Increased foraging in organic layers

Ph.D. Thesis by

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Summary

In this thesis, it was hypothesised that hens are capable of finding and utilizing a considerable amount of feed items from a forage area, dependent on the type of supplementary feed and forage vegetation offered. Thus, increased foraging in organic laying hens may be a way to increase the utilisation of local resources in organic egg production systems. This could increase the cycling of nutrients within the system, ease the transition to 100% organic feed supply, and benefit the economy of the egg production. Further, this may lead to a greater dispersion of the poultry in the open-air run that in turn may benefit the welfare of the hens and reduce the risk of nutrient leaching in the area closest to the henhouse. On this background, the main objective of this study was to provide better knowledge on the potential of utilizing the foraging of laying hens in a forage-based system. More specific the aims were: to estimate the intake of herbage from the open-air run, to determine selectivity of forage material in relation to restriction of nutrients in the supplementary feed, to estimate the possible contribution of foraging in meeting nutritional requirements of the poultry, and to suggest forage-based systems.

In this study, experimental work has been done concerning productivity and welfare in a forage-based system (Papers 1 and 4), egg quality (Paper 1), estimating feed intake from forage (Papers 1 and 3) and estimating selectivity of feed from forage (Papers 2, 3, and 4). These subjects were investigated in relation to different forage crops and two types of supplementary feed (a complete layer ration versus whole wheat and oyster shells). Three experimental setups were carried out in this study. In 2004, two short-term experiments (23 days each), each with 12 flocks with 20 hens and one cock in each flock, were carried out. The forage crops consisted of grass/clover versus a mixture of forbs in Experiment 1, and grass/clover versus chicory in Experiment 2. In 2005, a third experiment (130 days), with six flocks with 26 hens and one cock, were conducted. The flocks were moved regularly between grass/clover, pea/vetch/oats, lupin and quinoa. In Chapters 3 to 6, the results of the above papers are discussed against other relevant literature.

In Chapter 3, a discussion on herbage intake from forage is made, using the results from a sward-based method (Paper 1) and using the amount of herbage in the crops from hens slaughtered in the evening (Paper 3 and 4). The results indicate that hens consume a considerable amount of herbage irrespectively of type of forage and type of supplementary feed, even though nutrient-restricted hens were found to have approximately 50% higher intake. Thus, intake of grass/clover may be 10-30g

per hen per day for non-restricted hens and 20-40g per hen per day for nutrient-restricted hens. However, hens foraging the chicory plots particularly seemed to benefit from forage with approximately twice as high intake of this forage crop as of grass/clover.

Discussion on feed selection using the crop content and microhistological analyses of faeces is made in Chapter 4. Analyses of crop content indicate that selectivity among feed items was found to differ in relation to type of supplementary feed. Thus, in Papers 3 and 4 it was found that wheat-fed hens had more plant material, grit stone, soil, and oyster shells in the crops and less seeds than concentrate-fed hens. Moreover, wheat-fed hens gave priority to earthworms and larvae even though the amount of this feed item seemed to decrease after a few days in a given forage vegetation (Paper 4). Only to a minor degree did type of forage influence the amount of different feed items in the crops, though the crop rotation experiment (Experiment 3) indicated a higher intake of quinoa seeds compared with seed of lupin and pea/vetch/oats.

In Experiment 1 microhistological analyses of faeces indicated that nutrient-restricted hens had significantly more of the species of grass *Elytriga repens* in the faeces, whereas non-restricted hens had more white clover (*Trifolium repens).* Further, in the plots with the mixture of forbs, selectivity indices suggested that hens gave priority to plant species nearer the ground, since positive selectivity indices were found for the grasses, *Elytriga repens* and *Lolium perenne* as well as for the clover *Trifolium repens*. Negative selectivity indices were found for the much taller species of plants, *Fagopyrum esculentum* and *Phacelia tanacetifolia* (Paper 2).

