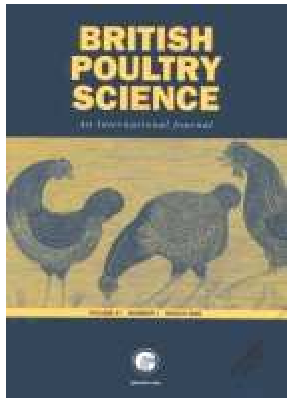

EFFECT OF FEEDING SILAGES OR CARROTS AS SUPPLEMENTS TO LAYING HENS ON PRODUCTION PERFORMANCE, NUTRIENT DIGESTIBILITY, GUT STRUCTURE, GUT MICROFLORA AND FEATHER PECKING BEHAVIOUR

Journal:	*British Poultry Science*
Manuscript ID:	CBPS-2006-164.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	25-Jan-2007
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Keywords:	Feather pecking, Microbiology, Laying hens, Nutrition

Effect of feeding silages or carrots as supplements to laying hens on production performance, nutrient digestibility, gut structure, gut microflora and feather pecking behaviour

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Short title: FORAGING MATERIAL FOR LAYING HENS

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Abstract. 1. An experiment was carried out to examine the suitability of using maize silage, barley-peas silage and carrots as foraging materials for egg-laying hens. Production performance, nutrient digestibility, gastrointestinal characteristics, including the composition of the intestinal microflora as well as feather pecking behaviour were the outcome variables.

2. The protein content of the foraging material (g/kg DM) was on average 69 g in carrots 94 g in maize silage and 125 g in barley-pea silage. The starch content was highest in the maize silage (312 g/kg DM), and the content of NSP varied from 196-390 g/kg, being lowest in carrots. Sugars were just traceable in the silages, whereas carrots contained on average 496 g/kg DM.

3. Egg production was highest in hens fed either carrots or maize-silage, whereas hens fed barley-pea silage produced less (219 *vs.* 208). Although the consumption of foraging material was high (33%, 35% and 48% of the total feed intake on "as fed" basis for maize silage, barley-pea silage and carrots, respectively) only a minor effect on AME_n and apparent digestibility was seen. At 53 weeks of age, hens fed maize silage had AME_n and apparent digestibility values close to the control group (12.61 and 12.82, respectively), whereas access to barley-pea silage and carrots resulted in slightly lower values (12.36 and 12.42, respectively). Mortality was reduced dramatically in the three groups given supplements (0.5-2.5%) compared to the control group (15.2%).

4. Hens receiving silage had greater relative gizzard weights than the control or carrot-fed groups. At 53 weeks of age, the gizzard-content pH of hens receiving silage was about 0.7-0.9 units lower than that of the control or carrot-fed hens. Hens fed both types of silage had higher concentrations of lactic acid (15.6 *vs.* 3.2 µmol/g) and acetic acid (3.6 *vs.* 6.1 µmol/g) in the gizzard contents than the other two groups. The dietary supplements had a minor effect on the composition of the intestinal microflora of the hens.

5. Access to all three types of supplements decreased damaging pecking in general (to feathers as well as skin/cloaca), reduced severe feather pecking behaviour and improved the quality of the plumage at 54 weeks of age.

6. In conclusion, access to different types of foraging material such as silages and carrots improved animal welfare.

INTRODUCTION

Non cage housing systems for egg production (single or multiple tier systems with or without access to range area) have expanded markedly in Denmark within the last 15 years, due mainly to changes in consumer preference. In Denmark, the market share of organic eggs was 14.1% of all eggs in retail in 2005 (Danish Poultry Council, 2006), a trend which reflects an increased interest and concern related to our environment, animal welfare and product quality. Unfortunately, it has been demonstrated in many cases that egg production in alternative housing systems is facing welfare problems in terms of feather pecking and cannibalism. Outbreaks of feather pecking and cannibalism can be caused by a number of factors such as genetic background of the high productive strains used (Kjaer *et al.*, 2001), dietary deficiencies (Ambrosen and Petersen, 1997) or environmental conditions (Savory, 1995; Kjaer and Sørensen, 2002). The Danish law requires that hens in organic egg production have access to some high-fibre feed ingredients such as silages, straw, grass, sugar beets or carrots. In general, feeding with forage seems to have a positive effect on behaviour, and available foraging material in the housing system motivates the hens to spend more time foraging and less feather pecking (Blokhuis, 1986; Nørgaard-Nielsen *et al.*, 1993; Aerni *et al.*, 2000).

Feeding poultry with whole cereals, oat hulls or wood shavings having a large particle size and more structure than pelleted feed stimulates the upper part of the intestinal system and increases gizzard weight and activity (Gabriel *et al.*, 2003; Hetland

and Svihus, 2001; Hetland *et al.*, 2003, Engberg *et al.*, 2004). Furthermore, an increased content of structural components, such as whole grains, is claimed to have a beneficial effect on gastrointestinal health, probably due to increased hydrochloric acid secretion and a fall in gizzard pH, which inhibits acid-sensitive potentially-pathogenic bacteria like Salmonella from entering the lower intestinal tract (Engberg *et al.*, 2004; Bjerrum *et al.*, 2005). Other coarsely-structured feeds may have a comparable effect on the gastrointestinal tract, as shown for maize silage by Idi *et al.* (2005).

The nutritive value of silages to poultry may be minimal, because it has a high content of dietary fibre (non-starch polysaccharides ~ NSP + lignin) which, due to the lack of endogenous fibre-degrading enzymes in the intestine (Scott *et al.*, 1982), is poorly digested by poultry (Longstaff and McNab, 1989; Carré *et al.*, 1990; Steenfeldt *et al.*, 1998; Lazaro *et al.*, 2003). A high intake of soluble NSP can depress performance and the digestibility of other nutrients in broilers (Choct and Annison, 1992*a*; Steenfeldt, 2001), in part because of increased microbial activity (Choct *et al.*, 1996; Smits *et al.*, 1998; Langhout *et al.*, 1999). In contrast to soluble fibre, the insoluble fibre fraction is not much fermented by the microflora (Choct *et al.*, 1996).

Diets with moderate amounts of insoluble fibre can improve starch digestibility in broilers or laying hens (Svihus and Hetland, 2001; Hetland *et al.*, 2002, 2003) and reduce pecking at other birds and mortality from cannibalism in layers (Hartini *et al.,* 2003). If the use of foraging material is to be recommended in practice in alternative production systems for laying hens, it is important to know how it may influence different aspects of production. The purpose of the present study was to examine the effect of using different foraging materials as feed supplements to laying hens on egg production, nutrient digestibility, gastrointestinal characteristics including the composition of the intestinal microflora, as well as aspects of feather pecking behaviour and plumage condition. In the present investigation maize silage, barley-pea silage and carrots were used as forages.

MATERIAL AND METHODS

Diet, foraging material and treatments

The basal layer diet was formulated to contain the nutrients recommended (NRC, 1994) for commercial layer diets but was based on Danish-grown raw ingredients, with the exception of soyabean meal (Table 1.)

