikken (Hetland *et al.*, 2002, 2003). Daarnaast zijn positieve resultaten bereikt met het verstrekken van ruwvoer (Steenfeldt *et al.*, 2001).
We veronderstellen dat hennen die voer met een lagere nutriëntendichtheid krijgen meer voer gaan opnemen en uiteindelijk een gelijke energieopname realiseren als hennen die een controlevoer met een gangbare nutriëntendichtheid krijgen. Uit een eerste experiment, uitgevoerd door (Krimpen *et al.*, 2006) 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. Bovendien besteedden de hennen die verdund voer kregen meer tijd aan voeropname, waardoor de kans op verenpikken vermoedelijk afneemt. Om meer inzicht te krijgen in het werkingsmechanisme en de effectiviteit van de voedingsfactoren nutriëntendichtheid, NSP-niveau en maalfijnheid van de NSP-fractie op de mate van verenpikken is in opdracht van het Productschap Diervoeder en het Productschap Pluimvee en Eieren een experiment uitgevoerd met leghennen tijdens de eerste 22 weken van de legperiode.

Doel

Het reduceren van de incidentie van verenpikken bij leghennen door middel van 1) nutriëntendichtheid, 2) niet-water oplosbaar NSP-gehalte en 3) maalfijnheid van de NSP-fractie van het voer.

Materiaal

Voor het experiment is gebruik gemaakt van 2 identieke afdelingen (9,0 x 9,0 m) , waarvan de een 24 en de ander 25 grondkooien bevatte 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. 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 via een voertrog en het water via 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 588 niet-gekapte hennen ingezet, verdeeld over 49 grondkooien (7 behandelingen met 7 herhalingen per behandeling. De hennen waren als eendagskuiken aangevoerd en op Zodiac 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 laag-energetisch opfokvoer (OE_Kuiken = 2500 KCal). Al tijdens de opfokperiode (vanaf 5 weken leeftijd) werd er al pikgedrag in de koppel waargenomen.

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, hetgeen een duidelijk hogere bezettingsgraad is dan dat gangbaar is voor scharrelkippen (9 hennen/m^2); dit om de kans op verenpikken te verhogen. De hennen werden random over de hokken verdeeld, waarbij gestreefd werd 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 (8 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:

- Gangbare nutriëntendichtheid versus lage nutriëntendichtheid (10% verdunning van het voer door toevoeging van zand).
- Gangbaar versus hoog inert niet-water oplosbaar NSP-gehalte door toevoeging van 10% haverdoppen. Verhogen van het NSP gehalte verlaagde echter ook de nutriëntendichtheid. Dit werd gecompenseerd door door toevoeging van extra vet (behandeling 2 en 3).
- Fijn versus grof malen van de niet-water oplosbare NSP-fractie. In het geval van fijne maling werden de haverdoppen vooraf fijngemalen op een 1 mm zeef en daarna buiten de hamermolen om aan de menger toegevoegd. In het geval van de grove maling werden de haverdoppen ongemalen buiten de hamermolen om aan de menger toegevoegd.
- Omdat een hoog vetgehalte (behandeling 2+3) een sederend (rustgevend) effect heeft op de hennen, zou het gedrag van de dieren die voer 2 of 3 kregen mogelijk ongewenst beïnvloed worden. Daarom is een extra behandeling (beh. 7) toegevoegd met een gangbare nutriëntendichtheid en een laag NSP-gehalte, maar met een hoog vetgehalte. Ter compensatie van het hoge vetgehalte is in dit voer zand toegevoegd. Door deze extra behandeling was het mogelijk om bij de analyse van de resultaten onderscheid te maken in effecten die toegeschreven kunnen worden aan het NSP-gehalte of aan het vetgehalte.

Deze opzet resulteerde dus in 7 behandelingen, zoals aangegeven in onderstaande tabel:

Nutriëntendichtheid	Gangbaar			Laag			Gangbaar
Niet-oplosbare NSP-gehalte	Gangbaar	Hoog		Gangbaar	Hoog		Gangbaar
Maalfijnheid NSP-fractie	n.v.t.	Gangbaar	Grof	n.v.t.	Gangbaar	Grof	n.v.t.
Vetgehalte	Gangbaar	Hoog	Hoog	Gangbaar	Gangbaar	Gangbaar	Hoog
Behandeling	1	2	3	4	5	6	7

De voeders werden zo samengesteld dat nutriëntendichtheid 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.

Waarnemingen

- De haverdoppen en alle voeders zijn geanalyseerd op de Weende analysekenmerken, op NDF, ADF en ADL en op oplosbaarheid van de NSP-fractie. Ook is van elk voer en van de haverdoppen de verdeling van de deeltjesgrootte vastgesteld.
- Wekelijks is de voeropname per hok bepaald. De hennen zijn als hok gewogen bij opzet, in week 4, 8 enz. tot en met week 22. Ook scoorden we op deze momenten de kwaliteit van het verenkleed.
- Wekelijks registreerden we eiproductie (aantal eieren, gewicht eieren, 2^e soort).
- Van elk hok is 1x per 4 weken (in de week van opleg, week 4 en 8 enz. tot en met week 22) de eettijd d.m.v. videocamera's vastgelegd. Ook registreerden we de mate van verenpikken. Doordat de dieren individueel gemerkt waren kon onderscheid gemaakt worden in dader en slachtoffer (gedragsobservaties).
- Tijdens het wegen van de hennen is de kwaliteit van het strooisel (uitgedrukt in het percentage mestplak per hok) gescoord.
- Toen de hennen 18 weken oud waren, is een novel-object uitgevoerd om van elke hen het gedrag te karakteriseren. Nadat een A4-tje in het hok was gelegd, werd de tijd bepaald die elke hen erover deed om in het papier te pikken (latentietijd).
- De kwaliteit van het verenkleed is elke 4 weken bepaald, te beginnen bij week 16, volgens de methode van Bilcik en Keeling (1999). Deze methode onderscheidt de volgende gebieden bij de hen: nek, rug, overgang rug-staart, staart en flanken.

- Mate van kannibalisme (op basis van uitgevallen dieren).
- Na afloop van week 40 werden 2 hennen per hok geëuthanaseerd, waarna het gewicht en de inhoud van de darmsegmenten (krop, spiermaag, dunne darm, dikke darm, blinde darm) is bepaald. Verwacht wordt dat het gewicht van de spiermaag negatief gecorreleerd is zijn met de mate van verenpikken.
- Aan het einde van het experiment is de voerpassagesnelheid bepaald, waarbij titanium als marker is gebruikt. Op t = 0,5, 1,5, 3,0, 4,5 en 6 uur na toediening van de titaniumcapsules werden hennen geëuthanaseerd, waarna de darminhoud van 5 segmenten van het maagdarmkanaal (krop, spiermaag, dunne darm, dikke darm en blinde darm) verzameld is en geanalyseerd op het titaniumgehalte. De resultaten hiervan zullen in een afzonderlijke publicatie worden gerapporteerd.
- Aan het einde van het experiment is bij 10 hennen per behandeling het bloed geanalyseerd op glucose, corticosteron, Heterofiel-lymfociet ratio en bloedcellen. De voorhersenen van deze dieren zijn geanalyseerd op serotonine- en dopamineturnover en op het noradrenalinegehalte.

Statistische verwerking

Het experiment was opgezet als een 2 x 3 factoriele proef plus een extra behandeling. De factor op 2 niveaus was energie (laag en normaal) en de factor op 3 niveaus was een combinatie van de factoren NSP en Grofheid (hoog NSP, fijn en grof, en laag NSP). De 2 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 2 vrijheidsgraden van de interactie zijn opgesplitst in een contrast energie x NSP en een contrast energie x grofheid. De extra behandeling met hoog vet was bedoeld om te vergelijken met de behandelingen fijn en grof hoog NSP bij normale energie. Het statistische model 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. Indien het vetrijke voer significant (P<0.05) verschilde van minstens 1 van de 2 behandelingen met hoog NSP bij normale energie, is dat aangegeven met een *.

Wekelijks of 4-wekelijks verzamelden we resultaten van dezelfde hennen, zodat er sprake was van herhaalde waarnemingen (longitudinale data). De technische resultaten bleken een exponentieel verloop te hebben. Dit verloop is per hok gemodelleerd met behulp van een non-lineaire parameterschatting. Vervolgens is binnen dezelfde REML-procedure nagegaan of de geschatte modelparameters van de verschillende behandelingsfactoren van elkaar verschilden (Energie-gangbaar versus Energie-laag; NSP-laag versus NSP-hoog, NSP-hoog-fijngemalen versus NSP-hoog-grofgemalen).

Resultaten

<u>Gedrag gerelateerde parameters</u>

Voergericht gedrag

Verlaging van het energiegehalte met 10% resulteerde in een verhoging van de maximale voeropname met 9,3%. Het NSP gehalte en de grofheid van NSP hadden geen effect op de voeropname. Verlaging van het energiegehalte met 10% resulteerde in een relatieve verhoging van de voeropnametijd met 14.2%, terwijl verhoging van het NSP gehalte leidde tot een relatieve verhoging van de voeropnametijd met 17.2%. Grof malen van de NSP fractie verhoogde de voeropnametijd met 7,9%. De eetsnelheid werd niet beïnvloed door het energiegehalte en de grofheid van de NSP fractie, maar verhoging van het NSP gehalte in het voer verlaagde de eetsnelheid (gram voeropname/eetminuut) met 21%. Zowel energieverlaging als NSP-verhoging beïnvloedden dus de mate van voergericht gedrag.

Mortaliteit en Kwaliteit van het verenkleed

Bij een gangbaar energiegehalte in het voer was het uitvalspercentage als gevolg van kannibalisme duidelijk lager als het voer een hoog in plaats van laag NSP-gehalte had (31.6 versus 44.1%). Bij het lage energiegehalte in het voer was het uitvalspercentage als gevolg van kannibalisme minder als hennen in plaats van voer met een hoog NSP gehalte voer met een laag NSP gehalte kregen (13,1 versus 28,6%).
Hoewel we incidenteel in bepaalde weken significante positieve effecten zien van energiegehalte en NSP gehalte of interacties van beide factoren op de kwaliteit van het verenkleed is hierin geen duidelijke trend te ontdekken. Ondanks dat er numeriek vaak wel verschillen tussen proefbehandelingen zichtbaar zijn, is de variatie in verenscores dermate groot dat niet resulteert in statistisch aantoonbare verschillen.

Pikgedrag
Het gemiddelde niveau van mild en ernstig verenpikken, kooipikken, agressief pikken en cloacapikken verschilde niet tussen de proefbehandelingen. Wel was de helling van de lineaire lijn, die de ontwikkeling van het pikgedrag in de loop van de tijd beschrijft, voor kooipikken, cloaca pikken en totaal pikken lager als hennen laag energie voer in plaats van gangbaar energievoer kregen. Hieruit kan geconcludeerd worden dat de verschillen in aantal pikinteracties voor deze gedragingen aantoonbaar toenamen in de loop van de proefperiode.

Overige gedragingen
De proefbehandelingen hadden in het algemeen geen duidelijke effecten op het gedragspatroon van hennen, zoals bepaald op basis van de scan sampling techniek in week 11 en 19 van de proefperiode. Het belangrijkste verschil was dat hennen die voer met grofgemalen NSP kregen in vergelijking met hennen die fijngemalen NSP kregen in week 11 meer tijd aan voeropname (31.1 versus 24.5%) en minder tijd aan verzorging van het verenkleed (12.4 versus 21.7%) besteedden.

Technische resultaten
Er waren geen relevante effecten van de proeffactoren op de ontwikkeling van het legpercentage, eigewicht, eimassa, voederconversie en lichaamsgewicht van de hennen. Wel lijkt de energieconversie (g voer gecorrigeerd voor energiehalte/g ei) iets gunstiger als laag energievoer in plaats van gangbaar energievoer wordt verstrekt.

Fysiologische, neurobiologische en overige paramaters
NSP-verhoging van het voer geeft gemiddeld een verhoging van het gewicht van de lege spiermaag met 30%. Verstrekking van grofgemalen NSP rijk voer gaf echter weer een duidelijk (30%) hoger gewicht van de lege spiermaag in vergelijking met fijngemalen NSP rijk voer. Verstrekking van NSP rijk voer leidde ook tot 18% meer inhoud van de spiermaag in vergelijking met voer met een gangbaar NSP gehalte. De inhoud van de caeca van hennen die energiearm voer kregen was 12,5% hoger dan die van hennen die voer met een gangbaar energiegehalte kregen. Het gewicht en de inhoud van de overige darmsegmenten werd niet beïnvloed door de proeffactoren.
De resultaten van de bloed(-plasma) analyses laten af en toe verschillen zien tussen de proefbehandelingen. Er kan echter nog geen logisch verband tussen de proeffactoren en de aangetoonde verschillen gevonden worden. Hennen die laag energievoer kregen hadden 43% minder dopamine turnover in de hersenen dan hennen die gangbaar energievoer kregen.
Uit de resultaten van de novel object test, uitgevoerd in de eerste week van het experiment, bleek dat de latentietijd van hennen die NSP-rijk voer kregen 13% lager was dan hennen die voer met een gangbaar NSP gehalte kregen. Dit kan geassocieerd worden met meer belangstelling voor de omgeving en een lager stressniveau. Tijdens de manual restraint test, uitgevoerd vlak voor sectie van de hennen, bleek een tendens tot meer verzet bij hennen die NSP-rijk voer kregen in vergelijking met hennen die voer met gangbaar NSP kregen.

Conclusie
De onderzochte voerfactoren (energieverlaging, NSP-verhoging, grofheid NSP fractie) hebben tot op zekere hoogte invloed gehad op het gedrag en andere parameters van de hennen. Verlaging van het energiegehalte verhoogt de hoeveelheid voeropname en de voeropnametijd en vermindert de mate van kannibalisme. Verhoging van het NSP gehalte verhoogt de voeropnametijd en het gewicht en de inhoud van de spiermaag en vermindert de eetsnelheid, de mate van kannibalisme, de dopamineturnover in de hersenen en de latentietijd in de novel object test. Het grofmalen van de NSP-fractie verhoogt de voeropnametijd.
Ondanks deze positieve effecten hadden de voerfactoren geen effect op de ontwikkeling van het verenkleed en de mate van pikgedrag. Daarnaast moet gesteld worden dat ondanks de positieve effecten van de voerbehandelingen het niveau van uitval, zelfs bij de meest gunstige behandelingen, nog steeds duidelijk hoger ligt dan het niveau dat in de praktijk gangbaar is. Uiteindelijk kan daarom gesteld worden dat de onderzochte voerbehandelingen onder de geldende proefomstandigheden (hennen die al pikgedrag vertoonden in de opfokperiode, onbehandelde snavels, hoge bezettingsgraad, hoge lichtintensiteit) onvoldoende in staat zijn om verenpikgedrag en kannibalisme te reduceren. Aanvullend onderzoek zal moeten aantonen of het verstrekken van (extra) verdunde en of NSP-rijke voeders vanaf de eerste levensdag wel in staat zijn om verenpikken en kannibalisme te voorkomen. Voor een goede vertaalslag naar de praktijk, dient daarna een validatiestudie onder (semi-) praktijkomstandigheden uitgevoerd te worden.

Inhoudsopgave

1 Introduction

Feather pecking in layers, that is often seen in modern alternative housing systems (Morgenstern, 1995, Mollenhorst, 2005), is a multi factorial problem, which can be caused by environmental, genetic or nutritional factors (Blokhuis, 1989). Some researchers reported that feather pecking behaviour is a substitute for normal feeding behaviour (Hoffmeyer, 1969, Blokhuis, 1989). Nutritional factors may positively or negatively affect feather pecking behaviour in laying hens (Krimpen *et al.*, 2005). Dietary deficiencies, resulting in a marginal supply of nutrients, such as protein (Ambrosen en Petersen, 1997), amino acids (Al Bustany en Elwinger, 1987a, Al Bustany en Elwinger, 1987b, Elwinger *et al.*, 2002), or minerals (Schaible *et al.*, 1947, Hughes en Whitehead, 1979), may increase feather pecking behaviour and cannibalism. In addition, laying hens seem to spend more time on feeding and foraging behaviour when they are fed mash diets in stead of crumbles or pellets, low energy diets, diets with high (in-)soluble Non Starch Polysaccharides (NSP) or roughages (Krimpen *et al.*, 2005). Laying hens that are fed low nutrient density diets, will normally compensate for the lower nutrient concentration by increased feed intake (Savory, 1980, Lee *et al.*, 2001, Krimpen *et al.*, Submitted). Therefore, egg performance will be maintained, even in early lay (Krimpen *et al.*, Submitted). Diets high in insoluble NSP content decreased eating rate (Krimpen *et al.*, Submitted) and increased the rate of digesta passage (Hartini *et al.*, 2003). Both an increased feeding and foraging time and/or an increased digesta passage rate may reduce feather pecking behaviour (Hartini *et al.*, 2003, Krimpen *et al.*, 2005).
Although energy and NSP concentration and particle size of NSP fraction may reduce feather pecking behaviour in laying hens, these nutritional factors were often confounded in experimental diets. Therefore, an experiment was conducted to investigate the pure effects of energy concentration, NSP concentration and particle size of NSP on eating behaviour, feather pecking behaviour, egg performance and some physiological and neurobiological parameters of laying hens. We hypothesise that eating time will be increased by feeding diets with low energy levels and/or high contents of coarsely ground insoluble NSP's, resulting in reduced feather pecking behaviour, without negatively affecting egg performance.

2 Materials and methods

2.1 Housing, birds and management

A total of 588 non beak trimmed 16 wk old layers (Isa Brown strain) were housed in two climate controlled rooms, both measuring 9 x 9 metres. The one room had 24 and the other 25 floor pens (90 x 150 centimetres), while a laying nest was placed at the outside of the pen. The pens were built of wire and hens could see their flock mates in other pens. Each pen contained 2 perches, a feeding trough (length of 100 cm), nipple drinkers. Hens were housed with twelve birds per pen (10.4 hens/m^2), and sand was used as litter. Average body weight at 18 weeks of age (start of the experiment) was 1713 g (±48.0). Hens were reared in another room in the same accommodation. Stocking density during rearing was 13 birds/m^2 and birds were fed *ad libitum.* The first 8 weeks of age they received a standard commercial diet ($OE_{Broiler}$= 10.9 MJ/kg). To stimulate feed intake capacity, birds were fed a diluted rearing diet ($OE_{Broiler}$= 10.5 MJ/kg) from 9 to 17 weeks of age. From an age of 18 weeks hens received the experimental laying diets until the end of the experiment, 22 weeks later. Laying hens were fed *ad libitum* and had free access to water. Room temperature was set at 20°C and two times a day, health status of the hens, room temperature and air humidity were monitored. At 16 weeks of age, the light schedule was set at 10L : 14D (10 lux). Weekly, the light period was extended by one hour till the birds had a 16L: 8D light schedule at the age of 22 weeks. Photoperiod lasted from 1:00 to 17:00h. Light intensity was increased in week 18 (20 lux) and week 20 (30 lux), but because of an outbreak of cannibalism in week 21 reduced to 20 lux and maintained until the end of the experiment. Throughout the experiment, litter quality was maintained monthly by adding new sand.
Feather pecking behaviour was latent available from 5 weeks of age. To stimulate stress level of birds, and thereby the chance on feather pecking behaviour, stocking density and light intensity were conscious set on higher levels than usual in practice.

2.2 Experimental design

At 18 weeks of age, hens were allotted to 1 of 6 dietary treatments, with normal and low energy concentration, normal and high NSP concentration, and fine and coarse particle size of NSP according to a 2 x 3 factorial arrangement, with 7 replicates per treatment. Sand was used as dilution material to reduce energy concentration in low NSP diets, whereas oat hulls were used as insoluble NSP source. To maintain energy concentration in normal energy/high NSP diets, extra fat was added to these diets. Therefore, NSP and fat concentrations were confounded in these diets. To separate the effect of NSP and fat, a seventh dietary treatment was added. This normal energy diet also contained a high fat content, but sand in stead of oat hulls was added as dilution source. This treatment was also replicated 7 times.
All diets were in mash. Characteristics and classification of the seven treatments are shown in table 1.

Table 1 Characteristics and classification of the treatments

Energy concentration	Normal			Low			Normal
NSP concentration	Normal	High		Normal	High		Normal
Coarseness NSP	not relevant	Normal	Coarse	not relevant	Normal	Coarse	not relevant
Fat concentration	Normal	High	High	Normal	Normal	Normal	High
Treatment number	1	2	3	4	5	6	7

Diet composition and the chemical contents are shown in table 2a and 2b, respectively.

Table 2a Diet composition (g/kg)

	Treatment 1	Treatment 2 -3	Treatment 4	Treatment 5-6	Treatment 7
Maize	383.4	383.4	345.1	345.0	383.4
Wheat	204.8	40.0	184.2	184.3	40.0
Soybean meal, extracted	137.9	108.9	124.1	124.1	108.9
Peas	84.6	91.9	76.1	76.1	91.8
Oyster shells	72.4	72.0	65.2	65.2	72.1
Rapeseed, extracted	30.0		27.0	27.0	0.0
Soybean, heat treated	25.0	116.1	22.5	22.5	116.0
Soybean oil	23.3	25.0	21.0	21.0	25.0
Limestone	20.0	20.0	18.0	18.0	20.0
Monocalciumphosphate	8.1	9.0	7.2	7.2	9.0
Premix laying hens	5.0	5.0	4.5	4.5	5.0
Salt	3.7	3.7	3.3	3.3	3.7
DL-Methionine	1.6	2.0	1.4	1.4	2.0
L-Lysine	0.4		0.4	0.4	
Palm oil		23.2			23.2
Sand			100.0		100.0
Oat huls		100.0		100.0	

Table 2b Analysed and calculated chemical contents of the diets (g/kg, as-fed basis)

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7
				Analysed contents			
Dry matter	911.0	920.5	926.9	929.9	925.0	916.1	934.6
Ash	123.3	124.3	124.8	223.0	115.9	114.0	227.6
Fat	41.7	67.3	63.6	43.7	44.3	39.5	63.5
Crude Fibre	26.6	57.9	55.0	22.7	62.1	60.4	21.7
Crude Protein	168.1	155.7	154.5	150.2	150.9	151.5	152.3
Starch	411.8	338.2	343.4	378.4	388.1	391.5	333.6
Sugar	33.6	29.8	29.0	29.3	30.1	28.6	29.5
Calcium	38.9	38.6	41.1	36.0	35.6	35.4	39.6
Magnesium	1.9	1.8	1.8	1.7	1.7	1.7	1.8
Phosphor	5.4	4.9	5.0	4.9	4.9	4.8	4.9
Copper (mg/kg)	17.3	11.3	10.3	9.5	10.8	11.3	12.9
Sink (mg/kg)	77.5	77.7	78.3	69.7	73.8	69.8	76.7
Iron (mg/kg)	209.7	230.2	263.6	244.3	229.8	232.8	275.6
Sodium	1.4	1.5	1.5	1.5	1.4	1.3	1.7
Potassium	7.0	7.1	7.1	6.2	6.8	6.6	6.8
NSP	132.6	201.9	193.7	105.2	195.7	190.9	114.1
NDF	67.7	127.9	129.9	63.0	140.0	138.7	56.6
ADF	26.6	61.7	60.8	29.8	68.0	64.1	25.0
ADL (lignin)	6.6	14.1	11.4	6.8	14.1	13.2	4.2
Cellulose	20.0	47.6	49.4	23.0	53.9	50.8	20.8
Hemi cellulose	41.1	66.2	69.1	33.2	72.0	74.7	31.5
				Calculated contents			
OElh (MJ/kg)	11.80	11.80	11.80	10.60	10.60	10.60	11.80
LYS	8.09	8.30	8.30	7.33	7.63	7.63	8.00
Dig. LYS	6.70	6.70	6.70	6.08	6.20	6.20	6.59
Dig. MET	3.61	3.86	3.86	3.25	3.27	3.27	3.84
Dig. CYS	2.18	1.93	1.93	1.96	1.99	1.99	1.90
Dig. M+C	5.80	5.80	5.80	5.22	5.27	5.27	5.75
Dig. THR	4.60	4.52	4.52	4.14	4.20	4.20	4.46
Dig. TRP	1.47	1.41	1.41	1.32	1.34	1.34	1.39

Diet 1 (normal energy and normal 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). Besides, diets of treatment 2 and 3 had higher fat and lower starch content, compared with diet 1. Addition of 100 g/kg sand to the control diet (treatment 4) increased ash content from 123 to about 225 g/kg (treatment 4 and 7), 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 fibre, NSP, (hemi-) cellulose and lignin increased. Oat hulls were hammer milled, along with the other raw materials (fine) or ungrounded added to the diet (coarse).

2.3 Observations

Particle size distribution
Particle size distribution of the diets was analysed. Seven particle size fractions were separated by using six sieves with diameters of 0.25, 0.50, 1.25, 2.50, 3.15 and 5.0 mm respectively.

Body weight, feed intake and egg production
All hens were weighed per pen at 18, 22, 26, 30, 34, 38 and 40 wks of age. Feed consumption and egg production per pen were recorded weekly. Egg weight per pen was based on the amount of 'normal' egg mass, i.e. all clean and dirty (blood- or faecal-stained), normal graded eggs. The remaining 'abnormal' egg mass consisted of broken, cracked, shell-less, double-yolked and very small (< 30 g) eggs. For the trait 'total egg mass' the entire egg mass production was calculated, assuming shell-less and cracked eggs to weigh the mean 'normal' egg weight of that specific pen and week.

Eating time
In week 1, 5, 9, 13, 17 and 21 of the experiment, video observations were made from which eating time per pen could be calculated. The day was divided in three blocks, from 9.00 am to 11.30 am, 11.30 am to 2.00 pm and from 2.00 pm to 4.30 pm. In each block on every day, eight pens were observed using 4 cameras. Each observation lasted one hour. The number of eating hens (between 0 and 12), was recorded continuously until the end of each observation by using Observer 4.1/5.0 software (Noldus, 1993). Then, the percentage of eating time per hen per pen per observation period was calculated. Eating rate on a weight base was calculated as feed intake (g/d) divided by daily eating minutes . Eating minutes per day were not determined, but calculated as the number of hours with light on (16 h) multiplied by the percentage of observed eating time. Besides, intercept and slope of the linear functions to model eating time and eating rate development over time were calculated.

