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Development of performance, behavior and gut health in laying hens in relation to dietary protein source

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Abstract
From this study, it can be concluded that addition of protein of animal origin to a layer diet will not guarantee for a reduction in feather pecking behavior and an improvement of gut health. Despite this, feather pecking behavior and percentage of wet litter area were reduced in two (Daka-40 and Sonac-50) of four treatments with protein of animal origin.

Keywords
Digestibility, Feather condition, Laying hen, Performance, Processed Animal Protein

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Ontwikkeling van technische resultaten, gedrag en darmgezondheid van leghennen in relatie tot eiwitbron in het voer

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VEE VLEES EIEREN PRODUCTSCHAPPEN

April 2010

Dit onderzoek is uitgevoerd met subsidie van het Productschap Pluimvee en Eieren (PPE)

Samenvatting

In opdracht van het Productschap Pluimvee en Eieren, Sonac, Daka Proteins en EFPRA is een onderzoeksproject uitgevoerd naar het effect van eiwit van dierlijke versus plantaardige herkomst op technische resultaten, gedrag en darmgezondheid van leghennen. Hieruit bleek dat het toevoegen van dierlijk eiwit aan het voer geen garantie is voor minder verenpikgedrag of betere darmgezondheid. Wel was er bij twee van de vier onderzochte voeders met dierlijk eiwit een lager niveau van verenschade. Hennen die deze twee voeders kregen, namen minder voer en daardoor o.a. ook minder mineralen op. Als gevolg hiervan was de strooiselconditie beter, wat het scharrel- en bodempikgedrag van de hennen stimuleerde. De vertraagde ontwikkeling in verenschade bij de hennen die de betreffende twee voeders kregen lijkt dus samen te hangen met de verminderde mineralenopname via het voer.

Verbod op eiwit van dierlijke herkomst
Sinds januari 2001 geldt binnen Europa een volledig verbod voor het verwerken van diermeel in diervoeders en dus ook in het voer van leghennen. Overigens spreken verwerkers van dierlijk eiwit zelf liever van Processed Animal Protein (PAP) in plaats van diermeel. Sinds het verbod op PAP komen er uit de praktijk geluiden over verminderde technische resultaten, die het gevolg zouden kunnen zijn van de wijzigingen in de voersamenstelling. Dit uitte zich in de legsector ondermeer in een lagere eiproductie, een slechtere schaalkwaliteit, meer uitval (m.n. door E. Coli), meer problemen met verenpikken en kannibalisme en een grotere gevoeligheid voor o.a. IB-besmettingen. Vleeskuikenshouders zagen de strooiselkwaliteit verminderen, wat resulteerde in meer pootproblemen. Het verbod op het gebruik van diermeel heeft ook kostprijsverhogend gewerkt op de prijs van het voer, mede door de ontstane schaarste aan hoogwaardige eiwitten en mineralen. In opdracht van het Productschap Pluimvee en Eieren (PPE), en medegefinancierd door Sonac, Daka Proteins en EFPRA, is daarom een dierexperiment uitgevoerd om vast te stellen wat de toegevoegde waarde is van dierlijke ten opzichte van plantaardige eiwitbronnen in het voer op legprestaties, darmgezondheid en gedrag van leghennen.

Proefopzet
In het experiment zijn vier proefvoeders waaraan verschillende varkensvleesmelen waren toegevoegd vergeleken met een controlevoer zonder dierlijk eiwit en dierlijk vet. In de proefvoeders was 50 g/kg eiwit van dierlijke herkomst toegevoegd. Omdat het eiwitgehalte van de dierlijke eiwitbronnen verschilde, varieerde ook de hoeveelheid PAP die aan de voeders werd toegevoegd. De PAP's waren:

- Sonac-50; ruw eiwitgehalte 50%; 10% toegevoegd aan het voer;
- Sonac-60; ruw eiwitgehalte 60%; 8,3% toegevoegd aan het voer;
- Daka-40; ruw eiwitgehalte 40%; 12,5% toegevoegd aan het voer;
- Daka-58; ruw eiwitgehalte 58%; 8,6% toegevoegd aan het voer.

Twee van de vier PAP's (Sonac-50 en Daka-40) behoorden vanwege een hoog botgehalte en een relatief laag eiwitgehalte tot de categorie 'vleesbeendermelen': de overige twee producten vielen onder de categorie 'vleesmelen'. De voeders hadden allemaal hetzelfde energiegehalte (2825 Kcal) en essentiële aminozuurgehalte (darmverteerbaar methionine- + cystinegehalte = 5,8 g/kg) en waren verder voedingskundig zoveel als mogelijk identiek. De verteerbaarheid en het omzetbare energiegehalte van deze partijen PAP's was vooraf vastgesteld door middel van verteringsonderzoek.
Voor dit onderzoek zijn 495 Isa Brown leghennen (18 – 40 wk) met intacte snavels ingezet. De hennen werden gehuisvest in 40 grondkooien (0,9 x 1,50 m; 12 hennen/kooi; 10,4 hennen/m^2). Elk voer is acht keer beproefd. Om het verenpikgedrag enigszins te stimuleren was de bezetting bewust wat hoger dan gebruikelijk bij scharrelhennen (norm = 9 hennen/m^2). De hennen hadden onbeperkt water en voer ter beschikking. De bodem van de grondhokken was volledig ingericht als scharrelruimte (met zand als strooiselmateriaal). Elk hok bevatte twee zitstokken, zodat er voldoende ruimte was om elke hen een zitplek te geven.
Tijdens het experiment werden voeropname en legprestaties wekelijks bepaald. Om de zes weken werd het verenpikgedrag, de kwaliteit van het verenkleed, het algemene gedrag en de kwaliteit van het strooisel gescoord.
Op vier momenten (wk 20, 24, 29 en 35) zijn uit elke behandeling drie willekeurige hennen genomen en door de GD onderzocht op de aanwezigheid van (chronische) darmontstekingen.

Resultaten
De gemiddelde technische resultaten per behandeling zijn weergegeven in tabel 1.

Tabel 1 Gemiddelde technische resultaten per behandeling

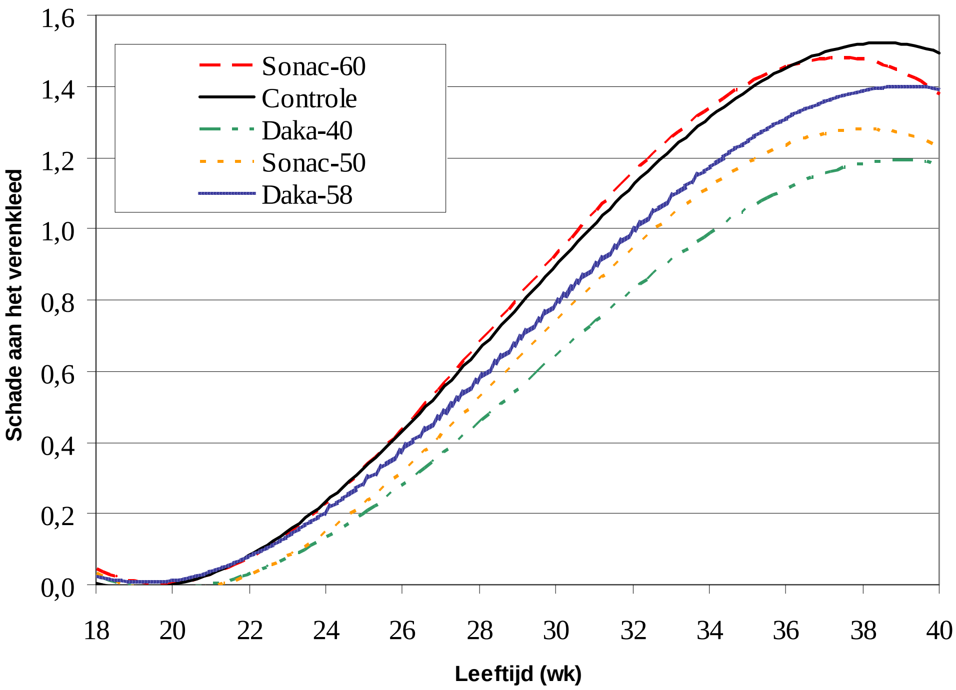
Kenmerk[1]	Voeropname (g/hen/d)	Leg percentage (%)	Ei gewicht (g)	Ei massa (g/hen/d)	Voerder conversie (g voer/g ei)	Hen Gewicht (g)	Nat strooisel (%)[2]
Controle	120.7[bc]	78.2	59.8[ab]	48.9[ab]	2.17[bc]	1820	33.0[a]
Sonac-50	118.9[c]	79.7	59.4[abc]	49.2[a]	2.13[c]	1795	24.8[b]
Sonac-60	124.5[a]	77.6	59.9[a]	48.4[abc]	2.30[a]	1788	32.5[a]
Daka-40	119.1[c]	76.5	58.7[bc]	46.4[c]	2.22[abc]	1767	21.1[b]
Daka-58	122.3[ab]	78.2	58.4[c]	47.6[bc]	2.26[ab]	1781	32.4[a]

[1] Waarden binnen een kolom met verschillende letters[a,b,c] verschillen aantoonbaar van elkaar (P<0,05)
[2] Uitgedrukt in percentage van het hokoppervlak

Hoewel alle voeders voedingskundig gelijkwaardig waren, verschilde de voeropname toch tussen de behandelingen. De twee voeders waaraan vleesbeendermeel (Sonac-50 en Daka-40) was toegevoegd werden duidelijk minder goed opgenomen dan de voeders waaraan vleesmeel (Sonac-60 en Daka-58) was toegevoegd. Het voeropnameniveau van de controlebehandeling zat tussen beide categorieën in. De hoeveelheid geproduceerde eimassa was bij de behandelingen met Daka-40 en Daka-58 lager in vergelijking met de controlegroep en de behandeling met Sonac-50. De behandeling met Sonac-60 zat hier tussenin en verschilde niet wezenlijk van de andere behandelingen. Legpercentage en hengewicht werden niet beïnvloed door de voerbehandelingen. Opvallend was dat het strooisel in de hokken waarin vleesbeendermeel (Sonac-50 en Daka-40) was verstrekt aantoonbaar droger was dan bij de andere drie behandelingen.