Table 1

The control group received the layer diet without any supplement, whereas hens in the other three treatments were fed supplemental foraging material, provided as maize-silage, or barley-pea-silage, or carrots. The supplements were given unprocessed (carrots as cleaned, uncooked roots), and the daily amounts were slightly increased during the experimental period in order to achieve an approximate *ad libitum* intake during the light hours of the day. The layer diet was provided as 3 mm pellets and fed *ad libitum* over the entire experimental period. The treatments will be referred to as LD (layer diet without supplements), LD+MS (layer diet + maize silage), LD+BS (layer diet + barley-pea silage) and LD+C (layer diet + carrots). The experiment was performed over a period of 238 d, from the age of 20 to 54 weeks.

Birds, housing and experimental design

A total of 800 ISA Brown pullets were obtained from a commercial company at 16 weeks of age and placed in 16 identical indoor floor pens with 50 birds/pen, each with a floor area of 8.9 m^2. The hens were not beak trimmed because this process would probably hamper the hens ability to fully exploit the foraging material. In addition, beak trimming is not allowed in organic egg production in Denmark (Danish Ministry of Justice, 1998:

Bekendtgørelse nr. 210 af 6/4-1998**)**. The lighting programme was 12L:12D at 16 weeks. Day length was gradually increased by 1 h per week to 16L:8D at 19 weeks. The 16L:8D programme was planned to continue until termination of the experiment at 54 weeks of age. However, a 23L:1D programme was introduced in error when the hens were 27 weeks of age. The mistake was discovered when the hens were 39 weeks; thereafter the light programme was corrected gradually by reducing day length by 1 h per week until a 16L:8D programme was reached at 46 weeks and continued for the rest of the experiment. The number of hens was reduced to 43 hens per pen after 5 weeks, when the hens were 23 weeks old. The 4 experimental treatments were allocated at random to the 16 floor pens, to provide 4 replicates. The bedding material was wood shavings. The basal diet was given *ad libitum* in one round feeder (34 cm diameter) and water was supplied by nipple drinkers. Each pen was equipped with 9 single nests in a battery, each 28 cm wide. Adjacent pens were separated by thin wooden walls 160 cm high and with wire mesh above (total 200 cm), which allowed auditory contact but prevented visual contact between hens from separate pens and treatments. Supplementation with maize silage or barley-pea silage or carrots started at 20 weeks of age, with fresh material fed each morning in a wooden box (height: 40 cm, length: 50 cm, width: 40 cm). Eggs were collected and recorded from nests every day, and the collection of one day per week was weighed. Feed consumption was recorded every second week. Egg production and feed consumption were calculated on a hen-day basis (taking into account the actual number of hens housed per floor pen at the time of recording). Mortality was noted daily, and body weight was recorded at 25, 39 and 54 weeks of age. The experiment complied with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of birds under study.

Digestibility experiments and calculations

At the age of 23 and 53 weeks, respectively, digestibility experiments were performed in battery cages with raised floors, each of the three batteries contained 12 cages (50x50x50 cm), each with a feeding trough outside and two water cups inside. Two hens were taken at random from each pen and placed in one cage, representing one replicate. The 4 treatments were assigned at random, each treatment being represented in each battery and on each tier. Supplements were given fresh in small portions, once in the morning and once in the afternoon and after the adaptation period of 7 d, the excreta were collected quantitatively on three consecutive days. Pelleted feed and supplements were weighed separately. Excreta were collected three times per day and stored in closed containers at 20^{o}C, immediately after collection to prevent microbial degradation. In addition, two hens per replicate were fed with the silages or carrots alone without access to layer diet to give an indication of the DC of organic matter and AME of the supplements.

The content of polysaccharide residues was calculated as anhydrosugars, and apparent digestibility coefficients were calculated according to the analysed content of nutrients (g/kg DM) in feed (layer diet plus supplements, analysed separately) and in excreta, taking into account the amount of feed eaten (g) and excreta voided (g) on a dry matter basis. The AME_n content was calculated as the difference between gross energy content in feed eaten (layer diet and supplement, separately) and the gross energy in excreta, and corrected to zero N-retention using a value of 34 kJ/g N retained (Hill and Anderson, 1958). N-retention was determined as (N in feed – N in excreta)/g feed intake.

Chemical analyses

The dry matter (DM) content of the layer diet, foraging material and excreta was determined by drying at 105°C for 8 h. Protein (N x 6.25) was determined by the Kjeldahl method 984.13 (AOAC, 1990*a*) using a Kjell-Foss 16200 autoanalyser and energy by a LECO AC 300 automated calorimeter system 789-500 (LECO, St. Joseph, Michigan, USA). Ash was analysed according to method 923.03 (AOAC, 1990*b*), and fat (hydrochloric acid-fat) was extracted with diethyl ether after acid-hydrolysis (Stoldt, 1952). Amino acids were analysed as described by Mason *et al.* (1980). The sugars (glucose, fructose and sucrose), and the oligosaccharides (raffinose, stachyose and versbascose) were extracted with 50% (v/v) ethanol at 60°C and quantified by gas-liquid chromatography (GLC) using the method of Bach Knudsen and Li (1991). Starch was analysed by the enzymatic-colorimetric method of Bach Knudsen (1997). Total NSP and their constituent sugars were determined as alditol acetates by gas-liquid chromatography for neutral sugars and by the colorimetric method for uronic acids using a modification of the Uppsala procedure (Theander *et al.*, 1994) as described by Back Knudsen (1997). Cellulose was determined as the difference in glucose content of NSP when the swelling step with 12 M sulphuric acid was included and omitted, respectively, and the content of cellulose, non-cellulosic (NCP) and soluble NSP was calculated as described by Bach Knudsen (1997). Klason lignin was measured gravimetrically as the residue obtained of the treatment with 12 M sulphuric acid (Theander *et al.*, 1994). All analyses were performed in duplicate.

Gastrointestinal characteristics and intestinal microflora

At 23 and 53 weeks of age, 5 hens from each pen were killed by cervical dislocation and the contents of the gizzard, jejunum, ileum, caeca and rectum were quantitatively collected and pooled by segment before further analyses. Jejunum and ileum were defined as the intestinal segments cranial and caudal to the Meckels diverticulum. The weight of the contents in gizzard, jejunum, ileum and caeca as well the weight of the empty gastrointestinal segments were measured. The pH-value of the contents in all segments was measured with a combined glass/reference electrode (GK 2401C, Radiometer, Denmark), and the dry matter of intestinal contents was analysed after freeze drying of the samples. In gastrointestinal contents, coliform bacteria, lactose-negative enterobacteria, lactic acid bacteria and enterococci were enumerated as described by Engberg *et al.* (2004). Concentrations of short chain fatty acids and lactic acid were analysed (Jensen *et al.*, 1995).