Feather condition scores and mortality
With 2-week intervals, plumage and skin condition per individual hen was 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 analyses. Besides, slopes of the linear lines to model feather condition scores over time were calculated. Mortality of birds was recorded on a weekly basis. Average mortality and the chance not to survive the experiment were analysed.

Behavioural recordings
Recordings of feather pecking (gentle and severe), aggressive pecking, vent pecking and cage pecking were made in week 4, 10, 18 and 21 of the experiment. Each pen was observed for 10 min, counting each peck. Results were presented as nr. of pecks per observed hen per 10 min. Duration of behaviour elements, as described in table 3, was scored during week 11 and 19 of the experiment by using scan sampling technique. For each pen, an observer scored the number of hens per behaviour class at 1-min intervals over a 15 min observation period. Based on these 15 observations, average number of hens per behaviour class were determined and recalculated to percentages of time spend on the different behaviours.

Table 3 Ethogram showing the behavioural measurements

Behaviour	Definition
Feeding	Pecking at food in trough
Foraging	Pecking at the litter and scratching (separately scored as ground scratching) or moving with the head in a lower position than the rump
Walking	Walking, running, jumping or flying (it may be accompanied by wing-flapping)
Preening	Preening behaviour as described by Kruijt (1964): e.g. autopecking, nibbling, stroking, combing, head-rubbing
Ground scratching	Bird, alternately, makes backward strokes with both legs in the litter as part of foraging behaviour. (Every stroke is recorded as one occurrence)
Dustbathing	Sitting and performing: vertical wing-shaking, body shaking, litter pecking and/or scratching, bill raking, side and head rubbing
Resting	Sitting or standing inactive (no movement of the legs)
Drinking	Bird drinks water from the nipple or the cup

Physiological and neurobiological parameters
At the end of the experiment, 25 hens per treatment (5 birds from 5 selected pens) were dissected at different time intervals. The first three birds per pen were dissected by injection of T61, whereas bird 4 and 5 of each pen were manually restraint for 5 min before killing by rapid decapitation. After dissection, weight and content of each of five GIT segments (crop, gizzard, ileum, colon and caeca) of all killed hens were determined.
Hens that were subjected to the manual restraint test were placed on the side for 5 minutes, and their behaviour and (latency time struggling, number of struggles, latency time vocalising and number of vocalisations) was scored (Hierden *et al.*, 2004). All activities were performed by two parallel functioning teams.
Blood and forebrains of the decapitated birds (2 birds from 5 selected pens) were collected. Blood was used for blood cell counts and determination of glucose and corticosterone concentrations, whereas forebrains were used fore serotonin and dopamine turnover and noradrenalin measurements.
Trunk blood of decapitated birds was collected in K_3EDTA coated tubes for plasma corticosterone measurements. Blood was centrifuged for 10 min at 3000 rpm at a temperature of 4^0C. Corticosterone concentrations were determined in unextracted, enzymatically pre-treated plasma. For blood cell counts, trunk blood was collected in K_3EDTA coated tubes and offered for automated blood cell counts (Post *et al.*, 2003).
The brains of the restrained birds were removed within five minutes after decapitation and immediately frozen in a dry ice pre-cooled tube containing n-heptane and stored at - 70^0C until the assays were performed (Hierden *et al.*, 2004).

2.4 Curve-fitting procedure and statistical analysis

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

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

where *Y* is the expected value of the performance parameter; *A* is performance value at $t = -\infty$; *B* is the increase of performance value over time, therefore on $t = \infty$ maximum value is *A + B*; *t* is point in time (week number-1); α is velocity of increase of the performance parameter. Egg weight was corrected for number of weighed eggs, because average weight of the first eggs per pen varied highly as a result of low number of eggs. A residual term was added because of records with weighing 0 (no eggs). A REML procedure in (Genstat_8_Committee, 2002) was used to estimate curve parameters per pen. The nonlinear parameters were estimated by using a two-step iterative procedure, starting from a first order Taylor approach (Lindstrom en Bates, 1990, Engel *et al.*, 2003). Following curve-fitting, the REML variance component analysis procedure tested the effect of the nutritional factors, using the model [2]:

$$Y_{ij} = \mu + energy_i + NSP_j + (energy \times NSP) + e_{ij} \quad [2]$$

where Y_{ij} = dependent variable; μ = overall mean; $energy_i$ = fixed effect of energy concentration i (i = 2; normal and low); NSP_j = fixed effect of NSP concentration j (j = 3; a combination of NSP and coarseness); the contrast of NSP represents low 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. The extra high fat diet was included to compare with the two energy normal/high NSP treatments. If the effect of high fat diet differs from one of these two treatments ($P<0.05$), it will be marked with a *. Model [2] was corrected for room and pen effects.

Model [2] was also used to test effects of eating time, eating rate, feather condition score, average mortality and chance not to survive the experiment, behaviour distribution, weight of GIT segments, blood data and brain data. Survival of hens over time was modelled by use of survival analysis with Weibull distribution. Then, for the hens in each pen the chance not to survive the experiment was calculated and these chances were statistically analysed by use of model [2]. Weight of GIT segments and brain data were not normal distributed and therefore transformed to 10log scale. The models for analysing weight of GIT segments, brain and blood data were corrected for room, section day, team and section order.

A GLMM (General Linear Mixed Model) procedure was used to test differences in latency time, number of hens with resistance, and number of vocalising hens per treatment.

3 Results

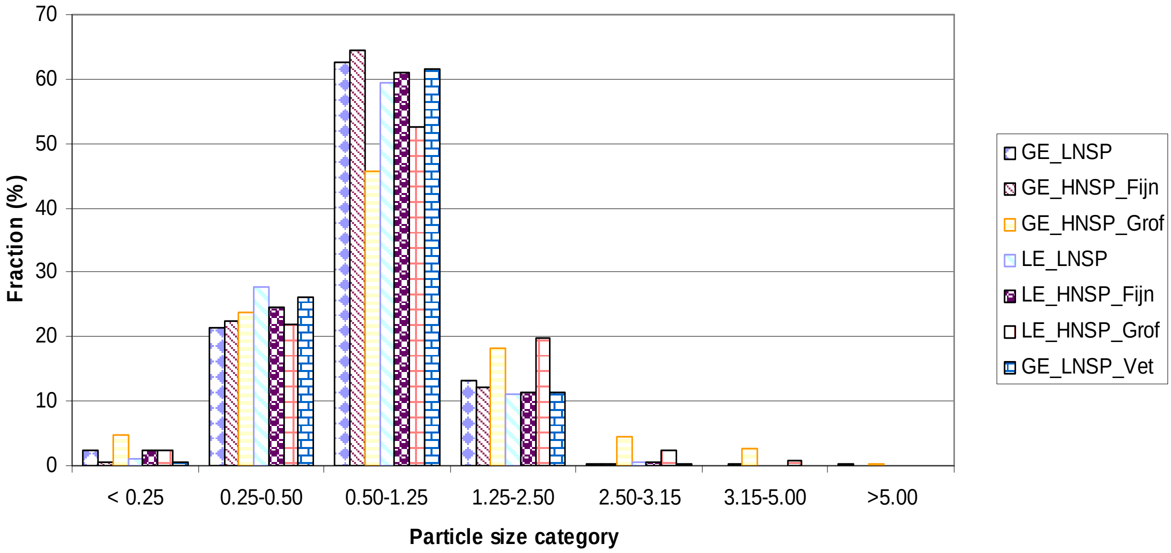
Results are divided in the sections 1) behaviour related parameters, 2) performance parameters and 3) physiological, neurobiological and some other parameters.

3.1 Behaviour related parameters

3.1.1 Particle size distribution

Particle size distribution of the experimental diets is shown in figure 1.

Figure 1 Particle size distribution per diet



The two coarsely ground high NSP diets had lower fractions in the category 0.50 – 1.25 mm (49.2% ±4.9 versus 61.8% ±1.8), but higher fractions in the categories > 1.25-2.50 mm (19.0% ±1.2 versus 11.8% ±0.8) compared with the other diets. Particle size distributions of the other diets were very similar to each other.

3.1.2 Feed intake parameters

Estimates of feed intake parameters per treatment, as described by an exponential curve, are given in table 4.

Table 4 Parameter estimates (A, B and α) of feed intake (g/hen/d) per treatment as described by an exponential curve

Treatment	Initial feed intake (g/d) (A)	Rate of increase (α)	Increase in feed intake (g/d) (B)	Asymptotic feed intake level (g/d) (A+B)
Normal Energy				
Low NSP	93.4	0.27	39.7	133.1
High NSP-Fine	95.1	0.34	34.6	129.7
High NSP-Coarse	98.0	0.39	31.6	129.7
High Fat	95.4	0.43*	31.2	126.5
Low Energy				
Low NSP	99.6	0.36	40.4	140.0
High NSP-Fine	96.4	0.48	37.0	133.3
High NSP-Coarse	100.3	0.28	55.5	155.8
Standard error	1.408	0.034	3.180	
P-Value				
Energy	0.060	0.394	0.021	
NSP	0.594	0.201	0.928	
Energy*NSP	0.225	0.439	0.140	
Coarseness	0.108	0.138	0.106	
Energy*Coarseness	0.807	0.011	0.025	

Initial feed intake of the low energy diets was on average 3.3 g/d (± 1.4) higher than the normal energy diets (P=0.06). Initial feed intake was not affected by NSP concentration and coarseness of NSP. Rate of increase in feed intake was not affected by coarseness of normal energy diets, but in low energy diets rate of increase of finely ground high NSP diet was 0.20 (± 0.034) higher compared with coarsely ground high NSP diet (P=0.011). Coarseness of NSP in diets with normal energy concentration had no effect on increase in feed intake, whereas increase in feed intake in coarsely ground low energy diets was 18.5 g/d higher compared with finely ground low energy diets (P=0.025). Asymptotic feed intake level of the low energy diets was 12.2 g/d (± 3.18) higher than the normal energy diets. Initial feed intake level and increase in feed intake of the high fat treatment differed not from the other fat-rich treatments (high NSP, fine and coarse). Rate of increase of the high fat treatment, however, was 0.09 (± 0.034) higher than the treatment with normal energy/finely ground NSP. Feed intake development per pen and per treatment is shown in appendix 1.

Estimates of energy intake parameters per treatment, as described by an exponential curve, are given in table 5.

Table 5 Parameter estimates (A, B and α) of energy intake (MJ/hen/d) per treatment as described by an exponential curve

Treatment	Initial energy intake (MJ/d) (A)	Rate of increase (α)	Increase in energy intake (MJ/d) (B)	Asymptotic energy intake level (MJ/d) (A+B)
Normal Energy				
Low NSP	1.11	0.33	0.39	1.50
High NSP-Fine	1.12	0.29	0.52	1.64
High NSP-Coarse	1.09	0.41	0.41	1.50
High Fat	1.05*	0.37	0.46	1.51
Low Energy				
Low NSP	1.10	0.44	0.39	1.49
High NSP-Fine	1.11	0.34	0.40	1.41
High NSP-Coarse	1.07	0.37	0.44	1.51
Standard error	0.038	0.079	0.080	
P-Value				
Energy	0.523	0.378	0.548	
NSP	0.811	0.451	0.255	
Energy*NSP	0.928	0.309	0.593	
Coarseness	0.204	0.181	0.574	
Energy*Coarseness	0.971	0.409	0.181	

Energy intake parameters were similar for nearly all treatments, except for the high fat treatment. Hens that were fed fat rich diet tended (P=0.106) to 0.06 (± 0.038) MJ/d lower initial energy intake than the hens fed the other normal energy diets. It can be concluded that 10% reduction in dietary energy concentration will be compensated by higher feed intake of the hens, resulting in similar energy intake.

3.1.3 Eating time and eating rate

Average eating time and parameters of linear functions to model eating time development over week 1-22 are presented in table 6.

Table 6 Average eating time (% of observed period) and intercept (a) and slope (b) of linear function to model eating time development over week 1 to 22

	Average eating time (%)	Intercept (%) (a)	Slope (%/week) (b)
Normal Energy			
Low NSP	15.1	15.5	0.005
High NSP-Fine	18.4	17.8	0.031
High NSP-Coarse	19.3	17.6	0.190
High Fat	19.0	16.4	0.245
Low Energy			
Low NSP	18.6	18.0	0.105
High NSP-Fine	19.7	15.5	0.408
High NSP-Coarse	21.9	22.9	-0.081
Standard error	2.84	1.85	0.142
P-Value			
Energy	0.001	0.081	0.408
NSP	< 0.001	0.128	0.347
Energy*NSP	0.316	0.679	0.797
Coarseness	0.075	0.006	0.103
Energy*Coarseness	0.475	0.003	0.001

Hens that were fed low energy diets spent on average 2.5% more time on feed intake than hens that were fed normal energy diets (P=0.001), which corresponded with a relative increase in eating time of 14.2%. Hens that were fed high-NSP diets spent on average 2.9% more time on feed intake than hens that were fed low-NSP diets (P<0.001), which corresponded with a relative increase in eating time of 17.2%. Coarse grinding of the NSP source increased average eating time numerically with 1.5% (P=0.075) (relative increase = 7.9%) compared with feeding finely ground NSP-high diets. Coarseness of NSP in normal energy diets did not affect intercept of linear function to model eating time, whereas in low energy diets intercept was 7.4% (± 1.85) higher in coarsely ground versus finely ground NSP diets (P=0.003). Likewise, coarseness of NSP in normal energy diets did not affect slope of linear function to model eating time, whereas in low energy diets slope was 0.49 (± 0.142) lower in coarsely ground versus finely ground NSP diets (P=0.003). Hens that were fed normal energy/high fat diets showed similar eating time compared with the normal energy/high NSP treatments. Observed eating time data per treatment per week are given in appendix 2.

Average eating rate and parameters of linear functions to model eating rate development over time are presented in table 7.

Table 7 Average eating rate (g feed intake/observation minute) and intercept (a) and slope (b) of linear function to model eating time development over time

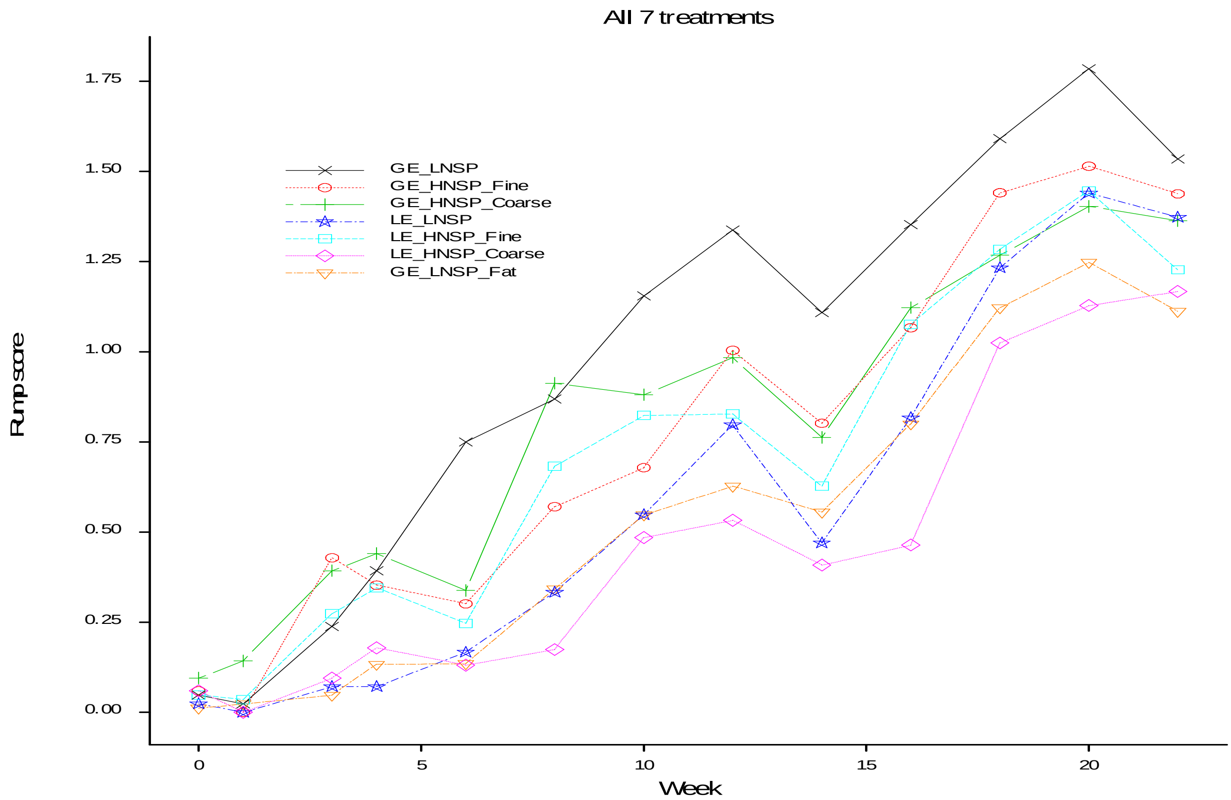
	Average eating rate (g/min)	Intercept (g/min) (a)	Slope (g/min/week) (b)
Normal Energy			
Low NSP	1.04	0.76	0.027
High NSP-Fine	0.80	0.66	0.016
High NSP-Coarse	0.77	0.70	0.006
High Fat	0.78	0.67	0.010
Low Energy			
Low NSP	0.86	0.70	0.016
High NSP-Fine	0.69	0.83	-0.007
High NSP-Coarse	0.75	0.53	0.016
Standard error	0.122	0.101	0.012
P-Value			
Energy	0.146	0.704	0.238
NSP	0.010	0.388	0.065
Energy*NSP	0.444	0.623	0.758
Coarseness	0.582	0.078	0.429
Energy*Coarseness	0.883	0.015	0.059

Eating rate of hens that were fed high-NSP diets was on average 0.20 g/min lower compared with normal energy fed hens (P=0.01), which corresponded with a relative decrease in eating rate of 21%. Coarseness of NSP in normal energy diets did not affect intercept of linear function to model eating rate, whereas in low energy diets intercept was 0.30 g/min (± 0.1.01) lower in coarsely ground versus finely ground NSP diets (P=0.015). The slope of the linear function was not affected by coarseness of NSP in normal energy diets, but in low energy diets was the slope numerically increased by feeding coarsely ground NSP-high diets compared with finely ground NSP-high diets (P=0.059).
Hens that were fed normal energy/high fat diets showed similar eating rate compared with the normal energy/high NSP treatments. Observed eating rate data per treatment per week are given in appendix 3.

3.1.4 Feather condition scores

In this section results of feather condition scores (FC) per body part and total feather condition score are shown. Development of FC of the rump per treatment over time is presented in figure 2. P-values of FC of the rump per treatment per week and P-values of the slope of the lines are given in table 8.

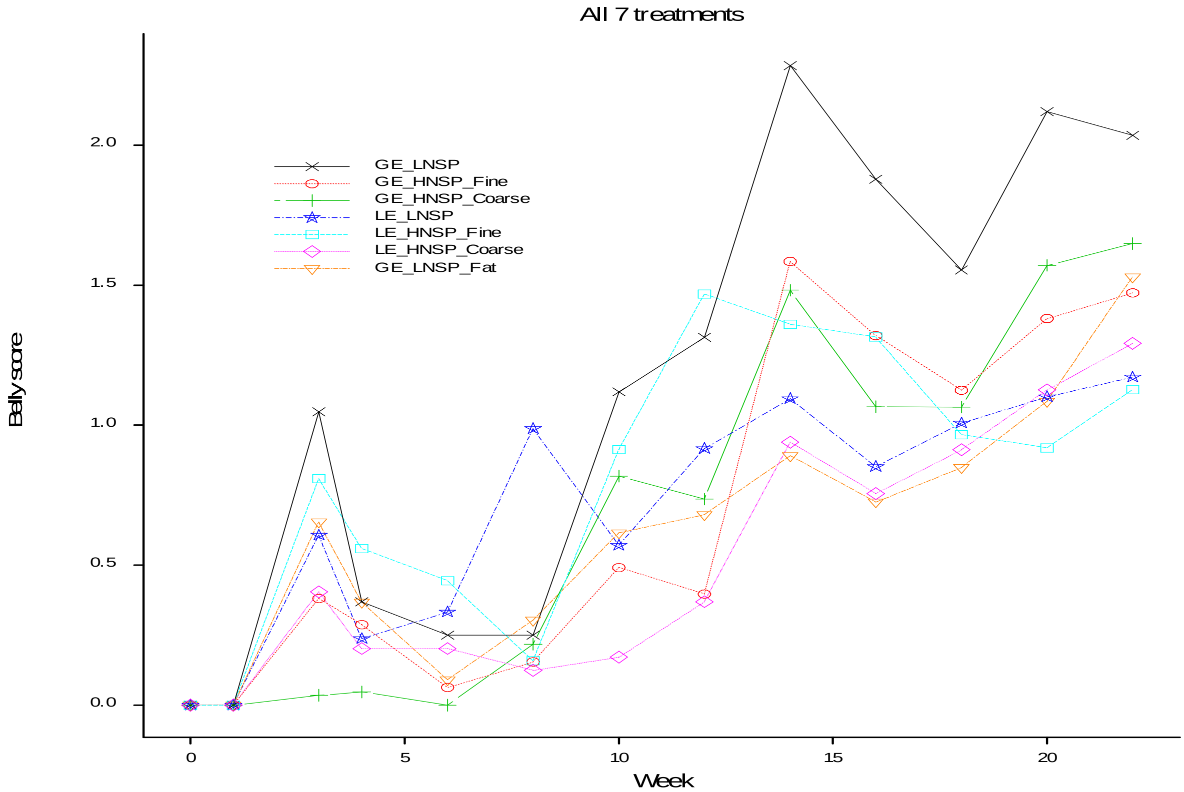
Figure 2 Development of feather condition of the rump per treatment over time



For all treatments, FC of the rump increased during the whole experimental period. Although development of FC differed numerical per treatment, slopes of the lines were not affected by treatment (see table 7). In most weeks, treatment did not affect FC of the rump. Relevant significant effects were only found in week 6 and 8. In week 6, FC of birds fed low energy diets was better compared with birds fed normal energy diets (0.13 versus 0.36, P=0.019). Likewise, in this week FC of birds fed high NSP diets was better compared with birds fed low NSP diets (0.18 versus 0.38, P=0.046). In week 8, FC of birds fed low energy diets was better compared with birds fed normal energy diets (0.29 versus 0.57, P=0.021). Birds that were fed normal energy/fat high diet had better FC over week 3, 4 and 8, compared with birds that were fed normal energy/NSP high diets.

Development of FC of the belly per treatment over time is presented in figure 3. P-values of FC of the rump per treatment per week and P-values of the slope of the lines are given in table 9.

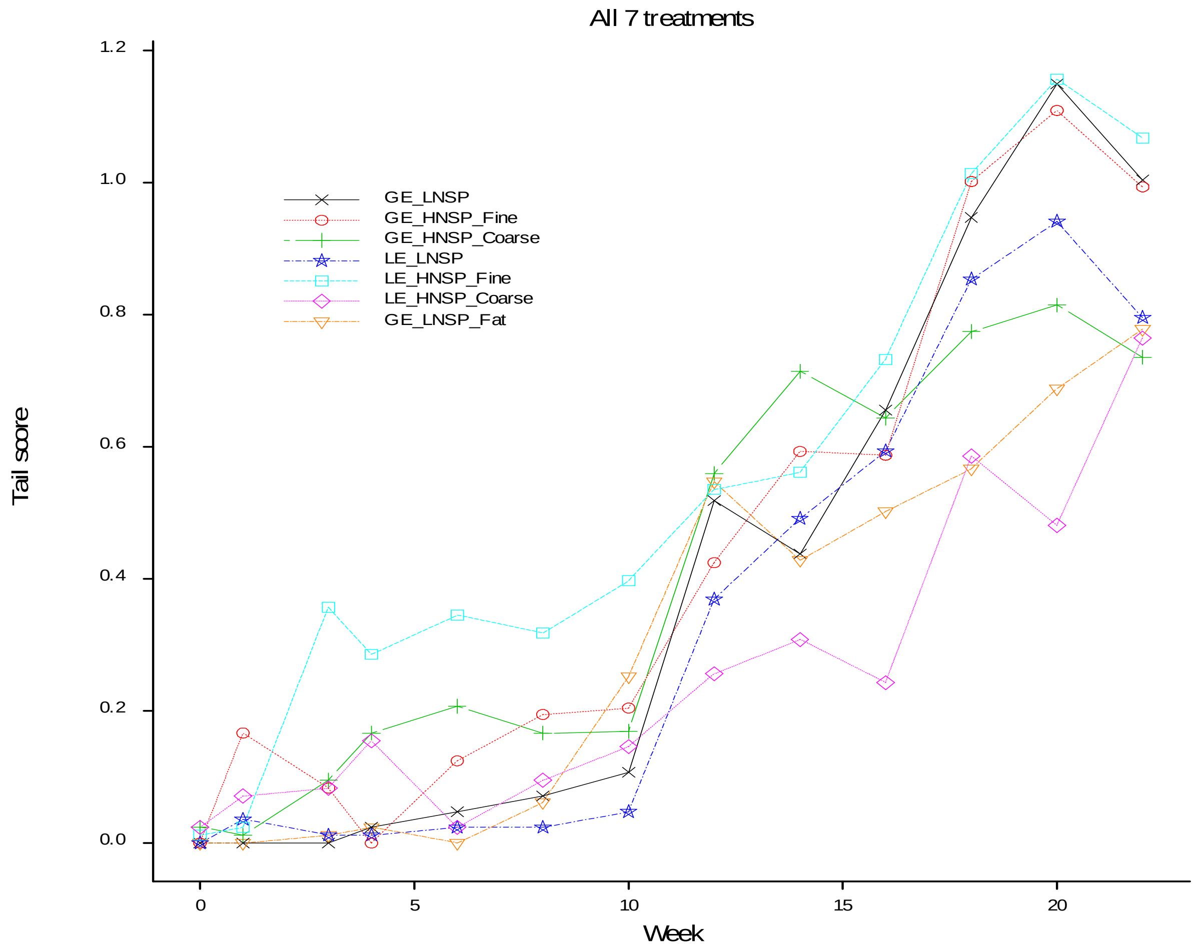
Figure 3 Development of feather condition of the belly per treatment over time



For all treatments, FC of the belly increased during the whole experimental period. Although development of FC differed numerical per treatment, slopes of the lines were not affected by treatment (see Table 8). In most weeks, treatment did not affect FC of the belly. Relevant significant effects were only found in week 8 and 12. In week 8, FC of birds fed high NSP diets was better compared with birds fed low NSP diets (0.11 versus 0.49, P=0.014). In week 12, FC of birds fed normal energy diets was not affected by coarseness of NSP, whereas in low energy diets FC of hens was better if coarsely ground NSP was fed compared with finely ground NSP (0.25 versus 0.97, P=0.050). Hens that were fed normal energy/fat high diet had worse FC over weeks 3 and 4 compared with birds that were fed normal energy/NSP high/coarse diet.