Schade aan het verenkleed
Het verloop van de ontwikkeling van schade aan het verenkleed per behandeling is weergegeven in figuur 1.

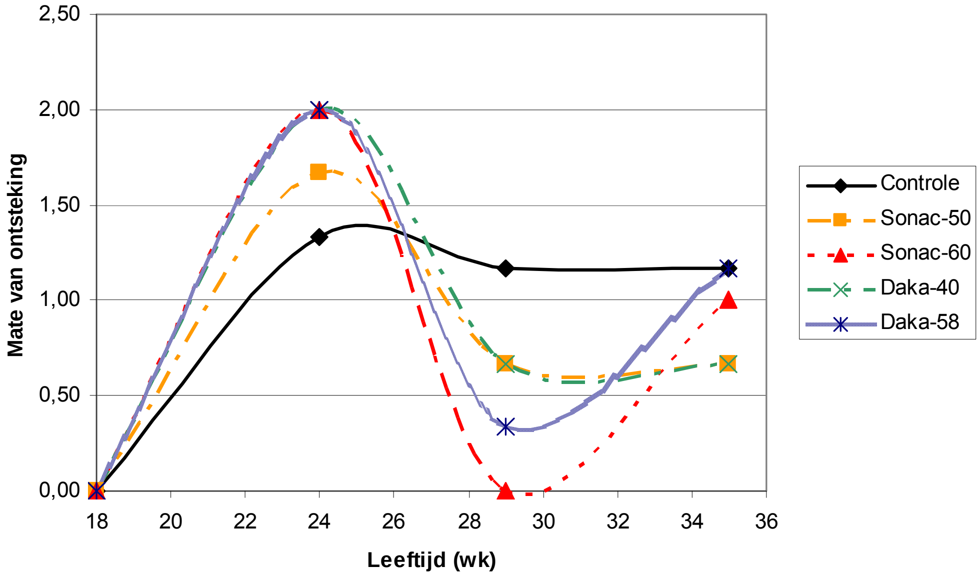
Figuur 1 Verloop schade verenkleed per behandeling. De schade is weergegeven in een schaal van 0 (volledige verenbedekking) tot 5 (volledig kaal of wonden groter dan 2 cm)



Hoewel verenpikgedrag in dit experiment relatief weinig voorkwam (maximale schadeniveau was 1,5 op een schaal van 0 tot 5), was er tussen proefbehandelingen toch een duidelijk verschil in de ontwikkeling van verenschade. Meer schade aan het verenkleed duidt op een hoger niveau van verenpikgedrag. Opnieuw bleek dat er een verschil was tussen de behandelingen met vleesbeendermeel en de overige behandelingen. Tot 36 weken leeftijd verliep de ontwikkeling in verenschade bij de hennen die Sonac-50 en Daka-40 verstrekt kregen trager dan bij hennen die Sonac-60 kregen. De overige twee behandelingen zaten hier tussen in en verschilden niet aantoonbaar van de andere groepen.

Darmschade
Het verloop van de darmschade per behandeling tijdens de proefperiode is weergegeven in figuur 2.

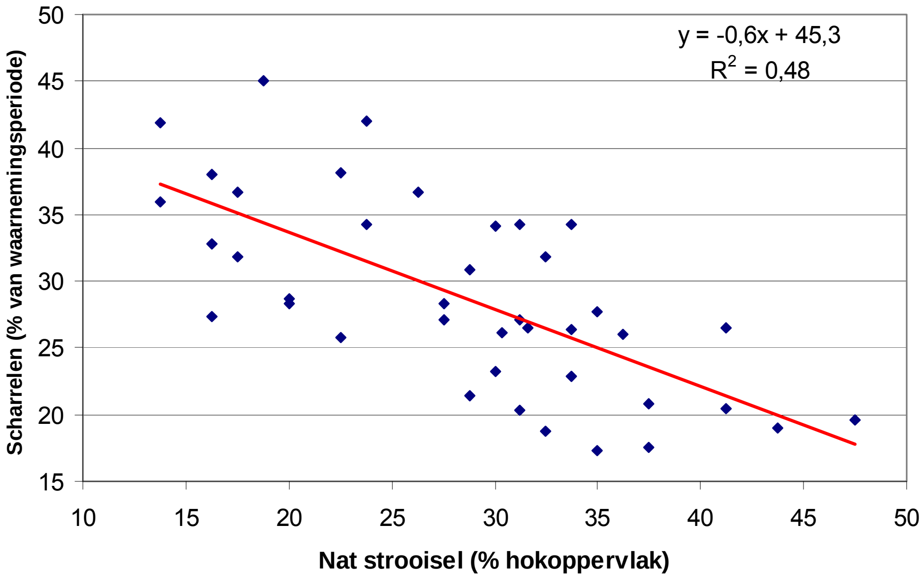
Figuur 2 Verloop van de gemiddelde darmschade per behandeling tijdens de proefperiode. De mate van darmschade is als volgt gescoord: 0 = geen schade, 1 = matige ontsteking, 2 = ontsteking, 2,5 = matige chronische ontsteking



De hennen die aan het begin van de proefperiode naar de GD waren ingestuurd, vertoonden geen enkele vorm van darmschade. In week 20 deed zich een coccidiose uitbraak voor. Als gevolg hiervan kwamen bij vrijwel alle ingestuurde hennen op 24 weken leeftijd nog ontstekingsreacties voor. Het toevoegen van PAP aan het voer had dus geen remmend effect op deze ontstekingen. Op 29 weken leeftijd was de darmschade in de meeste proefgroepen getalsmatig verminderd. Het grootste deel van de ingestuurde hennen had toen geen of slechts matige ontstekingen. In week 35 was het gemiddelde niveau van darmschade weer enigszins toegenomen. Tijdens dit experiment werd chronische darmontsteking geen enkele keer waargenomen. Ondanks dat de mate van darmontsteking tussen behandelingen in sommige weken getalsmatig verschilt kan op basis van de statistische analyse geconcludeerd worden dat het toevoegen van PAP aan het voer in dit experiment geen aantoonbaar positief effect heeft gehad op de ontwikkeling van de darmgezondheid.

Belang strooiselconditie
Uit dit onderzoek bleek dat het toevoegen van PAP geen garantie is voor minder verenpikgedrag. Toch bleek de ontwikkeling van verenschade trager te verlopen bij de behandelingen met Sonac-50 en Daka-40. Bovendien was de strooiselconditie van deze twee behandelingen aantoonbaar beter ten opzichte van de andere behandelingen. De conditie van het strooisel had een duidelijk effect op het gedrag van de hennen. Naarmate een groter deel van het hokoppervlak bedekt was met nat strooisel nam de tijd die besteed werd aan het scharrelen en het aantal pikken naar het strooisel af (Figuur 3). In dit experiment werd een duidelijke negatieve samenhang gevonden tussen de natheid van het strooisel en de opname van mineralen (natrium, kalium, chloor en calcium). De betere strooiselconditie van de behandelingen met Sonac-50 en Daka-40 kon toegeschreven worden aan de lagere opnameniveaus van mineralen als gevolg van de lagere voeropname.

Figuur 3 Relatie tussen natheid van het strooisel en het voorkomen van scharrelgedrag



Summary

On request of the Dutch Board of Meat and Eggs, Sonac, Daka Proteins and Efpra, an experiment was performed to investigate the development of performance, behavior and gut health in laying hens in relation to dietary protein source. A diet containing protein sources of only vegetable origin was compared with four diets, each containing one of four processed animal proteins (PAP's). First, faecal digestibility of nutrients of the PAP's (Daka-40, Daka-58, Sonac-50 and Sonac-60), was determined in Lohmann brown layers. Hens (n=132) were housed in 22 cages (6 hens/cage) and allotted to five dietary treatments. In the PAP diets (4 replicates/treatment), 100 g/kg CP of animal origin was added, thereby replacing soybean meal and maize of the basal diet (6 replicates/treatment). PAP sources differed largely in chemical composition and digestibility coefficients. Energy content (AME_n) varied from 7.59 (Daka-40) to 12.98 (Sonac-60) MJ/kg, and digestible lysine from 15.4 (Daka-40) to 28.3 g/kg (Sonac-50).
Subsequently, the impact of a control diet (without PAP) versus four PAP diets (50 g/kg CP of animal origin from the same batches as used in the digestibility study) on performance, behavior, and gut health was determined. All diets were isocaloric (AME_n = 11.8 MJ/kg) and isonitrogenous (dig. lysine = 6.8 g/kg). Hens were housed in 40 floor pens (12 hens/pen, 8 pens/treatment) from 20 to 40 wk of age. Performance traits of hens fed the PAP diets were reduced, similar or improved compared to hens that were fed the control diet. Differences seemed to be partly related to differences in feed intake and corresponding amino acid intake. Supplementation of PAP's did not generally reduce feather pecking behavior. Nevertheless, Daka-40 and Sonac-50 fed hens showed a delay in the development of feather damage and, simultaneously, an increase in litter condition, foraging and walking behavior, and floor pecks compared to hens fed Sonac-60. These shifts seemed to be partly related with the intake of digestible glycine, available phosphorus, calcium, potassium, and sodium. No evidence was found for positive effects of PAP-addition on gut health by PAP inclusion.