Behavioural and clinical recordings

Recordings of allo-pecking (pecking of one bird by another) were made at 24, 38 and 53 weeks of age. Every pen was observed for 20 min at each age. All pecks at other chickens were recorded. Each peck was counted, and recorded in groups (events) called bouts. A bout was defined as pecks in a continuous series directed to the same chicken and body part (plumage, beak or feet) and of the same type (see below). The inter-bout interval was 10 s. Pecks were classified: 1) severe feather pecking, 2) gentle feather pecking, 3) aggressive pecking, 4) beak pecking, 5) object pecking and 6) pecking at dust particles on dustbathing birds. The two last categories were rarely seen and discarded from further analyses. The distinction between non-aggressive and aggressive pecks was in most cases quite clear, aggressive pecks being vigorous, directed towards the head region and forcing the receiver to react (escape or fight) (Hoffmeyer, 1969; Wennrich, 1975). At 25, 39 and 54 weeks of age a random sample of 15 birds per pen were individually scored for the condition of plumage and skin, as well as for health status of feet and comb. Body weight was also recorded. Plumage and skin condition were scored individually (Tauson *et al.*, 2005), assigning scores of 1, 2, 3 and 4 points for each character. Higher scores means better health. Plumage condition was scored for each of 5 body parts (neck, breast, back, wings and tail). The sum of these 5 scores (‘total score’, 5 to 20 points) was also used for analyses. Less than 1% of birds had wounds (skin score of body part less than 4) and therefore these data were discarded from further analysis.

Statistical analyses

A randomised complete block design was used, a single pen representing the experimental unit (replicate). The results are presented as means and standard error of means (SEM) calculated by standard procedures. Analysis of variance by the general linear model (GLM) procedure (SAS Institute Inc., 1990) was used to determine the significance of treatment and block effects on the dependent variables (production, apparent digestibility, $AME_{n,}$, intestinal values, feather pecking behaviour and plumage score at 54 weeks of age). Normality of data was tested by a univariate procedure, and the means of the variables were tested for variance homogeneity by Bartlett's test. An outlier test based on externally Studentised residuals was performed. No significant outliers were found in the data, and no effect of block. The LS-Means were calculated and differences regarded as significant at $P<0.05$. With respect to production, apparent digestibility and $AME_{n,}$, differences were classified by the Ryan-Einot-Gabriel-Welsch (REGW) multiple range test (SAS Institute Inc., 1990). The results obtained from measurements in the intestinal content of gizzard, duodenum and jejunum, ileum, caeca and rectum (pH, dry matter and bacterial counts) were analysed separately for each segment. In cases in which the overall effect was significant ($P < 0.05$) means were compared pairwise (pdiff). Data on plumage condition at 25 and 39 weeks of age were tested using the Wilcoxon signed rank test (non-normally distributed data).

RESULTS

Chemical analysis of basal diet and foraging material

The silages and carrots were chemically analysed at the beginning of the experiment at 23 weeks of age (I) and at the end at 54 weeks of age (II), which represented the periods where the digestibility experiments were performed (Table 2).

Table 2

The analyses were performed at these times to examine any possible changes in chemical composition of the foraging material over time. The silages and carrots used were from the same harvest year (2000). The DM content of the silages was on average 323 g/kg DM (maize I and II) and 221 g/kg DM (barley-pea I and II), respectively, whereas carrots had a DM content as low as 84 and 125 g/kg DM (I and II). Protein content varied from 65 to 127 g/kg DM, the lowest values measured in carrots. Fat content was generally low, ranging between 12-32 g/kg DM. Starch content averaged 312 g/kg DM in maize silage and 126 g/kg DM in barley-pea silage, but was not detectable in carrots. Overall, the results of the nutrient analyses in samples from the two periods agreed closely.

Only traces of sugars were detected in the silages. In carrots, high concentrations of glucose, fructose and sucrose were measured, constituting 458 g/kg DM in sample I and 533 g/kg DM in sample II. The highest content of total NSP was found in the silages, containing average values of 333 g/kg DM in maize-silage and 376 g/kg DM in barley-pea silage. Cellulose and arabinoxylans (AX), the sum of arabinose and xylose residues, were the dominant NSP residues, constituting approximately 88% and 77% of the total NSP in maize- and barley-pea silage respectively. In barley-pea silage the content of uronic acid averaged 57 g/kg DM. Soluble NSP as a percentage of total NSP averaged 6% in the maize-silage and 12% in the barley-pea silage. The lignin content was high in the silages, with dietary fibre ranging between 403-483g/kg DM. In carrots the dominant NSP residues were uronic acids and glucose monosaccharides, cellulose content averaged 63 g/kg DM, total NSP content was 196 and 203 g/kg DM, and in contrast to the silages, soluble NSP was a very high proportion of total NSP, constituting more than 50%. The total dietary fibre content averaged 232g/kg DM. The two silages were similar with respect to the dietary fibre fraction, but differed with regard to protein and starch. The carrots contained less protein than the silages.

The protein content of the layer diet was close to that calculated (Table 1). The starch content was 365 g/kg DM and fat was 76 g/kg DM. The ME of the layer diet was 11.79 MJ/kg DM, calculated on the basis of the chemical analyses and close to the ME given in Table 1.

Production

Average egg weight was not influenced by the use of silages or carrots (Table 3). The number of eggs and total egg mass (kg) per hen was highest in the group given carrots, whereas hens with access to barley-pea silage (LD+BS) produced fewer eggs per hen than the other groups given foraging material (*P*<0.03).

Table 3

Rate of lay (%) was highest for hens fed carrots or maize-silage when compared to the control group, whereas hens fed barley-pea-silage produced less (*P*<0.04). The unintended change in the light programme at 27 weeks of age did not influence production during the remaining part of the experimental period. The consumption of the layer feed was reduced (*P*<0.004) by supplementation with carrots and barley-pea silage as compared to the LD group. The intake of maize silage, barley-pea silage and carrots was 33%, 35% and 48 %, respectively, as a percentage of total feed intake on "as fed" basis. On a dry matter (DM) basis, the figures were 15%, 11% and 8%. Due to the lower DM content of the foraging material the total feed consumption in kg per kg egg produced was higher in the groups given the silages and carrots compared to the LD group on "as fed" basis (*P*<0.0001). Litter samples were taken at the end of the experiment and the DM content was 78% (LD), 76% (LD+MS), 71% (LD+BS) and 68% (LD+C), where the DM content for the last two groups was lower than for the control (P<0.05). Supplemental foraging material reduced mortality dramatically (*P*<0.02), (Table 3). Approximately 50% of the mortality recorded in the LD group was cannibalism directed against the cloacal region. Body weight was higher in the LD group and in hens fed LD+C at 25 and 39 weeks and at 54 weeks the hens fed LD+C were heavier than all other hens.

Digestibility and AME_n

The coefficients of total tract apparent digestibility (DC) of different dietary constituents and the apparent metabolisable energy, nitrogen corrected (AME_n) are given in Table 4.

Table 4

Three weeks after the hens had been introduced to the supplements (23 weeks of age), there was no significant difference between treatments with respect to the DC of organic matter (average 0.749) and fat (average 0.853). The hens digested starch very efficiently in all groups with DC averaging 0.997, however, the DC of starch was lower for hens fed LD+BS compared to the other groups ($P<0.01$). The DC of total NSP was low, ranging from 0.131 (LD+C) to 0.309 (LD), whereas hens fed with carrots had a significantly lower NSP digestibility as compared to hens fed LD and LD+MS. The N-retention varied from 0.412 (LD+C) to 0.521 (LD+MS), ($P<0.01$). The highest AME_n was seen in hens fed the LD diet and LD+MS, both groups being better than the LD+C ($P<0.01$), reflecting the generally lower DC obtained in hens fed carrots as supplement at this stage of the experiment.