In Chapter 5, the possible contribution of metabolizable energy, lysine, methionine and calcium from forage is estimated using the requirements for laying hens and the results on productivity (Papers 1 and 4). After a period of adaptation it was estimated that hens, irrespectively of type of supplementary feed, may consume up to 0.25 MJ ME of their requirements of ME from forage; presumably a little higher for nutrient-restricted hens due to a higher foraging activity. Moreover, it was estimated that the forage area on average had supplied the nutrient-restricted hens with approximately 70% of their requirements according to the feeding standards for lysine and methionine, and approximately 25% of their requirements according to the feeding standards for calcium. Concentrate-fed hens were fully covered through the supplementary feed.

The production in forage-based systems is discussed in Chapter 6. On a short-term basis nutrient-restricted hens had a decline in egg production, except for hens foraging the chicory (Paper 1). Further, a tendency to a darker and redder yolk colour was found when hens were foraging the chicory plots. Also albumen DM was higher in eggs from hens foraging chicory. Eggshell parameters were not affected by forage crops or by type of supplementary feed, suggesting that nutrient-restricted hens were provided with the required amount of calcium through oyster shells and foraging material (Paper 1). After a period of adaptation nutrient-restricted hens were found to produce well in a crop rotation system, since laying rate was comparable to non-restricted hens. Egg weight and body weight was lower in nutrient-restricted hens, though increasing at the end of the experiment. In general, hens fed whole wheat had a lower intake of supplementary feed, even though a distinct increase in this feed was seen after a few weeks. At the end of the experiment, gizzards were found to be significantly larger for the wheat-fed hens than for the concentrate-fed hens (Paper 4). The effects of productivity and intake of supplementary feed were reflected in the nitrogen and phosphorus balances in the way that N and P surpluses were considerably lower for nutrient-restricted hens. The welfare of hens in forage-based systems was found to be excellent irrespectively of type of supplementary feed (Papers 1 and 4).

It was concluded that high-producing layer strains are able to consume considerable amounts of herbage and that forage can provide laying hens with important nutrients. Chicory and quinoa seem promising, just as focus on earthworms has potential. Moreover, it seems possible to lower the standards of important nutrients in the supplementary feed, provided that good forage is available and that the production system supports good welfare of the poultry. A crop rotation system was suggested.

Dansk sammendrag

Det var hypotesen i dette projekt, at æglæggende høner er i stand til at finde og udnytte en betydelig del af de tilgængelige fødeemner i et udeareal med dyrkede afgrøder, og at dette er afhængigt af hvilken type tilskudsfoder der tildeles og hvilke afgrøder der dyrkes i udearealet. Øget fouragering hos økologiske æglæggere kan således være en måde at øge udnyttelsen af lokale ressourcer i ægproduktionssystemet og dermed mindske importen af næringsstoffer til systemet. Dette vil forøge cirkulering af næringsstoffer indenfor systemet, lette overgangen til 100% økologisk fodring og medvirke til bedre økonomi i ægproduktionen. Endvidere kan et mere attraktivt udeareal føre til, at flere høns fordeler sig på hele udearealet, hvilket kan have en positiv effekt på hønsenes velfærd og samtidig reducere risikoen for nedsivning af næringsstoffer i nærområdet udenfor hønsehuset. På baggrund heraf var hovedformålet i dette ph.d.-projekt at tilvejebringe en bedre viden om potentialet for æglæggende høners fouragering i et system baseret på øget fouragering. De specifikke formål var; at estimere indtaget af plantemateriale fra udearealet, at bestemme hønernes selektivitet af fødeemner fra udearealet set i relation til restriktion af næringsstoffer i tilskudsfoderet, at estimere det mulige bidrag fra fouragering til dækning af hønernes næringsstofbehov, og at foreslå et system der er baseret på udnyttelsen af hønernes fouragering.

Der blev udført forsøg mht. produktivitet og velfærd i et system baseret på øget fouragering (Artikel 1 og 4), ægkvalitet (Artikel 1), metoder til estimering af planteindtag fra udearealet (Artikel 1 og 3), og metoder til bestemmelse af selektivitet af fødeemner fra et dyrket udeareal (Artikel 2, 3 og 4). Disse emner er undersøgt i relation til forskellige afgrøder og to forskellige typer af tilskudsfoder, dvs. fuldfoder versus et foder med lavt proteinindhold (hel hvede og østersskaller). Tre forsøgsopstillinger blev udført. Der blev gennemført to forsøg over en kort tidshorisont (23 dage) i 2004, hvor hvert forsøg bestod af 12 flokke á 20 høner og en hane. Afgrøderne bestod af kløvergræs versus en urteblanding i Forsøg 1, og kløvergræs versus cikorie i Forsøg 2. Endvidere blev der udført et tredje forsøg (130 dage) i 2005 med seks flokke á 26 høner og en hane. Her blev hønerne flyttet rundt på fire forskellige afgrøder, kløvergræs, ærte/vikke/havre, lupin og quinoa. I kapitlerne 3-6 er resultaterne fra ovenstående artikler diskuteret i forhold til relevant litteratur.