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1 Introduction

Nutritionists have utilized processed animal proteins (PAP) in poultry diets for many years (Kratzer and Davis, 1959; Skurray, 1974; Waldroup and Adams, 1994; Dale, 1997; Parsons, et al., 1997). The primary advantages associated with the utilization of PAP in poultry diets have been the high digestibility of amino acids, as well as the biological availability of phosphorus in PAP (Waldroup and Adams, 1994; Sell and Jeffrey, 1996; Parsons, et al., 1997). By 1950, identification of vitamin B_{12} as the animal protein factor, and its commercial synthesis, made it possible to develop diets without PAP for non-ruminants (Haugen et al., 1985). After the ruminant to ruminant ban (1989 in the Netherlands, 1994 EU-wide), inclusion of PAP in poultry rations increased because of the favorable supply and pricing. The relatively high inclusion level of PAP in poultry diets remained in place until the total ban on MBM in 2001.
Since the total ban, adverse effects like reduced egg production, increased susceptibility for chronic enteritis, and higher incidences of feather pecking behavior and cannibalism were reported in practice. Until now, however, it has not been demonstrated whether these supposed effects were directly related to the changes in dietary protein source. In 2005, liberalizations of the EU regulations were announced regarding the use of PAP in diets of pigs and poultry (TSE-step-by-step plan)(EU, 2005). Probably in the future, products of non-ruminant animals that are appropriate for human consumption (C3 material) can again be incorporated in animal diets. Due to the species-to-species ban, only meat of pork origin may be included in poultry diets. The aim of this study was to investigate the development of performance, behavior and gut health in laying hens in relation to vegetable versus animal (pork) protein source. To identify the nutritional value of the tested PAP sources, first a digestibility study was performed.

2 Materials and methods

2.1 Housing, birds and management of the digestibility study

At 17 weeks of age, a total of 160 laying hens (ISA Brown strain), that passed a visual health inspection, were allotted to one of five dietary treatments: one basal diet without PAP and four PAP enriched diets. The hens were housed in battery cages (65 x 75 cm) with six hens per cage in a climate-controlled poultry house. The basal treatment was replicated six times (36 hens), whereas the PAP enriched treatments were replicated four times (4 x 24 hens). Besides, 24 additional hens were kept as reserve animals until the moment that the experimental diets were provided. Temperature and relative humidity of the experimental room were continuously registered. The target temperature was maintained at 20 °C, whereas a L:D schedule of 16 h of light and 8 h of darkness was applied. To realize a constant feed intake during the light period, an intermittent L:D schedule of 15 min. of light / and 45 min. of dark was applied in this period. All diets were provided for *ad libitum* intake and water was also available continuously.

2.2 Experimental design of the digestibility study

The study was performed as a fecal digestibility study in laying hens from 24-27 weeks of age. Starting with hens of 17 weeks of age, the birds were all laying at 21 weeks of age. The experimental period started at 24 weeks of age and lasted until the end of 27 weeks of age (a period of four weeks). From week 26 onwards, the hens received the experimental diets. In this experiment, fecal digestibility of four different pork meat meals was determined: 1) Sonac-50, 2) Sonac-60, 3) Daka-40 and 4) Daka-58. These PAP sources varied in chemical composition, thereby covering the range of commercially available pork meat meal qualities. The analyzed nutrients of the different PAP sources are shown in Table 1.

Table 1 Analyzed nutrients of the tested pork meat meals (g/kg)

		Sonac-50	Sonac-60	Daka-40	Daka-58
DM	(g/kg)	977.5	963.9	968.7	974.8
Ash	(g/kg)	336.2	182.6	437.0	251.6
Organic Matter	(g/kg)	641.3	781.3	531.7	723.2
CP	(g/kg)	558.1	616.8	416.8	596.5
Fat	(g/kg)	95.9	116.5	99.1	118.2
Gross Energy	(MJ/kg)	16.10	19.84	13.57	18.87
Calcium	(g/kg)	116.0	52.0	160.5	82.2
Magnesium	(g/kg)	2.6	2.2	3.6	2.3
Phosphorus	(g/kg)	58.6	29.5	77.3	42.1
Sodium	(g/kg)	6.0	6.9	7.0	7.0
Potassium	(g/kg)	4.3	7.6	4.0	5.8
Amino acids					
LYS	(g/kg)	30.9	32.5	19.7	29.4
MET	(g/kg)	8.5	9.3	4.9	8.2
CYS	(g/kg)	3.7	5.3	1.5	2.7
M+C	(g/kg)	12.2	14.6	6.3	10.9
THR	(g/kg)	17.4	20.7	11.4	18.1
TRP	(g/kg)	4.0	5.1	2.1	4.2
ILE	(g/kg)	15.3	18.3	9.7	17.0
ARG	(g/kg)	38.2	37.7	28.3	38.9
PHE	(g/kg)	17.9	20.1	12.5	19.4
HIS	(g/kg)	10.8	11.3	6.2	10.6
LEU	(g/kg)	33.2	39.0	21.8	36.1
TYR	(g/kg)	12.0	15.5	6.6	13.8
VAL	(g/kg)	22.4	27.2	15.8	24.8
ALA	(g/kg)	40.8	41.6	33.3	44.0
ASP	(g/kg)	42.0	45.4	28.5	43.6
GLU	(g/kg)	68.9	76.7	49.7	74.4
GLY	(g/kg)	72.4	67.7	65.7	76.2
PRO	(g/kg)	46.0	41.6	36.1	48.7
SER	(g/kg)	20.2	23.7	14.8	21.3
Sum AA	(g/kg)	504.6	538.7	368.6	531.2

PAP sources showed large differences in chemical composition. CP content, for instance, ranged from 417 to 617 g/kg, whereas ash content ranged from 183 to 437 g/kg.
In the pre-experimental phase (from week 17 to week 23) a commercial layer meal diet was fed. From week 24 onwards, the respective experimental meal diets were fed. The main ingredients of the basal diet were maize (747 g/kg) and heat treated soybean meal (143 g/kg). The PAP diets were a mixture of the basal diet and one of the pork meat meals. Each PAP-enriched diet contained about 100 g/kg CP of animal origin. Therefore, inclusion levels ranged from 164 g/kg in the Sonac-60 and Daka-58 diet to 220 g/kg in the Daka-40 diet. Furthermore, some vitamins, minerals and synthetic amino acids were added to the diet to meet the NRC requirements (NRC, 1994). The basal diet was formulated as standard diet (Metabolisable Energy 11.9 MJ/kg, digestible lysine 5.5 g/kg). Titanium oxide (1 g/kg) was included in the diets as indigestible marker. Dietary ingredients and calculated nutrients of the diets (in g/kg) are shown in table 2.

Table 2 Dietary ingredients (g/kg) and calculated nutrients of the diet

Ingredient		Basal	Sonac-50	Sonac-60	Daka-40	Daka-58
Soy bean meal heat treated	(g/kg)	142.6	123.4	121.9	122.7	124.0
Maize	(g/kg)	746.8	646.9	639.0	643.4	650.0
Pork Meat meal Sonac-50	(g/kg)		186.5			
Pork Meat Meal Sonac-60	(g/kg)			163.9		
Pork meat meal Daka-40	(g/kg)				220.0	
Pork meat meal Daka-58	(g/kg)					163.5
Oyster shells	(g/kg)	30.4	30.0	30.0	6.6	30.0
Limestone	(g/kg)	60.8	4.2	37.3		25.4
Monocalcium phosphate	(g/kg)	7.2				
Salt	(g/kg)	4.3	3.0	1.9	1.3	1.1
Premix laying hens[1]	(g/kg)	5.0	5.0	5.0	5.0	5.0
Titanium oxide	(g/kg)	1.0	1.0	1.0	1.0	1.0
L-Lysine HCl	(g/kg)	0.9				
DL-Methionine	(g/kg)	0.9				
L-Tryptophan	(g/kg)	0.1				
Calculated nutrients						
DM	(g/kg)	887	899	899	898	899
Ash	(g/kg)	122	117	107	122	156
CP	(g/kg)	130	210	210	205	210
Fat	(g/kg)	37	46	53	52	49
Crude fiber	(g/kg)	22	19	19	21	21
Starch	(g/kg)	457	397	392	394	398
Sugar	(g/kg)	22	19	19	19	19
Ca	(g/kg)	36	36	36	40	36
P	(g/kg)	4,6	15,8	5,7	18,1	9,1
Available P	(g/kg)	2,3	8,8	2,3	10,2	4,8
LYS	(g/kg)	6,7	11,4	11,2	9,4	10,2
M+C	(g/kg)	5.5	6.6	7.1	5.4	5.9
THR	(g/kg)	4.9	7.7	8.1	6.8	7.4
TRYP	(g/kg)	1.4	1.7	1.6	1.5	1.7

[1] Provided the following nutrients per kg of diet: vitamin A, 12,000 IU; vitamin D3, 2,400 IU; vitamin E, 40 mg; vitamin B1, 4.8 mg; vitamin B2, 12 mg; d-panthothenic acid, 16 mg; niacinamide, 48 mg; vitamin B6, 5.6 mg; folic acid, 1.8 mg; vitamin B12, 25 µg; vitamin C, 100 mg; biotin, 0.1 mg; vitamin K3, 4.8 mg; choline chloride, 260 mg; copper, 8 mg (as $CuSO_4.5H_2O$), iron, 65 mg (as $FeSO_4.7H_2O$); manganese 65 mg (as MnO_2); zinc, 50 mg (as $ZnSO_4$); cobalt, 0.4 mg (as $CoSO_4.7H_2O$); iodine, 1 mg (as KI); selenium, 0.4 mg (as $Na_2SeO_3.5H_2O$)

2.3 Excreta collection and apparent digestibility calculations

In week 27, representative samples of excreta were collected semi quantitatively per cage over a period of 4 x 24 h to determine AME_n (Apparent Metabolisable Energy) and apparent digestibility of DM, ash, fat and amino acids. Excreta samples were frozen and stored at -18^{0}C until chemical analysis. The AME_n content was determined by correcting AME values to zero nitrogen values (Hill and Anderson, 1958). Therefore, nitrogen retention (nitrogen intake minus faecal nitrogen excretion) (De Jonge, et al., 2000) was multiplied by the enthalpy of oxidation of uric acid (36.5 kJ g^{-1}) and subtracted from AME.

