

Whole wheat versus mixed layer diet as supplementary feed to layers foraging a sequence of different forage crops

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In many cases health and welfare problems are observed in organic egg production systems, as are high environmental risks related to nutrient leaching. These disadvantages might be reduced if the layers are allowed to utilise their ability to forage to a higher degree thereby reducing the import of nutrients into the system and stimulating the hens to perform a natural behaviour. However, very little is known about the ability of modern high-producing layers to take advantage of foraging to cover their nutritional needs, and the aim of the present work was to clarify this subject. Six flocks, each of 26 hens and one cock, were moved regularly in a rotation between different forage crops for a period of 130 days. Half of the flocks were fed typical layer feed for organic layers and half were fed whole wheat. The forage crops consisted of grass/clover, pea/vetch/oats, lupin and quinoa. At the beginning of the experiment, wheat-fed hens had a lower intake of supplementary feed (wheat) and a lower laying rate, egg weight and body weight. However, after a period of 6 to 7 weeks, the intake of wheat increased to approximately 100 g per hen per day and the laying rate increased to the same level as for the hens fed layer feed. For both groups of hens egg weight and body weight increased during the remaining part of the experiment. Crop analysis revealed different food preferences for hens fed layer feed and wheat-fed hens. Wheat-fed hens ate less of the cultivated seeds, whereas the amounts of plant material, oyster shells, insoluble grit stone and soil were larger in the crops from wheat-fed hens. Floor eggs were significantly more frequent in the hens fed layer feed, whereas wheat-fed hens only rarely laid floor eggs. Irrespective of treatment, hens were found to have excellent health and welfare. We conclude that nutrient-restricted, high-producing organic layers are capable of finding and utilising considerable amounts of different feed items from a cultivated foraging area without negative effects on their health and welfare.

Keywords: animal welfare, crop content, feed intake, free-range behaviour, organic egg production

Introduction

The potential contribution of vegetation and earthworms, insects, etc. from the outdoor area has been overlooked in organic egg production systems to a great extent, presumably because the production systems of today do not support a proper utilisation of the outdoor areas by poultry. Typically, an organic egg production system is characterised by having a high animal density in the henhouse and immediately outside the house, since the hens do not use the outdoor area very much (Keeling *et al.*, 1988; Hegelund *et al.*, 2005). This may be due to an unattractive range area because there is no overhead cover and because feed is provided indoors (Bubier and Bradshaw, 1998). This involves a considerable risk of welfare problems (Bestman and Wagenaar, 2003), leaching of nutrients to the ground

water and parasitic infections (Permin *et al.*, 1998 and 1999). Moreover, the feeding strategies in organic egg production systems are widely based on purchased feed, synonymous with a huge import of nutrients to the system.

A higher degree of utilisation of local resources in the shape of cultivated forage vegetation in the hen yard could increase the utilisation of the hen yard and thus the welfare of the hens. In addition, if hens are capable of finding and utilising valuable feed items from the outdoor area, the nutrient standards of the imported feed can be adjusted, leading to a lower import of nutrients to the system. This could benefit the environment because of the increased circulation of nutrients within the system, but also the economy of the farmer. Feed costs constitute the largest expenditure in organic egg production systems (Walker and Gordon, 2003), so even a small reduction in feed costs will be noticeable. Historical studies have shown that access to forage vegetation reduces the intake of

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supplementary feed and thereby the feed costs (Sipe and Polk, 1941; Buckner *et al.*, 1945). Fuller (1962) also showed that the savings were larger if pullets were forced to forage by providing them only grain and minerals. Even though hens at that time were not as high-producing as the hybrids of today, a more recent study with ISA Brown hens indicates that supplementing maize silage or carrots reduced the intake of a commercial layer feed without compromising egg production (Steenfeldt *et al.*, 2001).

More recent studies of the nutritional effect of feed intake from pasture on hens are scarce. However, studies by Gustafson and Antell (2005) indicate that hens foraging on oilseed, sunflower and wheat cropping systems are capable of supporting their nutritional needs through weeds and other feed items found in the vegetation. This is supported by studies on the crop content, indicating that hens have a considerable intake of herbage and other accessible feed items (Wood *et al.*, 1963; Mwalusanya *et al.*, 2002).

In a previous study we investigated the short-term effect (3 weeks) of different forage crops (grass/clover, chicory and a mixture of forbs) and two types of supplementary feed (commercial layer feed *v.* whole wheat and oyster shells) on productivity in organic layers (Horsted *et al.*, 2006). The results indicated that the foraging areas contributed to the nutrition of the hens, even though hens fed whole wheat showed a declining egg production during the 3 weeks. However, hens with access to chicory showed only a lower egg weight compared with hens fed layer feed, whereas the laying rate was maintained. For all forage crops, hens on the wheat diet were not able to eat sufficient whole wheat to fulfil the requirement for metabolisable energy (ME). However, since gizzards have been found to be heavier during a long period with roughage supplementation (Steenfeldt *et al.*, 2001), hens might be able to increase their capacity for eating coarse feed.

It is the hypothesis that a rotation between different forage areas may be a way to optimise the contribution of nutrients from the range area over a long period of time depending on the type of supplementary feed, and that such an effect is reflected in the production, health and welfare parameters. Thus, the aim of the present study was to evaluate how feed intake, egg production, health

and welfare develop in a system where hens are moved in a rotation between different forage crops and where hens are restricted or unrestricted in nutrient supply. In the present study, hens were restricted in nutrient supply through the supply of whole wheat and oyster shells as only supplementary feed. This rather drastic nutrient restriction was chosen to evaluate the capacity of the hens to utilise the forage material.

Material and methods

Experimental design

Six flocks of 26 hens and one cock each were moved regularly in a rotation between different forage crops. Half of the flocks were fed supplementary feed consisting of layer feed for organic layers and the other half were fed a supplementary feed of whole wheat. Three plots of each type of forage plants were alternately distributed on the experimental field with 4 m between plots to minimise any inter-plot effects. Each plot measured 420 m² and was subdivided into two subplots according to feed type, resulting in subplots of 210 m² (12 × 17.5 m). At introduction, hens were randomly distributed in the subplots, and at shifts to new forage vegetations the established flocks were randomly allocated to the new subplots. The experimental period was 130 days (23 June to 31 October). The length of each period in the experiment was related to the length of time during which forage material was available in the plots. Thus, if the vegetation was of poor nutritional value when the hens were moved to a new forage area.