I Kapitel 3 diskuteres hønernes indtag af plantemateriale fra udearealet. Indtaget af plantemateriale estimeres dels ved en metode hvor plantemateriale er høstet før og efter hønernes adgang til et dyrket areal (Artikel 1) og dels ved en metode hvor hønernes indtag er estimeret ud fra mængden af

plantemateriale i kroen hos høns slagtet om aftenen (Artikel 3 og 4). Resultaterne indikerer, at høns konsumerer betydelige mængder af plantemateriale uanset typen af afgrøder og typen af tilskudsfoder. De høns der fik hel hvede og østersskaller havde dog ca. 50% højere indtag af plantemateriale. Indtaget af kløvergræs blev således estimeret til 10-30g pr høne pr dag hos høns der fik fuldfoder, og 20-40g pr høne pr dag for høns der fik hel hvede. Hønsenes indtag af cikorie var dog ca. dobbelt så højt som ved kløvergræs for begge tilskudsfoder.

Hønsenes selektion af fødeemner fra udearealet er i Kapitel 4 diskuteret i relation til analyser af indholdet i kroen og en mikrohistologisk analyse af ekskrementer. Analyserne af indholdet i kroen indikerer, at selektion mellem forskellige fødeemner var forskellig hos hønsene afhængig af typen af tilskudsfoder. I Artikel 3 og 4 blev det således fundet, at høns der fik hvede som tilskudsfoder havde mere plantemateriale, småsten, jord og østersskaller i kroen samt mindre frø i forhold til de høns der fik fuldfoder. Desuden havde hvede-holdene flere regnorm, larver og insekter i kroen, selvom dette kun var tilfældet efter kort tid på en ny afgrøde (Artikel 4). Afgrødetypen influerede kun i mindre omfang på mængden af de forskellige fødeemner i kroen, selvom forsøget med rotation mellem forskellige afgrøder (forsøg 3) tydede på et højere indtag af quinoafrø sammenlignet med indtaget af frø fra lupin- og ærte/vikke/havre-parcellerne.

I Forsøg 1 viste en mikrohistologisk analyse af ekskrementer, at de høns der fik hvede som tilskudsfoder havde signifikant mere kvikgræs (*Elytriga repens*) i ekskrementerne i forhold til de høns der fik fuldfoder, hvorimod det modsatte gjorde sig gældende mht. hvidkløver (*Trifolium repens*). Desuden viste beregnede selektivitetsindekser, at hønsene prioriterede de laveste plantearter i parceller med en urteblanding, idet positive selektivitetsindekser blev fundet for græsserne *Elytriga repens* og *Lolium perenne* samt for hvidkløver (*Trifolium repens*). Negative selektivitetsindekser blev fundet for de højere plantearter boghvede (*Fagopyrum esculentum*) og honningurt (*Phacelia tanacetifolia*) (Artikel 2).

I Kapitel 5 er det mulige bidrag af omsættelig energi (ME), lysin, methionin og calcium fra udearealet estimeret ved at benytte de teoretiske behov for æglæggende høner samt produktivitets-resultaterne (Artikel 1 og 4). Efter en tilpasningsperiode blev det estimeret, at hønsene, uanset typen af tilskudsfoder, kan indtage op til 0,25 MJ ME fra udearealet; sandsynligvis mere for de høns der fik hvede som tilskudsfoder pga. et højere aktivitetsniveau. Endvidere blev det estimeret, at udearealet i gennemsnit havde forsynet hvedeholdene med ca. 70% af normen for lysin og methionin samt 25% af behovet for calcium, set i forhold til standardnormerne til æglæggere. Fuldfoder-holdenes behov for disse næringsstoffer var fuldt dækket ind via fuldfoderet.