Apparent digestibility of nutrients (AD) was calculated using the following equation:

$$AD(\%) = (1 – [(Ti_{fd}/Ti_{fc} \times N_{fc}/N_{fd})]) \times 100 \quad (1)$$

where Ti_{fd} and Ti_{fc} represent titanium in feed and excreta, respectively, and N_{fd} and N_{fc} are the nutrients in feed and excreta, respectively.
AME_n and digestibility coefficients of the PAP sources were calculated from the differences of AME_n and digestibility coefficients between the basal and PAP enriched diets.

2.4 Analytical procedures

After defrosting, excreta samples were homogenized and subsequently representative samples were taken. Fresh excreta samples were analyzed on DM and Kjeldahl nitrogen. Dried manure samples were analyzed for DM, ash, faecal nitrogen, fat, gross energy, and amino acids. Feed was analyzed for DM, crude ash, crude fat, nitrogen, gross energy, and amino acids. All samples were analyzed in duplicate. For determination of the DM content, samples were freeze-dried according to ISO method number 6496 (1998). Following freeze-drying, samples were ground to pass a 1 mm screen and kept for analysis. Air-dry samples were dried in a forced air oven at 103^0C to a constant weight according to ISO method number 6496 (1998b). Kjeldahl nitrogen content was measured according to ISO method number 5983 (1997) in fresh samples. CP content was calculated as nitrogen * 6.25. Amino acids (except tryptophan) were separated by ion-exchange chromatography and detected photometrically after post-column derivatisation with ninhydrine using an automated amino acid analyzer according to ISO method number 13903 (International Organization for Standardization, 2005a). Tryptophan was determined by reversed phase C_{18} HPLC with fluorescence detection according to ISO method number 13904 (International Organization for Standardization, 2005b). Crude fat content was determined after acid hydrolysis according to ISO method number 6492 (1999). For determining crude ash content, samples were incinerated at 550^0C in a muffle furnace according to ISO method number 5984 (2002). Gross energy of diet and faeces was determined using an adiabatic bomb calorimeter (IKA-C5003, Janke&Kunkel, Staufen, Germany) according to ISO method number 9831 (International Organization for Standardization, 1998a). Titanium oxide was determined according to the method developed by Short et al. (1996) and further refined by Myers et al. (2004). This method is based on digestion of the sample in sulphuric acid and addition of hydrogen peroxide to produce an intense orange/yellow color that is read colorimetrically at 408 nm by use of an UV- visible spectrophotometer (Varian, CARY 50 probe).

2.5 Housing, birds and management of the performance study

A total of 480 non beak trimmed 21 wk-old layers (Isa Brown strain) were housed in two climate controlled rooms. Each room contained 20 floor pens (0.90 x 1.50 m), whereas each pen contained 12 hens. The experimental period was from 21 to 40 wk of age. The pens were built of wire and hens could see their flock mates in other pens. Each pen contained 4 perches, a feeding trough (length 100 cm), and 3 nipple drinkers. Sand was used as litter on the floor. A laying nest was placed outside each pen. Throughout the experiment, litter quality was maintained by monthly adding new sand. At the start of the experiment, mean body weight of the hens was 1484 g (± 52). To improve conditions for developing feather pecking behavior, stocking density was higher (10.4 hens/m^2) than usual in practice (9.0 hens/m^2). Feed and water were provided for ad libitum consumption. Temperature was set on a constant value of 21 ^{0}C. Hens received a L:D schedule of 16 h of light and 8 h of dark, whereas photoperiod lasted from 1:00 - 17:00 h. Health status of the hens was monitored daily.

2.6 Experimental design of the performance study

Pens were allotted to one of 5 dietary treatments, and each treatment had 8 replicates. In this study, a control diet without PAP was compared with four PAP diets, thereby using the same PAP batches as used in the digestibility experiment: Sonac-50 (treatment 2), Sonac-60 (treatment 3), Daka-40 (treatment 4) and Daka-58 (treatment 5). The nutritional values of these PAP sources (Table 1), that were determined in the digestibility study, were used for the optimization of the diets for the performance study. All diets were isocaloric (11.8 MJ/kg), had similar digestible essential amino acid profiles, and met NRC requirements (table 3) (NRC, 1994). Each PAP-enriched diet contained 50 g/kg protein of animal origin. As a consequence of differences in protein content, PAP inclusion ranged from 8.1% (Sonac-60) to 12.0% (Daka-40). Compared to the control diet, contents of soybean meal, rape seed meal, oyster shells and monocalcium phosphate were reduced in the PAP diets, whereas content of wheat middling's increased. Moreover, to balance the relatively low digestible lysine content in Daka-40 and Daka-58, part of the wheat in diet 4 and 5 was replaced by peas.

Table 3 Dietary ingredients and calculated nutrients of the diets (g/kg, as fed basis)

Ingredient		Control	Sonac-50	Sonac-60	Daka-40	Daka-58
Maize	g/kg	400.0	400.0	400.0	400.0	400.0
Wheat	g/kg	211.0	190.5	192.2	159.0	150.5
Soybean meal extr. DF < 50	g/kg	129.7	62.2	66.1	48.6	47.4
Peas CP < 220	g/kg	75.0	75.0	75.0	125.0	125.0
Oyster shells	g/kg	72.5	48.9	64.8	25.7	58.2
Rapeseed meal extr. CP < 380	g/kg	30.0	30.0	30.0	30.0	30.0
Wheat middlings	g/kg	0.0	65.5	50.9	52.3	65.8
PAP Sonac-50	g/kg	0.0	89.6	0.0	0.0	0.0
PAP Sonac-60	g/kg	0.0	0.0	81.1	0.0	0.0
PAP Daka-40	g/kg	0.0	0.0	0.0	120.0	0.0
PAP Daka-58	g/kg	0.0	0.0	0.0	0.0	83.8
Maize gluten meal	g/kg	24.9	0.0	0.0	0.0	0.0
Limestone	g/kg	20.0	20.0	20.0	20.0	20.0
Soybean oil	g/kg	17.9	9.7	10.0	9.9	9.8
Monocalcium phosphate	g/kg	8.2	0.0	1.1	0.0	0.0
Premix for laying hens[1]	g/kg	5.0	5.0	5.0	5.0	5.0
NaCl	g/kg	3.5	2.1	2.1	1.3	2.0
DL-Met	g/kg	1.2	1.4	1.5	1.9	1.7
L-Lys	g/kg	1.0	0,0	0.1	0.6	0.4
L-Tryp	g/kg	0.1	0.1	0.1	0.3	0.2
L-Thr	g/kg	0.0	0.0	0.0	0.4	0.2
Calculated nutrients						
DM	g	887	890	890	890	890
Ash	g	127	122	123	120	122
CP	g	160	170	170	170	170
NSP	g	135	141	140	140	142
Fat	g	41	41	42	44	42
Crude Fiber	g	27	29	30	29	31
Starch	g	419	412	410	412	409
Reducing Sugars[2]	g	28	25	25	24	25
NDF	g	90	105	100	101	105
ADF	g	35	37	36	38	39
ADL	g	6	7	6	6	6
ME	MJ	11.80	11.80	11.80	11.80	11.80
LYS	g	8.08	8.16	8.15	8.35	8.32
Digestible Lys	g	6.80	6.84	6.70	6.70	6.70
Digestible M+C	g	5.81	5.81	5.81	5.81	5.81
Digestible Thr.	g	4.70	4.70	4.73	4.70	4.70
Digestible Trp.	g	1.50	1.50	1.50	1.50	1.50
Calcium	g	38.00	38.00	38.00	38.00	38.00
Phosphorus	g	5.17	8.63	5.89	12.51	6.88
Available P	g	2.80	4.39	2.80	6.86	3.32
Na	g	1.40	1.40	1.40	1.40	1.40
Cl	g	2.75	2.54	2.45	2.47	2.50
K	g	6.31	5.93	6.06	5.90	6.03
Vit. A	i.e.	11.544	11.544	11.544	11.544	11.544
Vit. D3	i.e.	2.309	2.309	2.309	2.309	2.309
Vit. E	i.e.	38	38	38	38	38

[1] Provided the following nutrients per kg of diet: vitamin A, 12,000 IU; vitamin D3, 2,400 IU; vitamin E, 40 mg; vitamin B1, 4.8 mg; vitamin B2, 12 mg; d-panthothenic acid, 16 mg; niacinamide, 48 mg; vitamin B6, 5.6 mg; folic acid, 1.8 mg; vitamin B12, 25 µg; vitamin C, 100 mg; biotin, 0.1 mg; vitamin K3, 4.8 mg; choline chloride, 260 mg; copper, 8 mg (as $CuSO_4.5H_2O$), iron, 65 mg (as $FeSO_4.7H_2O$); manganese 65 mg (as MnO_2); zinc, 50 mg (as $ZnSO_4$); cobalt, 0.4 mg (as $CoSO_4.7H_2O$); iodine, 1 mg (as KI); selenium, 0.4 mg (as $Na_2SeO_3.5H_2O$).