Development of FC of the tail per treatment over time is presented in figure 4. P-values of FC of the rump per treatment per week and P-values of the slope of the lines are given in table 10.

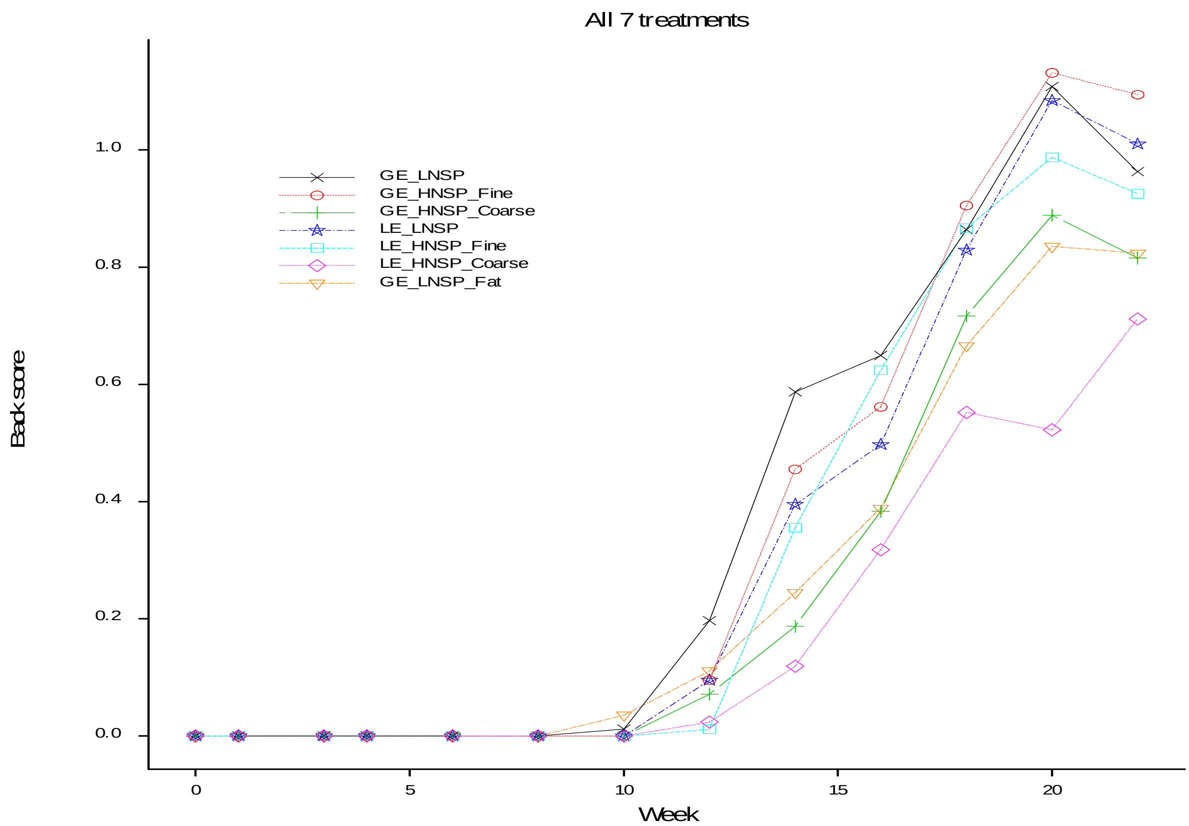
Figure 4 Development of feather condition of the tail per treatment over time



For all treatments, FC of the tail increased during the whole experimental period. Although development of FC differed numerical per treatment, slopes of the lines were not affected by treatment (see table 9). In most weeks, treatment did not affect FC of the tail. Relevant significant effects were only found in week 3, 6 and 8. In week 3 and 8, FC of birds fed high NSP diets was worse compared with birds fed low NSP diets (0.113 versus 0.006, P=0.043) and (0.128 versus 0.030, P=0.050) respectively. In week 6, coarseness of NSP did not affect FC of the tail in normal energy fed hens, but in low energy fed hens fine grinding of NSP reduced FC of the tail compared with coarse grinding (0.23 versus 0.01; P=0.027). Hens that were fed high fat diet had better FC than hens fed normal energy/finely ground NSP diet in week 1 and 20. Hens that were fed high fat diet had better FC than hens fed normal energy/coarsely ground NSP diet in week 6. In week 8, hens that were fed high fat diet had better FC than hens fed both finely and coarsely ground NSP/normal energy diets.

Development of FC of the back per treatment over time is presented in figure 5. P-values of FC of the back per treatment per week and P-values of the slope of the lines are given in table 11.

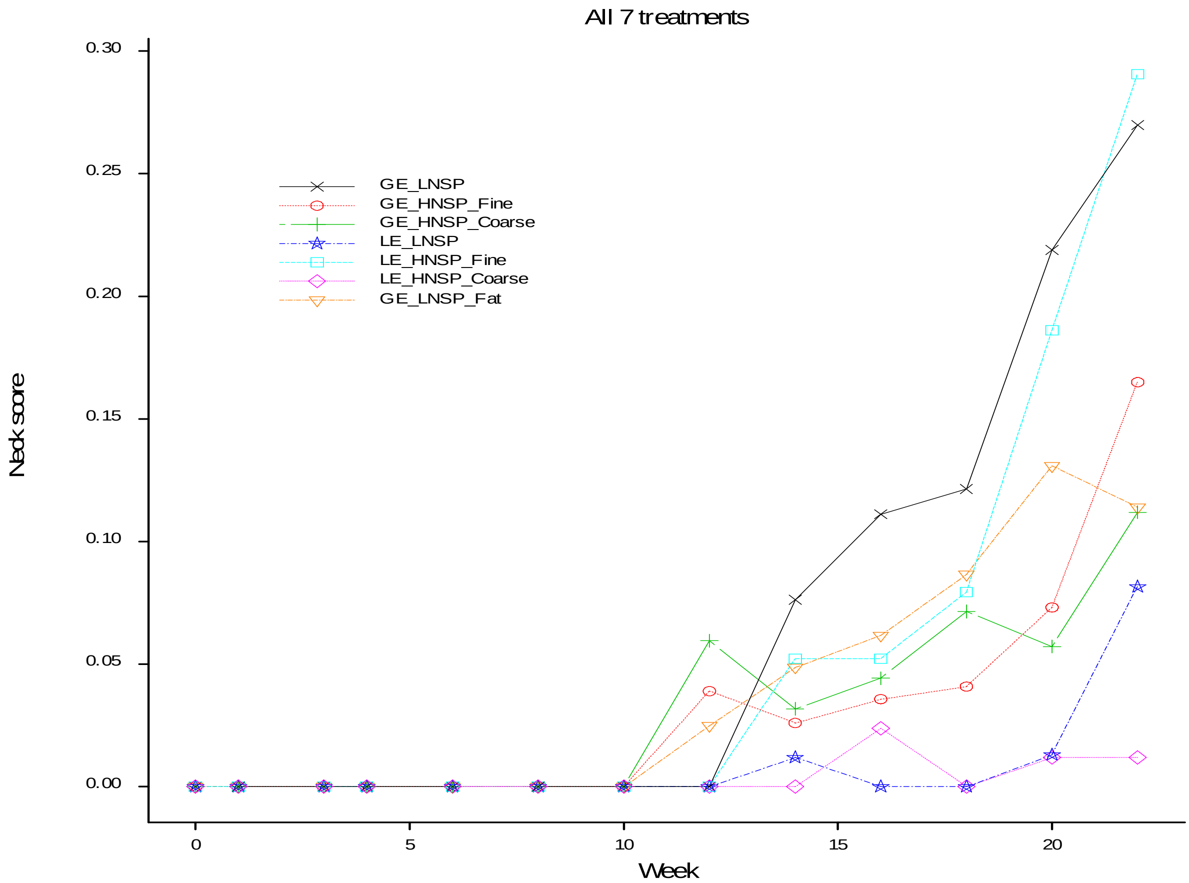
Figure 5 Development of feather condition of the back per treatment over time



For most treatments, FC of the back increased from week 10 onwards. Although development of FC differed numerical per treatment, slopes of the lines were not affected by treatment (see table 10). In most weeks, treatment did not affect FC of the back. Relevant significant effects were only found in week 12. In this week FC of hens fed high NSP diets was lower than FC of hens fed low NSP diets (0.033 versus 0.113, P=0.033). Hens that were fed high fat diet had better FC than hens fed normal energy/finely ground NSP diet in week 14, 20 and 22.

Development of FC of the neck per treatment over time is presented in figure 6. P-values of FC of the neck per treatment per week and P-values of the slope of the lines are given in table 12.

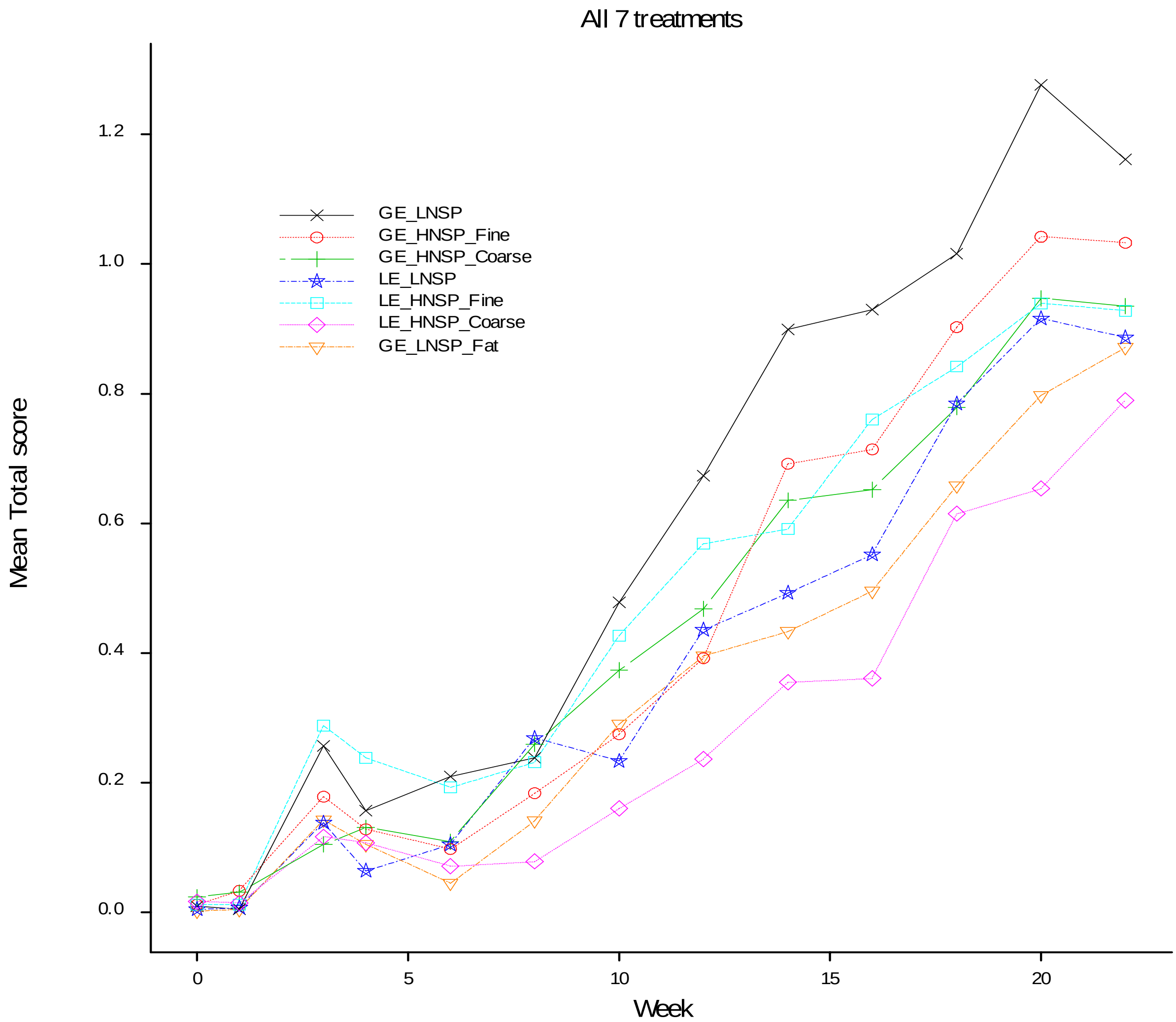
Figure 6 Development of feather condition of the neck per treatment over time



For most treatments, FC of the neck increased from week 10 onwards. Although development of FC differed numerical per treatment, slopes of the lines were not affected by treatment (see table 11). In most weeks, treatment did not affect FC of the neck. Relevant significant effects were only found in week 16. In this week FC of normal energy fed hens was better in high NSP diets compared with low NSP diets (0.024 versus 0.095, P=0.055), whereas NSP concentration did not affect FC of the neck in low energy diets. Hens that were fed high fat diet had worse FC than hens fed normal energy/coarsely ground NSP diet in week 20 (0.024 versus 0.010).

Development of mean total FC per treatment over time is presented in figure 7. P-values of mean total FC per week and P-values of the slope of the lines are given in table 13.

Figure 7 Development of mean total feather condition score per treatment over time



For all treatments, mean total FC increased from week 1 onwards. Although development of FC differed numerical per treatment, slopes of the lines were not affected by treatment (see table 12). In none of the weeks, mean total FC was significantly affected by energy or NSP concentration or by coarseness of NSP. In week 8, hens that were fed high fat diet had better FC than hens fed normal energy/coarsely ground NSP diet. In week 20, hens that were fed high fat diet had better FC than hens fed normal energy/finely ground NSP diet.

Table 8 Standard error and P-values of FC of the rump per week and of the slope of the lines per treatment

	Week													Slope
	0	1	3	4	6	8	10	12	14	16	18	20	22	
Standard error	0.018	0.020	0.071	0.070	0.079	0.101	0.127	0.096	0.095	0.113	0.106	0.125	0.110	0.006
P-Value														
Energy	0.342	0.284	0.136	0.148	**0.019**	**0.021**	0.201	0.160	0.125	0.252	0.640	0.911	0.711	0.508
NSP	0.454	0.256	0.351	0.470	**0.046**	0.636	0.494	0.184	0.582	0.168	0.450	0.213	0.506	0.249
Energy*NSP	0.879	0.597	0.619	0.503	0.084	0.537	0.464	0.778	0.604	0.841	0.549	0.208	0.249	0.312
Coarseness	0.866	0.290	0.327	0.665	0.720	0.418	0.771	0.738	0.668	0.526	0.769	0.875	0.758	0.971
Energy*Coarseness	1.000	**0.018**	0.780	0.569	0.611	0.197	0.901	0.870	0.534	0.400	0.279	0.375	0.195	0.300

Table 9 Standard error and P-values of FC of the tail per week and of the slope of the lines per treatment

	Week													Slope
	0	1	3	4	6	8	10	12	14	16	18	20	22	
Standard error	0.005	0.032	0.041	0.045	0.041	0.038	0.050	0.087	0.101	0.093	0.094	0.0.97	0.095	0.005
P-Value														
Energy	0.474	0.727	0.170	0.255	0.894	0.740	0.928	0.670	0.856	0.721	0.617	0.946	0.640	0.992
NSP	0.176	0.516	**0.043**	0.137	0.107	**0.050**	0.131	0.583	0.751	0.246	0.355	0.211	0.682	0.129
Energy*NSP	0.735	0.307	0.450	0.488	0.755	0.771	0.268	0.917	0.404	0.757	0.678	0.463	0.672	0.699
Coarseness	0.554	0.304	0.106	0.829	0.164	0.252	0.275	0.649	0.899	0.532	0.371	0.126	0.392	0.493
Energy*Coarseness	0.435	0.170	0.080	0.092	**0.027**	0.258	0.692	0.497	0.694	0.579	0.674	0.595	0.168	0.363

Table 10 Standard error and P-values of FC of the belly per week and of the slope of the lines per treatment

	Week											Slope
	3	4	6	8	10	12	14	16	18	20	22	
Standard error	0.174	0.104	0.080	0.118	0.140	0.158	0.185	0.162	0.117	0.125	0.131	0.007
P-Value												
Energy	0.689	0.566	0.139	0.341	0.248	0.507	0.178	0.358	0.836	0.479	0.583	0.310
NSP	0.223	0.826	0.231	**0.014**	0.306	0.104	0.347	0.290	0.218	0.353	0.650	0.643
Energy*NSP	0.260	0.365	0.519	0.070	0.541	0.423	0.569	0.617	0.481	0.488	0.632	0.528
Coarseness	0.202	0.159	0.389	0.744	0.644	0.282	0.606	0.319	0.890	0.754	0.355	0.533
Energy*Coarseness	0.874	0.760	0.621	1.000	0.086	**0.050**	0.898	0.788	0.477	0.195	0.091	0.323

Table 11 Standard error and P-values of FC of the back per week and of the slope of the lines per treatment

	Week						Slope
	12	14	16	18	20	22	
Standard error	0.029	0.074	0.082	0.087	0.085	0.081	0.004
P-Value							
Energy	0.071	0.454	0.975	0.763	0.982	0.896	1.000
NSP	**0.033**	0.117	0.342	0.372	0.057	0.272	0.144
Energy*NSP	0.370	0.818	0.703	0.558	0.097	0.264	0.376
Coarseness	0.717	0.065	0.276	0.572	0.191	0.501	0.275
Energy*Coarseness	0.682	0.873	0.771	0.585	0.378	0.130	0.341

Table 12 Standard error and P-values of FC of the neck per week and of the slope of the lines per treatment

	Week						Slope
	12	14	16	18	20	22	
Standard error	0.029	0.013	0.019	0.017	0.030	0.039	0.001
P-Value							
Energy	0.116	0.264	0.176	0.108	0.521	0.507	0.304
NSP	0.246	0.388	0.338	0.428	0.594	0.685	0.537
Energy*NSP	0.209	0.286	**0.055**	0.137	0.110	0.265	0.125
Coarseness	0.908	0.251	0.683	0.530	0.233	0.091	0.155
Energy*Coarseness	0.774	0.370	0.678	0.360	0.510	0.270	0.317

Table 13 Standard error and P-values of mean total FC per week and of the slope of the lines per treatment

	Week													Slope
	0	1	3	4	6	8	10	12	14	16	18	20	22	
Standard error	0.004	0.008	0.040	0.030	0.030	0.037	0.052	0.059	0.069	0.070	0.068	0.074	0.076	0.004
P-Value														
Energy	0.496	0.395	0.922	0.948	0.704	0.478	0.215	0.631	0.158	0.329	0.725	0.808	0.995	0.804
NSP	0.309	0.258	0.751	0.534	0.225	0.157	0.540	0.105	0.260	0.128	0.235	0.120	0.468	0.180
Energy*NSP	0.958	0.263	0.340	0.255	0.192	0.450	0.360	0.604	0.766	0.780	0.535	0.266	0.608	0.445
Coarseness	0.763	0.768	0.075	0.217	0.312	0.641	0.551	0.396	0.421	0.266	0.572	0.453	0.902	0.769
Energy*Coarseness	0.845	0.918	0.555	0.331	0.264	0.352	0.289	0.202	0.720	0.617	0.427	0.301	0.132	0.271

3.1.5 Mortality parameters

In table 14 average mortality due to cannibalism and the chance not to survive the whole experimental period per treatment are given.

Table 14 Average mortality due to cannibalism and the chance not to survive the whole experimental period per treatment

Treatment	Average mortality (%)	Chance not to survive the experiment (%)
Normal Energy		
Low NSP	44.1	22.6
High NSP-Fine	23.8	12.8
High NSP-Coarse	39.3	20.3
High Fat	28.6*	14.2
Low Energy		
Low NSP	13.1	6.5
High NSP-Fine	33.3	18.2
High NSP-Coarse	23.8	12.0
Standard error	3.07	3.20
P-Value		
Energy	0.126	0.113
NSP	0.731	0.768
Energy*NSP	0.071	0.083
Coarseness	0.835	0.899
Energy*Coarseness	0.206	0.157

In normal energy diets mortality decreased when hens were fed high-NSP diets (31.6 versus 44.1%), whereas in low energy diets mortality decreased when hens were fed low-NSP diets (13.1 versus 28.6%) (*P*=0.071). Hens that were fed normal energy/high-NSP/coarse diet had higher mortality compared with hens that were fed normal energy/high-fat diet (39.3 versus 28.6). 35.7 Feeding low energy diets numerically (*P*=0.126) reduced mortality compared with normal energy diets (23.4 versus 35.7%). Likewise, in normal energy diets the chance not to survive the experiment decreased when hens were fed high-NSP diets (22.6 versus 16.6%), whereas in low energy diets the chance not to survive the experiment decreased when hens were fed low-NSP diets (6.5 versus 15.1%) (*P*=0.083).

Development of cumulative mortality and chances not to survive the experiment per treatment are graphically shown in figures 8 and 9, respectively.

Figure 8 Development of cumulative mortality per treatment over the experimental period

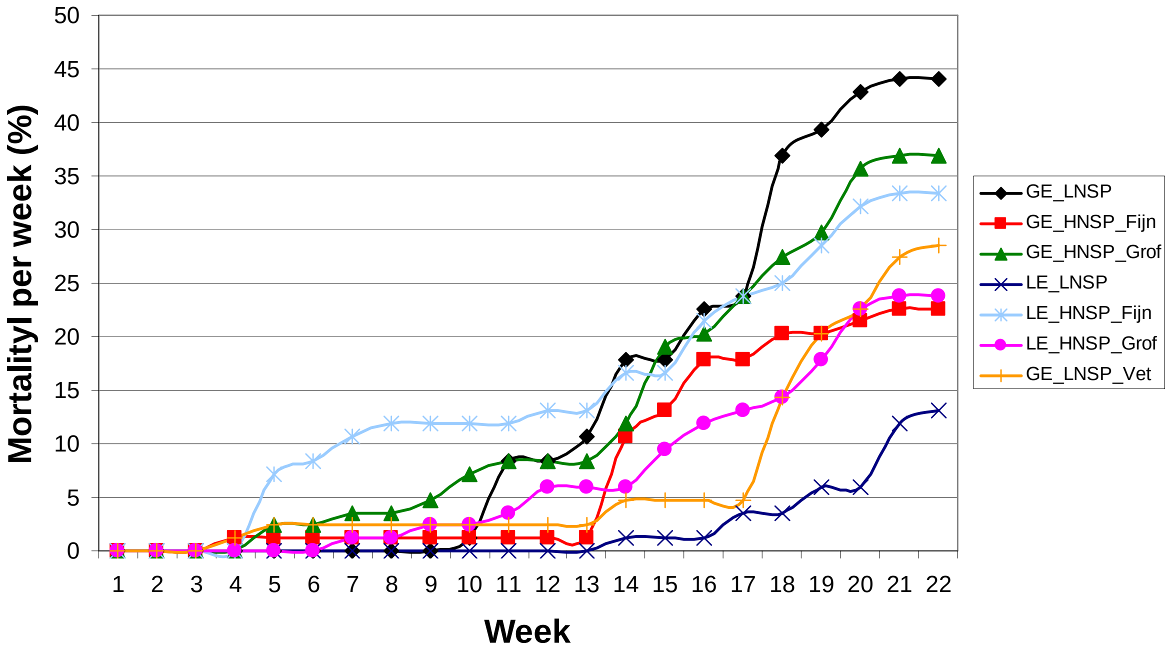
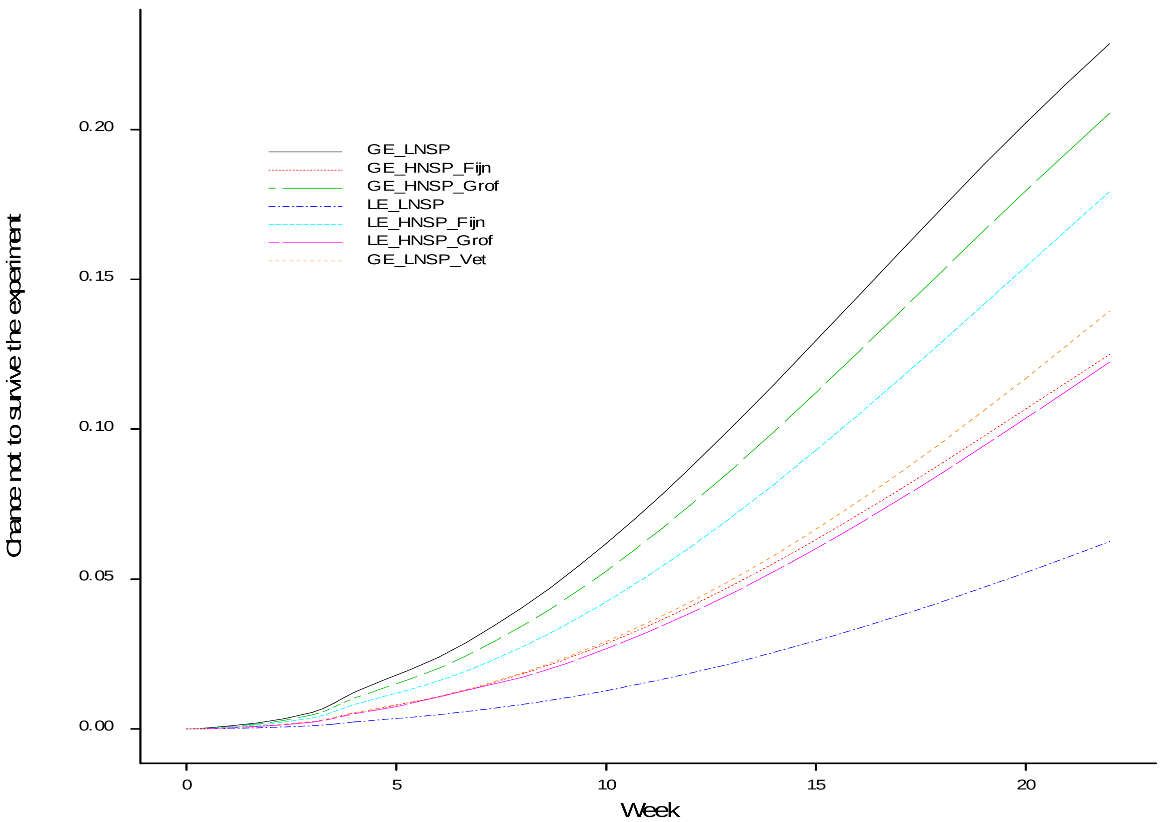


Figure 9 Development of chances not to survive the experimental development per treatment over the experimental period



3.1.6 Pecking behaviour

In this section results of pecking behaviour are shown. Mean level of gentle feather pecking behaviour (nr of pecks/observed hen/10 min) per treatment and the slope of the linear function to model gentle feather pecking behaviour over time are given in table 15.