Forage vegetation

The experiment was carried out as a rotation between four different forage crops as shown in Table 1. The grass/clover pasture recovered rapidly after removal of the hens, which is why hens could return to grass/clover plots after removal from one of the other forage plots. The grass/clover pasture was well established (6 years old) and consisted primarily of *Trifolium repens*, *Lolium perenne* and *Elytrigia repens*. To a lesser degree, *Taraxacum* sp. was observed in the plots. The other forage crops were sown in spring. For the pea/vetch/oats (*Pisum sativum/Vicia sativa* ssp.

Table 1 Periods at different forage plots, and dates of slaughtering of hens for crop analyses

Period no.	Period	No. of days	Forage crop	Slaughtering of hens (two per subplot)	
				Date	No. of hens
1	23.06.05–11.07.05	18	Grass/clover		
2	11.07.05–01.08.05	21	Pea/vetch/oats	19.07.05	12
3	01.08.05–15.08.05	14	Grass/clover	05.08.05	12
4	15.08.05–05.09.05	21	Lupin	18.08.05	12
5	05.09.05–23.09.05	18	Grass/clover		
6	23.09.05–27.10.05	34	Quinoa	04.10.05	12
7	27.10.05–31.10.05	4	Grass/clover	28.10.05	48

Sativa/Avena sativa) the cultivars 'Julia', 'Carole' and 'Markant', respectively, were used and for the lupin (*Lupinus angustifolius*) and quinoa (*Chenopodium quinoa*) the cultivars 'Prima' and 'Atlas', respectively, were used. The latter was a variety without bitter saponins that adversely affect palatability (Reichert *et al.*, 1986; Ridout *et al.*, 1989). Therefore, this cultivar was expected to be edible for the hens directly from the non-harvested plant without further treatment of the seeds. When hens were foraging the quinoa plots, some of the tall quinoa plants (about 1.5 m) were manually bent over daily to make the seeds accessible to the hens.

Pre-experimental handling of birds

The hens were of the strain 'Hyline Brown' and arrived from the breeders at approximately 17 weeks of age. The pullets were established in a house with access to an outdoor area with vegetation (weeds), where they were kept until the experiment commenced 3 weeks later. The pop holes were open at all times giving the hens access to the hen yard day and night. The flock was fed a commercial layer feed as well as oyster shells (32% calcium) and insoluble grit stone during this adaptation period. In addition, 3 kg whole wheat was spread out in the hen yard daily to introduce the hens to this feed. Feed and water was given *ad libitum* both inside and outside the henhouse. The hens were introduced to the experiment at 20 weeks of age.

Housing and feeding of experimental birds

In the experiment the hens were housed in henhouses of 4.6 m² with five nesting boxes (40 × 40 cm each) placed on the outside of the henhouse. Pop holes were open day and night, and the hens thus foraged from sunrise to sunset. Supplementary feed and water was given *ad libitum* outside the henhouse just as oyster shells (32% calcium) and insoluble grit stone, i.e. feed and water was always available. Table 2 shows the nutrient content in the layer feed and wheat as well as the quinoa seeds.

Recordings

Dry matter (DM), nutrient content and herbage mass per hectare were determined for the forage crops shown in Table 3 prior to introduction of the hens. Herbage was harvested in two randomly chosen patches (0.25 m²) in each subplot and cut approximately 2 cm above ground level. This method was not applicable in the quinoa, because of the heights of these plants. The grass/clover was analysed in only two of the four periods during which the hens had access to it (Table 3). The harvested biomass was weighed for each square and a representative sample from each plot taken and stored at -20°C until analysis. All samples were analysed for DM and crude ash, and a representative sample from each type of forage crop was taken for crude protein, crude fat, starch and sugar determinations. Crude protein was determined by the Kjeldahl method (Association of Official Analytical Chemists (AOAC), 1990) and ash according to method 923.03 (AOAC, 1990). Crude fat

Table 2 Dietary composition and nutrient content of layer feed, wheat and quinoa seed (wet weight)

	Layer feed	Wheat	Quinoa seed
Dietary composition (%)			
Quinoa			100
Wheat	40.75	100	
Oats	10.00		
Maize gluten 60%	6.32		
Ground limestone	6.32		
Maize	6.29		
Barley	5.00		
Soya bean, toasted	5.00		
Sunflower cake	5.00		
Fish meal	4.00		
Potato protein, concentrate	4.00		
Oyster shells	3.00		
Peas	2.62		
Mono calcium phosphate	1.03		
Sodium bicarbonate	0.28		
Vitamins	0.25		
Rock salt	0.11		
Bergazym P	0.03		
Nutrient content (%)			
Crude protein (N × 6.25)	19.6	10.6	16.0
Crude fat	3.7	1.8	5.4
Starch	38.7	59.0	50.7
Sugar	1.7	2.4	2.8
Calcium	3.4	<0.1	0.1
Phosphorus	0.7	0.4	0.6
Amino acids (g/kg)			
Lysine	8.8	2.6	8.3
Methionine	3.3	1.4	2.6
Cystine	3.0	2.4	2.6
Threonine	6.9	2.8	4.8
Metabolisable energy (MJ/kg)	11.0	12.4	13.2

was extracted with diethyl ether after acid-hydrolysis (Stoldt, 1952) and the sugars were extracted with 50% ethanol at 60°C and quantified by gas-liquid chromatography (Bach-Knudsen and Li, 1991). Starch was analysed by the enzymatic-colorimetric method (Bach-Knudsen, 1997).

All hens were weighed and an assessment of clinical indicators of health and welfare was made on the day the hens were introduced to the experiment and each time the hens were moved (in transportation crates) between plots. However, no welfare assessment was made when the hens were moved from the quinoa plots to the grass/clover plots (period 6 to 7, Table 1). Instead, an assessment was made after 21 days in the quinoa plots. At this time no green fodder was left in the plots, but quinoa seeds were abundant, so the hens continued in these plots for a further 9 days. The welfare assessment included an evaluation of plumage condition of neck, thorax, back, wings and tail (score 1–4), boils on feet (score 1–4), wounds on feet (score 1–3), keel bone (score 1–4), pubic bone (score 1–3) and colour and wounds of the comb (score 1–3) using

Table 3 Dry matter (DM) content, nutrient content and herbage mass per ha of the forage crops prior to introduction of the hens, means (s.d.)