[2] Mono- and disaccharides as glucose units.

2.7 Observations

Feed intake, body weight, and hen performance
Feed consumption and hen performance per pen were recorded weekly. All hens were weighed in a 4-week interval (average per pen). For the trait 'total egg mass' the entire egg mass production was calculated, assuming that shell-less and cracked eggs had the same weight as the mean 'normal' egg weight of that specific pen in that week. Pen wetness was assessed as percentage of floor area that was visually observed as wet.

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

Eating time
Video observations were recorded in a 6-week interval to calculate eating time of birds in a pen. Eating time was defined as percentage of time birds spend on feed intake during the observation period. An observation day was divided in three blocks, i.e. from 9.00 - 11.30 hrs, 11.30 - 14.00 hrs and from 14.00 until 16.30 hrs. An observation lasted one hour, but to avoid possible disturbances of the camera man at the start and end of the observation period, only the middle 30 observation minutes were analyzed. The number of eating birds (between 0 and 12), was recorded continuously by using Observer 4.1/5.0 software (Noldus, 1993). Eating rate was calculated as feed intake (g/d) divided by number of eating minutes per day. Eating minutes per day were estimated by multiplying the number of minutes with lights on (16 h x 60 min) times the percentage of observed eating time. Eating time and eating rate were averaged per pen per day.

Behavioral recordings
In a 4-week interval, behavioral recordings were scored according to the ethogram as described by Van Hierden (2002). Recordings of gentle feather pecking (without removal of feathers) and severe pecking (leading to feather loss), aggressive pecking, vent pecking and cage pecking were made. Each pen was observed for 10 min, counting each peck. Results were presented as the number of pecks per observed hen per 10 min. Behaviors were classed in five groups: eating, walking and foraging, preening, resting, and drinking. For each pen, an observer scored the number of hens per behavior class at 1-min intervals over a 15 min observation period. Based on these 15 observations, the average number of hens per behavior class was determined and recalculated to percentages of time spent on the different behaviors.

Gut health
At the age of 20, 24, 29 and 35 wk, three hens per treatment were send to the public animal health service for determining gut health status. Classes of scored gut health were 1) no deviation, 2) moderate enteritis, 3) enteritis, and 4) moderate chronic enteritis.

Statistical analysis
The REML variance component analysis procedure tested the effect of the nutritional factors on the determined traits, using the model (2):

$$Y_{ij} = \mu + week_i + treatment_j + week \times treatment + e_{ij} \qquad (2)$$

where Y_{ij} = dependent variable; μ = overall mean; $week_i$ = fixed effect of week i (i = 21, 22 …40); $treatment_j$ = fixed effect of the dietary treatments j (j = 1,2 …5); e_{ij} = the error term (Genstat, 2002). The effects of room and pen were added to the random term of the model. In case of behavioral observations, the model was corrected for the effect of period of the day.

Gut health classifications are analyzed by using the IRCLASS procedure in Genstat (Genstat, 2002). A quadratic model (2) on Ln-scale was used to model development of feather damage:

$$Ln(y_i + 0.1) = \beta_0 + B_{1_i} X + B_{2_i} X^2 \qquad (3)$$

where Y_i is the expected value of feather damage and X is wk of the experiment (1-20).
P-values for week, treatment and week x treatment are presented.

3 Results

Digestibility coefficients of DM, ash, organic matter, CP, fat, GE, and amino acids of the PAP sources are presented in table 4.

Table 4 Digestibility coefficients (%) of DM, ash, organic matter, CP, fat, GE, AME_n (MJ/kg) and digestibility coefficients of amino acids of the PAP sources (sd between brackets)

		Sonac-50	Sonac-60	Daka-40	Daka-58
Digestibility coefficients of nutrients					
DM	(%)	55.74 (4.31)	64.28 (6.26)	38.42 (7.76)	52.63 (3.67)
Ash	(%)	50.23 (8.50)	106.90 (6.46)	31.05 (7.03)	67.14 (3.72)
Organic matter	(%)	64.63 (3.41)	61.33 (6.76)	47.10 (8.69)	53.65 (4.99)
CP	(%)	90.06 (1.93)	83.37 (1.54)	81.43 (3.42)	85.26 (1.05
Fat	(%)	81.20 (2.32)	97.20 (6.18)	79.65 (8.02)	94.49 (3.80)
Gross Energy	(%)	66.61 (2.81)	65.44 (5.58)	55.95 (7.40)	62.01 (2.53)
AME_n	(MJ/kg)	10.72	12.98	7.60	11.70
Digestibility coefficients of amino acids					
LYS	(%)	91.5 (1.18)	86.2 (3.69)	78.1 (3.80)	81.0 (2.52)
MET	(%)	86.0 (1.71)	82.7 (1.81)	75.3 (4.23)	79.9 (1.25)
CYS	(%)	88.4 (2.78)	54.8 (6.07)	41.4 (12.27)	64.5 (7.29)
M+C	(%)	86.7 (1.99)	72.5 (3.35)	67.3 (5.80)	76.1 (2.62)
THR	(%)	84.8 (2.17)	79.2 (3.92)	68.0 (5.29)	77.8 (5.96)
TRP	(%)	90.7 (2.12)	72.5 (7.58)	67.0 (7.43)	71.5 (3.18)
ILE	(%)	87.7 (3.74)	76.8 (4.24)	67.9 (3.26)	77.1 (1.78)
ARG	(%)	94.2 (1.41)	87.5 (2.35)	87.2 (3.73)	91.5 (0.69)
PHE	(%)	90.8 (4.02)	81.9 (2.31)	76.0 (6.30)	80.1 (2.92)
HIS	(%)	92.6 (0.87)	83.2 (4.87)	77.3 (3.44)	82.4 (3.81)
LEU	(%)	89.7 (2.99)	78.8 (2.62)	74.4 (6.20)	79.3 (1.16)
TYR	(%)	92.2 (2.96)	79.4 (5.86)	72.9 (8.36)	77.7 (6.08)
VAL	(%)	88.6 (2.87)	76.7 (1.89)	71.7 (4.71)	76.8 (1.36)
ALA	(%)	83.0 (5.10)	73.1 (2.06)	68.5 (5.31)	71.0 (2.08)
ASP	(%)	86.3 (1.35)	75.5 (5.92)	63.7 (3.73)	70.1 (5.96)
GLU	(%)	90.7 (0.74)	82.6 (2.57)	78.9 (3.88)	81.3 (3.70)
GLY	(%)	68.1 (4.33)	61.3 (2.11)	62.0 (3.65)	58.7 (5.12)
PRO	(%)	91.1 (1.62)	84.9 (3.88)	81.6 (5.33)	85.8 (4.43)
SER	(%)	90.7 (0.54)	80.7 (3.56)	77.9 (4.15)	83.3 (3.90)
Sum AA	(%)	86.4 (1.57)	77.7 (2.82)	73.3 (3.94)	76.5 (2.29)

The digestibility coefficients of the four PAP sources showed a large variation. DM digestibility ranged from 38.4% (Daka-40) to 64.3% (Sonac-60). Digestibility of CP ranged from 81.4% (Daka-40) to 90.1% (Sonac-50). Digestibility of GE ranged from 56.0% in Daka-4- to 66.6% in Sonac-50. AME_n amounted for Sonac-50, Sonac-60, Daka-40 and Daka-58 10.72, 12.98, 7.60 and 11.70 MJ/kg, respectively. Digestibility of amino acids was in general lowest in Daka-40 and highest in Sonac-50.

Performance traits of hens per treatment are summarized in table 5.

Table 5 Performance traits per treatment in ISA Brown laying hens over 21 to 40 weeks of age

Performance trait	Feed intake (g/hen/d)	Rate of lay (%)	Egg weight (g)	Egg mass (g/hen/d)	FCR (feed/egg) (g/g)	Hen weight (g)	wet litter (%)
Control	120.7^{bc}	78.2	59.8	48.9^{ab}	2.17^{bc}	1820	33.0^{a}
Sonac-50	118.9^{c}	79.7	59.4	49.2^{a}	2.13^{c}	1795	24.8^{b}
Sonac-60	124.5^{a}	77.6	59.9	48.4^{abc}	2.30^{a}	1788	32.5^{a}
Daka-40	119.1^{c}	76.5	58.7	46.4^{c}	2.22^{abc}	1767	21.1^{b}
Daka-58	122.3^{ab}	78.2	58.4	47.6^{bc}	2.26^{ab}	1781	32.4^{a}
SE	1.509	1.214	0.595	0.754	0.058	20.78	3.07
P-value							
Treatment	**0.005**	0.163	**0.014**	**0.001**	0.049	0.156	**<0.001**
Week	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Treatment x week	0.321	0.939	**<0.001**	0.998	0.998	0.758	0.427

Feed intake differed between treatments (*P*=0.005) and was highest in the Sonac-60 treatment (124.5 g/hen/d) and lowest in the Sonac-50 (118.9 g/hen/d) and Daka-40 treatments (119.1 g/hen/d). Feed intake levels of the control diet and the Daka-58 treatment were in between. Egg weight of the two Daka treatments (58.4-58.7 g/egg) were significantly (*P*=0.014) lower, compared to the control diet and the Sonac-60 treatment (59.8-59.9 g/egg), whereas egg weight of the Sonac-50 treatment was in between (59.4 g/egg). Differences between treatments, however, were not constant over time (interaction treatment x week; *P*<0.001). Egg mass levels differed between treatments (*P*=0.005) and ranged from 46.4 g/hen/d (Daka-40) to 49.2 g/hen/d (Sonac-50). The amount of feed to produce one kg of eggs also differed between treatments (*P*=0.049) and ranged from 2.13 kg (Sonac-50) to 2.30 kg (Sonac-60). Rate of lay and hen weight were not significantly affected by dietary treatments. All performance traits were significantly affected by week, although no significant week x treatment interactions were found, except for egg weight. The percentage of wet litter in the pens was significantly lower in the Sonac-50 and Daka-40 fed hens compared to the other treatments. Average pen wetness was extremely high in wk 23 (56%), but ranged then from 19.5% (wk 28) to 25.6% (wk 30).