Table 15 Mean level of gentle feather pecking behaviour (nr/observed hen/10 min) per treatment and the slope of the linear function to model gentle feather pecking behaviour over time

Treatment	Mean level	Slope
Normal Energy		
Low NSP	0.098	-0.0020
High NSP-Fine	0.118	0.0005
High NSP-Coarse	0.162	0.0077
High Fat	0.099	0.0019
Low Energy		
Low NSP	0.108	0.0017
High NSP-Fine	0.112	0.0061
High NSP-Coarse	0.114	-0.0002
Standard error	0.0147	0.00295
P-Value		
Energy	0.414	0.900
NSP	0.221	0.338
Energy*NSP	0.336	0.523
Coarseness	0.307	0.919
Energy*Coarseness	0.328	0.127

Mean level and slope of gentle feather pecking behaviour were not affected by dietary treatments. However, hens that were fed normal energy/high coarsely ground NSP had higher mean level and slope than birds fed fat high diet.

Mean level of severe feather pecking behaviour (nr of pecks/observed hen/10 min) per treatment and the slope of the linear function to model severe feather pecking behaviour over time are given in table 16.

Table 16 Mean level of severe feather pecking behaviour (nr/observed hen/10 min) per treatment and the slope of the linear function to model severe feather pecking behaviour over time

Treatment	Mean level	Slope
Normal Energy		
Low NSP	0.134	0.0049
High NSP-Fine	0.133	0.0021
High NSP-Coarse	0.091	-0.0009
High Fat	0.143	0.0070
Low Energy		
Low NSP	0.153	0.0016
High NSP-Fine	0.165	0.0035
High NSP-Coarse	0.131	-0.0002
Standard error	0.0223	0.00239
P-Value		
Energy	0.268	0.902
NSP	0.642	0.499
Energy*NSP	0.749	0.487
Coarseness	0.260	0.358
Energy*Coarseness	0.905	0.913

Mean level and slope of severe feather pecking behaviour were not affected by dietary treatments. However, hens that were fed normal energy/high coarsely ground NSP had lower mean level and slope than birds fed fat high diet.

Mean level of cage pecking behaviour (nr of pecks/observed hen/10 min) per treatment and the slope of the linear function to model cage pecking behaviour over time are given in table 17.

Table 17 Mean level of cage pecking behaviour (nr/observed hen/10 min) per treatment and the slope of the linear function to model cage pecking behaviour over time

Treatment	Mean level	Slope
Normal Energy		
Low NSP	0.175	0.0174
High NSP-Fine	0.029	0.0004
High NSP-Coarse	0.157	0.0237
High Fat	0.107	0.0022
Low Energy		
Low NSP	0.048	-0.0029
High NSP-Fine	0.078	-0.0025
High NSP-Coarse	0.062	-0.0077
Standard error	0.0433	0.00597
P-Value		
Energy	0.281	0.013
NSP	0.590	0.626
Energy*NSP	0.360	0.849
Coarseness	0.390	0.317
Energy*Coarseness	0.268	0.112

Mean level of cage pecking behaviour was not affected by dietary treatments. The slope of the linear function to model cage pecking of low energy treatments over time was lower compared with normal energy treatments (-0.004 versus 0.014; P=0.013), indicating that differences in cage pecking behaviour between both energy treatments increased over time.

Mean level of vent pecking behaviour (nr of pecks/observed hen/10 min) per treatment and the slope of the linear function to model vent feather pecking behaviour over time are given in table 18.

Table 18 Mean level of vent pecking behaviour (nr/observed hen/10 min) per treatment and the slope of the linear function to model vent pecking behaviour over time

Treatment	Mean level	Slope
Normal Energy		
Low NSP	0.158	0.0166
High NSP-Fine	0.027	0.0010
High NSP-Coarse	0.151	0.0230
High Fat	0.096	0.0028
Low Energy		
Low NSP	0.049	-0.0047
High NSP-Fine	0.062	-0.0048
High NSP-Coarse	0.067	-0.0062
Standard error	0.0443	0.00602
P-Value		
Energy	0.338	0.011
NSP	0.645	0.731
Energy*NSP	0.468	0.818
Coarseness	0.334	0.258
Energy*Coarseness	0.374	0.196

Mean level of vent pecking behaviour was not affected by dietary treatments. The slope of the linear function to model vent pecking of low energy treatments over time was lower compared with normal energy treatments (-0.005 versus 0.014; P=0.011) , indicating that differences in vent pecking behaviour between both energy treatments increased over time.

Mean level of aggressive pecking behaviour (nr of pecks/observed hen/10 min) per treatment and the slope of the linear function to model aggressive pecking behaviour over time are given in table 19.

Table 19 Mean level of aggressive pecking behaviour (nr/observed hen/10 min) per treatment and the slope of the linear function to model aggressive pecking behaviour over time

Treatment	Mean level	Slope
Normal Energy		
Low NSP	0.030	0.0002
High NSP-Fine	0.045	0.0022
High NSP-Coarse	0.022	0.0013
High Fat	0.043	0.0031
Low Energy		
Low NSP	0.039	0.0050
High NSP-Fine	0.036	0.0016
High NSP-Coarse	0.034	0.0019
Standard error	0.0092	0.00116
P-Value		
Energy	0.720	0.268
NSP	0.994	0.572
Energy*NSP	0.754	0.104
Coarseness	0.382	0.846
Energy*Coarseness	0.455	0.725

Mean level and slope of aggressive pecking behaviour were not affected by dietary treatments.

Mean level of total pecking interactions (nr of pecks/observed hen/10 min) per treatment and the slope of the linear function to model total pecking interactions over time are given in table 20.

Table 20 Mean level of total pecking interactions (nr/observed hen/10 min) per treatment and the slope of the linear function to model total pecking interactions over time

Treatment	Mean level	Slope
Normal Energy		
Low NSP	0.420	0.0196
High NSP-Fine	0.322	0.0058
High NSP-Coarse	0.426	0.0312
High Fat	0.381	0.0148
Low Energy		
Low NSP	0.348	0.0037
High NSP-Fine	0.375	0.0064
High NSP-Coarse	0.346	-0.0048
Standard error	0.0517	0.00662
P-Value		
Energy	0.610	0.036
NSP	0.805	0.819
Energy*NSP	0.667	0.914
Coarseness	0.634	0.478
Energy*Coarseness	0.393	0.067

Mean level of total pecking interactions was not affected by dietary treatments. The slope of the linear function to model total pecking interactions of low energy treatments over time was lower compared with normal energy treatments (0.002 versus 0.019; P=0.036) , indicating that differences in total pecking interactions between both energy treatments increased over time.

3.1.7 Behaviours of hens

Time budget of hens per treatment in week 11 and 19, observed by using scan sampling technique, is shown in table 21.

Table 21 Time budget of hens per treatment in week 11 and 19, observed by using scan sampling technique

	Feed intake (%)		Foraging (%)		Drinking (%)		Preening (%)		Walking (%)		Dust bathing (%)		Resting (%)		Ground scratching (%)	
Week	11	19	11	19	11	19	11	19	11	19	11	19	11	19	11	19
Normal Energy																
Low NSP	24.8	19.7	23.2	34.1	12.3	10.0	19.8	23.8	2.2	6.3	0.1	n.o.[1]	16.8	6.2	1.0	n.o.
High NSP-Fine	21.0	24.2	27.0	28.5	7.1	9.4	23.7	23.7	3.4	4.1	0.0	n.o.	16.1	9.9	1.8	n.o.
High NSP-Coarse	30.5	30.1	26.5	28.2	5.5	7.0	15.0	23.0	3.8	6.0	2.3	n.o.	13.6	5.7	2.9	n.o.
High Fat	26.4	26.6	34.1	16.5	7.8	7.5	16.1	31.9	1.9	5.1	0.5	n.o.	12.7	12.3	0.7	n.o.
Low Energy																
Low NSP	24.0	29.6	30.4	31.1	7.3	8.1	22.5	19.8	1.2	1.9	2.1	n.o.	9.9	9.4	2.6	n.o.
High NSP-Fine	28.0	29.8	29.2	20.2	7.1	7.0	19.7	24.5	3.5	4.5	0.0	n.o.	9.2	10.9	3.1	n.o.
High NSP-Coarse	31.7	26.7	30.7	18.5	7.8	13.3	9.8	26.3	2.2	4.2	0.1	n.o.	12.2	10.6	5.7	n.o.
Standard error	4.97	7.04	6.82	9.00	2.78	3.64	5.56	8.34	1.93	3.03	1.28		5.25	4.68	1.81	
P-Value																
Energy	0.384	0.326	0.247	0.178	0.578	0.760	0.494	0.985	0.455	0.273	0.951		0.096	0.269	0.062	
NSP	0.266	0.482	0.710	0.113	0.088	0.950	0.228	0.615	0.187	0.745	0.544		0.851	0.604	0.152	
Energy*NSP	0.434	0.310	0.626	0.584	0.073	0.385	0.292	0.556	0.906	0.320	0.048		0.664	0.969	0.848	
Coarseness	0.062	0.789	0.923	0.873	0.819	0.447	0.018	0.930	0.719	0.704	0.196		0.953	0.504	0.154	
Energy*Coarseness	0.411	0.367	0.843	0.911	0.563	0.089	0.879	0.834	0.518	0.609	0.197		0.462	0.558	0.576	

[1]) n.o. = not observed

Hens that were fed coarsely ground high-NSP diets spent in week 11 more time on feed intake than hens that were fed finely ground high-NSP diets (31.1 versus 24.5%; *P*=0.06). At the same time, coarse grinding of the NSP source reduced preening behaviour (12.4 versus 21.7%; *P*=0.018), compared with fine grinding. Feeding low energy diets did not affect drinking time in week 11, whereas drinking time increased in normal energy/low-NSP fed hens compared with normal energy/high-NSP fed hens. Dietary energy dilution numerically (*P*=0.096) reduced in week 11 resting behaviour (10.4 versus 15.5%), but increased ground scratching behaviour (3.8 versus 1.9; *P*=0.062). Time budgets in week 19 were not affected by dietary treatments.

3.2 Performance parameters

3.2.1 Rate of lay parameters

Estimates of rate of lay parameters per treatment, as described by an exponential curve, are given in table 22.

Table 22 Parameter estimates (A, B and α) of rate of lay (%) per treatment as described by an exponential curve

Treatment	Initial rate of lay (%) (A)	Rate of increase (α)	Increase in rate of lay (%) (B)	Asymptotic rate of lay (%) (A + B)
Normal Energy				
Low NSP	10.6	0.93	85.5	96.1
High NSP-Fine	13.7	0.98	82.2	96.0
High NSP-Coarse	10.0	0.92	86.2	96.2
High Fat	6.0*	0.88*	87.9*	94.0
Low Energy				
Low NSP	8.4	0.90	91.4	99.8
High NSP-Fine	9.4	1.00	86.1	95.5
High NSP-Coarse	5.6	0.85	89.3	94.9
Standard error	1.797	0.055	2.059	
P-Value				
Energy	0.044	0.682	0.090	
NSP	0.565	0.761	0.353	
Energy*NSP	0.965	0.971	0.654	
Coarseness	0.166	0.225	0.251	
Energy*Coarseness	0.998	0.598	0.891	

Rate of lay parameters were not affected by NSP concentration and coarseness of NSP. Initial rate of lay of hens that were fed low energy diets was 4.4% (± 1.80) lower (*P*=0.044), whereas increase in rate of lay was numerically 4.2% (± 2.06) higher (*P*=0.090) compared with hens that were fed normal energy diets. Therefore, asymptotic rate of lay level was not affected by dietary energy concentration. Hens that were fed the fat-rich diet had 7.7% lower initial rate of lay but 5.7% higher increase in rate of lay compared with hens that were fed the finely ground normal energy diet, resulting in an almost similar asymptotic rate of lay level. Feeding high fat diets reduced rate of increase in rate of lay compared with the both high NSP normal energy treatments. Rate of lay development per pen and per treatment is shown in appendix 4.

3.2.2 Egg weight parameters

Estimates of egg weight parameters per treatment, as described by an exponential curve, are given in table 23.

Table 23 Parameter estimates (A, B and α) of egg weight (g) per treatment as described by an exponential curve

Treatment	Initial egg weight (g) (A)	Rate of increase (α)	Increase in egg weight (g) (B)	Asymptotic egg weight level (g) (A + B)
Normal Energy				
Low NSP	48.8	0.25	16.9	65.7
High NSP-Fine	49.7	0.23	16.8	66.5
High NSP-Coarse	49.6	0.21	17.0	66.5
High Fat	48.1	0.25	18.4	66.5
Low Energy				
Low NSP	48.5	0.24	17.4	65.9
High NSP-Fine	48.2	0.26	17.8	66.0
High NSP-Coarse	49.4	0.24	17.6	67.0
Standard error	0.733	0.016	0.650	
P-Value				
Energy	0.379	0.339	0.207	
NSP	0.398	0.466	0.649	
Energy*NSP	0.946	0.522	0.684	
Coarseness	0.656	0.401	0.989	
Energy*Coarseness	0.555	0.966	0.868	

Egg weight parameters were not affected by energy and NSP concentration of the diet or by coarseness of NSP. Hens that were fed normal energy/high fat diets showed similar egg weight performance compared with the normal energy/high NSP treatments. Egg weight development per pen and per treatment is shown in appendix 5.

3.2.3 Egg mass parameters

Estimates of egg mass parameters per treatment, as described by an exponential curve, are given in table 24.

Table 24 Parameter estimates (A, B and α) of egg mass (g) per treatment as described by an exponential curve

Treatment	Initial egg mass (g/d) (A)	Rate of increase (α)	Increase in egg mass (g/d) (B)	Asymptotic egg mass level (g) (A + B)
Normal Energy				
Low NSP	5.1	0.62	56.6	61.8
High NSP-Fine	6.9	0.63	55.4	62.3
High NSP-Coarse	5.1	0.63	60.0	65.1
High Fat	2.9	0.61	60.5	63.5
Low Energy				
Low NSP	3.9	0.61	58.4	62.2
High NSP-Fine	4.5	0.68	57.0	61.5
High NSP-Coarse	3.4	0.63	58.1	61.5
Standard error	2.06	0.063	2.435	
P-Value				
Energy	0.083	0.722	0.119	
NSP	0.437	0.474	0.252	
Energy*NSP	0.932	0.609	0.388	
Coarseness	0.324	0.657	0.445	
Energy*Coarseness	0.828	0.579	0.923	

Egg mass parameters were not affected by energy and NSP concentration of the diet or by coarseness of NSP. Hens that were fed normal energy/high fat diets showed similar egg mass performance compared with the normal energy/high NSP treatments. Egg mass development per pen and per treatment is shown in appendix 6.

3.2.4 Feed conversion ratio and energy conversion ratio

Average feed conversion ratio and energy conversion ratio per treatment and development of these ratios over the experimental period are given in table 25 and figures 10 and 11.

Table 25 Average feed conversion ratio and energy conversion ratio per treatment and relative values compared with the control treatment (GE_LNSP)

Treatment	Average feed conversion ratio	Relative (%)	Average energy conversion ratio	Relative (%)
GE_LNSP	2.20	100.0	2.20	100.0
GE_HNSP_Fine	2.16	98.0	2.16	98.1
GE_HNSP_Coarse	2.22	100.7	2.22	100.8
LE_LNSP	2.32	105.4	2.11	95.8
LE_HNSP_Fine	2.26	102.7	2.05	93.4
LE_HNSP_Coarse	2.46	111.7	2.23	101.5
GE_LNSP_Fat-High	2.17	98.6	2.17	98.6

Figure 10 Feed conversion ratio per treatment per week over the experimental period

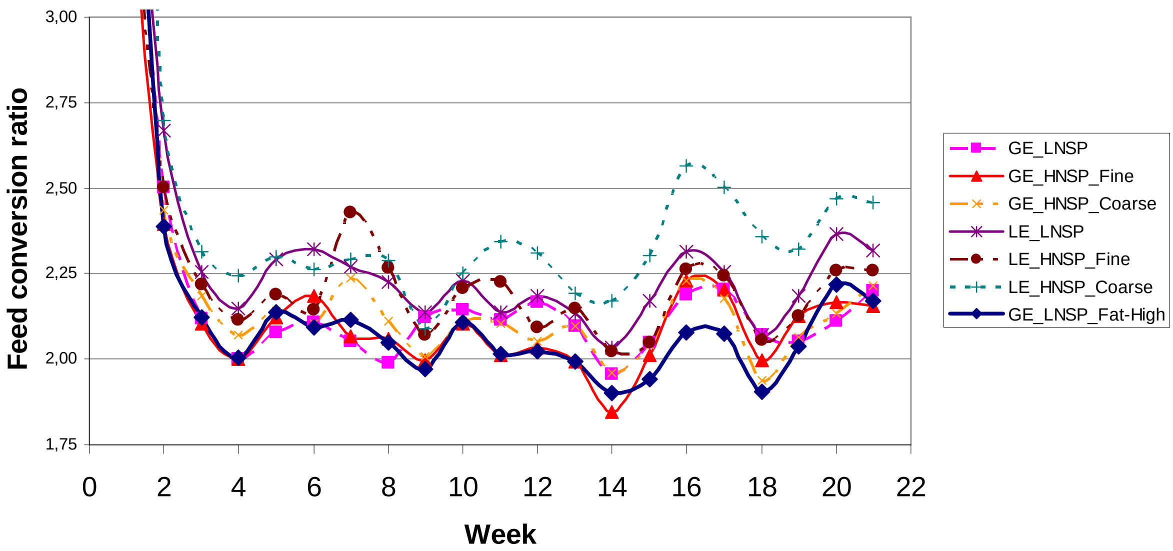
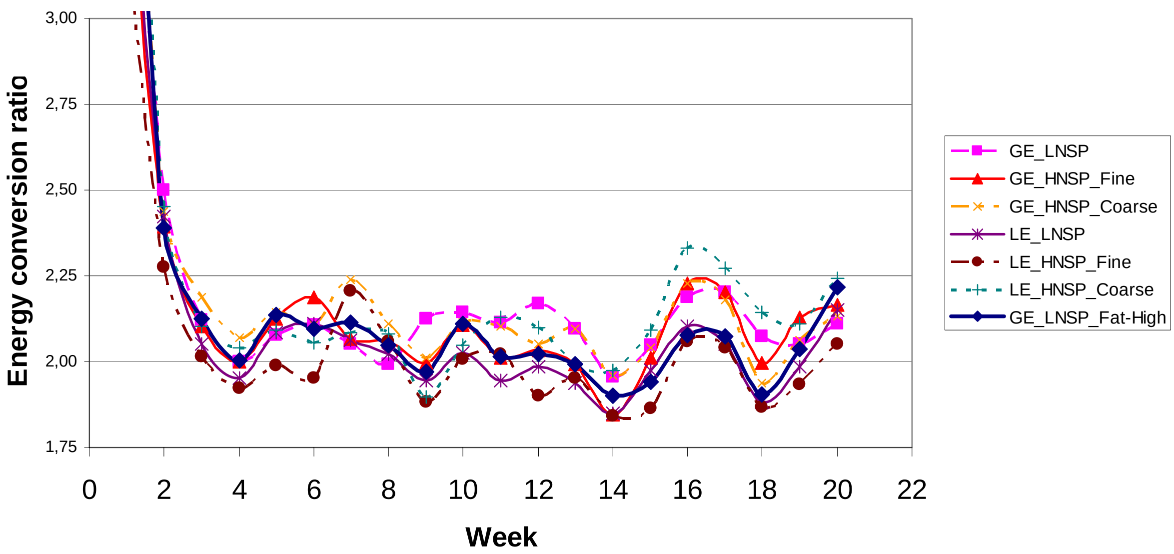


Figure 11 Energy conversion ratio per treatment per week over the experimental period



Hens that were fed normal energy diets had on average similar feed conversion ratios. Feed conversion ratios of the low energy diets were on average 2.7% (LE_HNSP_Fine) to 11.7% (LE_HNSP_Coarse) higher than of hens that were fed the control diet (GE_LNSP). Energy conversion ratios (feed conversion ratio, corrected for differences in energy concentration of the diets) of hens that were fed LE_LNSP or LE_HNSP_Fine were on average 4.2% and 6.4% better compared with hens that were fed the control diet.

3.2.5 Body weight parameters

Estimates of body weight parameters per treatment, as described by an exponential curve, are given in table 26.

Table 26 Parameter estimates (A, B and α) of body weight (g) per treatment as described by an exponential curve

Treatment	Initial body weight (kg) (A)	Rate of increase (alpha)	Increase in body weight (g) (B)	Asymptotic body weight level (g) (A + B)
Normal Energy				
Low NSP	1727	0.39	249	1976
High NSP-Fine	1718	0.31	281	1999
High NSP-Coarse	1721	0.36	284	2005
High Fat	1699	0.41	282	1981
Low Energy				
Low NSP	1710	0.40	284	1994
High NSP-Fine	1735	0.26	272	2007
High NSP-Coarse	1704	0.37	278	1982
Standard error	11.7	0.082	14.7	
P-Value				
Energy	0.528	0.806	0.710	
NSP	0.674	0.168	0.538	
Energy*NSP	0.350	0.813	0.266	
Coarseness	0.443	0.174	0.832	
Energy*Coarseness	0.334	0.591	0.943	

Body weight parameters were not affected by energy and NSP concentration of the diet or by coarseness of NSP. Hens that were fed normal energy/high fat diets showed similar body weight performance compared with the normal energy/high NSP treatments. Body weight development per pen and per treatment is shown in appendix 7.

3.3 Physiological, neurobiological and other parameters

3.3.1 Weight and content of GIT segments

Empty weight and content of crop, gizzard, ileum, colon and caeca, as expressed in g/kg hen, are shown in table 27.

Table 27 Weight of GIT segments (g/kg hen)

Treatment	Crop empty	Crop content	Gizzard empty	Gizzard content	Ileum empty	Ileum content	Colon empty	Colon content	Caeca empty	Caeca content
Normal Energy										
Low NSP	5.0	5.8	19.2	12.8	29.4	15.1	2.8	1.6	5.2	2.4
High NSP-Fine	4.5	4.8	21.9	14.9	29.0	14.8	2.7	2.1	5.2	2.3
High NSP-Coarse	5.0	7.1	29.3	15.9	29.6	14.3	2.7	1.8	4.9	2.4
High Fat	4.6	6.5	19.3	13.3	29.5	15.7	2.6	1.9	4.8	2.5
Low Energy										
Low NSP	4.9	5.4	19.5	13.2	29.9	14.0	2.6	1.7	5.1	2.7
High NSP-Fine	4.9	4.5	21.8	14.9	30.3	15.0	2.6	1.8	4.6	2.6
High NSP-Coarse	4.8	6.6	27.6	15.8	30.4	14.4	2.8	1.8	5.2	2.7
Standard error	1.05	1.32	0.68	1.35	0.65	0.97	0.15	0.22	1.04	1.09
P-Value										
Energy	0.995	0.759	0.194	0.954	0.056	0.686	0.688	0.572	0.466	0.036
NSP	0.809	0.837	<0.001	<0.001	0.728	0.908	0.884	0.148	0.088	0.885
Energy*NSP	0.420	0.829	0.286	0.739	0.611	0.359	0.245	0.416	0.555	0.180
Coarseness	0.149	0.093	<0.001	0.174	0.464	0.538	0.430	0.446	0.449	0.547
Energy*Coarseness	0.087	0.509	0.272	0.996	0.675	0.936	0.315	0.288	0.004	0.159

Treatments had no effect on weight of empty crop and on crop content. Feeding high NSP diets increased empty gizzard weight of hens by 30% (25.2 versus 19.4 g/kg ± 0.68) compared with hens fed low NSP diets. Empty gizzard weight was also affected by coarseness of NSP. Hens fed coarsely ground NSP had 30% higher empty gizzard weight compared with hens fed finely ground NSP (28.5 versus 21.9 g/kg ± 0.68). Feeding high NSP diets also increased gizzard content by 18% (15.4 versus 13.0 ± 1.35) compared with hens fed low NSP diets. Feeding low energy diets numerically (P=0.056) increased empty ileum weight of the hens compared with feeding normal energy diets (30.2 versus 29.3 g/kg hen). Ileum and colon content and empty colon weight were not affected by dietary treatments. In normal energy fed hens empty caeca weight was not affected by coarseness of NSP, whereas in low energy fed hens empty caeca weight was lower in hens fed finely ground NSP compared with coarsely ground NSP (P=0.004). Caeca content was 12.5% higher in hens fed low energy diet compared with normal energy diet (2.7 versus 2.4 g/kg, P=0.036).

3.3.2 Blood plasma parameters and blood cell counts

Blood plasma parameters (corticosterone and glucose in mg/l) and blood cell counts per treatment are shown in table 28.