At 53 weeks of age, the DC of organic matter was lowest for the group receiving LD+BS. The DC of fat was significantly lower when hens were fed LD+C as compared to LD + MS (0.804 *vs* 0.861). No significant difference was observed between groups with respect to DC of starch and total NSP. The overall digestibility of total NSP was low, however, the different residues of NSP were digested to a varying degree (data not shown). The major NSP residues arabinose, xylose and galactose appeared to be digested to some extent in all groups (average DC arabinose: 0.320, xylose: 0.202 and galactose: 0.248). Uronic acids, being an important NSP-residue in carrots were poorly digested in hens fed the LD+C diet with a DC close to zero. In general, all groups digested the soluble part of NSP to a higher extent compared with the insoluble part, being on average 0.334 (S-NSP) and 0.219 (I-NSP) at 23 weeks of age, and 0.392 (S-NSP) and 0.208 (I-NSP) at 53 weeks of age (Table 4). The N-retention was found to be in the range of 0.363 (LD+C) to 0.419 (both group LD and LD+BS) with no significant difference. The AME_n varied from 12.1 to 12.8 MJ/kg DM and no significant difference between groups was observed. During the 30 weeks between the two digestibility experiments, the daily intake of the three supplements increased from 31 (MS), 29 (BS) and 45 (C) g/day to 74, 50 and 120 g/day, respectively. The results indicate that high supplemental intake of silages or carrots had only minor effects on the apparent digestibility of the dietary constituents and AME_n. The DM content in excreta (Table 4) was lowest in hens fed LD+C. With respect to the digestibility of the supplements fed without the layer diet (data not shown), on average 55 % of organic matter in the foraging material was digested. The AME_n obtained reached an average value of 11 MJ/kg DM. The results indicate that the supplements to some extent contribute with nutrient and energy to the hens.

Gastrointestinal characteristics and intestinal microflora

Birds receiving supplements of maize silage or barley pea silage had higher relative gizzard weights as compared to the control and birds fed carrots (Table 5).

Table 5

This difference was evident after three weeks on the experimental diets ($P < 0.05$) and was even more pronounced at 53 weeks of the experiment ($P < 0.001$). No significant differences between the dietary treatments were observed at any of the sampling times with respect to the relative weights of the small intestinal segments and the caeca. The relative weight of gizzard contents (Table 5) was likewise higher in birds receiving silage as compared to the other two dietary groups, ($P < 0.01$ and < 0.001 at 23 and 53 weeks of age, respectively). There was also a difference between groups regarding the relative weight of contents in the upper small intestine (duodenum/jejunum). At both sampling times, birds fed with LD+MS had the highest, and birds fed solely with LD had the lowest amounts of small intestinal contents (*P* < 0.05 and < 0.01 at 23 and 53 weeks of age, respectively). Following intake of barley-pea silage, the relative weight of the contents in the upper small intestine increased over time to reach values similar to maize silage at 53 week of age. A similar picture was observed considering the relative weight of ileal contents (Table 5). At 23 weeks, the relative weight of caecal contents was similar in all dietary groups, whereas at 53 weeks, the amount of caecal digesta tended to be lower (*P* = 0.07) in hens receiving supplements of barley pea silage as compared to the other groups. At both sampling times a difference between treatments regarding the percentage of dry matter in the gizzard contents (*P* < 0.01) was observed (Table 5). The dry matter content was lowest in hens supplemented with carrots, and increased slightly when fed with barley pea silage. The highest DM contents in gizzard contents was observed in birds receiving maize silage and in birds fed solely LD (Table 5).

The pH in the contents of the gizzard (Table 6) was not different at 23 weeks of age, whereas at 53 weeks of age a decrease of the pH about 0.7-0.9 units was found in birds fed with both types of silages as compared to birds supplemented with carrots or birds receiving no foraging material (*P* < 0.05).

Table 6

In the upper part of the small intestine, the pH was lower in birds receiving maize silage and carrots as compared to the other groups (*P* < 0.01). At 23 weeks, the pH of the caecal content was not influenced significantly by the dietary treatment, however, at 53 weeks, the caecal pH value was about 0.4 units higher in birds fed the LD as compared to the birds supplemented with the different foraging material (*P* < 0.05).

Counts of intestinal bacteria were conducted on two occasions (23 weeks and 53 weeks of age) and showed that differences between the dietary groups with respect to bacterial composition became more distinct with the progress of the experiment. Because of the large amount of data, only the results obtained at the last sampling are presented (Table 6). No difference between the diets was observed with respect to the number of anaerobic bacteria in the different gastrointestinal segments. Coliform bacteria and lactose negative enterobacteria in the small intestine were more numerous in hens fed with pellets alone as compared to birds supplemented with forages, but these differences were not statistically significant.

Lactic acid bacteria in the contents of the small intestine tended to be fewer in the group fed with barley pea silage compared to the other groups. The numbers of enterococci in the gizzard and small intestine of hens receiving only LD were in the range of 0.6 (gizzard) up to 2 log units (small intestine) higher than in birds supplemented with forage ($P < 0.05$).

The concentration of lactic acid (Table 7) was highest in gizzard contents of birds fed both types of silages as compared to hens fed LD+C and LD ($P < 0.01$).

Table 7

In the upper small intestine the highest lactic acid concentrations were found in small intestinal contents of hens LD+MS followed by LD+BS, LD and LD+C ($P < 0.02$). Birds fed with both types of silages had higher concentrations of acetic acid in the gizzard contents than the other two groups ($P < 0.05$). The amounts of acetic acid found in the contents of the upper small intestine were very low in all groups and did not exceed 1.5 μmol/g contents. In ileal contents, acetic acid concentration was lower in birds receiving supplements of carrots than in the other dietary groups. In caecal contents, the concentrations of both acetic and propionic acid generally tended to be higher in birds fed with maize silage than in the other dietary groups.

Feather pecking and plumage condition

No effects of treatments were found at 24 and 38 weeks of age. At 53 weeks of age the incidence of feather pecking bouts was significantly higher in the control compared to the groups receiving foraging material (Table 8).

<u>Table 8</u>

The number of feather pecks was higher in the control compared to hens fed carrots and with hens fed barley-pea silage and maize silage intermediate. These differences were caused by more severe feather pecking in the control than in forage treatments. Gentle feather pecking bouts were higher in control and barley than in the others, whereas gentle feather pecking pecks did not differ significantly amongst treatments. Treatments did not affect aggressive nor beak pecking. Plumage condition deteriorated over time and birds in the control treatment had in general the worst quality, even though this was only statistically significant for the combined score at 54 weeks of age (Table 8). As regards quality of neck , the barley-pea group had a significantly better score at 54 weeks than control hens. Skin quality was in general very good in all treatments and ages (data not shown).