Behavior traits per treatment are summarized in table 6. Hens that were fed PAP diets with the both meat meal sources (Sonac-60 and Daka-58) spent on average more time eating compared to the other three treatments, although differences were not significant in all observation weeks (interaction treatment x week; *P*= 0.003). Average time spent on walking and foraging ranged from 24.8% in Sonac-60 fed hens to 40.1% in Daka-40 fed hens. The time spent on this behavior gradually reduced during the experiment. Differences between treatments, however, were not constant over time (interaction treatment x week; *P*= 0.036). Hens that were fed Daka-40 spent less time on resting and dust bathing behavior compared to the other treatments, although differences between treatments were not significant in all observation weeks (significant treatment x week interactions). Preening and drinking behavior were not affected by dietary treatments. Pecking behavior, eating time and eating rate per treatment are summarized in table 7.

Table 6 Behavior traits (% of time) per treatment, observed by using a scan sampling technique, in ISA Brown laying hens over 21 to 40 weeks of age

Behavioral trait	Eating	Walking & Foraging	Preening	Resting	Drinking	Dust bathing
Control	24.7	29.5	8.5	28.0	7.3	2.1
Sonac-50	25.2	34.3	8.4	24.4	6.2	1.6
Sonac-60	28.9	24.8	10.8	27.1	7.0	1.5
Daka-40	25.1	40.1	7.3	19.8	7.4	0.3
Daka-58	29.9	24.1	9.7	26.3	7.4	2.6
SE	0.90	2.74	1.81	2.16	0.80	1.37
P-value						
Treatment	**0.018**	**< 0.001**	0.393	**0.015**	0.642	0.382
Week	**< 0.001**	**< 0.001**	**<0.001**	**< 0.001**	**<0.001**	**< 0.001**
Treatment x week	**0.003**	**0.036**	0.391	**0.003**	0.196	**<0.001**

Table 7 Pecking behavior (number of pecks/hen/ 10 min.), eating time (% of observation period) and eating rate (g/min) per treatment in ISA Brown laying hens over 21 to 40 weeks of age

Behavioral trait	Gentle FP	Severe FP	Cage Pecking	Ground Pecking	Eating time	Eating rate
Control	0.07	0.002	0.32	1.92	21.18	0.60
Sonac-50	0.08	0.002	0.33	2.29	21.71	0.62
Sonac-60	0.05	0.005	0.39	1.68	24.58	0.51
Daka-40	0.10	0.007	0.30	2.64	20.13	0.59
Daka-58	0.06	0.006	0.19	1.62	25.19	0.59
SE	0.043	0.0045	0.093	0.189	2.358	0.049
P-value						
Treatment	0.720	0.747	0.310	**<0.001**	0.168	0.394
Week	**0.016**	**0.051**	0.125	**<0.001**	**< 0.001**	0.150
Treatment x week	0.124	0.177	0.209	**<0.001**	0.599	0.896

Compared to other types of pecking behavior, the level of severe feather pecking was very low in all treatments. Dietary treatments did not affect gentle and severe feather pecking, head pecking and cage pecking. There was a general trend that feeding diets supplemented with Daka-40 or Sonac-50 increased ground pecking behavior compared to the other treatments. This effect, however, was not constant over time, as was shown by a significant treatment x wk interaction (P<0.001). Ground pecking behavior was similar for all treatments during the first week of the experiment. Moreover, number of ground pecks of Sonac-50 fed hens did not differ from the control diet fed hens in wk 28 and 31, whereas number of ground pecks of Daka-40 fed hens only slightly differ from the control fed hens in wk 31 and 37. Eating time ranged from 20.1 to 24.6%, but was not affected by dietary treatments. Eating rate was not significantly affected by treatments. Development of feather damage from wk 21 to wk 36 (linear part of the curve fitting model), and from wk 36 to wk 40 (quadratic part of the curve fitting model) is shown in table 8.

Table 8 Level of feather damage, and the scope of the linear curve, from start to wk 36, and from wk 21 to wk 40 per treatment in ISA Brown laying hens

	Wk 21 – Wk 36		Wk 36 – Wk 40	
	Feather damage[1]	Linear term of quadratic model relative to control	Feather damage[1]	Quadratic term of quadratic model relative to control
Control	0.62	0.000[ab]	1.48	0.00000[ab]
Sonac-50	0.52	-0.020[a]	1.17	0.000557[ab]
Sonac-60	0.64	0.022[b]	1.36	-0.001182[a]
Daka-40	0.46	-0.035[a]	1.14	0.001195[b]
Daka-58	0.56	-0.003[ab]	1.34	0.000002[ab]
SE	0.092	0.0189	0.149	0.000898
P-value				
Treatment	0.312	0.063	0.128	0.127
Week	**<0.001**	**<0.001**	---	---
Treatment x week	0.550	0.482	---	---

[1] Feather damage scores ranges from 0 (intact plumage) to 5 (fully denuded area)

Average level of feather damage from wk 21 to 36, and from wk 36 to 40 was not significantly affected by dietary treatments. The linear term of the curve fitting model, however, was significantly (*P*=0.063) increased in the Sonac-60 fed hens, compared to the Daka-40 and Sonac-50 fed hens, indicating that the development of feather damage in the latter two treatments over this period was reduced compared to the Sonac-60 fed hens. From wk 36 to 40, however, there was a trend (*P*=0.127) to a higher quadratic term of the curve fitting model in the Daka-40 fed hens compared to Sonac-60 fed hens, indicating that the development of feather damage in the latter over this period was reduced compared to the Daka-40 fed hens.

Gut health scores per treatment per wk are summarized in table 9.

Table 9 Gut health scores per treatment per wk in ISA Brown laying hens (% of dissected hens; 3 hens/treatment/week)

Treatment	No enteritis	Moderate enteritis	Enteritis	Moderate chronic enteritis
		Wk 24		
Control	33.33	0.00	66.67	0.00
Sonac-50	0.00	33.33	66.67	0.00
Sonac-60	0.00	0.00	100.00	0.00
Daka-40	0.00	0.00	100.00	0.00
Daka-58	0.00	0.00	100.00	0.00
		Wk 29		
Control	33.33	33.33	0.00	33.33
Sonac-50	33.33	66.67	0.00	0.00
Sonac-60	100.00	0.00	0.00	0.00
Daka-40	33.33	66.67	0.00	0.00
Daka-58	66.67	33.33	0.00	0.00
		Wk 35		
Control	33.33	33.33	0.00	33.33
Sonac-50	66.67	0.00	33.33	0.00
Sonac-60	33.33	33.33	33.33	0.00
Daka-40	66.67	0.00	33.33	0.00
Daka-58	33.33	33.33	0.00	33.33
P-value				
Treatment		0.998		
Week		0.996		
Treatment x week		**0.001**		

In wk 20, an outbreak of coccidiosis occurred. As a result of this, only one of the fifteen dissected hens in wk 24 showed to have a healthy gut. In wk 29 and 35, more hens had recovered from the coccidiosis, as shown by a larger percentage of hens with a healthy gut. Gut health was affected by an interaction of treatment x week (P<0.001). In wk 24, gut health of the control diet and Sonac-50 fed hens was significantly better, compared to the other three treatments, whereas gut health was not affected by dietary treatments in wk 29 and wk 35.