Produktionen i et fouragerings-baseret system er diskuteret i Kapitel 6. På kort sigt havde hvede-holdene et fald i ægproduktionen med undtagelse af de hold der fouragerede i cikorie (Artikel 1). Der fandtes desuden en tendens til en mørkere og mere rødlig farve på æggeblommen, samt et højere tørstofindhold i æggehviden når hønsene fouragerede i cikorien. De undersøgte egenskaber af æggeskallen var ikke påvirket af afgrøde eller typen af tilskudsfoder, hvilket tyder på, at de høns der fik hel hvede som tilskudsfoder fik dækket behovet for calcium via østersskaller og føde fra udearealet (Artikel 1). Efter en tilpasningsperiode blev det fundet, at hvede-holdene havde en god produktivitet når de blev flyttet mellem forskellige afgrøder (Artikel 4). Således var antallet af lagte æg efter denne periode på samme niveau som hos de høns, der havde adgang til fuldfoder. Ægvægten og kropsvægten var dog lavere, selvom den var stigende i den sidste halvdel af forsøget. De høns, der blev fodret med hel hvede havde et lavere indtag af dette foder set i forhold til fuldfoderholdenes indtag af fuldfoder. Der blev dog fundet en betydelig stigning i indtaget af hvede efter nogle få uger i forsøget. Ved afslutningen af forsøget fandtes kråserne at være signifikant større hos hvede-holdene sammenlignet med fuldfoder-holdene (Artikel 4). Effekten af produktivitet og indtag af tilskudsfoder var reflekteret i kvælstof- og fosforbalancerne på den måde, at N- og P-overskuddet var betydeligt lavere hos de høns, der fik tildelt hel hvede. Endeligt var hønsenes velfærd i de fouragerings-baserede systemer rigtig god uanset typen af tilskudsfoder.

Det er konkluderet, at højtydende æglæggere er i stand til at konsumere betydelige mængder af plantemateriale og at fouragering i et dyrket udeareal kan forsyne hønerne med vigtige næringsstoffer. Cikorie og quinoa synes at være gode afgrøder, ligesom fokus på regnorm o.l. er interessant i sådanne systemer. Det synes at være muligt at nedsætte mængden af vigtige næringsstoffer i tilskudsfoderet, såfremt rigelig føde er tilgængelig i udearealet og at systemet i øvrigt understøtter god velfærd. Et system hvor hønsene flyttes mellem forskellige afgrøder blev foreslået.

1. Introduction and aim

Organic production and processing are based on a number of general principles and ideas. Included in this, principle aims related to biological farming cycles within the farming systems, as well as principles related to livestock conditions of life, have been formulated. To exemplify, IFOAM (2000) list the following principles in this context: “To encourage and enhance biological cycles within the farming system, involving microorganisms, soil flora and fauna, plants and animals”, “To use, as far as possible, renewable resources in locally organised production systems”, “To create a harmonious balance between crop production and animal husbandry” and “To give all livestock conditions of life with due consideration for the basic aspects of their innate behaviour”.

Besides these principles and ideas, specific regulations in relation to organic poultry production are listed in the EU legislation. Thus, it is stated that hens must have access to an open-air run, which mainly must be covered with vegetation and provided with protective facilities (Council Regulation (EC) No 1804/1999, 1999). This regulation that partly is based on some of the guidelines formulated by the above International Federation of Organic Agriculture Movements (IFOAM) is, however, merely an administrative interpretation of these ideas of organic farming that, depending on the individual countries, have wider goals (Hermansen, 2003). In Denmark it is specified that hens, at any time, must have access to an open-air run of minimum 4 m^2 per hen and that roughage such as fresh green fodder, silage, root crops etc., must be available for all hens during daytime. Moreover, the open-air run must be covered with grass or crops, just as regulations have been stipulated as regards changing of the pens and establishments of shelter etc. (The Danish Plant Directorate, 2006).