DISCUSSION

The results show beneficial effects of supplemental feeding of silage and carrots. The hens given access to silages or carrots showed great interest in the supplements after few days of introduction, and the intake of layer diet was reduced in the groups given carrots or barley-pea silage compared to the control. The daily intake of both types of silage was close to 60 g per hen, and the intake of carrots exceeded 100 g per hen, illustrating that the supplement constituted a large proportion of the total feed intake on “as feed” basis. The daily intake of essential nutrients necessary for optimal egg-production was sufficient, in spite of a reduced intake of layer diet. Hvidsten and Herstad (1972) reported that silage of barley grain or barley meal could be used to supplement a layer diet with good results on egg production and feed utilisation. Tkalcic *et al.* (2000) fed ensiled maize grains mixed with a diet based on soyabean meal, meat meal, wheat meal and limestone in order to study the effect on egg-production. They concluded that groups fed diets with 60% of the dry matter intake from ensiled maize silage grain had better laying performance than the control group fed a complete commercial diet, indicating that inclusion of ensiled maize grains instead of dry maize grains had a positive effect on production, in line with the present experiment.

The daily protein intake from the layer diet was 22.2 g (LD), 20.3 g (LD+MS), 18.3 g (LD+BS) and 19.5 g (LD+C) per hen. Protein from the supplements contributed further with 1.8 g (LD+MS), 1.6 g (LD+BS) and 0.8 g (LD+C) daily per hen. Based on the calculated methionine content in the layer diet (Table 1), the daily methionine intake per day per hen from the layer diet ranged from 572 mg (LD) to 472 mg (LD+BS) emphasising that methionine intake was sufficient for egg production, as the recommended requirement is 300 mg/hen /day (NRC, 1994). As earlier reported by Hammershøj and Steenfeldt (2005), the content of methionine in maize-silage and carrots is very low, analysed to be 1.30 and 0.84 g/kg DM, respectively, suggesting that the contribution of foraging material to the total dietary essential amino acid concentration is negligible. A major concern in modern animal husbandry, both with regard to commercial and organic productions, is the excretion of nitrogen and phosphorus to the environment. The N-retention was less efficient when hens were fed with carrots as supplements compared to the other groups. Since no difference in N-retention was found between hens fed LD, LD+MS and LD+BS at any ages, we conclude that the high intake of silages did not increase excretion of nitrogen to the environment.

Dietary fibre (NSP+ lignin) in feed is a complex group of components differing widely in chemical composition, physical properties and physiological activity (Theander *et al.*, 1989; Bach Knudsen, 1997). The content of dietary fibre was high in the foraging material used in the present experiment, especially in the two silages, where the insoluble fibre constituted the main part (~ 90% of NSP). In carrots, the soluble fraction makes up the bulk of the total NSP. It is known that high amounts of soluble NSP in diets can increase intestinal viscosity, often having negative effects in broiler chickens (Bedford and Classen, 1992; Choct and Annison, 1992*b*; Smits *et al.*, 1998; Steenfeldt, 2001). However, adult birds seem to be less sensitive to increased intestinal viscosity created by soluble NSP in cereal-based diets (Salih *et al.*, 1991; Lázaro *et al.*, 2003). The results from the present digestibility experiment indicate that the hens are able to digest a part of the total NSP from diets and supplements, in agreement with studies performed with adult hens or cockerels (Longstaff and McNab, 1989; Carré *et al.*, 1990; Steenfeldt *et al.*, 1995; Lázaro *et al.*, 2003). Furthermore, in the present study the soluble part of NSP was digested to a higher extent than the insoluble fraction, in agreement with Carré *et al.* (1990). It is believed that the main part of the soluble NSP from the supplements are fermented during the passage through the intestinal system, especially in the caeca, and may contribute with energy through production of short chain fatty acids. In an experiment with broiler chickens Jørgensen *et al.* (1996), concluded that NSP fermentation contributed 3-4% of the metabolisable energy intake. Longstaff and McNab (1989) fed adult cockerels with carrots (freeze-dried and tube-fed) and overall average digestibility of soluble uronic acids to be 49.9%, whereas the corresponding figure for insoluble uronic acids was minus 111%. It was concluded that polysaccharide digestibility is very difficult to quantify, due to differences in polysaccharide solubility, when different fibre sources pass through the intestinal system.

The large amount of supplements eaten by the hens in the present experiment had only minor effects on AME_n and total tract apparent digestibility of the measured nutrients. (Table 4). In some recent studies the inclusion of different insoluble fibre sources (oat hulls, wood shavings, cellulose) in poultry diets had positive effects on starch digestibility in both broilers and laying hens (Hetland and Svihus, 2001; Svihus and Hetland, 2001; Hetland *et al.*, 2003).

Hens fed with carrots had a higher final body weight than the other groups, suggesting that large amounts of easily-fermented components as sugars and soluble NSP contribute some energy to the hens, which confirm the results of Lázaro *et al.* (2003). In the present study, hens fed with both types of silage developed larger gizzards than hens receiving carrots or no supplementation, due to increased mechanical stimulation by the increased amounts of dietary fibre retained in the gizzard and to a coarser feed structure (Engberg *et al.*, 2002, 2004; Hetland *et al.*, 2003; Idi *et al.*, 2005). In addition, the pH of the gizzard contents was lower in silage-fed birds, suggesting an increased secretion of hydrochloric acid as a response to the coarse structure of the silage (Engberg, *et al.*, 2004; Idi *et al.*, 2005). The relative weight of contents increased both in the gizzard and in the upper part of the small intestine following intake of the silages, which might indicate a prolonged passage time in these segments of the digestive tract. Hetland *et al.* (2004) propose that accumulation of insoluble fibres in the gizzard results in a slower passage rate of especially the fibre fraction. A full gizzard is likely to make the birds feel more satiated resulting in birds appearing more calm, which, in turn, may contribute to a lower feather pecking pressure.

The composition of the microflora in the digestive tract of the hens was influenced only marginally by the dietary treatments. Coliform bacteria and lactose negative bacteria tended to decrease in small intestinal contents of forage fed birds compared to non-supplemented hens (Table 6). These results are in accordance with earlier findings in broilers, where a coarser feed structure, such as feeding whole wheat, reduced the counts of these gram-negative bacteria compared to pellet feeding (Engberg *et al.*, 2004, Bjerrum *et al.*, 2005). This may be explained by a lower gizzard pH and longer retention time of feed in the gizzard, providing a barrier for acid labile bacteria, including pathogens like E. coli and Salmonella (Bjerrum *et al.*, 2005).

With respect to the lactic acid producing bacteria, only the enterococci were significantly influenced in the entire digestive tract, with the highest counts in hens fed the control diet without forage (Table 6). However, the increase of enterococcus counts in control hens was not reflected by the lactic acid concentration, which was highest in the upper digestive tract (gizzard and duodenum jejunum) of hens fed both types of silages. This might be explained by the contribution of lactic acid in addition to the acetic acid already present in both types of silage.

The lower pH in the caecal contents of hens given foraging material, compared to the controls, may indicate that forage provides some carbohydrate, which escapes enzymatic hydrolysis in the small intestine and is fermented by the caecal microflora. Although not significant, higher concentrations of acetic, propionic and butyric acid in caecal contents of forage-fed hens (Table 7) may point in this direction. Whether the increased caecal fermentation followed by a pH decrease has a significant beneficial influence on gut health, or following absorption of short chain fatty acids contributes to the energy supply of the bird, is a matter of speculation (Jozefiak *et al.*, 2004).