4 Discussion

4.1 Nutritional value of PAP sources

The tested PAP sources largely varied in chemical composition, digestibility of nutrients and AME_n value, which is in line with earlier findings in literature (Hendriks, et al., 2002; Hendriks, et al., 2004). Especially, the ash and protein contents of the PAP's showed large variations. Ash content ranged from 182.6 g/kg in Sonac-60 to 437.0 g/kg in Daka-40, whereas protein content ranged from 416.8 g/kg in Daka-40 to 616.8 g/kg in Sonac-60. Depending on the ratio of bone to soft tissue used in processing, the finished product is designated as 'meat meal' (containing >55% CP and <4.4% phosphorus) or 'meat and bone meal' (containing <55% CP and >4.4% phosphorus) (Ravindran and Blair, 1993). Based on this definition, Sonac-60 and Daka-58 were classified as meat meals, and Sonac-50 and Daka-40 as meat and bone meals.
The bone fraction is one of the constituents that can affect the composition and protein quality of MBM. Bones contain a high collagen content of about 83% (Eastoe and Long, 1960). Due to its high collagen content and poor amino acid (AA) balance, any increase in bone content of the raw materials may have a negative effect on protein quality. Collagen and gelatine (refined collagen) are deficient in most essential AA, such as tryptophan, sulphur AA, and isoleucine, while they are surfeit in hydroxyproline, proline, and glycine (Boomgaardt and Baker, 1972; Berdanier, 1998). The AA profile of the tested Daka-40 showed that this source contained a high amount of collagen. In comparison, soft offal – muscle, gut and stomach – contain much higher levels of essential AA and will produce meat meals of greater nutritional value. Increased bone or ash content negatively affect protein and energy concentrations (Dale, 1997; Mendez and Dale, 1998; Wang and Parsons, 1998a). In line with these findings, in the current study, a linear negative regression was found between ash content of the PAP's and the AME_n value ($P<0.001$, $R^2=84.0$). CVB, the Dutch office for feed evaluation, developed a linear regression equation to calculate AME_n values of PAP's for adult cocks, based on ash and fat content of PAP's (CVB, 2005):

$$AME_n \text{ (MJ)} = (14200 - 19.15 \times \text{ash content} + 2.51 \times \text{fat content}) / 1000 \qquad (4)$$

Calculated AME_n values, based on this equation, showed a good relationship with the determined AME_n values in the current study ($P<0.001$; $R^2 = 81.5$). Probably, R^2 could be further increased if adult cocks would have been used in the current study.
In the current study a linear negative relationship was found between ash content and protein content ($P<0.001$, $R^2=88.2$). The effect of ash content on protein quality is not totally clear from literature. It is expected that an increase of ash content would negatively affect protein quality, due to the changes in AA concentrations and AA digestibility. Indeed, protein efficiency ratio (chick weight gain per unit of CP intake) decreased from 1.7 to 1.0 as ash content increased from 24 to 35% in two samples of MBM (Johnson and Parsons, 1997; Johnson, et al., 1998). Mean AA digestibility of the 24 and 35% ash MBM samples, however, were not significantly different ($P > 0.05$) (70.8 and 76.3%, respectively), showing that increased bone ash had no negative effect on AA digestibility in the two MBM samples evaluated in the previous studies. Also in the current study, digestibility coefficients of CP of the PAP's were not affected by the ash content ($P=0.739$). Shirley and Parsons (2001) concluded that the negative relationship between protein quality and ash content of MBM is almost entirely due to negative effects on AA balance or profile of the MBM, and not because of reduced AA digestibility. In a study with broilers, however, digestibility of amino acids, with the exception of aspartic acid, threonine, serine, tyrosine, histidine, and cystine, was negatively correlated with ash content, with samples with high ash levels having lower digestibility (Ravindran, et al., 2002).
Differences in pressure during processing of the PAP's, however, might result in differences in AA digestibility. It was shown that true digestibility of most AA, particularly of cysteine and lysine, were significantly decreased by increasing pressures (Shirley and Parsons, 2000). Probably, the relatively low cysteine digestibility in Sonac-60 (55%), Daka-40 (41%), and Daka-58 (65%), compared to Sonac-50 (88%) is partly caused by a high pressure during processing.

4.2 Effect of PAP sources on performance

Feed intake level of the two meat meal diets (Sonac-60 and Daka-58) was higher compared to the intake level of the two meat and bone meal diets (Sonac-50 and Daka-40). Nutritional values of the PAP's were determined in the digestibility study and these values were used by calculating the diets

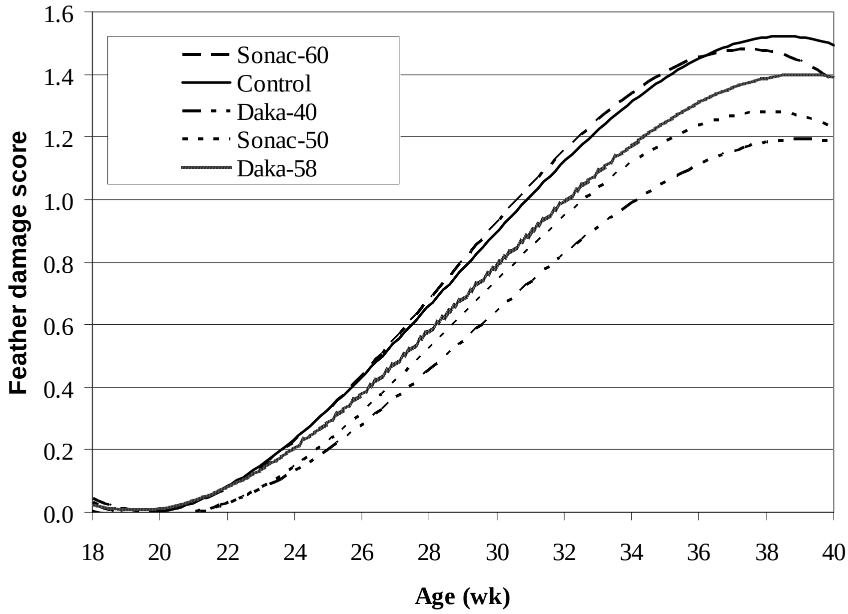
for the performance study. Therefore, it was assumed that the nutritional values of the different diets were similar. Hence, these differences in feed intake were not expected, because it is generally known that laying hens adjust their feed intake to their nutritional need (Van Krimpen, et al., 2007; Van der Meulen, et al., 2008; Van Krimpen, et al., 2008; Van Krimpen, et al., 2009). It is unknown, which specific property in the meat and bone meals was responsible for the reduced feed intake levels.
Egg performances differed between treatments and were most favorable in the Sonac-50 treatment and most adverse in the Daka-40 treatment. Although contents of the digestible essential AA were equated for all diets, AA intake levels differed between treatments due to differences in feed intake level. Because of different feed intake levels and different dietary contents, also intake levels of the non-essential AA differed between treatments. Probably, egg mass production was partly affected by different intake levels of (non)-essential AA. Significant positive linear regressions between intake of digestible ASP (R^2=18.6), CYS (R^2=18.1), GLU (R^2=16.0), HIS(R^2=20.8), ILE (R^2=15.6), LEU (R^2=12.2), PHE (R^2=12.5), SER (R^2=14.9), TYR (R^2=14.2), VAL (R^2=14.1) and egg mass production (g/h/d) were found. Compared to the control diet, egg weights of the PAP-rich diets were similar (Sonac-60) or (slightly) reduced (Sonac-50, Daka-40, Daka-58). Several other studies showed reduced egg weights in PAP-fed hens (Damron, et al., 2001; Bozkurt, et al., 2004). It is suggested that this effect might be associated with the AA concentration of the used PAP's (Parsons, et al., 1997; Wang and Parsons, 1998b; Shirley and Parsons, 2001). In the current study, however, differences in egg weight could only partially be explained by variations in intake of digestible AA. Significant positive linear regressions were found for CYS (R^2=6.9), GLU (R^2=5.1), HIS (R^2=4.3), and SER (R^2=4.5), but levels of declared variance were very low. It can be stated that egg performances in the current study differed between treatments. Performance traits of the PAP-fed hens were reduced, similar or improved compared to the control diet fed hens. Differences seemed to be partly related to differences in feed intake and AA intake.

4.3 Effect of PAP sources on behavior

The UK's Farm Animal Welfare Council stated, though without any conclusive evidence, that 'lack of animal protein in the diet predisposes the flock to injurious pecking leading to cannibalism and death' (Farm Animal Welfare Council UK, 1997). So far, consistent effects of dietary protein source on the incidence of pecking damage in layers have not been demonstrated experimentally, although there have been anecdotal reports of outbreaks of feather pecking and cannibalism after changes in the diet from mainly animal to mainly plant protein (McKeegan, et al., 2001). To prevent feather pecking behavior, feed producers often add some animal protein (e.g. fish meal, meat and bone meal or milk protein sources) to the diet (Hadorn, et al., 1998). It has been suggested that any suppressive effect on feather pecking behavior induced by animal protein is due to something beneficial found only in these protein sources, for instance vitamin B_{12}. However, it is also conceivable that a detrimental compound in plant protein sources could increase feather pecking behavior (McKeegan, et al., 2001). As an example, phytoestrogens could elevate plasma oestradiol concentrations and affect bird behavior. Indeed, greater numbers of vigorous pecks/pulls were observed in pullets up to 24 wk of age that were fed diets based on soybean meal protein compared to pullets that were fed diets based on fish meal protein, although levels were only significantly higher from week 13 to 16 (McKeegan, et al., 2001). Pecking damage scores, and plasma oestradiol and progesterone levels, however, were unaffected by diet in that experiment. Laying hens, that were fed diets with exclusively vegetable protein sources, such as extracted soybean meal, peas, faba beans and extracted sunflower seed, tended to a higher mortality rate due to feather pecking compared with laying hens fed a diet with 4% meat and bone meal (Richter and Hartung, 2003). Laying hens fed diets with 4% meat and bone meal had a better plumage condition at 56 weeks of age compared to laying hens fed diets with only vegetable protein sources or a diet with three sources of animal protein (3% meat and bone meal, 3% blood meal and 1.5% fish meal) (Pfirter and Walser, 1998). Mortality (including cannibalism), however, was unaffected by feeding diets with either animal (herring and meat meal) or vegetable (soybean meal extracted) protein sources (Hadorn, et al., 1998; Hadorn, et al., 1999). On the other hand, feeding diets based on either vegetal (soybean meal), animal (blood meal, fish meal and hydrolysed feather meal) or semi-purified (casein) protein to growing bantams did not result in differences in pecking damage scores between treatments (Savory, 1998; Savory, et al., 1999). Although some evidence suggests a higher incidence of feather pecking in laying hens fed vegetable protein diets, no uniform confirmation can be found in literature.
In the current study, behavioral traits and level of feather damage significantly differed between treatments. These differences, however, did not coincide with the absence or presence of PAP in the diet. The most striking results were a delay in feather damage in the Daka-40 and Sonac-50 fed hens,

compared to the Sonac-60 fed hens. In Figure 1, the fitted values of development of feather damage over time is presented by use of polynomial functions.