Period no.	Forage crop	DM and nutrient content (% DM)						DM per ha (kg)
		DM (%)	Crude ash	Crude protein [†]	Crude fat	Starch	Sugars	
1	Grass/clover	14.9 (0.8)	10.7 (0.9)	16.7	2.2	2.3	7.8	3020 (432)
2	Pea/vetch/oats	23.7 (2.0)	9.3 (2.4)	19.1	2.2	11.0	3.3	9107 (1822)
4	Lupin	33.8 (6.7)	11.5 (1.4)	15.5	2.2	1.5	1.7	4513 (821)
5	Grass/clover	15.3 (0.9)	13.0 (1.6)	25.6	2.9	2.1	6.3	1507 (459)

[†] N × 6.25.

the standardised methods described by Tauson *et al.* (1984) and Gunnarson *et al.* (1995).

Intake of layer feed or wheat was measured Monday and Thursday mornings each week by weighing the feeding silos. Intake of oyster shells was made up each time hens were moved from one forage crop plot to another. Eggs were collected daily in the afternoon and the number of floor eggs recorded (incl. eggs in the forage crops). Daily average egg weight for each subplot was calculated.

Two hens randomly chosen from each subplot were slaughtered in the evening on the day of slaughter (Table 1), since the crop could be expected to be full at this time of day (Mongin, 1976). Immediately after slaughter the hens were dissected and the crop removed and stored in a freezer at -18°C for later analysis.

Analysis of crop content

The individual crop was thawed and the contents separated by forceps into the fractions: 'supplementary feed (layer feed or wheat)', 'plant material', 'seeds from cultivated plants', 'seeds from wild species of plants', 'insects', 'earthworms, larvae and pupae', 'oyster shells', 'grit stones' and 'soil'. All fractions were dried in a forced air-drying oven at 60°C for approximately 24 h or until the samples were completely dry. Since DM determinations of crop contents in the study by Antell and Cizuk (2006) did not differ from air-dried crop contents, calculation on crop contents in this study is based on air-dried fractions of crop contents.

Statistical methods

In the experiment the forage crop and time of experiment (age of hens) were confounded and as a consequence these effects could not be estimated unbiased. However, the interaction between supplementary feed and time was of interest and, therefore, the data set was analysed in one model including period and its interaction with supplementary feed. In the presentation and the interpretation of the results this confounding has been acknowledged. Data on egg production, feed intake, body weight and crop content were subject to analysis of variance using the MIXED procedure in Statistical Analysis Systems Institute (SAS, 1990). Intake of supplementary feed and egg production parameters were based on the following model 1:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + A_{k(i)} + \varepsilon_{ijkl}$$

where Y_{ijkl} = intake of supplementary feed, laying rates, rate of floor eggs, and egg weights per subplot per time interval; μ = mean; α_i = supplementary feed ($i = 1, 2$); β_j = observation days, weeks or periods ($j = 1-35, 1-19, 1-8$, respectively); $(\alpha\beta)_{ij}$ = interaction supplementary feed × days, weeks or periods; $A_{k(i)}$ = random effect of replication; ε_{ijkl} = error. Three days before terminating of the experiment 48 hens were slaughtered during one day (12 hens at four different times). Thus, the last recordings on variables measured at group level (laying rate, floor egg rate and feed intake) were uncertain, for which reason these recordings were not included in the statistical analysis. The statistical analysis of body weight was based on model 2:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + A_{k(i)} + B_{l(ki)} + \varepsilon_{ijklm}$$

where Y_{ijklm} = body weight; μ = mean; α_i = supplementary feed ($i = 1, 2$); β_j = periods ($j = 1-8$); $(\alpha\beta)_{ij}$ = interaction supplementary feed × periods; $A_{k(i)}$ = random effect of replication; $B_{l(ki)}$ = random effect of the individual hen; ε_{ijklm} = error.

The crop content was analysed statistically by the above model 1, where Y_{ijkl} = quantity of each fraction in the crop of the individual hen; μ = mean; α_i = supplementary feed ($i = 1, 2$); β_j = period ($j = 1-4$); $(\alpha\beta)_{ij}$ = interaction supplementary feed × period; $A_{k(i)}$ = random effect of replication; ε_{ijkl} = error. In all cases residuals were approximately normally distributed.

The health and welfare parameters were tested using a chi-square test. A Wilcoxon rank-sum test was used to examine differences in pubic bones for the two groups of hens. These analyses were performed in SAS (1990). The FREQ procedure was used for the χ^2 -test, whereas Wilcoxon rank sum test was performed using the NPAR1WAY procedure.

Results

Overall results on productivity and the level of significance of variables influencing these values are shown in Table 4. A significant interaction between feed type and day of recording of feed intake (week/period) was found, in that the difference in supplementary feed intake was most pronounced at the beginning of the experiment and narrowed towards the end of the experiment (Figure 1). There was a tendency for the intake of oyster shells to change over

Table 4 Egg production, intake of supplementary feed and body weight; average of the entire experimental period (LS-means and s.e.) and level of significance for the effect of feed and period

	Unit	Supplementary feed		s.e.	Significance (P)		
		Layer feed	Wheat		Feed type	Week/ period	Feed type × week/period
Feed consumption							
Supplementary feed	g	125	94	2.3	< 0.001	< 0.001 [†]	< 0.001
Oyster shells	g	3.2	9.3	1.7	= 0.07	= 0.05	ns
Egg production							
Laying rate (incl. floor eggs)	%	85	76	3.8	ns	< 0.001	< 0.001
Egg weight	g	64.8	57.9	0.4	< 0.001	< 0.001	< 0.001
Floor eggs	%	4.7	0.3	0.7	< 0.05	< 0.01	< 0.001
Body weight	g	2032	1737	14	< 0.001	< 0.001	< 0.001

[†] The statistical analysis of layer feed and wheat is based on measurements of intake twice each week.

time, with the lowest intake at the beginning of the experiment (data not shown). Moreover, the wheat-fed hens tended to have a higher intake of this feed item (Table 4), but no time × supplementary feed interaction existed.