Feeding supplements dramatically reduced mortality in the groups given foraging material (0.5-2.5% *vs.* 15% in the control). Approximately 50% of the mortality recorded in the LD group was identified as cannibalism directed against the cloacal region. Layer diets deficient in protein or methionine and cystine can increase feather pecking and cannibalism (Ambrosen and Petersen, 1997). However, in the present experiment the layer diet contained sufficient essential nutrients to cover requirements, meaning that deficiency in the layer diet cannot explain the high incidence of this kind of cannibalism. Rather it seems that access to supplemental foraging material decreased damaging pecking in general (to feathers as well as skin/cloaca). This reduction in feather pecking was also seen with pheasant chickens given access to green clover, beech branches or range area (Hoffmeyer, 1969; Kjaer, 2004). In a number of studies with laying hens, access to different kinds of foraging material or dietary insoluble fibre resulted in reduced feather pecking and improved plumage condition (Blokhuis, 1986; Nørgaard-Nielsen *et al.*, 1993; Wechsler and Huber-Eicher, 1998; Aerni *et al.*, 2000; Köhler *et al.*, 2001). In addition, El-Lethey (2000) found improved immune response in groups of laying hens with access to foraging material (long-cut straw) compared to controls fed diets without foraging material, which might indicate lower stress. This corresponds well with the fact that divergent selection on feather pecking (Kjaer *et al.*, 2001) seems to affect a range of immune variables (Buitenhuis *et al.*, 2006). The type of feather pecking in this study was predominantly of the severe kind causing damage to the plumage and even feathers pulled out. Therefore it was quite surprising that so few hens had skin damage. Much pecking was directed to the cloacal region and, in many cases, this led to cannibalism and death of the hen prior to the records of pecking damages at 25, 39 and 54 weeks of age. Hughes and Duncan (1972) suggested that there was a distinction between cannibalistic pecking at the cloacal region (vent pecking) and at other parts of the body. All three types of forage reduced severe feather pecking behaviour and increased the quality of the plumage, whereas the effect on gentle, non damaging feather pecking was less clear. The significantly higher total feed consumption of the hens fed silages or carrots could indicate that more time was spent on feeding compared to the control LD, suggesting less time was spent feather pecking in these groups..

In conclusion, access to three types of foraging material, silages and carrots, had beneficial effects on feather pecking behaviour and mortality. In addition, egg-production was improved indicating that high fibre sources, like the silages and carrots, have some nutritional value. Caution must be taken with regard to the use of carrots, because a high intake increases the water content of the excreta, which in turn may result in wet litter increasing the risk of infection with bacteria and parasites. However, moderate amounts of carrots can be recommended. With regard to production, the use of maize silage as supplement was overall a better choice than the barley-pea silage. The very high content of mainly insoluble NSP, including cellulose, in barley-pea silage was probably the reason for the limited feed intake. The results also showed that the influence of forage supplementation on gastrointestinal characteristics depends on the type of foraging material. In contrast to carrots, silages are fermented feeds and provide large amounts of fibre that stimulate gizzard function. Both silage and carrots seem to stimulate caecal fermentation. On the basis of the present results, the provision of foraging material to laying hens can be recommended, and may benefit some aspects of animal welfare.

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Table 1. *Composition of the layer diet (g/kg).*

Ingredients	Diet
Wheat	383
Barley	160
Oat	100
Soyabean meal	136
Fish meal	20
Meat-and-bone-meal	37
Animal fat	27
Soyabean oil	20
Calcium carbonate	70
Dicalcium phosphate	5
Sodium chloride	1
Sodium bicarbonate	2.5
Alfalfa meal	32
DL-Methionine (40%)	4
Vitamin and mineral premix[1]	2.5
Calculated content:	
ME, MJ per kg DM	11.5
Protein (Nx6.25, g/kg)	174
Lysine (g/kg)	8.3
Methionine (g/kg)	4.4
Methionine + cystine (g/kg)	7.2
Calcium (g/kg)	33.5
Total phosphorus (g/kg)	5.4

[1]The vitamin and mineral premix provided per kg of diet: Vitamin A 4.1 mg; Vitamin D_3 0.08 mg; Vitamin E 30 mg; Vitamin K_3 2.5 mg; Vitamin B_1; 1 mg; Vitamin B_2, 5 mg; Vitamin B_6, 2.5 mg; D-pantothenic acid 9 mg; Niacin 30 mg; Betaine anhydrate 338 mg; Folic acid 1 mg; Biotin 0.05 mg; Vitamin B_{12} 0.02 mg; Fe 80 mg; Zn 120 mg; Mn 100 mg; Cu 6 mg; I 0.5; Se 0.3 mg.

Table 2. *Chemical composition of layer diet, maize[1]- and barley/pea silage[1] and carrots[1] (g/kg DM).*

Constituents	Layer diet	Maize silage	Barley/pea silage	Carrots
Dry matter	914.4	317.1-328.8	212.1-230.4	83.7-125.2
Ash	126.6	42.4-37.8	73.3-73.2	63.4-53.8
Protein (Nx6.25)	186.9	98.8-89.4	122.5-126.9	72.8-65.0
HCL-fat	75.9	32.4	29.2-28.3	11.6-16.4
Gross energy (MJ/kg DM)	181.0	19.0-18.7	18.3-18.5	16.1-16.7
Starch	364.9	304.8-319.2	123.5-125.6	t
Sugars				
Glucose	0.4	t	t	150.0-155.5
Fructose	1.0	t	0.5	157.5-142.5
Sucrose	15.6	t	t	150.0-234.5
Raffinose	2.2	t	t	t
Stachyose	6.4	t	t	t
Verbascose	t	t	t	t
Cellulose	33	171-166	201-180	60-66
NCP[2]				
Rhamnose	1	t	3-1	4-5
Fucose	t	t	t	1
Arabinose	23	22-21	16	20-11
Xylose	38	11-98	86-77	3
Mannose	4	3	7	4
Galactose	10	8	9	31-16
Glucose	17	13-12	12	3-4
Uronic acid	9	16	56-58	70-94
NSP[3]	133(29)[4]	342(13)-323(28)	390(47)-361(42)	196(112)-203(112)
Lignin	23	75-80	93-103	30-35
DF (NSP+lignin)	156	417-403	483-464	226-238

[1]Values given represent analyses from the supplement samples taken at hen age 23 (I) and 54 (II) weeks.
[2]Non-cellulosic polysaccharides.
[3]Non-starch polysaccharides (NCP+cellulose).
[4]Values in parentheses are the soluble fraction of NCP.
t=trace.