Table 28 Blood plasma parameters (corticosterone and glucose) and blood cell counts per treatment

Treatment	Cortico sterone	Glucose	Basoph iles (%)	Eosinoph iles (%)	Hemato cryte	Haemogl obins	Hetrop hiles (%)	Lympho cytes (%)	Monocy tes (%)	MCH	MCHC	MCV	PLT	RBC	RDW	Ratio H/L
Normal Energy																
Low NSP	12.4	272.5	0.17	7.0	33.6	7.1	31.7	56.5	11.4	27.5	21.2	129.5	8.2	2.60	10.9	0.66
High NSP-Fine	8.5	268.2	0.13	12.4	33.4	6.6	35.0	50.1	14.0	25.6	19.8	129.4	11.1	2.56	10.8	1.32
High NSP-Coarse	11.0	259.1	0.40	13.3	32.7	7.2	48.4	30.6	20.7	28.3	22.1	127.7	10.4	2.56	11.5	2.26
High Fat	11.4	258.5	0.20	11.8	33.6	6.8	37.8	49.5	12.1	27.3	20.8	131.3	8.7	2.49	10.8	1.38
Low Energy																
Low NSP	6.0	276.0	0.24	13.9	30.7	6.7	41.6	38.4	16.5	27.9	21.9	127.8	6.5	2.43	11.5	1.52
High NSP-Fine	15.9	270.8	0.13	8.8	33.2	7.2	44.9	37.8	16.5	27.2	21.3	127.6	10.2	2.61	11.2	1.64
High NSP-Coarse	8.5	255.5	0.05	9.4	33.1	6.6	37.9	45.6	14.5	25.2	19.9	127.1	10.6	2.60	11.4	1.84
Standard error	2.85	7.47	0.124	3.87	1.50	0.20	5.27	7.30	3.15	1.10	0.69	2.16	1.52	0.11	0.28	0.49
P-Value																
Energy	0.960	0.907	0.208	0.864	0.436	0.310	0.403	0.329	0.857	0.528	0.768	0.340	0.574	0.766	0.108	0.380
NSP	0.572	0.057	0.956	0.704	0.526	0.738	0.142	0.147	0.201	0.280	0.285	0.630	**0.010**	0.505	0.809	**0.043**
Energy*NSP	**0.037**	0.612	0.217	0.061	0.183	0.111	0.219	0.089	0.157	0.521	0.393	0.929	0.548	0.180	0.404	0.258
Coarseness	0.302	**0.046**	0.367	0.819	0.753	0.886	0.459	0.335	0.360	0.636	0.353	0.536	0.931	0.991	0.055	0.154
Energy*Coarseness	**0.029**	0.612	0.086	0.966	0.845	**<0.001**	**0.018**	**0.023**	0.090	**0.013**	**0.001**	0.743	0.672	0.974	0.292	0.355

Plasma corticosterone concentrations after 5 minutes of manual restraint were not affected by NSP concentrations in normal energy fed hens, whereas hens fed low energy/low NSP diets had lower corticosterone concentrations compared with hen fed low energy/high NSP diets (6.0 versus 12.2 mg/l, ± 2.85) (*P*=0.037). Further more, plasma corticosterone concentrations were not affected by coarseness of NSP in normal energy fed hens, whereas hens fed low energy/coarsely ground NSP diets had lower corticosterone concentrations compared with hen fed low energy/finely ground NSP diets (8.5 versus 15.9 mg/l) (*P*=0.029). Hens fed high NSP diets had numerically lower (*P*=0.057) plasma glucose concentration than hens fed low NSP diets (263.4 versus 274.3 mg/l, ± 7.47). Plasma glucose concentration was lower in hens fed coarsely in stead of finely ground high NSP diets (257.3 versus 269.5 mg/l; *P*=0.046). In normal energy fed hens blood contained more haemoglobins when NSP source was coarsely ground (7.2 versus 6.6 ± 0.20), whereas in low energy fed hens blood contained more haemoglobins when NSP source was finely ground (6.6 versus 7.2) (P<0.001).

3.3.3 Neurobiological parameters

Serotonine and dopamine turnover, and noradrenalin concentration in the forebrains are shown in table 29.

Table 29 Serotonine and dopamine turnover, and noradrenalin concentration (mg/g) in the forebrains

Treatment	Serotonin turnover	Dopamin turnover	Noradrenaline
Normal Energy			
Low NSP	1.132	2.873	0.547
High NSP-Fine	1.210	3.035	0.569
High NSP-Coarse	1.042	2.079	0.611
High Fat	0.906	2.211	0.629
Low Energy			
Low NSP	0.640	1.328	0.599
High NSP-Fine	1.041	2.244	0.590
High NSP-Coarse	0.805	1.165	0.631
Standard error	1.146	1.197	1.048
P-Value			
Energy	0.058	**0.014**	0.447
NSP	0.376	0.956	0.488
Energy*NSP	0.310	0.508	0.703
Coarseness	0.309	0.052	0.402
Energy*Coarseness	0.794	0.612	0.980

Hens fed low energy diets had lower serotonin turnover compared with normal energy fed birds (0.829 versus 1.128 versus 0.829 mg/g; *P*=0.058). Likewise, hens fed low energy diets had lower dopamine turnover compared with normal energy fed birds (1.58 versus 2.77; *P*=0.014). Feeding coarsely ground NSP resulted in lower dopamine turnover compared with feeding finely ground NSP (1.62 versus 2.64; *P*=0.052). NSP concentration did not affect brain parameters.

3.3.4 Latency time

Latency time to a novel object, determined in week 1 of the experiment, is shown in table 30.

Table 30 Latency time to novel object

	Latency time (s)
Normal Energy	
Low NSP	311
High NSP-Fine	262
High NSP-Coarse	267
High Fat	284
Low Energy	
Low NSP	304
High NSP-Fine	275
High NSP-Coarse	260
Standard error	19.6
P-Value	
Energy	0.966
NSP	<0.001
Energy*NSP	0.668
Coarseness	0.709
Energy*Coarseness	0.454

Latency time is significantly (*P*<0.001) affected by NSP content of the diet. Hens that were fed high NSP diets reacted on average 41.5s earlier to the novel object than hens that were fed low-NSP diets (266.0 versus 307.5s). Latency time was not affected by energy concentration and coarseness of NSP.

3.3.5 Manual restraint test

Results of the manual restraint test are shown in table 31.

Table 31 Number of hens with resistance and number of vocalising hens

	Hens with resistance (n of 10)	Vocalising hens (n of 10)
Normal Energy		
Low NSP	1.0	5.7
High NSP-Fine	1.4	8.1
High NSP-Coarse	1.3	9.9
High Fat	1.1	7.4
Low Energy		
Low NSP	0.6	3.2
High NSP-Fine	1.6	8.9
High NSP-Coarse	1.3	9.1
Standard error	0.28	0.80
P-Value		
Energy	0.815	0.941
NSP	0.107	0.130
Energy*NSP	0.337	0.430
Coarseness	0.578	0.618
Energy*Coarseness	0.734	0.977

Number of hens with resistance during the manual restraint test was numerically higher (*P*=0.107) in the high-NSP treatments, compared with the low-NSP treatments (1.4 versus 0.8). Likewise, number of vocalising hens was numerically higher (*P*=0.130) in the high-NSP treatments, compared with the low-NSP treatments (9.0 versus 4.5). Number of hens with resistance or number of vocalising hens was not affected by energy concentration and coarseness of NSP.

4 Discussion

4.1 Effect of dietary energy reduction

The reduction in dietary energy is often confounded with changes in the concentration of other ingredients and nutrients, like amino acid and NSP levels, and until now, the pure effects of energy dilution and NSP supplementation on feed intake behaviour are unknown. In this experiment the mere effects of energy dilution without or with (coarsely of finely ground) NSP addition on behaviour and performance were investigated. Feed intake of hens that were fed diets diluted with 10% low NSP (sand) or high NSP (oat hulls) raw materials (on a weight base) was increased by 9.3%, resulting in an almost similar nutrient intake compared to normal energy diets. Furthermore, feeding these low energy diets significantly prolonged eating time by 14%; eating rate, however, was not affected. These results are in line with findings in laying hens in early lay (Krimpen *et al.*, 2006) and in accordance with the results of Savory (1980) who fed male Japanese quail diluted (with 40% cellulose) and undiluted diets. Those receiving the diluted mash consumed about 40% more feed (14.9 vs 10.8 g/d) and spent a higher proportion of time on feed intake (23.8 vs 9.1%). An increase in feed intake and eating time may compensate for redirected foraging behaviour, resulting in less feather pecking behaviour (Krimpen *et al.*, 2005). However, feather pecking behaviour and feather condition scores in the current experiment were not affected by energy reduction, although less mortality due to cannibalism occurred in low energy treatments.
Feeding low energy diets to hens resulted in similar egg production compared with hens that were fed normal energy diet. These results are in accordance with other experiments (Lee *et al.*, 2001, Krimpen *et al.*, 2006, Meulen *et al.*, Submitted). Feeding laying hens a 5% nutrient diluted diet did not affect egg performance compared to hens that were fed a standard diet (Lee *et al.*, 2001). In a recent trial with laying hens (34-37 weeks of age) in our facilities, a reduced dietary energy concentration (by adding 10, 20, 25 or 30% sand) did not affect egg performance of the hens (Meulen *et al.*, Submitted). The hens fully compensated for the effect of added sand in the diet by increasing their daily feed intake. In the current experiment, energy to egg conversion ratio's of the hens that were fed low energy diets were numerically better, indicating that the presence of sand may have had a beneficial effect on performance. However, the mode of action of this effect is not clear. Such positive effects may be explained by sand being useful in degradation of the feed particles, as well as stimulating gut motility. Anyway, empty ileum weight and caeca contents were increased by supplementing low energy diets. We can conclude that feeding low energy diets increased eating time and decreased incidence of cannibalism in laying hens, although dietary energy reduction did not improve feather condition scores and pecking behaviour.

4.2 Effect of dietary NSP concentration

Feed intake parameters were not affected by NSP concentration of the diet. In an earlier experiment we found that hens that were fed diets diluted with 10% NSP-rich raw materials increased their feed intake by 8% (Krimpen *et al.*, 2006). NSP concentration in these diets, however, was confounded with energy concentration, resulting in a 10% reduction in energy concentration. Eating time was prolonged by 17.2% on feeding these high-NSP diets, whereas eating rate was decreased by 21%. These findings are in line with earlier results (Krimpen *et al.*, 2006). In contrast to energy reduction, increase of dietary NSP concentration had a large effect on eating rate, possibly due to differences in specific gravity of the raw materials. For instance, sand has a specific gravity of 1600 kg/m^3, against 780 kg/m^3 for oak wood (Jansen, 1977). Therefore, less volume of feed has to be consumed for reaching the same amount of feed intake when the hens are supplemented with sand-rich low-NSP diets, compared with high-NSP diets. (Savory, 1980) also suggested that the difference in meal length was related to dietary bulk. Birds fed diets high in insoluble NSP spent more time eating and appear calmer than those fed low-fibre diets (Hetland *et al.*, 2004).
NSP concentration had no effect on egg production traits. This was confirmed by Hartini *et al.*, (2003) who performed a number of feeding experiments in which they substituted wheat by millrun, barley, rice hulls or oats as (in)soluble NSP sources on an isocaloric and isonitrogenous basis, and also found no detrimental effects on performance. A better performance may be due to an increased nutrient digestibility. Feeding diets supplemented with insoluble NSP's increased nutrient digestibility, possibly due to a better gizzard development and more reflux activity in the fore-gut (Hetland *et al.*, 2004). Feeding high NSP diets significantly increased gizzard weight and content in the current experiment, which was in line with earlier findings (Krimpen *et al.*, 2006).
We can conclude that supplementing NSP high diets positively affected eating time, eating rate, gizzard development and results of some stress test. However, incidence of cannibalism, pecking behaviour and feather condition scores were not clearly improved.

4.3 Effect of particle size of NSP fraction

Coarse grinding of NSP fraction increased eating time with 7.9% and significantly affected gizzard development. Eating rate was not affected by particle size of NSP. Performance of birds was also not affected by particle size of NSP. Coarse particles accumulate in the gizzard, stimulating gizzard weight and activity, like an increased reflux of bile acids, resulting in an improved starch digestibility and an enhanced emulsification of liberated lipids (Hetland *et al.*, 2003). Fine oat hulls will pass the gizzard immediately after intake, whereas coarse oat hulls were still found in the gizzard 48 hours post feeding. The fact that insoluble fibre accumulates in the gizzard may indicate a slower feed passage rate on gizzard level when the coarse fibre content of the diet increased. It is thought that accumulation of insoluble fibre in the gizzard triggers a temporary satiety, but once passed the gizzard, it passes through the gut quickly. This could make the bird feel more satisfied between feeding bouts, but more hungry after gizzard emptying (Hetland *et al.*, 2004). In conclusion, feeding coarsely ground NSP did not affect performance but increased eating time and gizzard development. However, incidence of cannibalism, pecking behaviour and feather condition scores were not clearly improved by coarseness of NSP.

4.4 Impact of rearing conditions

Early rearing conditions affect the behaviour of hens later in life. Some authors concluded that more attention should be given to the development of feather pecking during the rearing of laying hen chicks [Bestman, 2006 #359; Huber Eicher, 2001 #2]. [Huber Eicher, 2001 #2] made observations on commercial farms and found that 40% of the flocks developed feather pecking when they were 5 weeks of age and this frequency increased to 77.3% when the same flocks were 14 weeks old. [Bestman, 2006 #359] found that if 20% of the pullets in a flock showed subtle signs of feather pecking at 16 weeks of age then the majority of the flock had bald patches due to feather pecking at 30 weeks of age. It is suggested that minimising differences between the rearing and laying environment via a seamless transition is likely to contribute to making a flock less prone to injurious feather pecking [Van de Weerd, 2006 #362]

5 Conclusions

- Energy reduction, NSP addition and coarse grinding of NSP increased average eating time with 14.2%, 17.2% and 7.9%, respectively. Eating rate was not affected by energy concentration and coarseness of NSP, but NSP addition decreased eating rate with 21.0%.
- Dietary treatments did not affect feather condition scores convincingly, although energy reduction and NSP addition improved feather condition scores in some individual weeks. Hens fed normal energy diets showed lower mortality rates if high-NSP diets were supplemented (31.6 versus 44.1%), whereas in low energy diets mortality decreased when hens were fed low-NSP diets (13.1 versus 28.6%) (P=0.071).
- Hens that were fed low energy diets compensated for 10% reduction in energy concentration by 9.3% higher asymptotic feed intake (143.0 versus 130.8 g/d). Egg performance and body gain of the hens were not affected by dietary treatments.
- It is concluded that hens that were fed low energy, high (coarsely ground) NSP diets spend more time on feed intake, compared with hens that were fed normal energy or low NSP diets. However, these effects were not necessarily reflected by a reduced feather pecking behaviour.

Practical implications

The investigated nutritional factors were to a certain extent effective in modifying behaviour and some other measured parameters of laying hens. Reduction of energy concentration increased feed intake, eating time and reduced the incidence of cannibalism. Increase of NSP concentration increased feed eating time and weight and content of gizzard, and reduced incidence of cannibalism, dopamine turnover and latency time in novel object test. Coarse grinding of NSP reduced eating time.
In spite of these positive effects, the nutritional factors did not improve feather condition scores and feather pecking behaviour. Furthermore, level of mortality was high compared with practical conditions. Finally, it can be concluded that the nutritional factors were not sufficient in reducing feather pecking behaviour and cannibalism under the tested conditions (birds showed already pecking behaviour during rearing, non debeaked, high stocking density and high light intensity). More research is necessarily to test the effect of higher levels of dietary dilution and NSP, supplemented from the first day of live, in preventing feather pecking and cannibalism.

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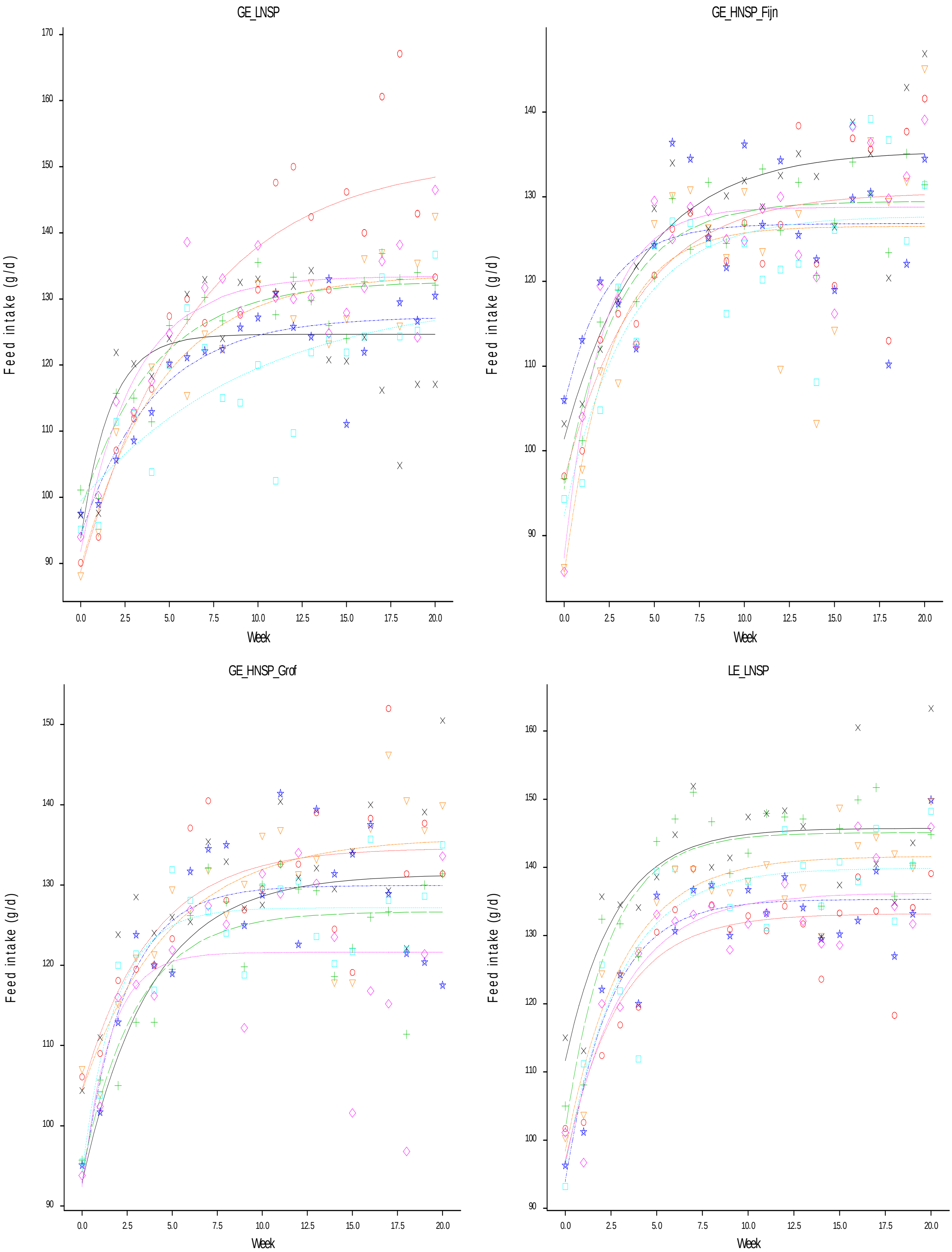
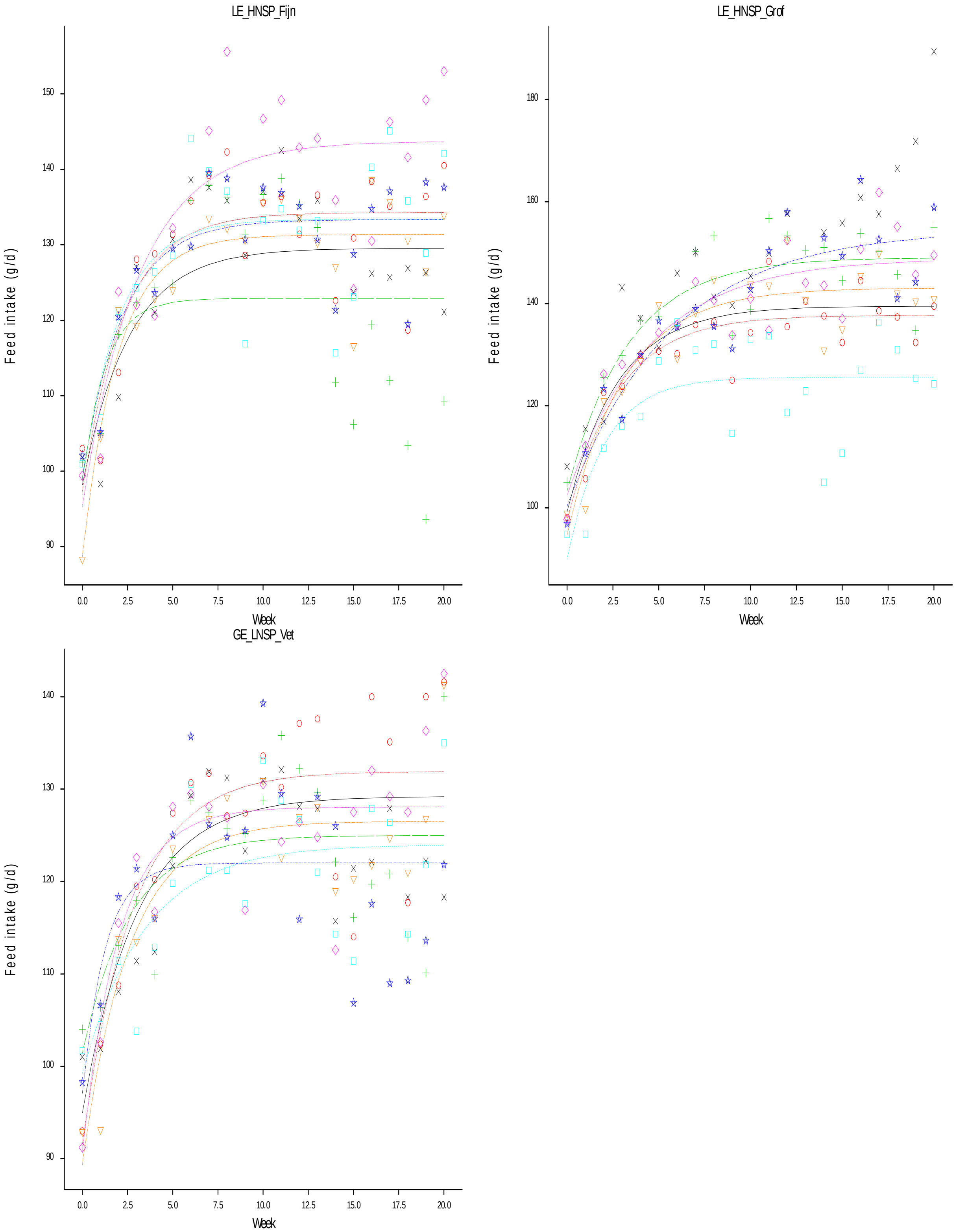
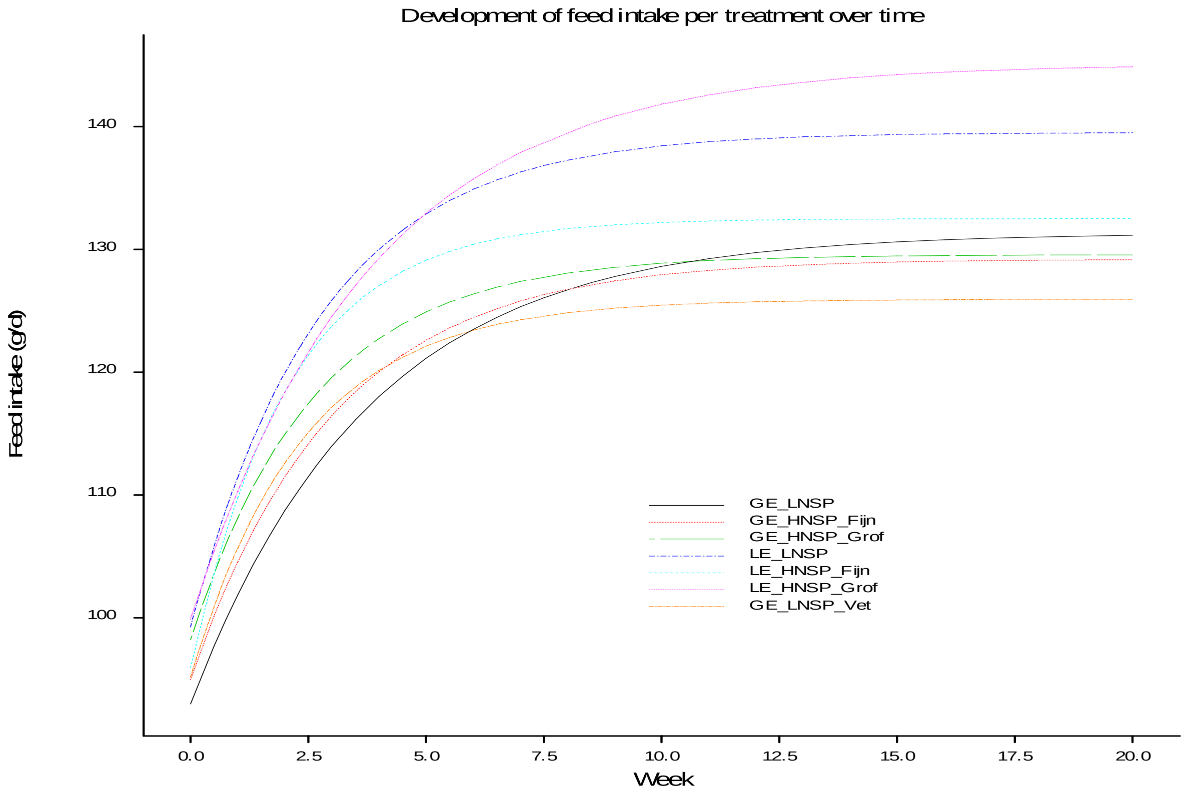
Appendices