Figure 1 Development of feather damage, ranging from 0 (intact plumage) to 5 (fully denuded area), per treatment over time, fitted by polynomial functions



Hens of the former groups also spent more time on walking and foraging, and less time on eating and resting behavior, whereas more pecks were directed to the floor. Feather pecking behavior has been hypothesized to arise from ground pecking behavior or feeding behavior that is redirected towards feathers in the absence of adequate foraging incentives (Hoffmeyer, 1969; Blokhuis, 1986). In line with this, some authors showed that the level of feather pecking is inversely related to the time spent feeding and foraging (Huber Eicher and Wechsler, 1998a; Huber Eicher and Wechsler, 1998b; El lethey, et al., 2001). Increasing feeding related behavior meets the natural drive of laying hens to spend a lot of time on these type of behaviors (Dawkins, 1989). Based on these findings, it could be hypothesized that the delay in feather damage in the Daka-40 and Sonac-50 fed hens could be the result of a shift to more feeding related behavior, as was shown by an increase in foraging and floor pecking behavior in those treatments. These favorable effects, however, were not found in all PAP treatments, indicating that these effects could not be attributed to a general PAP effect. Potentially, these effects could be related to differences in the intake of some particular nutrients from the tested PAP diets. No significant relationships, however, were found between the intake of nutrients and the level of feather damage. On the other hand, significant negative regressions between digestible nutrient intake and foraging and walking behavior were found for the intake of the AA HIS (R^2=22.2), ILE (R^2=17.1), LYS (R^2=22.1), METH+CYS (R^2=32.2), THR (R^2=34.8), TRYP (R^2=41.4), and VAL (R^2=32.5). Apparently, a higher intake of these AA reduced the need of performing feed searching behavior, as expressed by foraging and walking. In contrast, Sonac-60 fed hens consumed significantly higher levels of dig. METH+CYS and THR, compared to Daka-40 and Sonac-50 fed hens.

The intake of available phosphorus was positively related to the expression of foraging and walking behavior (R^2=33.4). Just Daka-40 and Sonac-50 fed hens consumed significantly higher levels of available phosphorus compared to Sonac-60 fed hens (817 and 522 vs. 349 mg/hen/d). A sound explanation for this phenomenon was not found. Potentially, the increased phosphorus intake resulted in improved bone strength and leg health (Webster, 2004), leading to more locomotion behaviors. After exclusion of the control diet (which AA profile strongly differed from the PAP-diets) from the calculations, in the remaining dataset a sound positive regression was also found between the intake of digestible GLY and foraging and walking behavior (R^2=44.4). Just Daka-40 and Sonac-50 fed hens consumed significantly higher levels of dig. GLY compared to Sonac-60 fed hens (1049 and 999 vs. 912 mg/hen/d). Glycine has been considered as an important inhibitory neurotransmitter in the forebrain (Hernandes and Troncone, 2009). The impact of an increased dietary glycine supplementation on the glycine content in the forebrain, and on subsequent feather pecking behavior is unknown. It is shown, however, that low peripheral 5-HT concentration and low 5-HT turnover in the forebrain are related to fearfulness and feather pecking behavior in laying hens (Van Hierden, et al., 2004; Bolhuis, et al., 2009). Increasing dietary tryptophan was shown to increase 5-HT turnover in the forebrain and to decrease gentle feather pecking (Van Hierden, et al., 2004). The ratio of plasma tryptophan to the sum the other large neutral AA (Trp-LNAA ratio) was suggested to affect brain tryptophan and serotonin activities in humans (Markus, et al., 2002). In the current study, however, variations of the dietary Trp-LNAA ratio could not explain differences in feather damage and pen wetness, although a weak negative linear relation between this ratio and walking and foraging behavior was found (R^2=23,9).

Another explanation for the increase in foraging and floor pecking behavior in the Daka-40 and Sonac-50 fed hens could be related to the improved litter condition in those treatments. In the current study, a linear negative regression between pen wetness and foraging and walking behavior was found (R^2 = 46.2%). Clearly, performing foraging behavior is more attractive in dry compared to wet litter. Moreover, loose litter is required for pecking and scratching behavior, preventing birds redirecting their ground pecks to the feathers of other birds (Blokhuis, 1986; Blokhuis and Van der Haar, 1992). Nutritional factors, like dietary protein and mineral contents, could affect litter condition (Veldkamp, et al., 2007; Enting, et al., 2009). In our study, we found significant linear regressions between pen wetness and intake of calcium (R^2 = 45.5), chloride (R^2 = 36.4), potassium (R^2 = 56.4), sodium (R^2 = 45.5). Intake of CP showed a weak relation with pen wetness (R^2 = 10.4). Compared to the Sonac-60 fed hens, just Daka-40 and Sonac-50 fed hens showed significantly lower intake levels of calcium (4730 vs. 4524 and 4520 mg/h/d), potassium (754 vs. 703 and 705 mg/h/d), and sodium (174 vs. 167 mg/h/d).

From this study, it can be concluded that dietary supplementation of PAP's did not generally reduce feather pecking behavior. Despite this, addition of Daka-40, and to lesser extend Sonac-50, to the diet caused a delay in the development of feather damage, and a shift in behavioral traits. These shifts seemed to be partly associated with differences in the intake of digestible glycine, available phosphorus, calcium, potassium, and sodium.

4.4 Effect of PAP sources on gut health

In practice, dietary PAP supplementation was expected to reduce the incidence of chronic enteritis. Chronic enteritis, however, was not observed in the current study at all. Moreover, no general PAP-effect on gut health was observed. The level of enteritis in Wk 24, after the coccidiosis outbreak in wk 20, was not reduced in the PAP-fed hens, whereas gut health scores were not affected by dietary treatments in wk 29 and 35. Theoretically, lower inclusion rates of vegetable protein should decrease the proportion of fermentative degradable carbohydrates in the diet, resulting in a reduced fermentative activity in the gut and herewith positively affecting gut health (Veldkamp, et al., 2008). Moreover, adverse effects of phytoestrogens of plant origin on fowl reproductive development have been reported. Laying hens that were fed diets containing high levels of coumestrol (an isoflavone) exhibited late sexual maturation, depressed egg production and low egg weight (Mohsin and Pal, 1977). Importantly, elevated plasma oestradiol concentrations have been reported in laying hens fed diets containing soybean meal (compared to a diet containing fishmeal as the main protein source) (Maurice, et al., 1979; Akiba, et al., 1982). The discovery of carnosine as well as Angiotensin Converting Enzyme (ACE) inhibitory peptides in meat and fish products has led to the hypothesis that meat and bone meal may have the potential to be a significant source of bioactive peptides (Ovelgonne, et al., 2007). Bioactive peptides are specific protein fragments that have influence on metabolic processes and ultimately may have a positive effect on health. Bioactive peptides could have specific bio-functions, affecting a range of physiological and metabolic processes, such as immune response, behavior, hormonal and neurological response, and gastrointestinal function (Ovelgonne, et al., 2007). No evidence for improved gut health because of PAP-addition to the diet, however, was found in this study.

5 Conclusions

The most important conclusions were as follows:

- The chemical composition of the tested pork meat meals showed a large variation
- Also the digestibility coefficients and the AME_n varied largely between pork meat meals. AME_n ranged from 7.60 MJ/kg in Daka-40 to 12.96 in Sonac-60.
- Feed intake was reduced in hens fed meat and bone meal supplemented diets (Sonac-50, Daka-40), compared to meat meal supplemented diets (Sonac-60, Daka-58).
- Egg mass levels differed between treatments and ranged from 46.4 g/hen/d (Daka-40) to 49.2 g/hen/d (Sonac-50).
- Hens that were fed PAP diets with the both meat meal sources (Sonac-60 and Daka-58) spent on average more time eating compared to the other three treatments, although differences were not significant in all observation weeks.
- Hens that were fed Daka-40 spent less time on resting and dust bathing behavior compared to the other treatments, although differences between treatments were not significant in all observation weeks (significant treatment x week interactions).
- The development of feather damage in Daka-40 and Sonac-50 fed hens over week 18 - 36 was reduced compared to Sonac-60 fed hens.
- The percentage of wet litter in the pens was significantly reduced in the Sonac-50 and Daka-40 fed hens compared to the other treatments.
- In wk 24, gut health of the control diet and Sonac-50 fed hens was significantly better, compared to the other three treatments, whereas gut health was not affected by dietary treatments in wk 29 and wk 35.

Practical applications
Because of large variation in chemical composition and digestibility coefficients of meat meals, it is essential for nutritionist to know the origin and quality of meat meal that will be processed in layer diets. From this study, it can be concluded that addition of protein of animal origin to a layer diet will not guarantee for a reduction in feather pecking behavior and an improvement of gut health. Despite this, feather pecking behavior and percentage of wet litter area were reduced in two (Daka-40 and Sonac-50) of four treatments with protein of animal origin. Hens of those treatments consumed less feed and as a consequence among others less minerals, which resulted in improved litter condition. An increase in dry litter area was linearly related to the occurrence of foraging and ground pecking behavior. Therefore, the retarded development of feather damage in the Daka-40 and Sonac-50 fed hens seemed to be the result of reduced intake of sodium, potassium, chloride and calcium.

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