Table 3. *Production, body weight and mortality of laying hens fed a layer diet with or without supplements of maize silage, barley pea silage or carrots.*

	Layer diet (LD)	Layer diet + maize silage (LD+MS)	Layer diet + barley/pea silage (LD+BS)	Layer diet + carrots (LD+C)	SEM[1]	*P*-value
Egg production, per hen						
Number of eggs	214[ab]	218[a]	208[b]	219[a]	1.61	0.04
Egg mass, kg	13.2[ab]	13.3[ab]	12.8[b]	13.6[a]	0.10	0.03
Egg weight, g	61.5	61.1	61.5	61.9	0.16	0.36
Rate of lay. %	89.9[ab]	91.4[a]	87.2[b]	92.0[a]	0.67	0.04
Feed consumption, per hen						
Layer diet, kg	31.0[a]	28.2[ab]	25.5[b]	27.2[b]	0.63	0.004
Layer diet, g/day	130.1[a]	118.5[ab]	107.3[b]	114.1[b]	2.63	0.004
Silage/carrots, kg	-	14.1[b]	13.8[b]	25.6[a]	1.66	0.0001
Silage/carrots, g/day	-	59.3[b]	58.1[b]	107.6[a]	6.99	0.0001
LD + silage/carrots, g/day	130.1[a]	177.7[b]	165.4[b]	221.7[a]	8.64	0.0001
Silage/carrots, % of total feed intake	-	33.4[b]	35.1[b]	48.5[a]	2.06	0.0001
Feed consumption, kg per kg egg						
Layer diet	2.35[a]	2.12[ab]	2.00[b]	2.00[b]	0.05	0.02
Silage/carrots	-	1.06[b]	1.08[b]	1.88[a]	0.12	0.0001
LD + silage/carrots	2.35[a]	3.18[b]	3.08[b]	3.88[a]	0.15	0.0001
Body weight, g						
25 weeks	1750	1742	1718	1726	18.1	0.59
39 weeks	1889[a]	1796[b]	1795[b]	1915[a]	23.4	0.0001
54 weeks	1813[b]	1787[b]	1805[b]	1917[a]	24.8	0.001
Mortality, % of hens housed	15.3[a]	1.5[b]	2.5[b]	0.5[b]	2.10	0.02

Means in each row followed by different superscript letters [a b] differ significantly.
[1]Standard error of mean.

Table 4. *Apparent metabolisable energy (MJ/kg DM), coefficients of total tract apparent digestibility and nitrogen retention in laying hens fed a layer diet with or without supplements of maize silage, barley pea silage or carrots.*

	Layer diet (LD)	Layer diet + maize silage (LD+MS)	Layer diet + barley/pea silage (LD+BS)	Layer diet + carrots (LD+C)	SEM[4]	*P*-value
23 weeks of age						
Organic matter	0.763	0.760	0.758	0.734	0.01	0.10
Fat	0.872	0.853	0.847	0.833	0.01	0.31
Starch	0.999[a]	0.998[a]	0.992[b]	0.997[a]	0.01	0.01
Total NSP[1]	0.309[a]	0.276[ab]	0.288[a]	0.131[b]	0.02	0.03
S-NSP[2]	0.414	0.429	0.353	0.381	0.02	0.87
I-NSP[3]	0.280[a]	0.245[a]	0.273[a]	0.041[b]	0.02	0.03
N-retention	0.472[ab]	0.521[a]	0.472[ab]	0.412[b]	0.02	0.005
AME_n	13.39[a]	13.41[a]	13.14[ab]	12.78[b]	0.09	0.04
DM in excreta	22.39	17.86	19.88	19.14	0.69	0.11
53 weeks of age						
Organic matter	0.721[a]	0.703[ab]	0.689[b]	0.706[ab]	0.01	0.03
Fat	0.848[ab]	0.861[a]	0.848[ab]	0.804[b]	0.01	0.03
Starch	0.993	0.994	0.983	0.985	0.01	0.08
Total NSP[1]	0.220	0.239	0.264	0.244	0.01	0.70
S-NSP[2]	0.270	0.497	0.543	0.258	0.04	0.34
I-NSP[3]	0.206	0.185	0.201	0.240	0.02	0.60
N-retention	0.419	0.400	0.419	0.363	0.02	0.74
AME_n	12.82	12.61	12.36	12.42	0.08	0.19
DM in excreta	22.31	20.06	21.06	17.00	0.82	0.11

Means in each row followed by different superscript letters [a, b] differ significantly.
[1]Non-starch polysaccharides (NCP+cellulose).
[2]Soluble NSP.
[3]Insoluble NSP.
[4]Standard error of mean.

Table 5. *Relative weights of digestive tract segments and their contents and dry matter content in gastrointestinal contents of hens fed with or without supplements of maize silage, barley pea silage or carrots at 23 and 53 weeks of age.*

	Layer diet (LD)	Layer diet + maize silage (LD+MS)	Layer diet + barley/pea silage (LD+BS)	Layer diet + carrots (LD+C)	SEM[1]	*P*-value
Relative segment weight, g/kg BW						
23 weeks						
Gizzard	20.62 [b]	24.72 [a]	24.87 [a]	22.51 [ab]	1.06	0.04
Duodenum/jejunum	20.14	20.86	19.93	19.97	0.60	0.68
Ileum	7.56	8.53	8.60	8.67	0.52	0.42
Caeca	4.51	4.50	4.57	4.73	0.38	0.97
53 weeks						
Gizzard	16.3 [b]	30.5 [a]	33.0 [a]	19.0 [b]	1.21	0.001
Duodenum/jejunum	16.3	16.1	15.2	15.7	0.64	0.67
Ileum	6.50	7.98	7.35	7.05	0.44	0.17
Caeca	4.67	4.14	4.11	4.30	0.38	0.71
Relative weight of contents, g/kg BW						
23 weeks						
Gizzard	8.35 [b]	11.69 [a]	11.27 [a]	9.89 [ab]	0.60	0.01
Duodenum/jejunum	7.20 [c]	9.69 [a]	7.64 [bc]	9.26 [ab]	0.63	0.04
Ileum	5.64 [b]	6.71 [ab]	5.74 [b]	7.54 [a]	0.41	0.02
Caeca	3.26	3.24	3.64	3.40	0.40	0.89
53 weeks						
Gizzard	8.2 [b]	14.0 [a]	15.9 [a]	8.3 [b]	0.67	0.001
Duodenum/jejunum	9.8 [c]	14.9 [a]	13.5 [ab]	11.2 [bc]	0.76	0.004
Ileum	5.50	7.59	6.55	6.58	0.65	0.21
Caeca	3.80	3.18	2.21	3.78	0.43	0.07
Dry matter content, %						
23 weeks						
Gizzard	34.13 [a]	34.02 [a]	29.24 [b]	24.19 [c]	1.59	0.002
Duodenum/jejunum	19.00 [a]	18.72 [a]	18.39 [a]	15.81 [b]	0.70	0.03
Ileum	22.88	20.87	21.83	19.47	0.82	0.07
Caeca	22.79	22.53	23.68	21.53	0.95	0.48
Colon/rectum	25.36	24.11	23.80	23.60	0.75	0.38
53 weeks						
Gizzard	33.56 [a]	29.46 [b]	23.96 [c]	21.71 [c]	1.09	0.001
Duodenum/jejunum	21.29 [a]	18.66 [b]	18.54 [b]	19.20 [b]	0.49	0.01
Ileum	29.76	19.88	20.17	19.87	3.69	0.21
Caeca	28.56	26.14	25.02	24.16	1.31	0.15
Colon/rectum	22.44	20.98	19.23	20.32	0.91	0.14

Means in each row followed by different superscript letters [a b c] differ significantly.
[1]Standard error of mean.