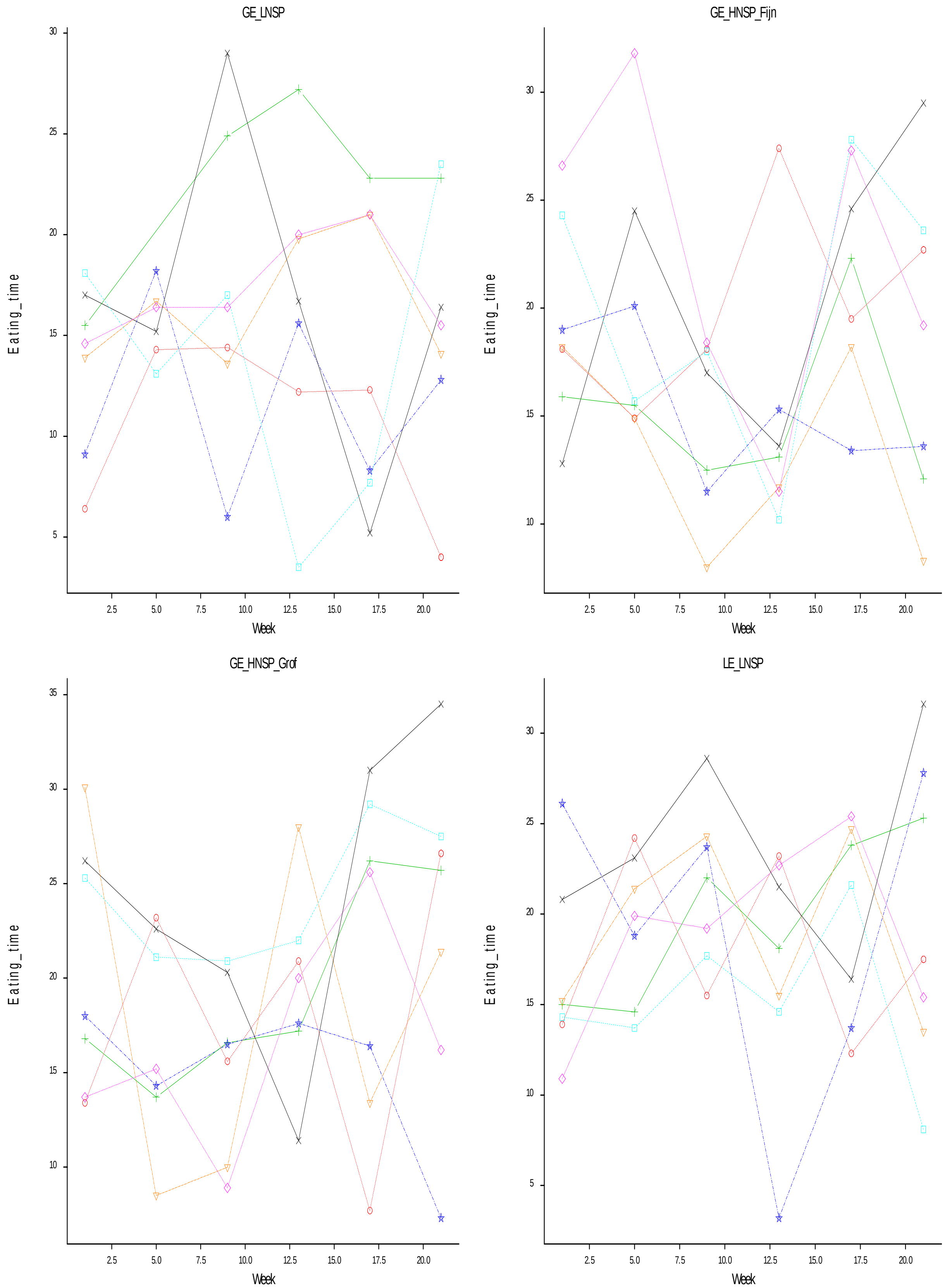
Appendix 1 Feed intake development (g/hen/d) per pen and per treatment

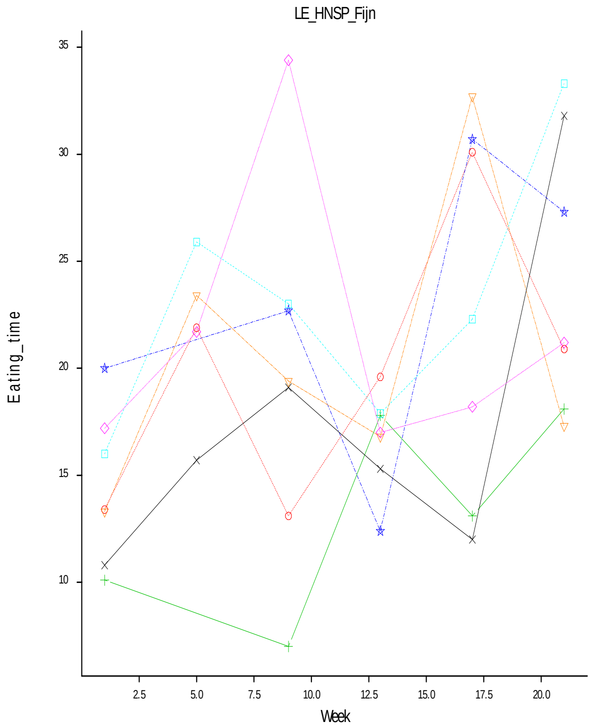


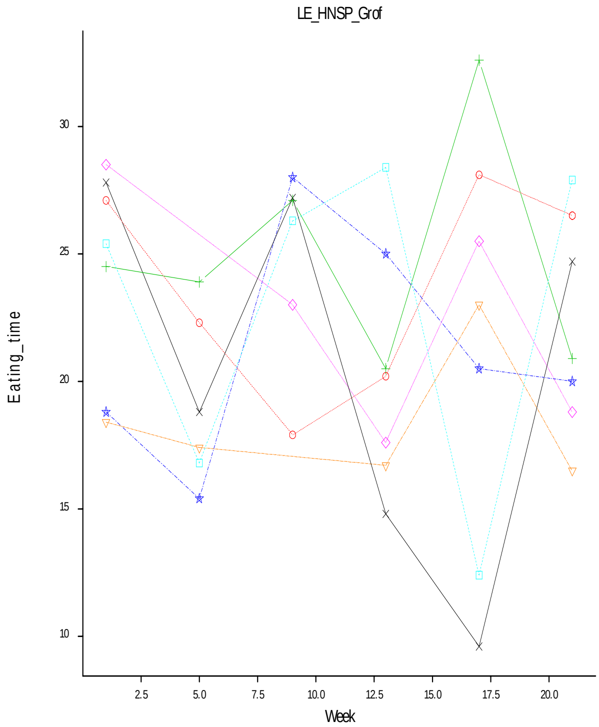

LE_HNSP_Fijn
Feed intake (g/d)
90
100
110
120
130
140
150
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Week
LE_HNSP_Grof
Feed intake (g/d)
100
120
140
160
180
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Week
GE_LNSP_Vet
Feed intake (g/d)
90
100
110
120
130
140
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Week

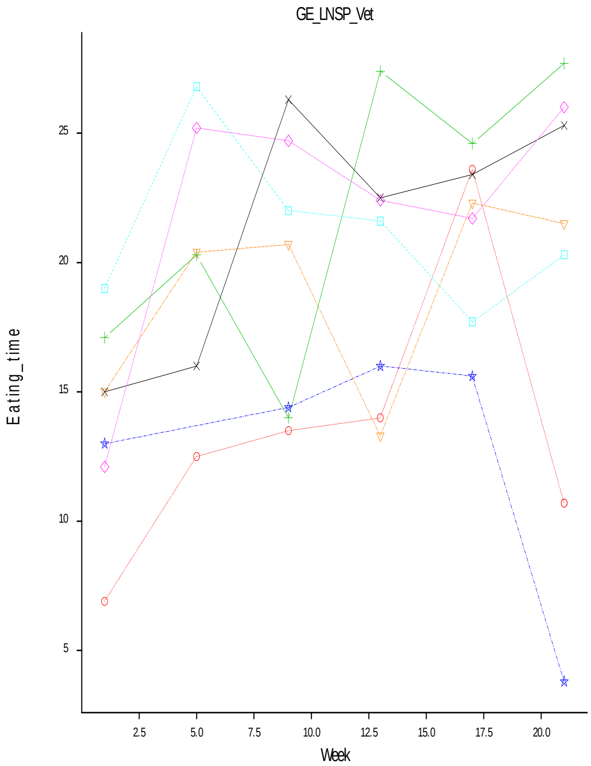

Development of feed intake per treatment over time
Feed intake (g/d)
140
130
120
110
100
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Week
GE_LNSP
GE_HNSP_Fijn
GE_HNSP_Grof
LE_LNSP
LE_HNSP_Fijn
LE_HNSP_Grof
GE_LNSP_Vet

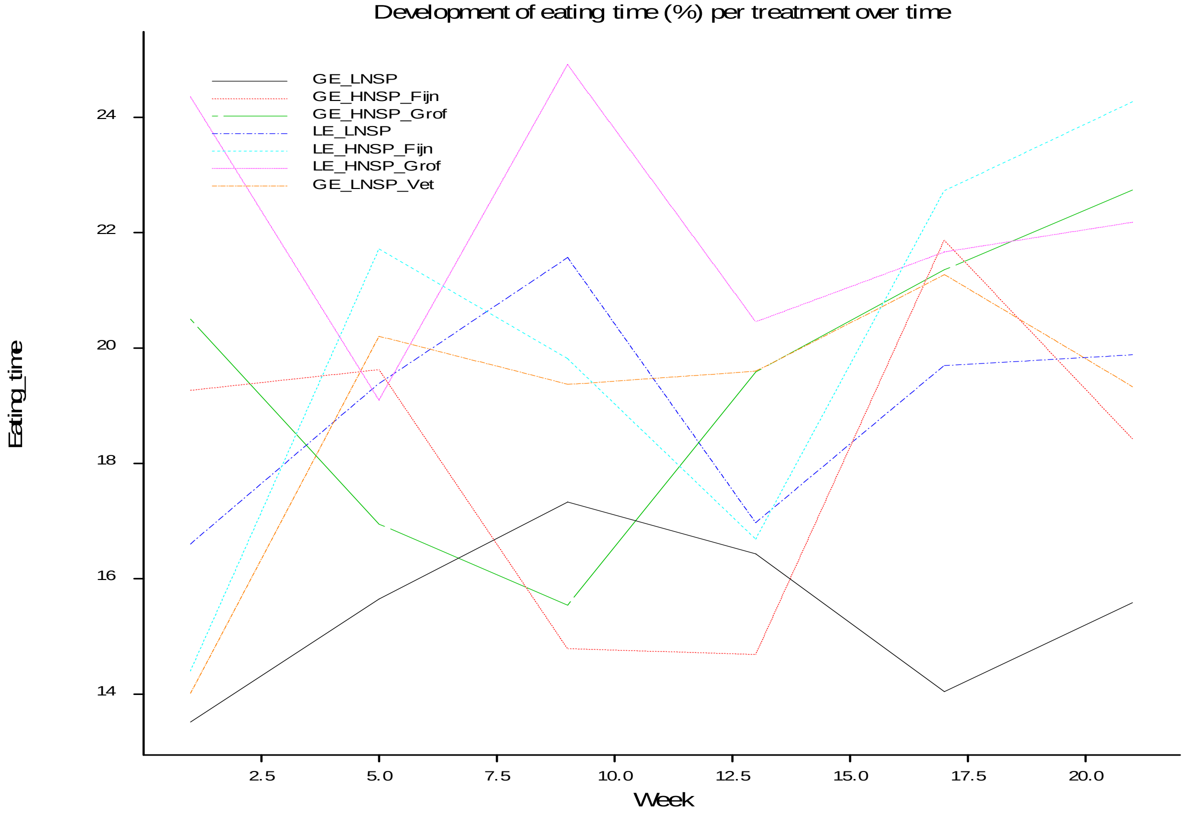
Appendix 2 Eating time (% of observed period) development per pen and per treatment



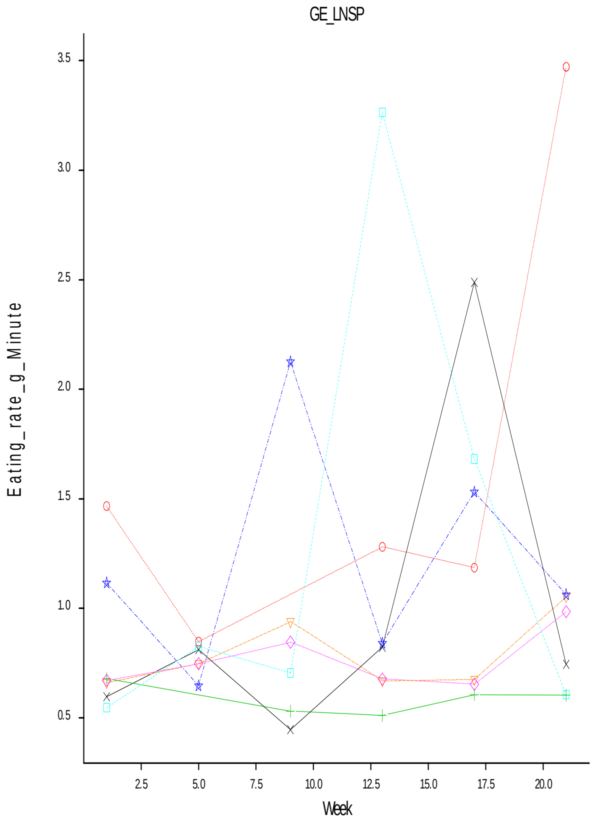

LE_HNSP_Fijn
Eating_time
Week
35
30
25
20
15
10
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

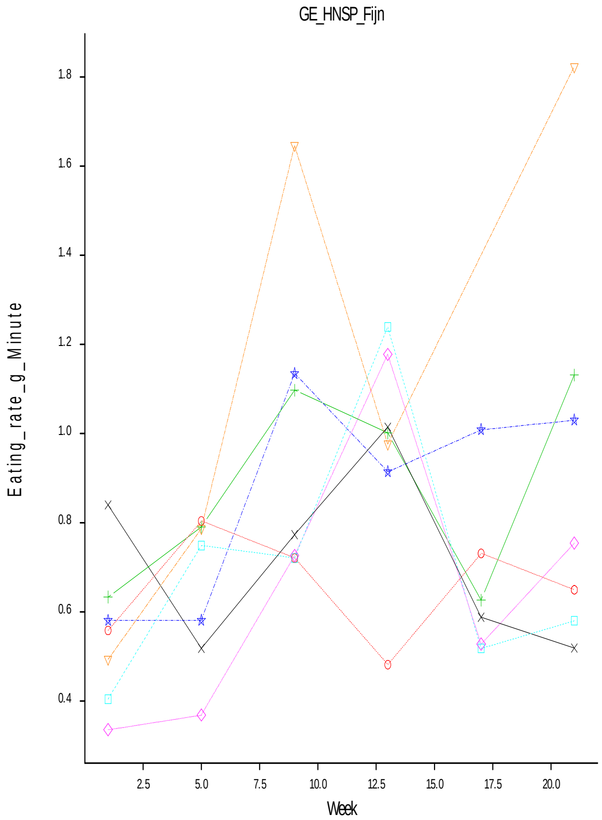

LE_HNSP_Grof
Eating_time
Week
30
25
20
15
10
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

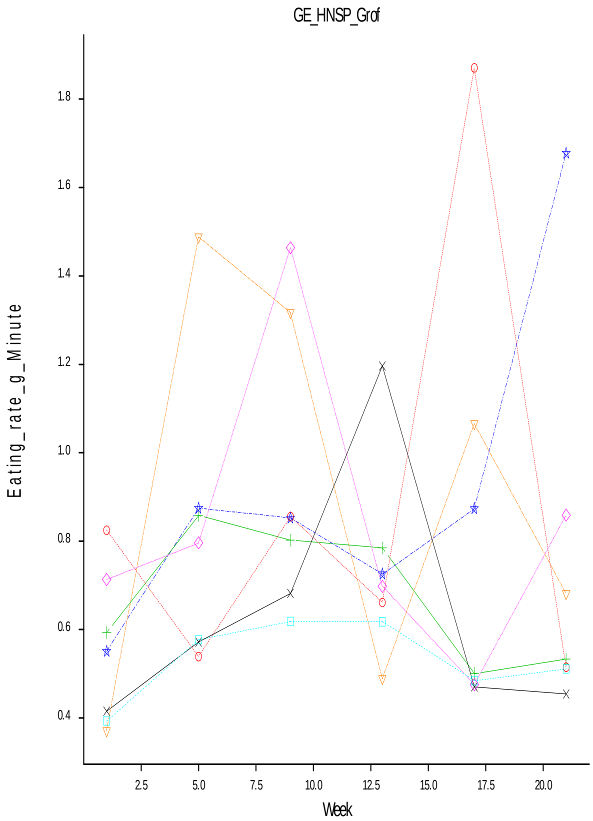

GE_LNSP_Vet
Eating_time
Week
25
20
15
10
5
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

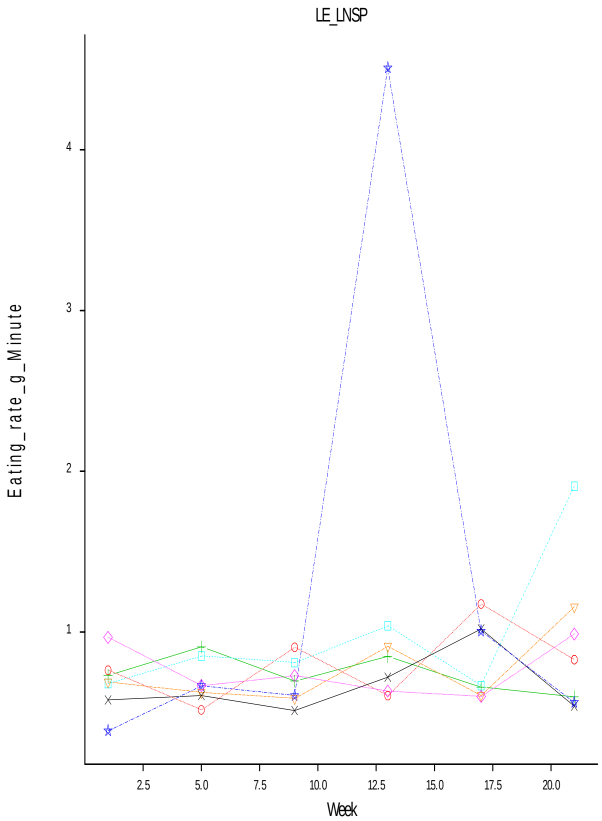

Development of eating time (%) per treatment over time
GE_LNSP
GE_HNSP_Fijn
GE_HNSP_Grof
LE_LNSP
LE_HNSP_Fijn
LE_HNSP_Grof
GE_LNSP_Vet
Eating_time
24
22
20
18
16
14
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Week

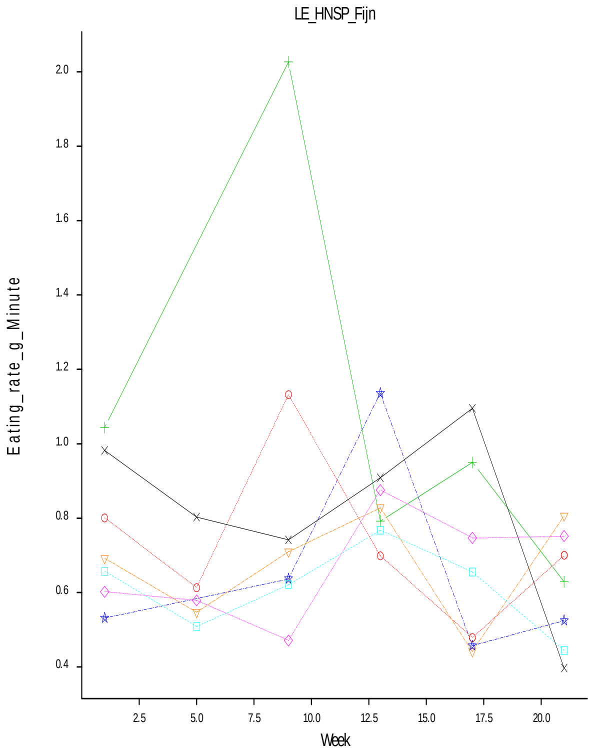
Appendix 3 Eating rate (g/min) development per pen and per treatment