Cultivation of the open-air run for the purpose of increasing poultry’s forage intake from this area is rarely seen though it has been shown that hens are able to consume considerable amounts of roughage (Steenfeldt et al., 2001) and that supplement of roughage has resulted in reductions of some welfare problems (Wechsler and Huber-Eicher, 1998; Aerni et al., 2000). Increased foraging of the poultry could be a way to follow up the principle of increasing the utilization of local resources and thus the cycling of nutrients in the system through a lesser import of feed to the farm. This would further ease the transition to a 100% organic feed supply. Moreover, the lesser import of feed to the system may benefit the finances of the farmer since feed costs account for the largest part of the total costs in organic egg production systems (Walker & Gordon, 2003; Danish Poultry Council, 2004).

Utilizing the innate foraging behaviour of laying hens in a cropping system may further benefit the welfare of the hens and reduce the leaching of nutrients to the groundwater in the area around the henhouse. Thus, it is a commonly recognized problem that hens in traditional organic egg production systems to a great extent do not utilize the available open-air run, but stay inside the henhouse or predominantly use the area right in front of the henhouse (Keeling et al., 1988; Zeltner & Hirt, 2003; Hegelund et al., 2005). This results in a high animal density, in the henhouse as well as outside the henhouse, involving a risk of health and welfare problems such as feather pecking (Green et al., 2000; Bestman & Wagenaar, 2003). Further, the high density outside the house enhances the risk of nutrient leaching to the groundwater and parasitic infections (Permin et al., 1998; 1999). It has been hypothesized that this lack of use to a great extent was due to the open-air run being unattractive to the hens for which reason concerns on this subject have been taken among egg producers (Hermansen et al., 2005). Moreover, the feeding strategies in organic egg production systems are widely based on purchased feed, traditionally fed inside of the henhouse, which adversely affect the hens' use of the open-air run (Bubier & Bradshaw, 1998). Thus, cultivation of the open-air run may enhance the attractiveness of the area resulting in improved distribution of the hens in the open-air run.

Newer studies dealing with forage intake from the open-air run in laying hens are scarce, though a study by Gustafson & Antell (2005) indicates that hens integrated into oilseed, sunflower and wheat cropping systems are capable of supporting their nutritional needs through weeds and other feed items found in the vegetation. Elwinger et al. (2004) suggest that the foraging activity in the open-air run was higher when hens were restricted in nutrients. Moreover, studies on crop content from especially low producing scavenging hens further indicate that hens have potential for a considerable intake of herbage and other feed items accessible in the available area (Tadelle, 1996; Mwalusanya et al., 2002; Sonaiya; 2004). However, forage intake from the open-air run was mainly of interest in the pre-industrial period where utilization of forage was an integrated part of the poultry production systems. Therefore, knowledge about this subject is primarily described in older studies in the period from the 1930s to the beginning of the 1950s. Thus, several studies have reported savings of feed or improved egg production when access to open-air runs with different forage crops was given. Moreover, some studies showed that laying pullets on good pasture did not need protein concentrates (reviewed by Heuser, 1955 and Cowlishaw & Eyles, 1957). Thus it was concluded that pasture will supply a considerable proportion of the protein and vitamins required by laying birds though the quality of the pasture as well as the number of hens to the acre were important factors. However, strains at that time were not as high producing as the strains used to day, suggesting that the distinct selection for egg production and feed conversion rate might have influenced the hen's characteristics in such a way that she has lost some of her ability to function in a free range system (Sørensen, 1996).

In the present study it is the hypothesis that hens are capable of finding and utilizing a considerable amount of feed items from the open-air run, and that the intake of these feed items is dependent on the type of supplementary feed and forage vegetation offered. Thus, the main objective of this thesis is to provide a better knowledge on the potential of utilizing the innate behaviour of foraging in laying hens in relation to the development of new production systems in organic poultry production. The specific aims are:

- to estimate the intake of herbage from the open-air run
- to investigate if nutrient restriction in the supplementary feed influence feed selectivity from the forage area
- to estimate the possible contribution of foraging in meeting the nutritional requirements of high producing layers
- to suggest forage-based systems

2. Materials, methods and thesis outline

Three experimental setups were carried out in this study; two short-term experiments in 2004 (of 23 days each) and one experiment lasting 130 days in 2005. The experimental conditions and the choices made in relation to this are given below.