All egg production parameters and body weight were significantly influenced by the interaction between feed and period as illustrated in Figures 1 and 2. The differences in laying rate (inclusive floor eggs) between hens fed layer feed or whole wheat were most pronounced at the beginning of the experimental period. In the later periods, there were no differences in laying rate, except for the last few days of the experiment, where wheat-fed hens had a decline in laying rate.

During the whole experiment, egg weights for the wheat-fed hens were lower than for the hens fed layer feed, but most pronounced at the beginning of the experiment. Egg weights were lowest during the lowest laying rate, just as egg weights increased with the increase in laying rate and wheat intake. Egg weight subsequently stabilised at approximately 60 g per egg, with a slight increase during the remainder of the experiment (Figures 1 and 2). As indicated in Figure 2, body weight for the wheat-fed hens approximately followed the progress of the egg weight, indicating that wheat-fed hens at the later stages were capable of gaining body weight along with the increase in egg weight. Also the measurements on pubic bone approximately followed the progress of egg weight (Figure 2). Except for the first and the last assessment, the hens fed layer feed received a significantly ($P < 0.001$) higher score for pubic bone than wheat-fed hens. At the last assessment a tendency ($P = 0.07$) to a higher score was found for the hens fed layer feed.

All hens received maximum scores (four points) for plumage condition for four out of five body parts (neck, thorax, back, wings, tail), irrespective of supplementary feed type and day of assessment. Only plumage condition on the thorax was given a slightly lower score during the experiment, irrespective of treatment. At the last three assessments, the groups of hens fed layer feed had a significantly higher proportion of hens given maximum scores for plumage condition on the thorax ($\chi^2 = 7.2, 10.8, 8.8$, respectively, d.f. = 1, $P < 0.01$). However, mean values were above three for both types of supplementary feed on

all days of assessments. Maximum scores were also found for foot health. A few hens were given a minor reduction in the score for colour and wounds of the comb but no differences between types of supplementary feed were found. For the characteristic 'colour of the comb', a slightly lower score was given at the beginning of the experiment, whereas no distinct pattern was seen in relation to the characteristic 'wounds to the comb' (data not shown).

Table 5 shows the content of different feed items in the crop at four times in the experiment (periods) together with the level of significance in relation to supplementary feed, periods and the interaction. The amount of supplementary feed in the crop interacted with period. Thus, the amount of layer feed gradually increased over the periods. This was likewise the case for the amount of wheat in the crops except for the fact that the amount of wheat was slightly higher on the 2nd day of slaughter (grass/clover) than on the 3rd day of slaughter (lupin).

Wheat-fed hens had significantly more supplementary feed in the crop than hens fed layer feed. Moreover, plant material, oyster shells, insoluble grit stone and soil were significantly more abundant in the crops from the wheat-fed hens, whereas the seeds of cultivated plants were significantly more abundant in the crops from the hens fed layer feed.

The period significantly influenced the overall amount of supplementary feed, since the amount of feed in the crops increased over the periods, except for the above interaction. The period also significantly influenced the overall amount of seeds of cultivated plants in the crops, primarily due to a large amount of quinoa seeds in the crops when hens were foraging these plots. Insects were found significantly more often in the crops in the periods when hens were foraging the lupin and quinoa plots. Amount of earthworms, larvae and pupae in the crops were particularly abundant in hens foraging grass/clover or the quinoa.

Discussion

Feed intake

Average daily consumption of layer feed was 125 g per hen per day (Table 4), which is within the estimated range