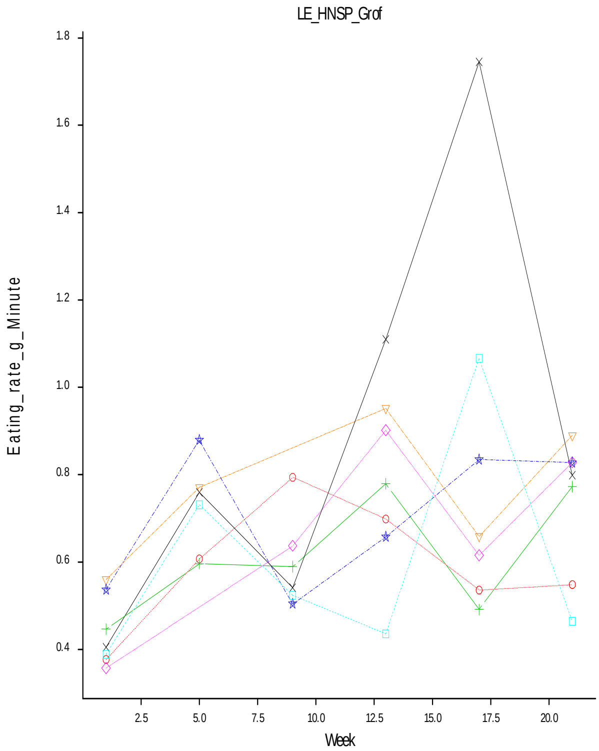


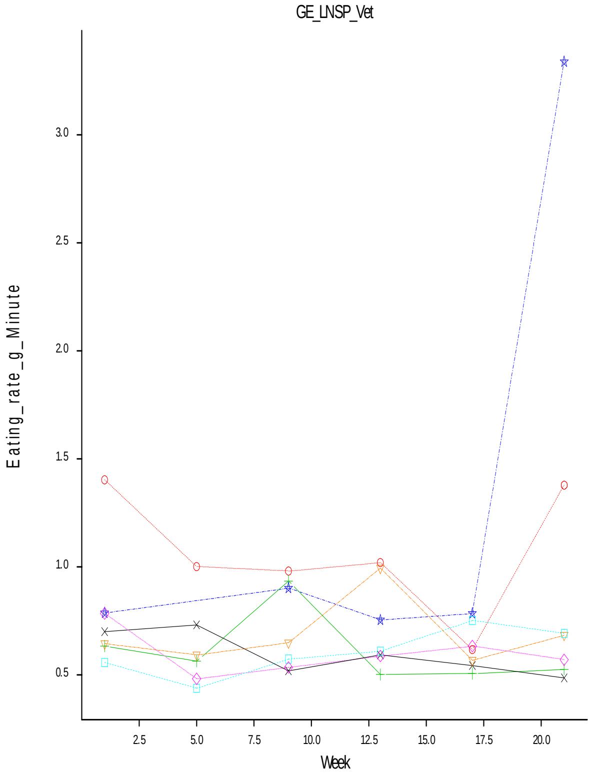


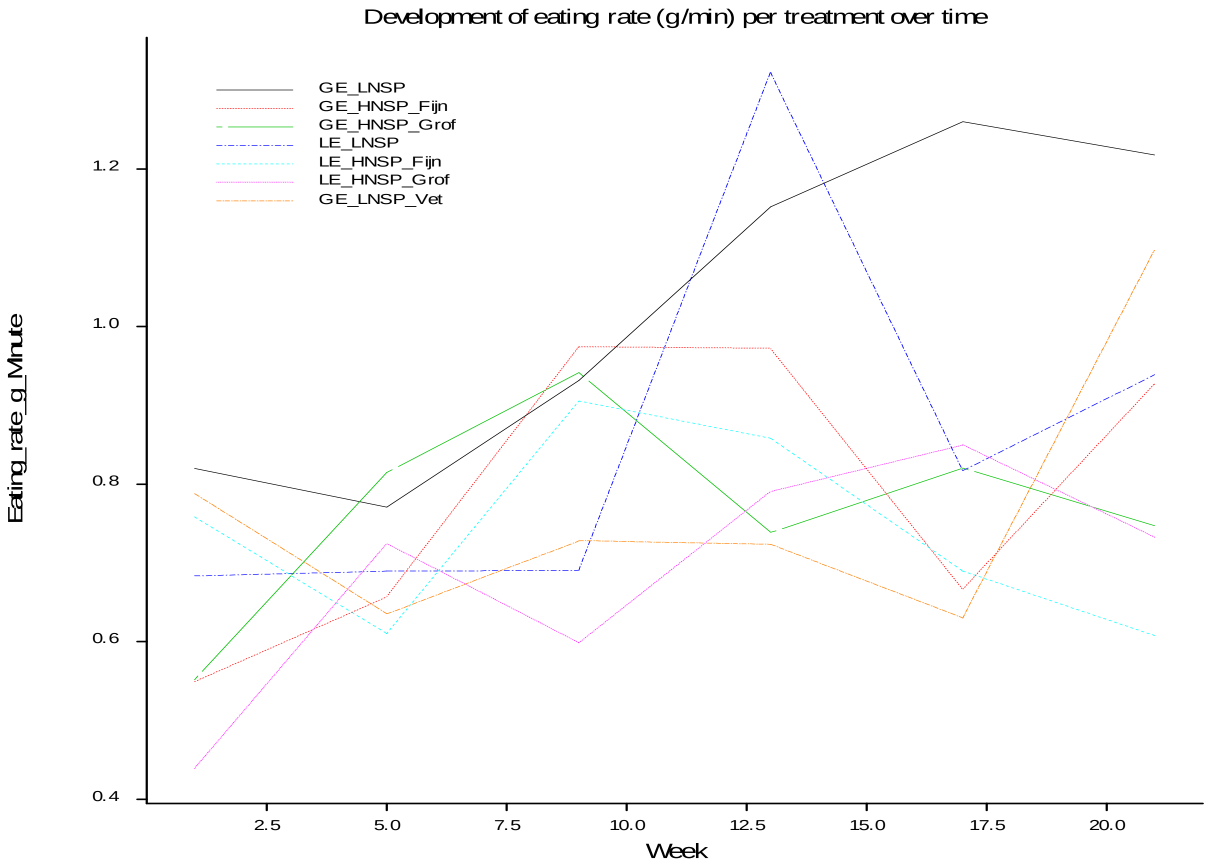




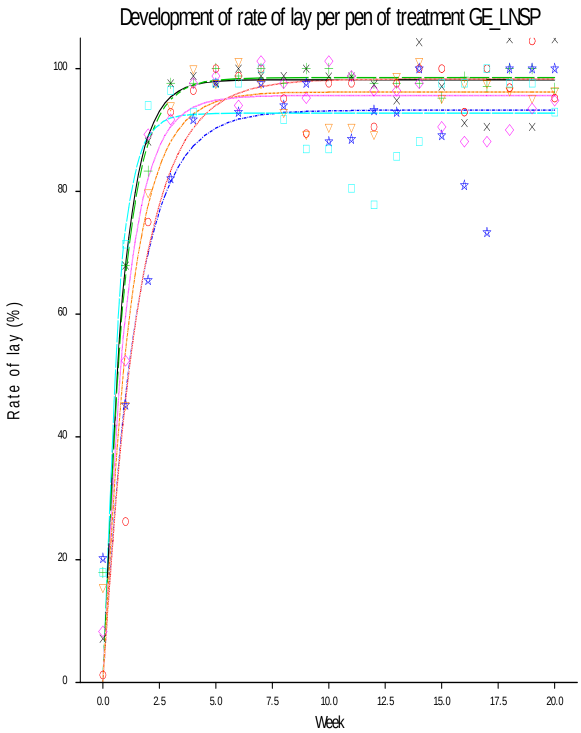

LE_HNSP_Fijn
Eating_rate_g_Minute
Week

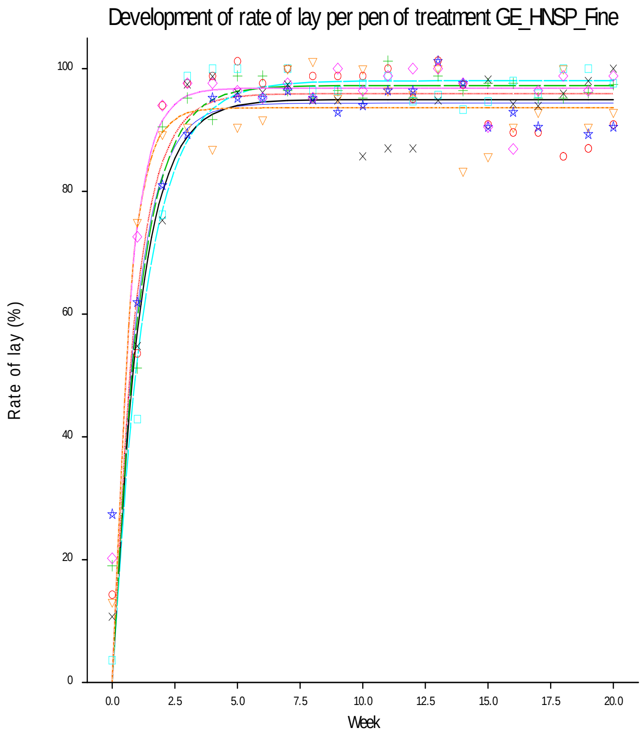

LE_HNSP_Grof
Eating_rate_g_Minute
Week

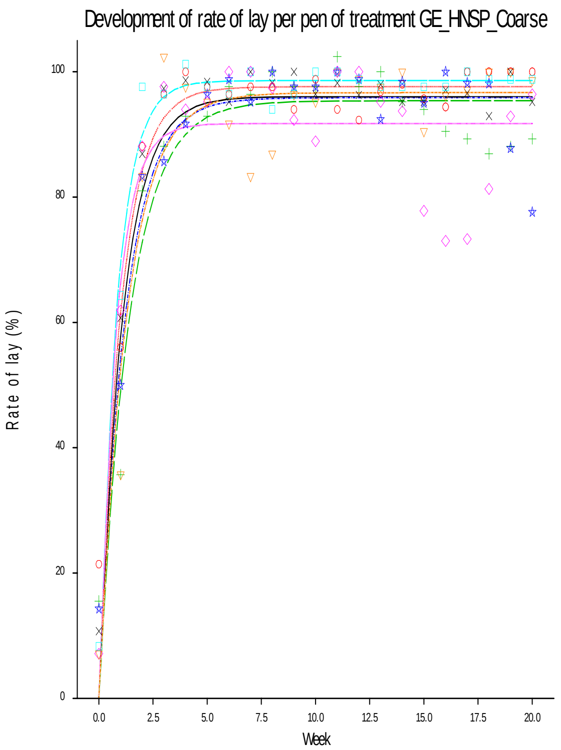

GE_LNSP_Vet
Eating_rate_g_Minute
Week

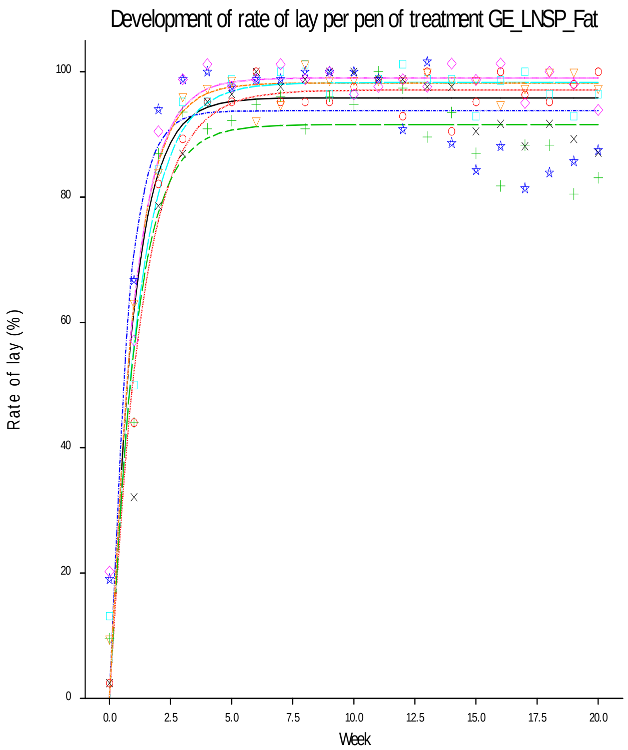

Development of eating rate (g/min) per treatment over time
GE_LNSP
GE_HNSP_Fijn
GE_HNSP_Grof
LE_LNSP
LE_HNSP_Fijn
LE_HNSP_Grof
GE_LNSP_Vet
Eating_rate_g_Minute
0.4
0.6
0.8
1.0
1.2
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Week

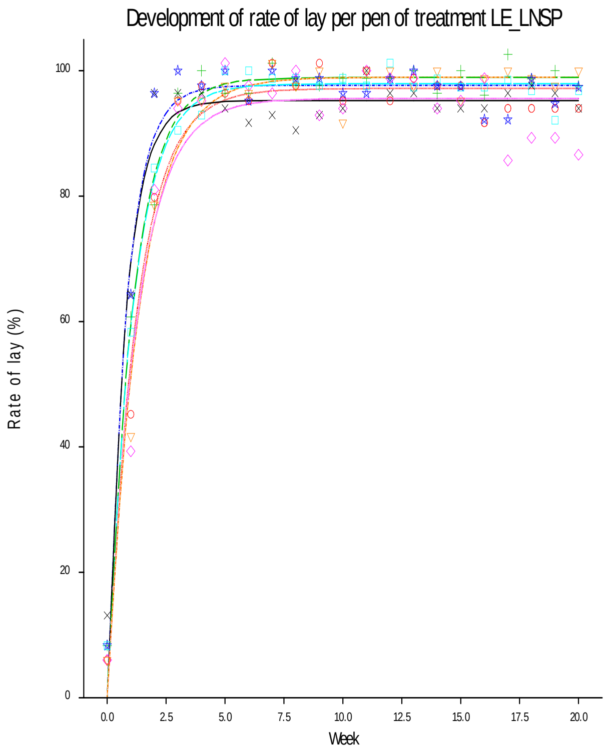
Appendix 4 Rate of lay (%) development per pen and per treatment

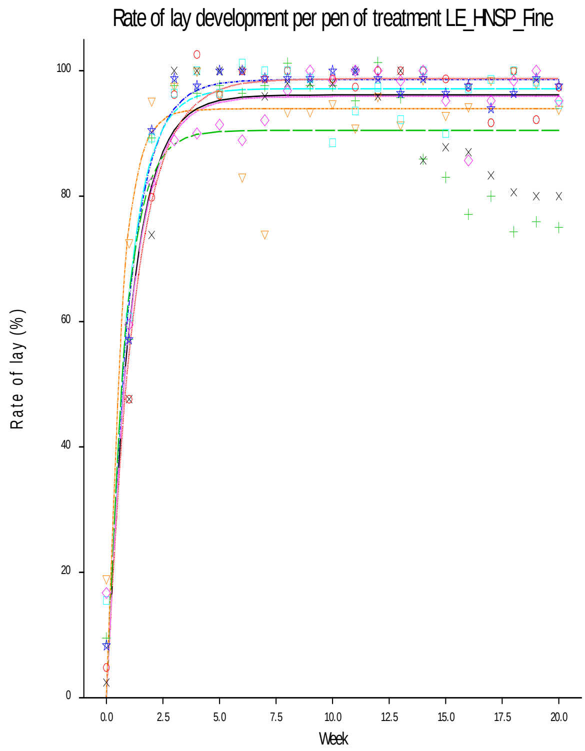


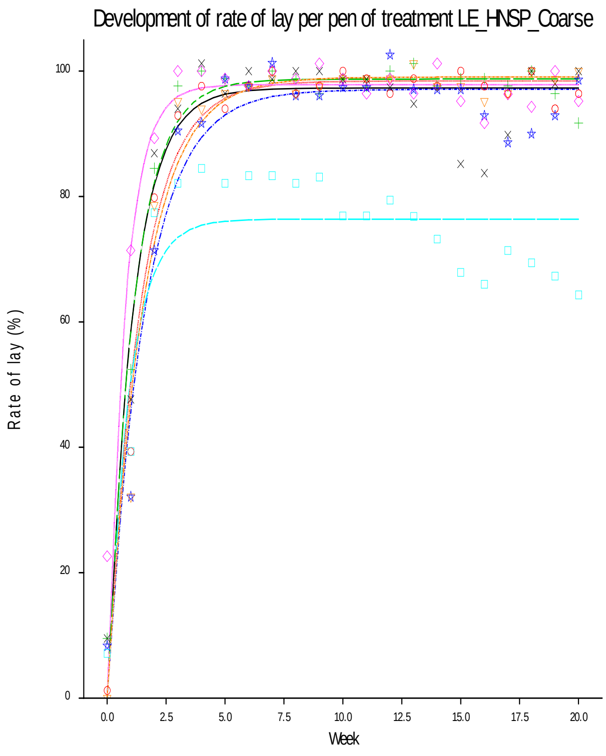


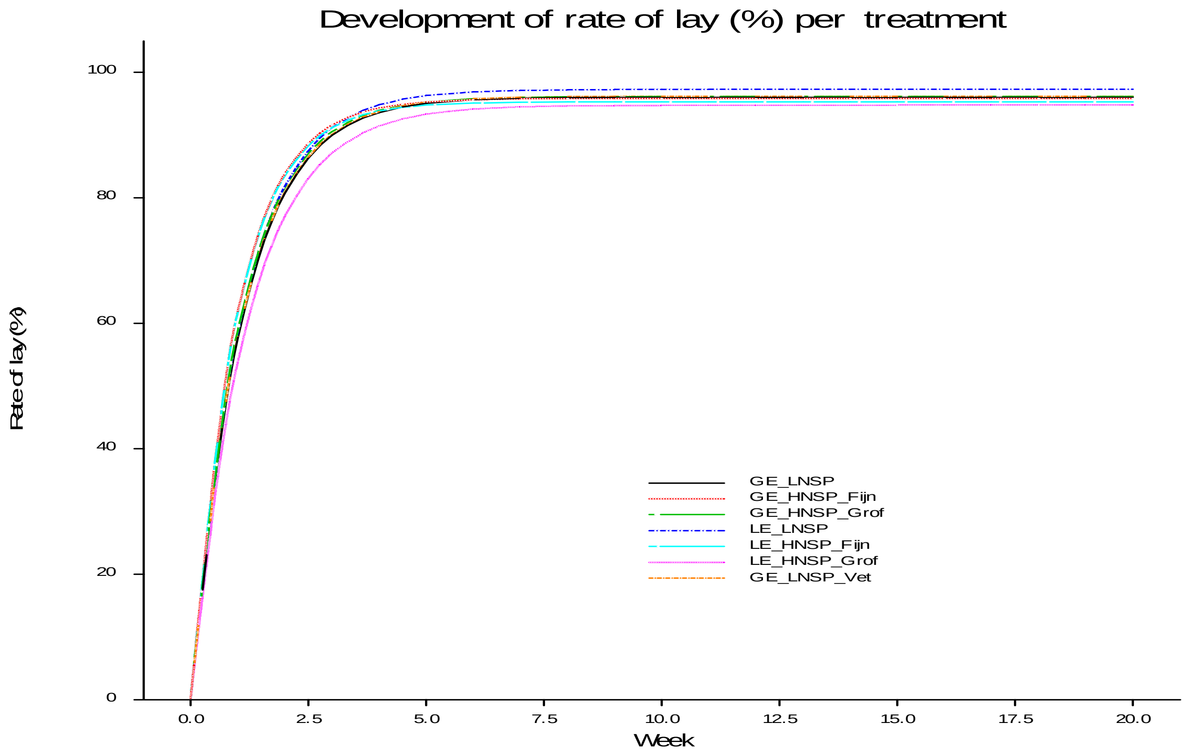




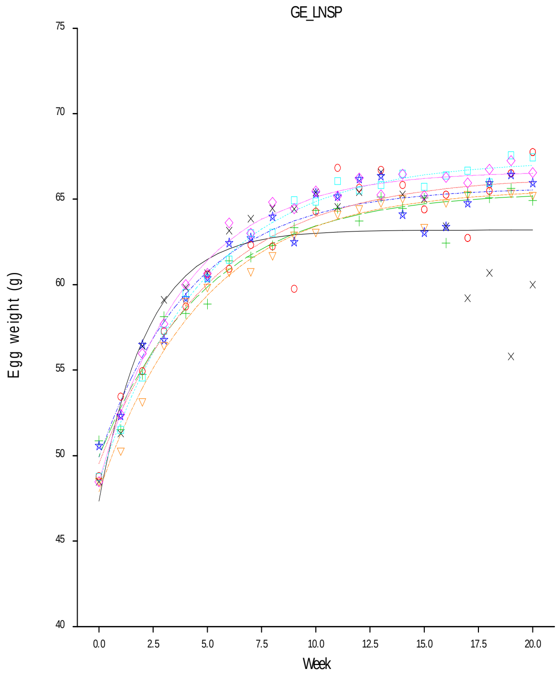

Development of rate of lay per pen of treatment LE_LNSP
Rate of lay (%)
Week
0
20
40
60
80
100
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

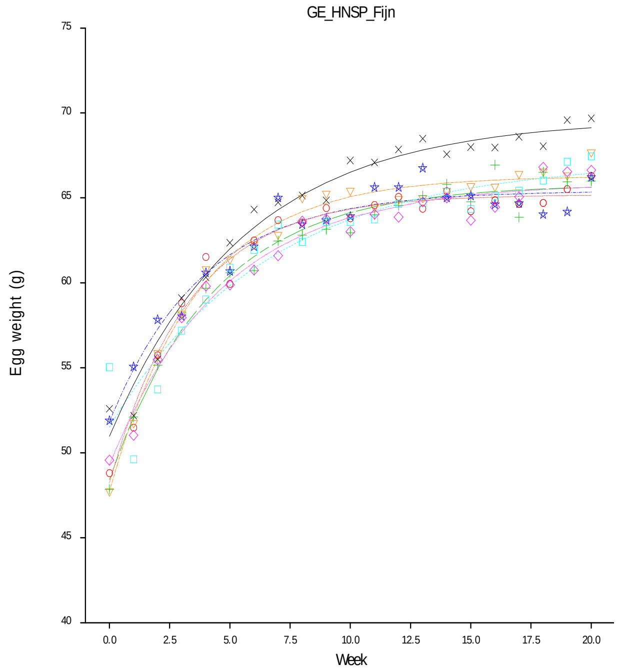

Rate of lay development per pen of treatment LE_HNSP_Fine
Rate of lay (%)
Week
0
20
40
60
80
100
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

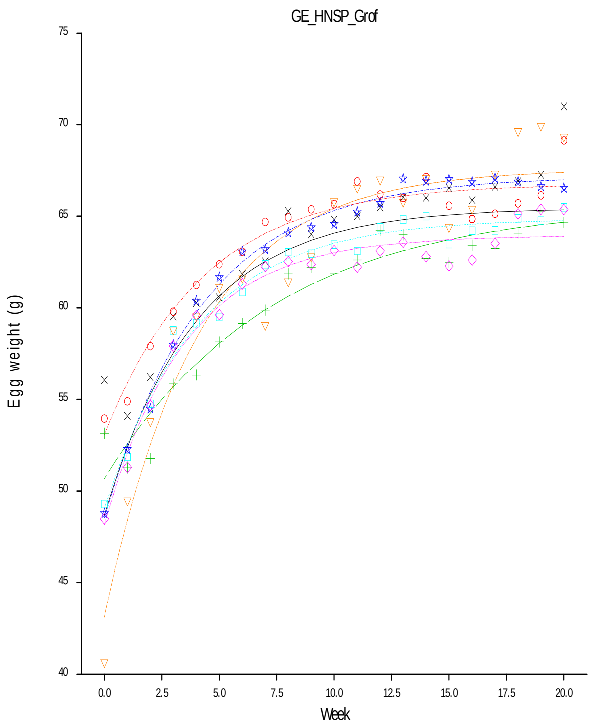

Development of rate of lay per pen of treatment LE_HNSP_Coarse
Rate of lay (%)
Week
0
20
40
60
80
100
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

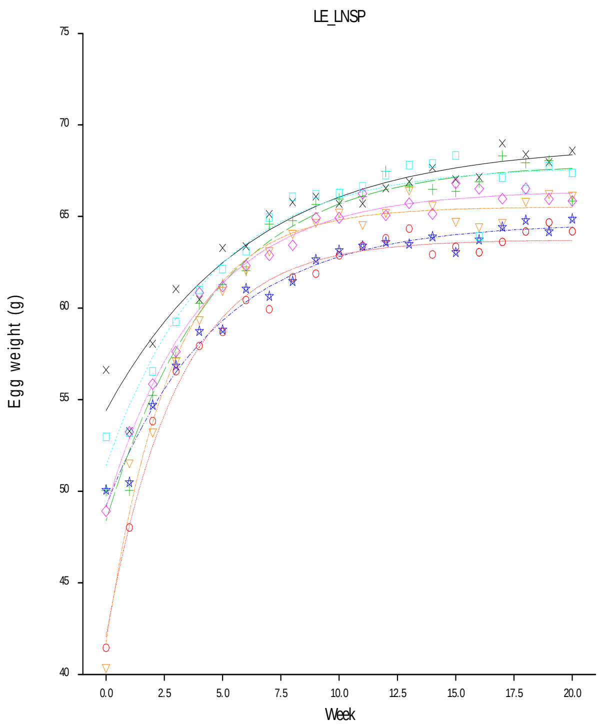

Development of rate of lay (%) per treatment
Rate of lay (%)
100
80
60
40
20
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Week
GE_LNSP
GE_HNSP_Fijn
GE_HNSP_Grof
LE_LNSP
LE_HNSP_Fijn
LE_HNSP_Grof
GE_LNSP_Vet

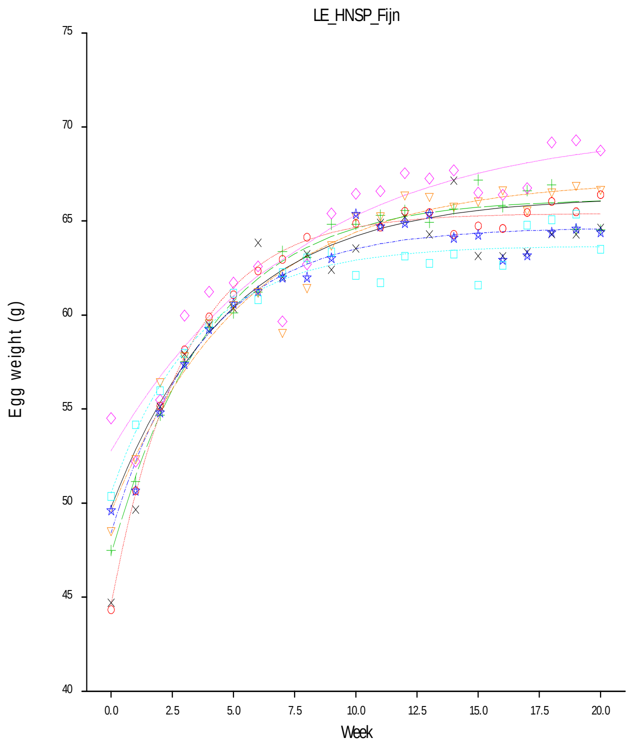
Appendix 5 Egg weight development (g) per pen and per treatment