2.1 Experimental design

Each of the experiments in 2004 (Experiments 1 and 2) consisted of two different forage crops and two types of supplementary feed (typically concentrate for organic layers and whole wheat) with three replications, i.e. a total of 12 groups, each including 20 hens and one cock. In each experiment, three plots of each forage crop were alternately established on the experimental field with four metres between the plots to minimize the possible effect of one forage crop on the other, e.g. where one forage crop attracts insects that are a potential feed for hens. Each plot, which totalled 420 m^2, was then subdivided into two subplots according to type of supplementary feed, resulting in subplots of 210 m^2 (12 x 17.5 metres). Due to the budget of the project, the number of hens was limited to 20 per flock and the number of replications to three. The size of the subplots was based on an estimated intake of maximum 20g roughage DM per hen per day in an indoor floor system (Steenfeldt et al., 2001). Moreover, it was assumed that unfertilized grass in the summer grows approximately 6g DM per m^2 per day (more in spring and less in late summer). This corresponds to 70 m^2 for 20 hens and one cock. However, free-range hens were expected to have a higher intake of herbage, just as nutrient restriction may influence the intake of herbage. Further, other crops than grass may have different growth rates. Thus, number of square metres was decided to 10 m^2 per hen, i.e. 210 m^2 per flock. An experimental period of approximately three weeks was chosen to meet the possibility of a declining value of the forage crops due to the foraging activity of the hens.

The experiment in 2005 (Experiment 3) was carried out as a rotation between different crops, with the same size of the forage plots as for the experiments in 2004. Also, the same kind of supplementary feed was provided, just as we had three replications, i.e. six flocks of hens. In this experiment we had slightly larger flock sizes with 26 hens and one cock in each flock.

2.2 Types of supplementary feed in the experiments

For the development of new systems for organic poultry production it was considered important to provide better knowledge about the capacity of the birds. Therefore, we chose to restrict half of the hens in nutrient supply through whole wheat and oyster shells, whereas the other half were fed a typical pelleted concentrate for organic layers. Some studies indicate a higher feed intake from forage when hens are nutrient-restricted (Fuller, 1962). Moreover, wheat is the basis feed in organic poultry rations and can be grown locally for which reasons it was chosen for the nutrient-restricted hens. Oyster shells were given ad lib to ensure that a calcium source was available to prevent the hens from compensating the calcium deficiency by using the skeletal body pool of calcium.

2.3 Types of forage crops in the experiments

The type of forage crops chosen in 2004 was grass/clover, a mixture of forbs and chicory. Grass/clover was chosen because it has a continuous growth during the summer, i.e. it is an excellent buffer in a system with other crops with different times of maturing. Moreover, a well-established grass/clover is fairly resistant to the scrapping of the hens, for which reason it will recover quickly in a period where hens are absent. The mixture of forbs consisted of buckwheat, tansy-leaf phacelia and flax (*Fagopyrum esculentum, Phacelia tanacetifolia and Linum usitatissimum*). These forbs are used by game managers and are known to attract a lot of insects in the flowering season for which reason this mixture of forbs was expected to be valuable to free-range hens too. Chicory, both the roots and the leaves, has proven to be an excellent crop in several ways when it has been used as roughage or forage to pigs or ruminants, respectively (Hansen et al., 2006; Marley et al., 2003). Thus, it was interesting to test this crop as forage for laying hens.

In the 2005 experiment hens were moved in a rotation between different forage crops, for which reason different times of maturing were necessary. In a crop rotation system for poultry, grass/clover was considered an important crop because of the characteristics mentioned above. In addition, we chose pea/vetch/oats, lupins and quinoa because of the higher protein content in their seeds compared to whole wheat. Moreover, pea/vetch/oats were expected to mature before the lupin and quinoa, just as a pea/vetch/oats could be used as green fodder if maturing made slow progress. The lupin (*Lupinus angustifolius*) cultivar chosen was 'Prima', which has been shown to be attractive to hens (Hammershøj & Steenfeldt, 2005). In contrast, no publication on the quinoa cultivar 'Atlas' was found. However, we chose this cultivar since it was a variety free of bitter saponin, which affects palatability adversely (Reichert et al., 1986; Ridout et al., 1989). Besides, a variety containing saponin was found not to benefit growth in broilers (Jacobsen et al., 1997). In addition, nutrient content in quinoa seeds may vary in relation to cultivars, growth conditions etc. (Galway et al., 1990; Jacobsen, 2003). According to Repo-Carrasco et al., (2003) also the leaves of quinoa have a high content of good quality proteins just as they are rich in vitamins and minerals.