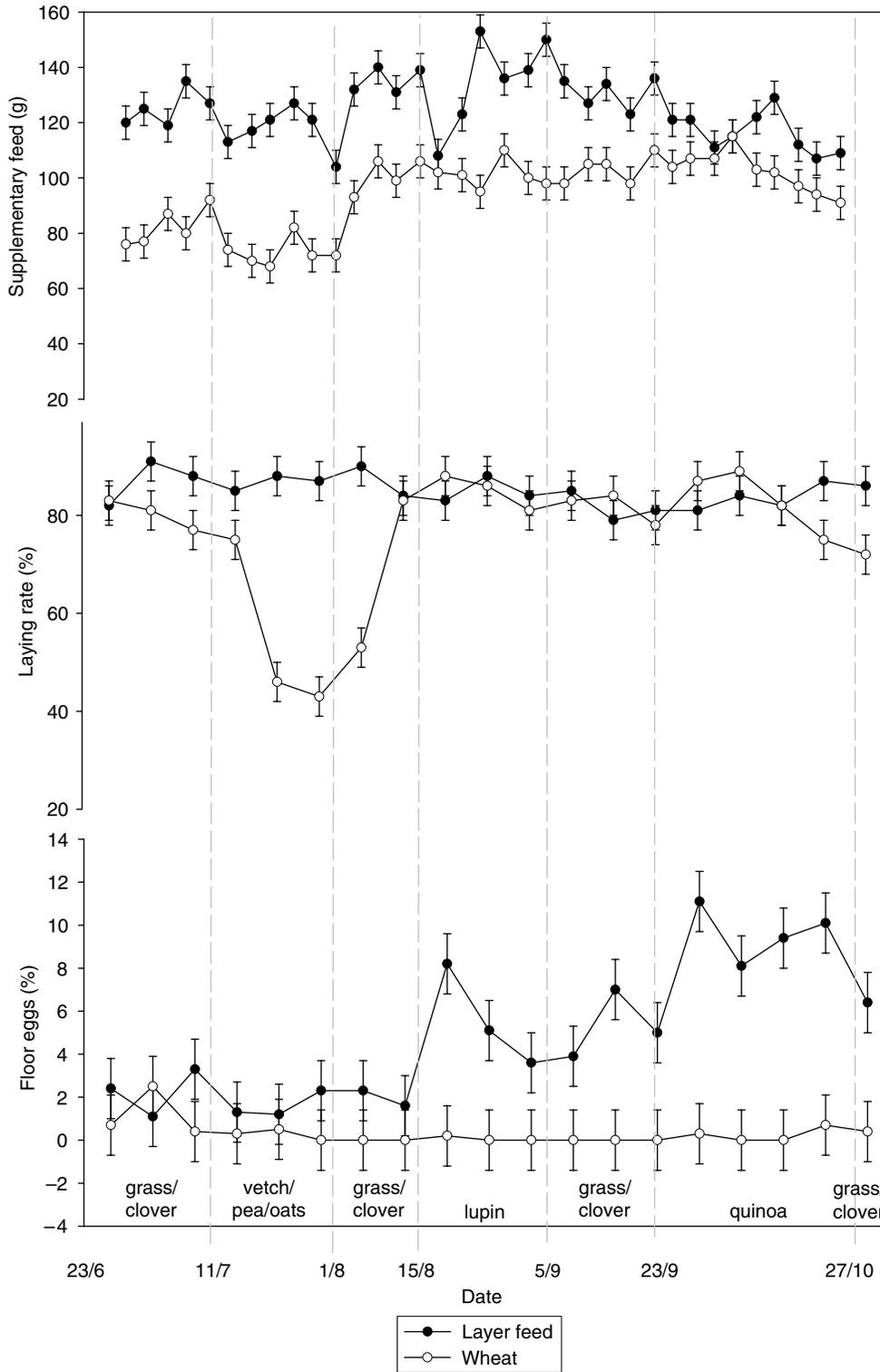


Figure 1 Intake of supplementary feed (g per hen per day), laying rate (%) and floor eggs (%), respectively (least-square means and s.e.).

(125 to 135 g feed per hen per day) for the hybrid used in the present study under free-range conditions (Hy-Line®, 2006), although hens in a free-range system often have been found to have a higher feed intake than expected (Keeling *et al.*, 1988; Hegelund *et al.*, 2006). For the

wheat-fed hens the average daily intake of wheat in the whole experimental period was 94 g wheat per hen per day, which is slightly above what we found in two experiments with experimental periods of 3 weeks (Horsted *et al.*, 2006). However, during the first two periods of

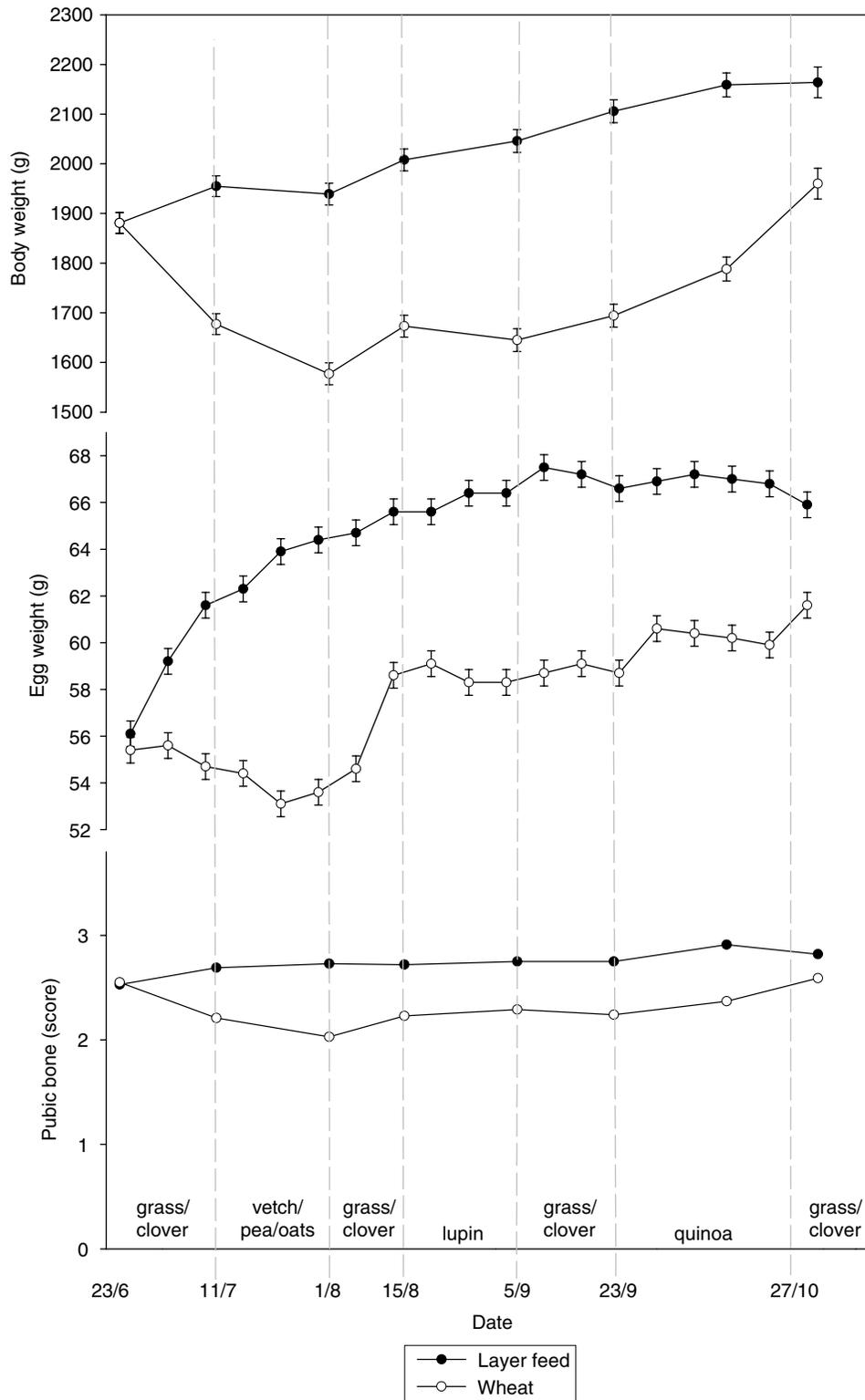


Figure 2 Body weight (g per hen), egg weight (g per egg) and pubic bone (score), respectively (least-square means and s.e.).