Table 6. *Counts of total anaerobic bacteria, coliform bacteria, lactose negative enterobacteria, lactic acid bacteria, enterococci (log cfu/g digesta) and pH in the digestive tract of hens fed with or without supplements of maize silage, barley pea silage or carrots.*

	Layer diet (LD)	Layer diet + maize silage (LD+MS)	Layer diet + barley/pea silage (LD+BS)	Layer diet + carrots (LD+C)	SEM[1]	*P*-value
Anaerobic bacteria						
Gizzard	7.68	7.15	7.54	7.54	0.25	0.49
Duodenum/jejunum	8.52	8.06	8.23	7.97	0.26	0.50
Ileum	8.59	8.51	8.77	8.52	0.14	0.52
Caeca	10.06	9.79	9.95	10.21	0.13	0.21
Colon/rectum	9.15	8.76	8.96	8.84	0.18	0.48
Coliform bacteria						
Gizzard	4.54	3.72	4.22	4.14	0.32	0.38
Duodenum/jejunum	5.73	4.28	5.13	4.90	0.44	0.19
Ileum	7.29	7.07	6.59	6.42	0.27	0.17
Caeca	7.39	7.15	7.28	7.31	0.17	0.78
Colon/rectum	7.08	7.48	7.24	7.10	0.27	0.71
Lactose negative enterobacteria						
Gizzard	3.76	3.43	3.47	3.46	0.18	0.54
Duodenum/jejunum	4.67 [a]	3.06 [b]	3.85 [a]	3.85 [a]	0.34	0.04
Ileum	6.40	5.76	5.46	5.82	0.26	0.19
Caeca	6.06	5.92	5.52	6.08	0.28	0.50
Colon/rectum	6.18	5.91	5.65	6.08	0.25	0.47
Lactic acid bacteria						
Gizzard	7.63	7.49	7.49	7.69	0.41	0.98
Duodenum/jejunum	8.47	8.38	7.99	8.43	0.20	0.32
Ileum	8.96	8.73	8.46	8.79	0.16	0.23
Caeca	9.53	9.43	9.56	9.68	0.10	0.40
Colon/rectum	8.93	8.84	8.60	9.15	0.13	0.06
Enterococci						
Gizzard	5.64 [a]	3.89 [b]	4.04 [b]	5.05 [a]	0.27	0.002
Duodenum/jejunum	6.85 [a]	4.70 [c]	4.70 [c]	5.74 [b]	0.27	0.001
Ileum	7.72 [a]	6.49 [b]	6.01 [b]	6.55 [b]	0.32	0.02
Caeca	7.96	7.43	6.97	7.95	0.25	0.05
Colon/rectum	7.41	6.85	6.82	7.16	0.29	0.44
pH						
Gizzard	4.82 [a]	3.91 [b]	3.90 [b]	4.64 [a]	0.21	0.02
Duodenum/jejunum	6.27 [a]	5.92 [b]	6.22 [a]	6.08 [ab]	0.06	0.01
Ileum	7.48	7.44	7.57	7.17	0.18	0.47
Caeca	6.53 [a]	6.18 [b]	6.14 [b]	6.14 [b]	0.10	0.04
Colon/rectum	7.19	7.14	7.21	7.17	0.26	0.99

Means in each row followed by different superscript letters [a b] differ significantly.
[1]Standard error of mean.

Table 7. *Concentrations of lactic - and acetic acid in the digestive tract and caecal concentrations of butyric and propionic acid (µmol/g digesta) of hens fed with or without supplements of either maize silage, barley pea silage or carrots.*

	Layer diet (LD)	Layer diet + maize silage (LD+MS)	Layer diet + barley/pea silage (LD+BS)	Layer diet + carrots (LD+C)	SEM	*P*-value
Lactic acid						
Gizzard	4.7 [b]	16.9 [a]	14.3 [a]	1.6 [b]	2.81	0.01
Duodenum/jejunum	16.4 [b]	25.0 [a]	19.6 [ab]	13.5 [b]	2.08	0.01
Ileum	13.9	22.3	12.8	17.5	3.83	0.34
Colon/rectum	13.5	16.6	14.1	17.0	3.65	0.84
Acetic acid						
Gizzard	3.5 [b]	5.9 [a]	6.2 [a]	3.6 [b]	0.71	0.03
Duodenum/jejunum	1.5	0.2	1.3	0.7	0.65	0.41
Ileum	9.7 [a]	11.5 [a]	9.7 [a]	6.8 [b]	0.82	0.01
Colon/rectum	26.1	24.5	24.0	25.0	4.43	0.99
Caeca						
Acetic acid	70.0	91.5	77.0	74.3	7.30	0.24
Propionic acid	25.0	32.0	27.0	27.3	2.67	0.35
Butyric acid	13.6	17.1	16.5	18.8	2.06	0.39

Means in each row followed by different superscript letters [a b] differ significantly.
[1]Standard error of mean.

Table 8. *Allo-pecking at 53 week of age (LSmeans of bouts/pecks per bird per hour) and plumage condition at 54 weeks of age of hens fed with or without supplements of either maize silage, barley pea silage or carrots.*

	Layer diet (LD)	Layer diet + maize silage (LD+MS)	Layer diet + barley/pea silage (LD+BS)	Layer diet + carrots (LD+C)	SEM[3]
Feather pecking					
-Total, bouts	0.92[a]	0.21[b]	0.44[b]	0.20[b]	0.09
-Total, pecks	1.58[a]	0.57[ab]	0.95[ab]	0.36[b]	0.15
-Severe, bouts	0.56[a]	0.01[b]	0.03[b]	0.07[b]	0.08
-Severe, pecks	0.60[a]	0.03[b]	0.04[b]	0.09[b]	0.08
-Gentle, bouts	0.36[a]	0.20[b]	0.42[a]	0.13[b]	0.04
-Gentle, pecks	0.98	0.54	0.91	0.27	0.15
Aggressive pecking					
-Bouts	0.21	0.18	0.19	0.23	0.02
-Pecks	0.22	0.21	0.21	0.24	0.02
Beak pecking					
-Bouts	0.25	0.23	0.10	0.17	0.04
-Pecks	0.98	0.78	0.32	0.51	0.17
Plumage condition [1]					
-Total score [2]	13.9 [b]	18.3 [a]	19.2 [a]	16.3 [ab]	1.24
Separate body parts					
-Neck	3.2	3.8	3.9	3.6	0.19
-Breast	2.4	3.1	3.5	3.1	0.26
-Back	2.4	3.8	4.0	3.0	0.38
-Wings	2.4	3.8	3.9	3.6	0.21
-Tail	2.6	3.8	3.9	3.1	0.33

Means in each row followed by different superscript letters [a b] differ significantly ($P < 0.05$).
[1]Maximum of 4 points for perfect plumage, minimum of 1 point for a very poor plumage.
[2]Total score is the sum of body part scores, max. 20 and min. 5 points.
[3]Standard error of mean.