2.4 Layer strains in the experiments

In the experiments in 2004, the strain Lohmann Silver was chosen in the light of some case studies at seven organic egg producers (Hermansen et al., 2005). This relatively new strain in Denmark was very popular with the participating producers. Thus, they believed that more Lohmann Silver hens used the open-air run than did other brown egg layers, just as the first experiences suggested that this strain had a good plumage condition during the production period. The strain was at that time considered as ‘the hen’ for organic egg production.

In the experiment in 2005 the strain Hyline Brown was chosen because it was the most widespread strain in organic egg production in Denmark. Besides, producers suggested that Lohmann Silver was less superior to the previous year, since feather pecking was found to occur in this strain, too.

2.5 Housing conditions in the experiments

The supplementary feed and water were provided ad lib just outside the henhouse. The houses that were developed to these experiments were 4.6 m^2 each and made of waterproof plywood on an iron frame and without a floor, since they should be moved regularly. Thus, pushing a specially developed trailer under the iron frame could move the houses. Windows were placed at each end of the house to allow daylight. Human access was allowed through a door at one end of the house and five nest boxes (40×40 cm each) were placed on the outside at the other end of the house. Perches were placed inside the house in 80-90 cm’s height. On both sides hatches stretched the full length of the house to avoid corners that could potentially function as nesting areas. There was no artificial light in the houses, just as the hatches were open day and night, giving the hens access to forage all day throughout the experiments.

2.6 Experimental activities

The experimental activities included:

- Recordings of egg production. In all experiments egg production was recorded on a daily basis, i.e. number of eggs, floor eggs and daily average egg weight (Papers 1 and 4).
- Measurements of egg quality. Six eggs from each subplot were collected at introduction and at termination of Experiments 1 and 2, and subsequently analysed for yolk colour, albumen DM, eggshell percentage and eggshell strength (Paper 1).
- Recordings on intake of supplementary feed. In Experiments 1 and 2 (2004), the intake of supplementary feed was recorded for the period as a whole (Paper 1), whereas in experiment 3 the intake of supplementary feed was recorded twice a week. Intake of oyster shells was recorded each time hens were moved to new forage (Paper 4).
- Chemical analyses of supplementary feed (Papers 1 and 4).

- Weighing and analysis of herbage (Papers 1 and 4)
- Estimation of herbage removed by harvesting herbage in small plots prior to introduction of the hens, and at termination (Paper 1).
- Welfare assessments. A welfare assessment of all hens was carried out at introduction and termination of each experiment (Papers 1 and 4). In Experiment 3, a further six welfare assessments were carried out during the experiment (Paper 4). The assessment included bodyweight, plumage condition, foot health, comb colour and wounds, pubic bone and keel bone.
- Collection of faeces for microhistological analysis. Faeces were collected three days after introduction in Experiment 1 (Paper 2).
- Slaughtering of hens for analysis of crop content (Papers 3 and 4) and gizzards (Paper 4). Two hens were slaughtered from each subplot in the evening and the following morning in Experiments 1 and 2 (Paper 3). This was done two times in each experiment. In Experiment 3, two hens from each subplot were slaughtered in the evening on four dates during the experiment. Further, hens were slaughtered at four different times of day at the end of the experiment (Paper 4).

2.7 Outline of thesis

The papers in the present study are discussed in four chapters (3-6) in relation to relevant literature. In Chapter 3 ‘Feed intake from forage’, it was considered important to include a discussion on the capacity and the adaptability of the digestive organs, since the amount of course feed consumed obviously is dependent on this. Subsequently, results from the methods used on herbage intake from forage were discussed.

The capability of the hens to select feed items was considered important in relation to the contribution of feed items from forage; particularly for hens restricted in nutrients in order to increase the intake of valuable feed items from forage. Thus, to make it probable that hens have capability to select different feed items from forage (Chapter 4) a short introduction in relation to choice feeding is made. Selection of feed items from forage is subsequently discussed in relation to the results from the methods used in this study.