the present experiment (grass/clover and pea/vetch/oats), the average intake was only about 80 g per hen per day, whereas 6 to 7 weeks after introduction to the experiment there was a significant increase ($P < 0.001$) in the intake

of wheat to approximately 100 g per hen per day. This level of intake continued throughout the remaining part of the experiment, with minor fluctuations. It cannot be concluded whether this interaction (feed type \times week/

Table 5 Amount of feed items (air-dry weight) in the crops from hens slaughtered in the evening while foraging the plots with pea/vetch/oats, grass/clover, lupin, and quinoa, respectively, LS-means, s.e. and significance (P) of effect of supplementary feed and period

Supplementary feed	Unit	Layer feed	Wheat	Significance (P)			
				s.e.	Supp. feed	Period	Supp. feed × period
<i>Feed item</i>							
Supplementary feed	g	10.0	20.2	1.2	< 0.001	< 0.001	< 0.05
Pea/vetch/oats-period	g	8.0	11.1	2.4			
Grass/clover-period	g	9.6	19.6	2.4			
Lupin-period	g	9.9	17.7	2.4			
Quinoa-period	g	12.7	32.4	2.4			
Plant material	g	2.1	3.6	0.4	< 0.05	ns	ns
Seeds of cultivated plants	mg	1710	357	288	< 0.01	< 0.01	ns
Pea/vetch/oats-period	mg	1140	70	577			
Grass/clover-period	mg	0	0	577			
Lupin-period	mg	1705	8	577			
Quinoa-period	mg	3993	1348	577			
Weed seeds	mg	38	31	13	ns	ns	ns
Insects	mg	197	208	78	ns	< 0.01	ns
Pea/vetch/oats-period	mg	0	30	155			
Grass/clover-period	mg	3	12	155			
Lupin-period	mg	585	595	155			
Quinoa-period	mg	198	195	155			
Earthworms etc.	mg	249	239	76	ns	< 0.01	ns
Pea/vetch/oats-period	mg	0	5	153			
Grass/clover-period	mg	798	368	153			
Lupin-period	mg	62	52	153			
Quinoa-period	mg	135	532	153			
Oyster shells	g	0.8	3.4	0.5	< 0.01	ns	ns
Grit	g	0.5	2.1	0.4	< 0.01	ns	ns
Soil	g	0	17.1	2.0	< 0.001	ns	ns

period) was due to the type of forage allocated in the particular period, a change in the intake capacity of the hens or a change in nutrient supply. However, we hypothesise that hens were not able to eat sufficient forage material and whole wheat at the beginning of the experiment, since the digestive organs were not adapted to coarse feed at this stage, whereas the digestive organs adapted to coarser feed during the experiment making a higher wheat intake possible. According to Williams *et al.* (1997) and Ferket (2000), the coarse nature of whole wheat probably enhances the development of the gizzard, allowing improved grinding, gut motility and nutrient utilisation. Moreover, it has been shown that chickens trained to eat rapidly had larger amounts of feed in the crops than untrained chicks, which probably reflects the higher capacity of the crop in trained chickens (Lepkovsky *et al.*, 1960).

In the present experiment, the content of supplementary feed was significantly higher in crops from wheat-fed hens, but because of different retention times in the crops for wheat and layer feed (Heuser, 1945) the contents of these feed items do not reflect the differences in feed intake as indicated by Table 4 and Figure 1. Plant material, oyster shells, insoluble grit stones and soil were significantly higher in crops from wheat-fed hens, indicating that hens

on the wheat diet were trying to fulfil their nutrient requirement through a higher intake of other feed items. Thus, plant material was found to have a relatively high content of protein (Table 3), just as it contains a considerable amount of different vitamins (Heuser, 1955). Oyster shells have a high content of calcium (32%) and soil may contain roots and micro-organisms that contain small amounts of amino acids (Pokarzhevskii *et al.*, 1997). Surprisingly, the content of seeds from cultivated plants was higher in the crops from hens fed layer feed compared with wheat-fed hens, although peas, lupins and quinoa are rich in protein, which the wheat-fed hens were lacking. This might be due to the fact that wheat-fed hens prioritise feed items of animal origin, even though no difference in the amount of earthworms and insects was found in the crops (Table 5). However, the amount of earthworms and insects at the soil surface might diminish with the time spent foraging the area, and thus not be reflected in our measurements. The higher amount of soil in the crops from wheat-fed hens can support such an interpretation.

ME, lysine and methionine requirements

The discussion of the ME, lysine and methionine requirements of hens covers the last 11 weeks of the entire experiment when the hens' digestive tracts seemed to have

adapted to the diet. Using the formula given by National Research Council (NRC, 1994), the requirements for ME were 1.42 and 1.58 MJ for hens fed wheat and layer feed, respectively. The ambient temperature was on average 12.6°C for the period. Since particularly the wheat-fed hens showed a very high foraging activity (not recorded) irrespective of weather conditions, it is important to realise that the above formula does not include energy requirement for this kind of activity. In the period considered, the wheat and the layer diet provided approximately 1.26 and 1.39 MJ per hen per day, respectively. Thus the supplementary feed accounted for 88.7 and 88.0% of the energy requirement for wheat and hens fed layer feed, respectively. The remaining energy needed (including that required for activity) must have been obtained through the intake of forage material. The measured ME contents (Chwalibog, 1993) of lupin and grass/clover biomass (period 5) are 3.62 and 6.13 MJ per kg green fodder, respectively. If the remaining ME requirement of hens had to be covered by green fodder alone, they would have to consume at least 44 to 52 g DM lupine biomass and 26 to 31 g DM grass/clover biomass. These quantities that correspond very well with our previous studies (Horsted *et al.*, 2006), do not include the energy requirement for activity. However, it is plausible that hens fed layer feed primarily obtained their remaining ME requirement from the seeds of cultivated plants (Table 5).