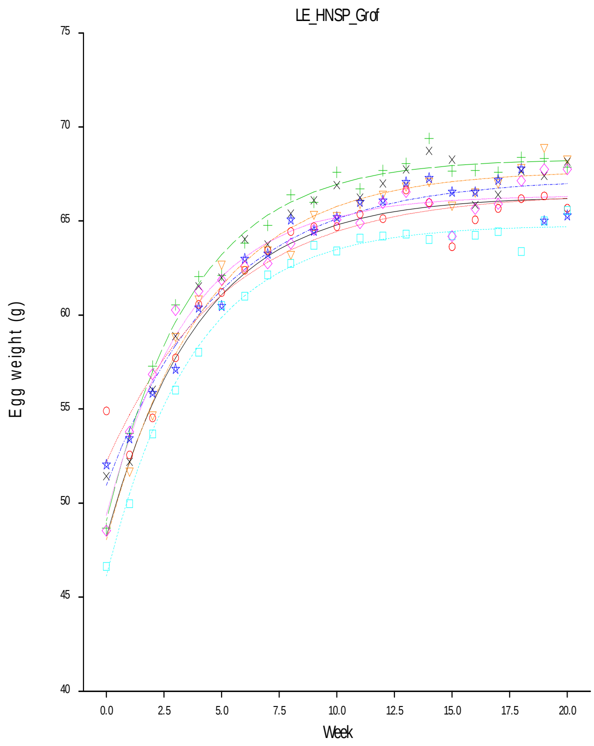


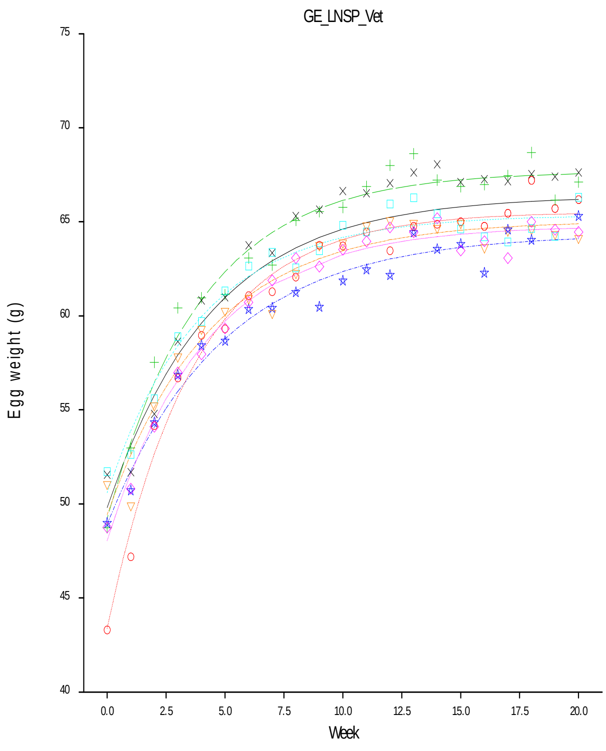


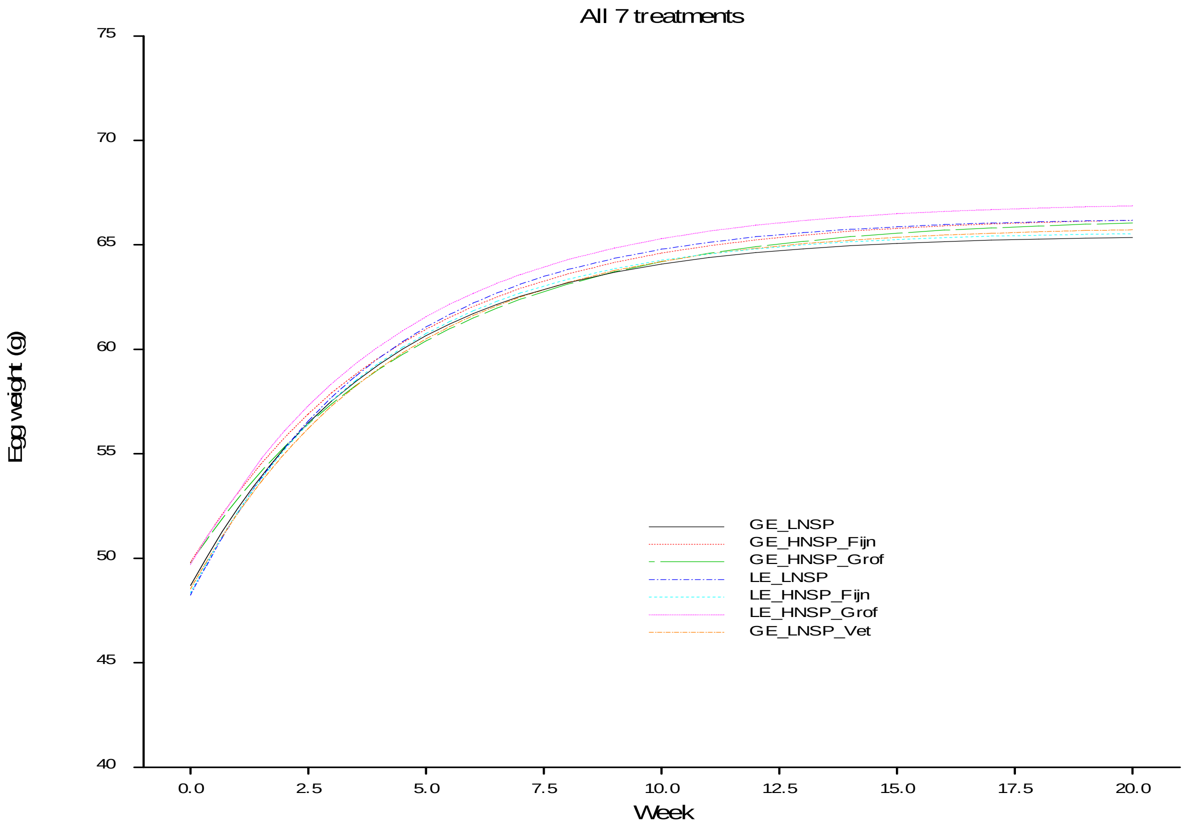




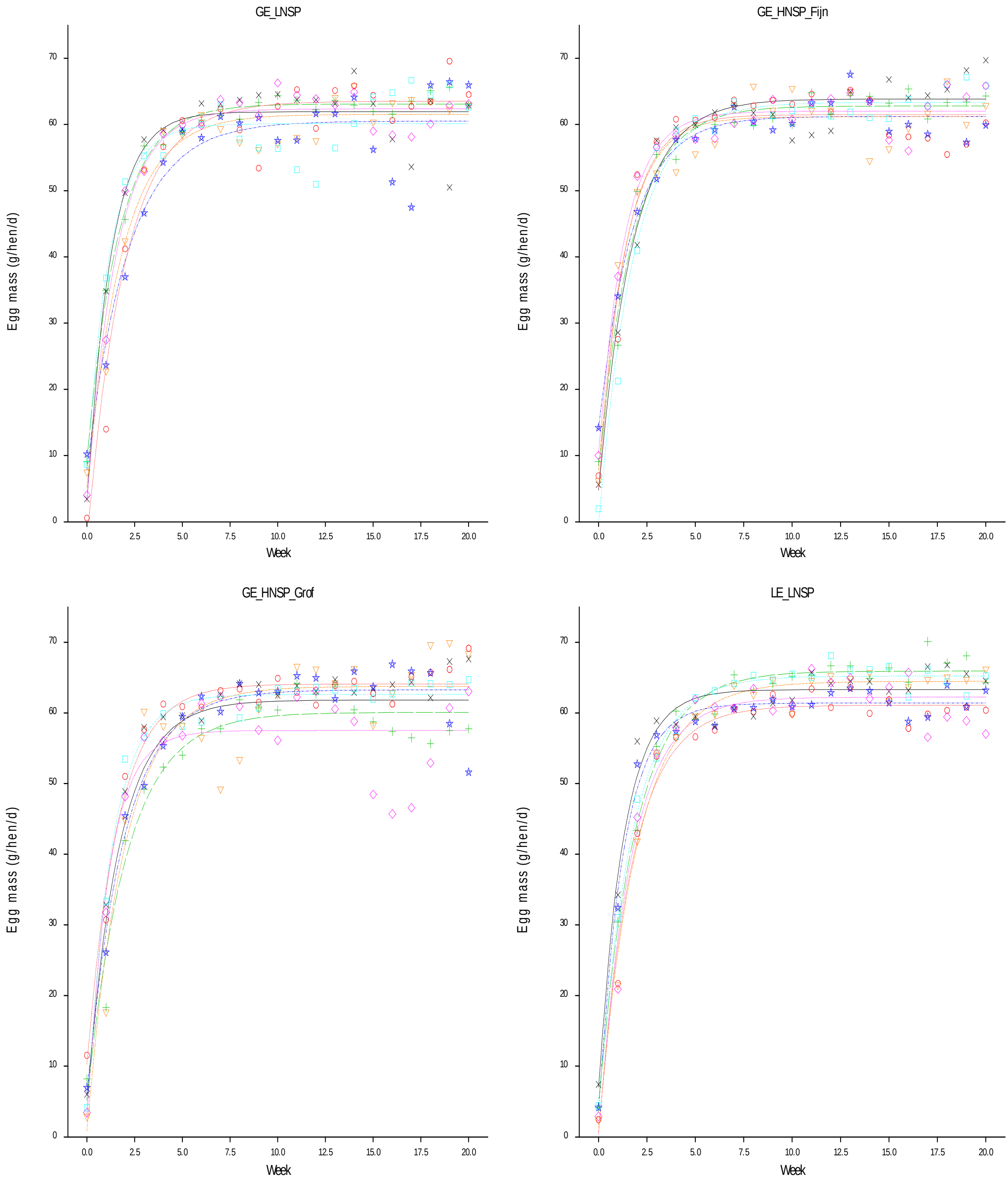

LE_HNSP_Fijn
Egg weight (g)
Week
75
70
65
60
55
50
45
40
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

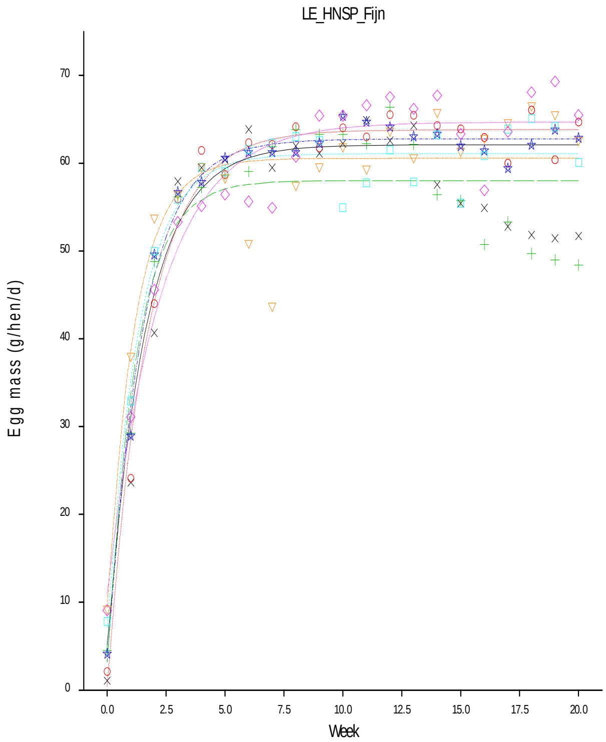

LE_HNSP_Grof
Egg weight (g)
Week
75
70
65
60
55
50
45
40
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

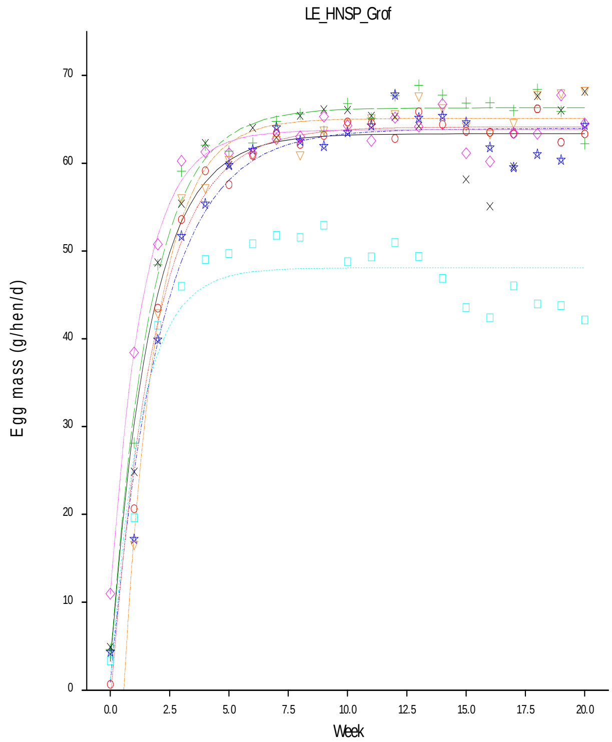

GE_LNSP_Vet
Egg weight (g)
Week
75
70
65
60
55
50
45
40
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

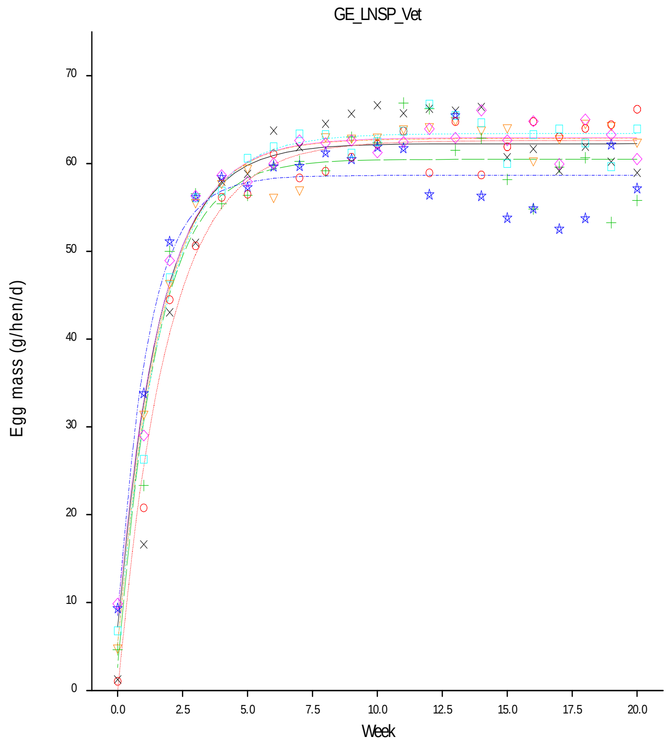

All 7 treatments
Egg weight (g)
75
70
65
60
55
50
45
40
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Week
GE_LNSP
GE_HNSP_Fijn
GE_HNSP_Grof
LE_LNSP
LE_HNSP_Fijn
LE_HNSP_Grof
GE_LNSP_Vet

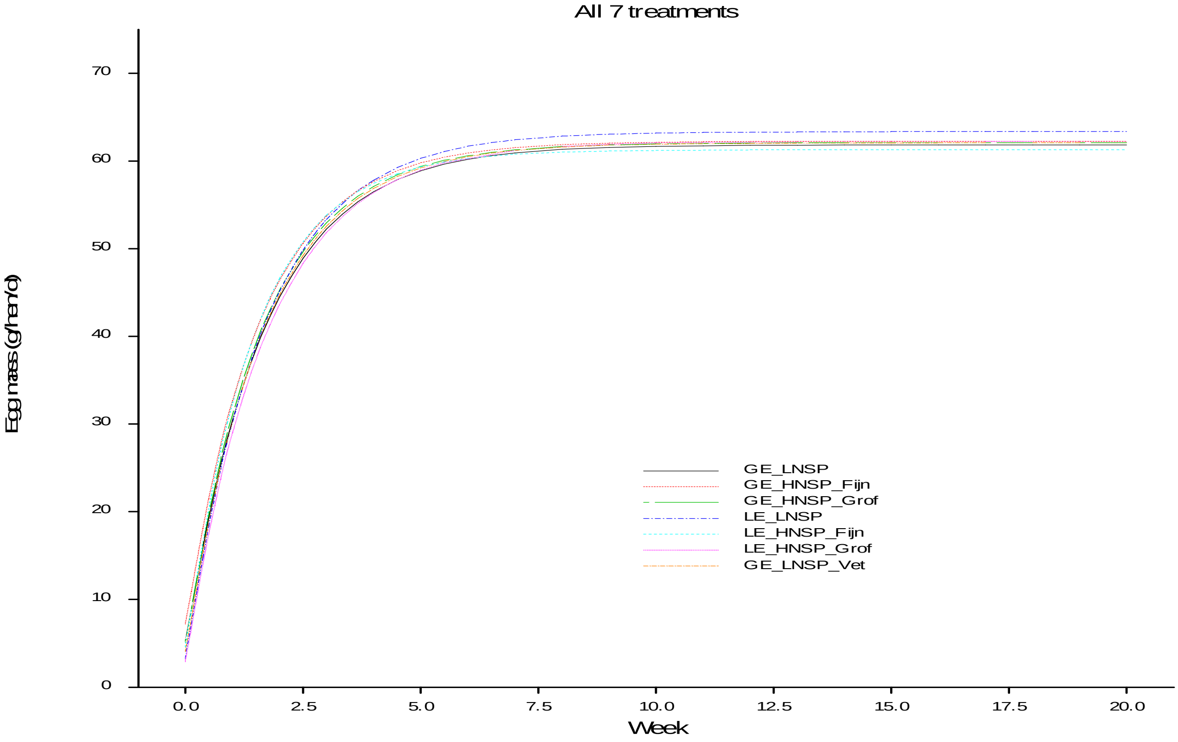
Appendix 6 Egg mass development (g/hen/day) per pen and per treatment



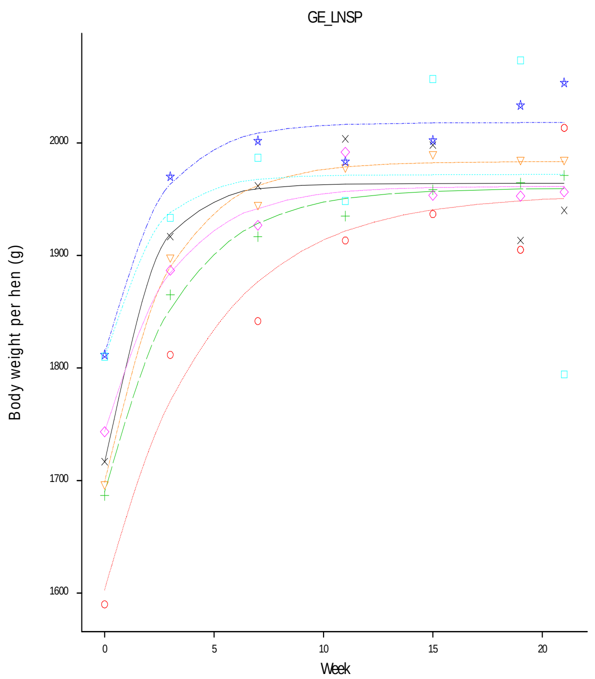

LE_HNSP_Fijn
Egg mass (g/hen/d)
Week

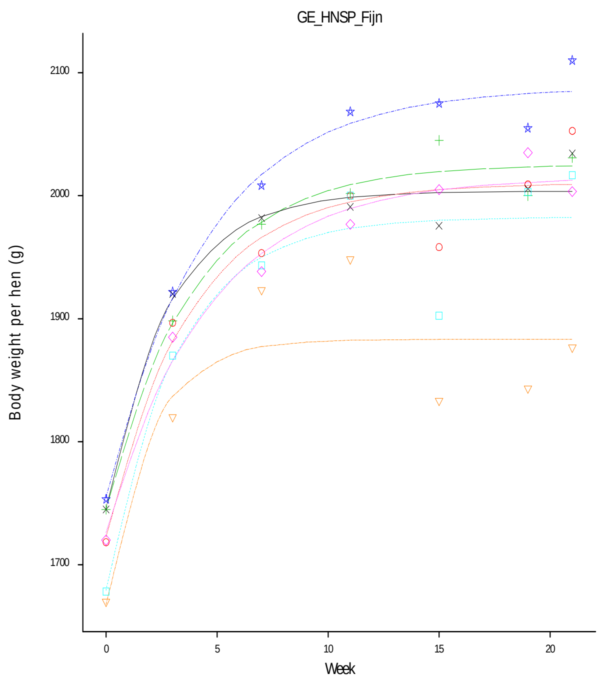

LE_HNSP_Grof
Egg mass (g/hen/d)
Week

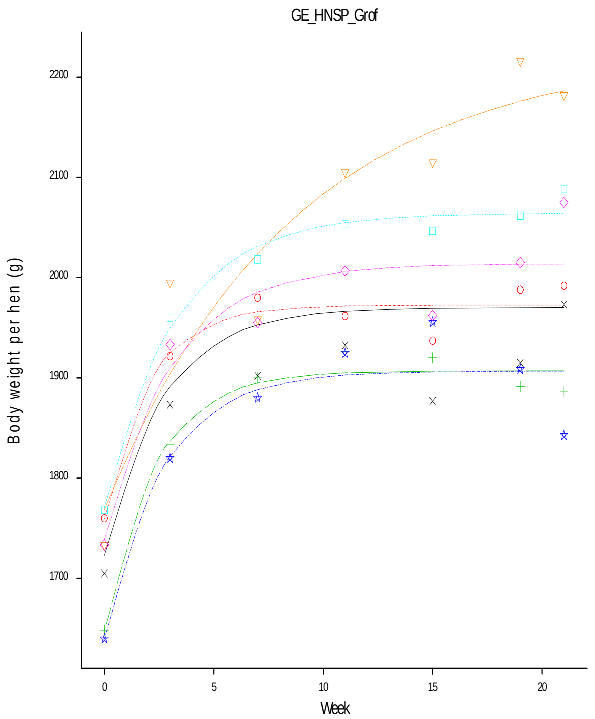

GE_LNSP_Vet
Egg mass (g/hen/d)
Week

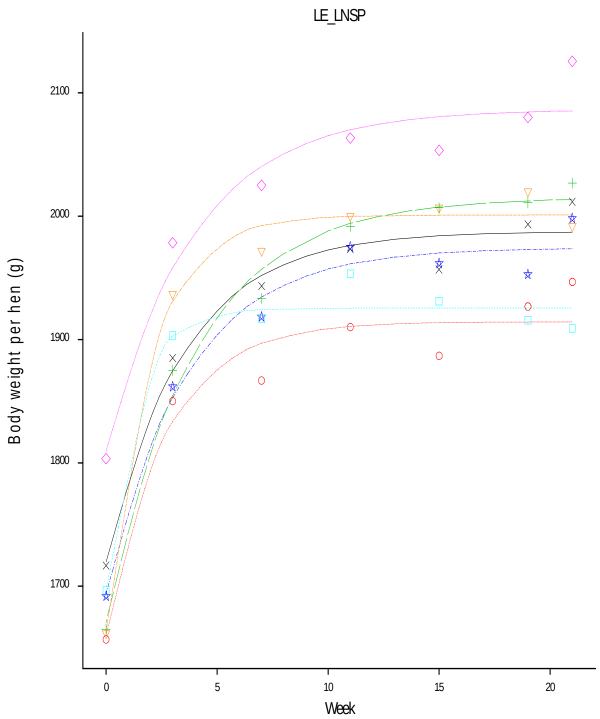

All 7 treatments
Egg mass (g/hen/d)
Week
0
10
20
30
40
50
60
70
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
GE_LNSP
GE_HNSP_Fijn
GE_HNSP_Grof
LE_LNSP
LE_HNSP_Fijn
LE_HNSP_Grof
GE_LNSP_Vet

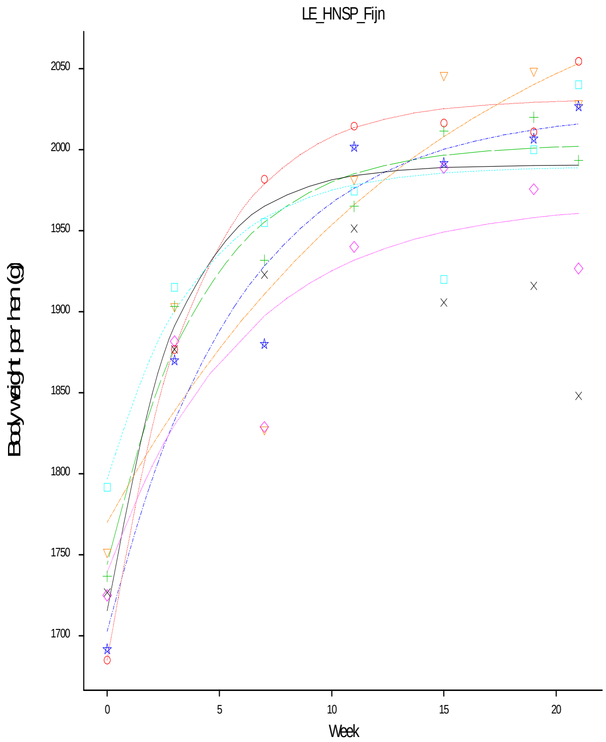
Appendix 7 Body weight development (g/hen) per pen and per treatment

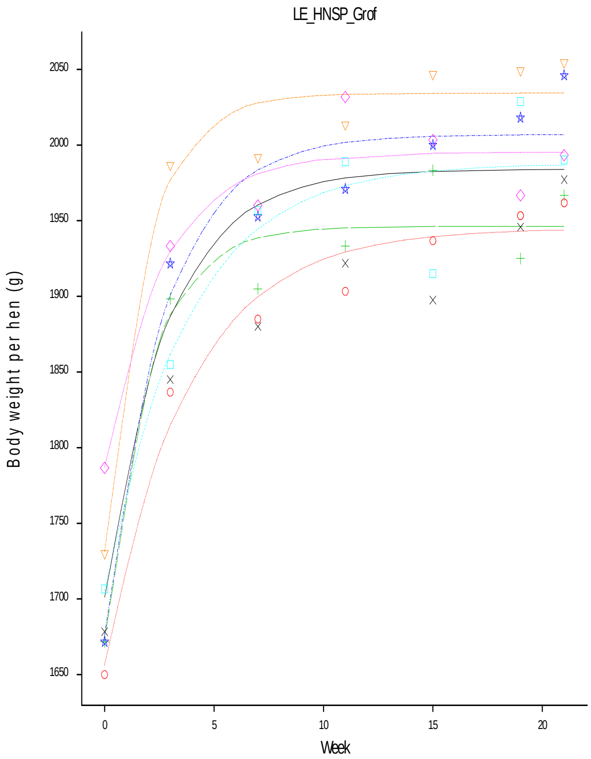


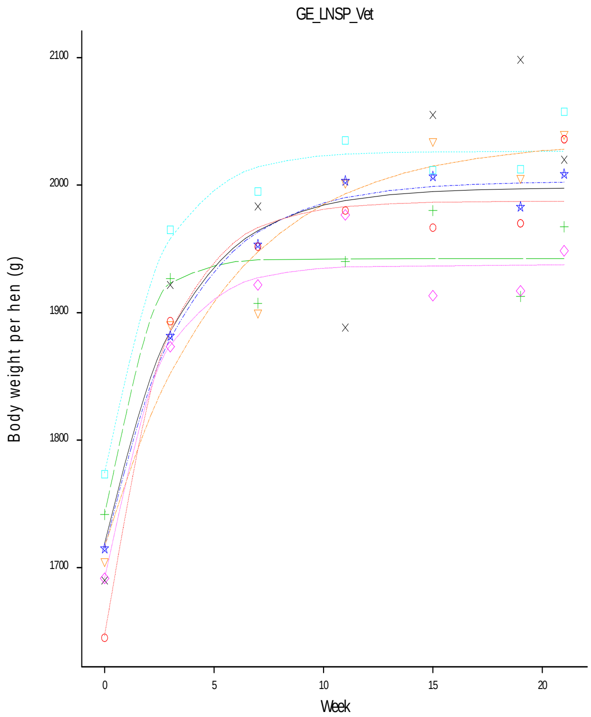


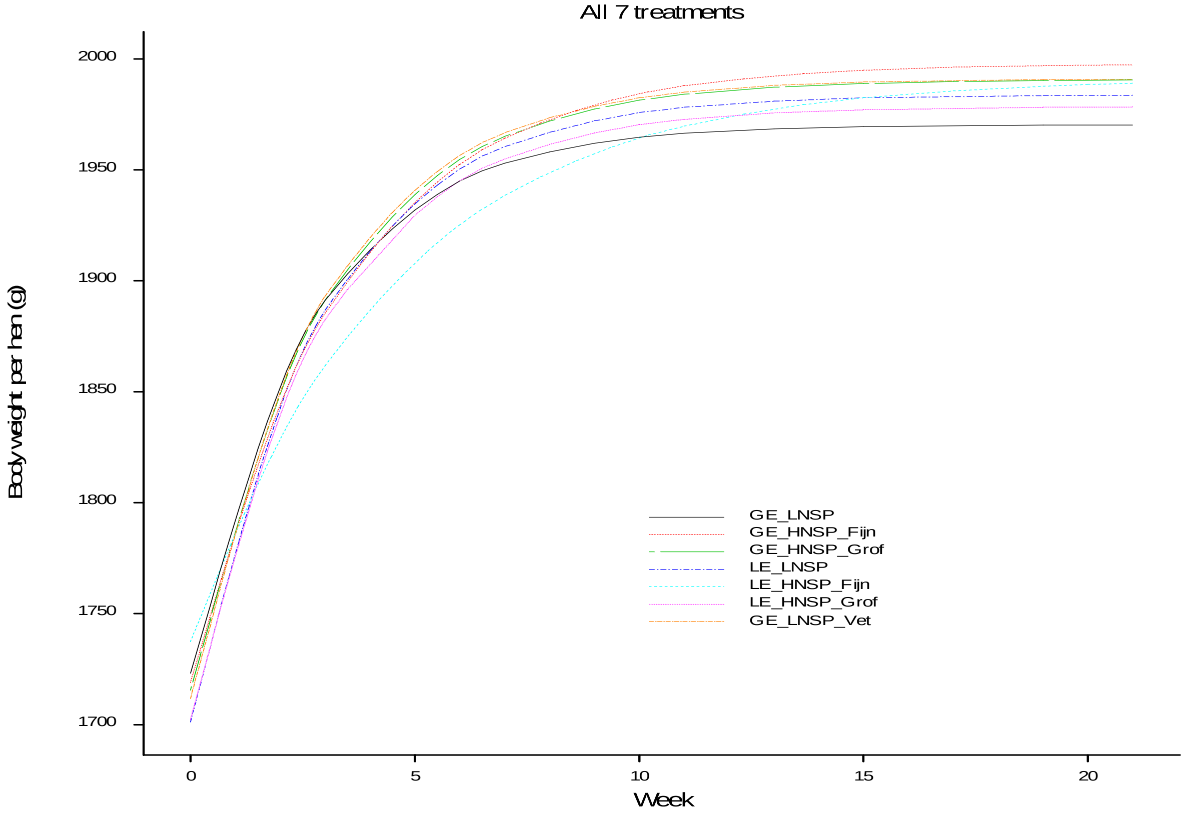





LE_HNSP_Fijn
Body weight per hen (g)
Week


LE_HNSP_Grof
Body weight per hen (g)
Week


GE_LNSP_Vet
Body weight per hen (g)
Week


All 7 treatments
Bodyweight per hen (g)
2000
1950
1900
1850
1800
1750
1700
0
5
10
15
20
Week
GE_LNSP
GE_HNSP_Fijn
GE_HNSP_Grof
LE_LNSP
LE_HNSP_Fijn
LE_HNSP_Grof
GE_LNSP_Vet