The NRC estimates for lysine and methionine intake of 0.76 and 0.33 g per hen per day, respectively, are based on brown-egg layers receiving 110 g feed per hen per day. However, Lohmann Tierzucht (2006) estimates an intake of 0.87 g lysine and 0.44 g methionine per hen per day for brown egg layers producing a daily egg mass of 57.5 g and kept in a free-range system. The wheat-fed hens in our study had a wheat intake of approximately 102 g per hen per day in the period after introduction to lupin. Thus the wheat diet provided 0.27 g lysine and 0.14 g methionine per hen per day, giving an undersupply of 69 and 68%, respectively. Since wheat-fed hens in this period actually gained body weight and as egg weight also increased slightly, it seems plausible that these hens obtained an even higher amount of amino acids from the forage material. In contrast, the hens fed layer feed received the required amounts of lysine and methionine in the layer feed with an intake of 1.11 g lysine and 0.42 g methionine. This gives an oversupply of both amino acids, since these hens also had a considerable intake of foraging material (Table 5).

Laying rate

Wheat-fed hens showed a decline in laying rate at the beginning of the experiment, in accordance with Horsted *et al.* (2006). However, in the present study this initial decline was followed by a further drastic reduction to approximately 45%, when hens were foraging the pea/vetch/oats plots. A remarkable and relatively rapid

increase in laying rate to the same level as for the hens fed layer feed was subsequently seen. This occurred along with the increase in wheat intake and the moving of the hens to new forage vegetation, indicating that wheat-fed hens were lacking ME rather than protein in the period with the lowest laying rate. This seems paradoxical, since wheat contains more ME and less protein than layer feed (Table 2). As pointed out above, the hens were presumably not able to eat sufficient forage material and whole wheat at the beginning of the experiment, whereas the digestive organs became adapted to a coarser feed during the experiment.

Floor eggs

In some cases hens do not lay their eggs in the available nest boxes (Appleby, 1984). In our study, the term floor eggs were used for all eggs not laid in the nest boxes. Overall, hens fed layer feed had a larger proportion of floor eggs than the wheat-fed hens (Table 4). As illustrated by the first significant peak in Figure 1, this was particularly pronounced after the hens had been introduced to the lupins, due to the fact that some of the hens fed layer feed laid their eggs in the vegetation. However, in the subsequent part of the experiment, all floor eggs were collected on the floor inside the houses. In contrast, wheat-fed hens only rarely laid floor eggs. Even though we did not make any behavioural recordings, this difference might be related to different behaviour. We observed hens fed layer feed laying their eggs during a relatively short period in the morning, whereas wheat-fed hens laid eggs during a longer period until early afternoon. Bad habits could be an explanation too, since Cooper and Appleby (1996) found that the same hens laid 80% of the floor eggs. These authors further suggest that nesting motivation and perception of the nest box may vary among hens.

Health and welfare assessments

Deficiency in protein and some amino acids can have a negative effect on plumage condition due to feather-pecking (Ambrosen and Petersen, 1997; Elwinger *et al.*, 2002). However, in our study the plumage condition was found to be excellent, with maximum scores for four out of five parts of the body at each assessment. Only the score for plumage condition on the thorax fell with the number of days in the experiment. This was presumably due to the deterioration of the thorax feathers when hens were using the nest boxes. Thus we observed the lowest score for a few broody hens. Wheat-fed hens had a significantly lower score at the end of the experiment, probably due to their spending more time in the nest boxes during the final days of the experiment. A relationship to the above-mentioned incidences of floor eggs cannot be excluded.

According to Elwinger *et al.* (2002), a higher incidence of pecking injuries to the combs of laying hens can be related to an organic diet low in protein and amino acids. Overall, the hens in our study were given a close-to-maximum

score, since only few hens were found to have small wounds on the comb and no differences were observed between types of supplementary feed (Table 4). No hens had boils on their feet and few had wounds or keel bone deviations. The good health and welfare of the hens in our study might have been related to their finding sufficient protein and amino acids in the forage plots to avoid feather-pecking behaviour. Moreover, the system in which the hens were kept seemed to promote good health and welfare, presumably as a consequence of small flock sizes, permanent access to outdoor areas, low densities in outdoor areas and permanent access to forage material (Wechsler and Huber-Eicher, 1998).

We conclude that high-producing layers have a huge capacity for finding and utilising considerable amounts of feed items from a cultivated forage area. In fact, we found that wheat-fed hens were able to cover two-thirds of their lysine and methionine requirement from foraging material. However, an adaptation period is needed to develop the digestive system and for behavioural adaptation. Thus, the productivity of wheat-fed hens decreased at the beginning of the experiment, but with the subsequent increase in the intake of wheat, the laying rate increased to the same level as for the hens fed layer feed. Moreover, there was an increase in egg weight and body weight for both groups of hens during this period. The health and welfare was found to be good during the entire experiment and was not affected by supplementary feed or different periods. To propose adjustments to nutrient levels in supplementary feed, when hens have access to different foraging vegetations, the level of nutrient restriction needs to be further studied since crop analyses revealed different food preferences for hens fed layer feed or wheat.

References

Ambrosen T and Petersen VE 1997. The influence of protein level in the diet on cannibalism and quality of plumage of layers. *Poultry Science* 76, 559-563.

Antell S and Ciszuk P 2006. Forage consumption of laying hens – the crop as an indicator of feed intake and AME content of ingested feed. *Archiv für Geflügelkunde* 70 (in press).

Appleby MC 1984. Factors affecting floor laying by domestic fowl: a review. *World Poultry Science Journal* 40, 241-249.

Association of Official Analytical Chemists 1990. Association of Official Analytical Chemists Official methods of analysis. Association of Official Analytical Chemists, Arlington, VA.

Bach-Knudsen KE 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology* 67, 319-338.

Bach-Knudsen KE and Li B 1991. Determination of oligosaccharides in protein-rich feedstuffs by gas-liquid chromatography and high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry* 39, 689-694.

Bestman MWP and Wagenaar JP 2003. Farm level factors associated with feather pecking in organic laying hens. *Livestock Production Science* 80, 133-140.

Bubier NE and Bradshaw RH 1998. Movement of flocks of laying hens in and out of the hen house in four free range systems. *British Poultry Science* 39, S5-S6.

Buckner GD, Insko WM Jr and Henry AH 1945. Influences of spring bluegrass and mature bluegrass pastures on laying hens and on the eggs produced. *Poultry Science* 24, 446-450.

Chwalibog A 1993. Husdyrnæring – Bestemmelse af næringsværdi og næringsbehov, 2. udgave. Jordbrugsforlaget, København.

Cooper JJ and Appleby MC 1996. Individual variation in prelaying behaviour and the incidence of floor eggs. *British Poultry Science* 37, 245-253.

Elwinger K, Tufvesson M and Hartmann C 2002. Genotype-environment interactions in layers housed in an organic feed environment. Eleventh European poultry conference, 6–10 September, Bremen, *Archiv für Geflügelkunde* 66, 32-33.

Ferret P 2000. Feeding whole grains to poultry improves gut health. *Feed-stuffs (USA)* 4 September, 12-14.

Fuller HL 1962. Restricted feeding of pullets. 1. The value of pasture and self-selection of dietary components. *Poultry Science* 41, 1729-1736.

Gunnarson S, Odén K, Algers B, Svedberg J and Keeling L 1995. Poultry health and behaviour in a tiered system for loose housed layers. Sveriges Lantbruksuniversitet, Institutionen för husdjurshygien: Rapport 35, Skara, Sweden.

Gustafsson G and Antell S 2005. Ekosystemtjänster i äggproduktionen, hur kan det se ut? Ekologiskt lantbruk konferens 22–23 November, pp. 290-291. Sveriges Lantbruksuniversitet, Centrum för Uthålligt Lantbruk, Uppsala, Sweden.

Hegelund L, Sørensen JT, Kjær JB and Kristensen IS 2005. Use of range area in organic egg production systems: effect of climatic factors, flock size, age and artificial cover. *British Poultry Science* 46, 1-8.

Hegelund L, Sørensen JT and Hermansen JE 2006. Welfare and productivity of laying hens in commercial organic egg production systems in Denmark. *NJAS - Wageningen Journal of Life Sciences* 54, 147-155.

Heuser GF 1955. Feeding poultry, second edition. John Wiley & Sons, Inc, New York.

Heuser GF 1945. The rate of passage of feed from the crop of the hen. *Poultry Science* 24, 20-24.

Horsted K, Hammershøj M and Hermansen JE 2006. Short-term effects on productivity and egg quality in nutrient-restricted versus non-restricted organic layers with access to different forage crops. *Acta Agriculturae Scandinavica, Section A* 56, 42-54.

Hy-Line® 2006. Hy-Line® Variety Brown. Commercial management guide, alternative systems. www.swedegg.se/pdf/varietybrown.pdf. Accessed 9 May 2006

Keeling LJ, Hughes BO and Dun P 1988. Performance of free-range laying hens in a polythene house and their behaviour on range. *Farm Building Progress* 94, 21-28.

Lepkovsky S, Chari-Bitron A, Lemmon RM, Ostwald RC and Dimick MK 1960. Metabolic and anatomic adaptations in chickens "trained" to eat their daily food in two hours. *Poultry Science* 39, 385-389.

Lohmann Tierzucht 2006. Management guide for laying hens in deep litter, aviary and free-range systems. www.ltz.de/bilder/content/ltz_haltung_englisch.pdf. Accessed 18 May 2006

Mongin P 1976. Composition of crop and gizzard contents in the laying hen. *British Poultry Science* 17, 499-507.

Mwalusanya NA, Katule AM, Mutayoba SK, Minga UM, Mtambo MMA and Olsen JE 2002. Nutrient status of crop contents of rural scavenging local chickens in Tanzania. *British Poultry Science* 43, 64-69.

National Research Council 1994. Nutrient requirements of poultry. National Academy Press, Washington, DC.

Permin A, Bisgaard M, Frandsen F, Pearman M, Kold J and Nansen P 1999. Prevalence of gastrointestinal helminths in different poultry production systems. *British Poultry Science* 40, 439-443.

Permin A, Nansen P, Bisgaard M and Frandsen F 1998. *Ascaridia galli* infections in free-range layers fed on diets with different protein contents. *British Poultry Science* 39, 441-445.

Pokarzhievskii AD, Zaboyev DP, Ganin GN and Gordienko SA 1997. Amino acids in earthworms: Are earthworms ecosystemivorous? *Soil Biology and Biochemistry* 29, 559-567.

Reichert R, Tatarynovich JT and Tyler RT 1986. Abrasive dehulling of quinoa (*Chenopodium quinoa*): effect on saponin content as determined by an adapted hemolytic assay. *Cereal Chemistry* 63, 471-475.

Ridout CL, Price KR, DuPont MS, Parker ML and Fenwick GR 1989. Quinoa (*Chenopodium quinoa* Willd.) saponins – analysis and preliminary investigations into the effects of reduction by processing. *Journal of the Science of Food and Agriculture* 54, 165-176.

Sipe GR and Polk HD 1941. Japanese tendergreen mustard, Italian rye grass, and oats as a source of green feed for laying hens. *Poultry Science* 20, 406-412.

Statistical Analysis Systems Institute 1990. SAS/STAT[®] users guide. SAS Inst. Inc, Cary, NC.

Steenfeldt S, Engberg RM and Kjær JB 2001. Grovfoder til æglæggende høner. In *Forskning og udvikling i økologisk ægproduktion* (ed. P Sørensen), pp. 49-59. Darcof report no. 11, Research Centre Foulum, Tjele, Denmark.

Stoldt W 1952. Vorschlag zur Vereinheitlichung der Fettbestimmung in Lebensmitteln (suggestion to standardise the determination of fat in food-stuffs). *Fetten, Seifen, Anstrichmittel* 54, 206-207.

Tauson R, Ambrosen T and Elwinger K 1984. Evaluation of procedures for scoring the integument of laying hens – independent scoring of plumage condition. *Acta Agriculturae Scandinavica* 34, 400-408.

Walker A and Gordon S 2003. Intake of nutrients from the pasture. *Proceedings of the Nutrition Society* 62, 253-256.

Wechsler B and Huber-Eicher B 1998. The effect of foraging material and perch height on feather pecking and feather damage in laying hens. *Applied Animal Behaviour Science* 58, 131-141.

Williams BA, Van Osch LJM and Kwakkel RP 1997. Fermentation characteristics of the caecal contents of broiler chickens fed fine and coarse particle diets. *British Poultry Science* 38, S41-S42.

Wood GM, Smith RT and Henderson DC 1963. A crop analysis technique for studying the food habits and preferences of chickens on range. *Poultry Science* 42, 304-309.