In Chapter 5 a discussion on the possible contribution of important nutrients from forage is made. The requirement for metabolizable energy (ME) is assumed to be considerable in a forage-based system, due to a high activity level, especially when hens are nutrient-restricted. Therefore, it was chosen to focus on the contribution of ME from forage. Moreover, ME together with amino acids has been suggested to be the dominant deficiency in scavenging chickens (Kyvsgaard & Urbina, 1996). The first two limiting amino acids in commercially prepared poultry rations are lysine and methionine (Bønsdorf, 1996) for which reason these amino acids were chosen for this discussion. Finally, the contribution of calcium was discussed as this nutrient is important for the eggshell formation (Clunies et al., 1992).

In Chapter 6, the consumption of supplementary feed in the papers is discussed in relation to the regulation of feed intake. Moreover, the performance of hens in a forage-based system is discussed in relation to the feeding of different types of supplementary feed. Further, it is suggested how the achieved results of productivity and intake of supplementary feed achieved in the present papers influence the nutrient balances. Finally, the welfare of the hens is discussed in relation to restriction of nutrient supply in a forage-based production system.

3. Feed intake from forage

The intake of roughage from an open-air run depends on the motivation of the hens to forage, i.e. hens restricted in nutrients presumably have a higher motivation to forage (Elwinger et al., 2004). Moreover, it also depends on the capacity of the digestive tract, for which reason a period of adaptation may be required to increase the feed intake from forage. Only limited information exists regarding roughage intake from standing crops, just as methods to estimate voluntary intake of forage are not well developed. In this chapter the results on different methods on forage intake are given and interpreted in the light of our current understanding of the capacity of the digestive tract of poultry.

3.1 Capacity of the digestive tract to forage

3.1.1. Capacity of the crop

Even though some hydrolysis of starch occurs in the crop (Bolton, 1965), the main function of the crop is to store and soften ingested food before it is transported to the proventriculus and the gizzard (Duke, 1986). The capacity of storage seems to increase with increased intake of feed. Thus, Lepkovsky et al. (1960) found that chickens trained to eat rapidly had larger quantities of feed in the crop per unit time than untrained chickens, just as crops were heavier in trained chickens, presumably reflecting the greater capacity of these crops. Our results support that the capacity of the crop can be increased, since the amount of supplementary feed in the crop was significantly influenced by number of days in the experiment (Paper 4). Thus, more supplementary feed was found in the crop at the last day of slaughter and least at the first day of slaughter (Paper 4). This was in particular pronounced in hens fed whole wheat and oyster shells as supplementary feed, illustrated by a significant interaction between type of supplementary feed and day in experiment. Similar results were seen in a short-term experiment (Paper 3) where the content of wheat was higher on the second day of slaughter, whereas the content of concentrate remained constant. The results suggest that the rate of adaptation, besides the amount of feed, is dependent on the type of feed too.

The capacity of the crop is closely related to the capacity of the gizzard since the crop supplies the feed to the gizzard in successive quantities as required (Browne, 1922). According to Heuser (1945) there is always feed in the gizzard when there is feed in the crop, suggesting that a more or less empty gizzard will cause the feed to pass directly through the crop to the gizzard. Mongin (1976) found, though, the same amount of dry matter in the gizzard at different times of the day. However, crop content differed significantly during the day, suggesting that the upper limit of the gizzard was higher, resulting in a continuous flow of feed through the gizzard. Thus, differences in crop content were presumably related to differences in feed intake during the day (Mongin & Sauveur, 1974). Compared with the study by Mongin (1976), dry matter content in the gizzard was much higher at four different times of day in our study (Paper 4). Especially for hens fed whole wheat compared with concentrate-fed hens. However, the gizzard content, exclusive of insoluble grit stone, was significantly affected by time of day so that the amount was smaller in the morning. This indicates that feed consumed during the morning passes more rapidly through the crop to the gizzard, whereas an accumulation of feed in the crop occurs during the day, which leaves the crop full at the end of the day.

3.1.2. Capacity of the gizzard

Steenfeldt et al. (2001) found that hens fed maize silage or barley/pea silage had larger gizzards and a higher content of feed in the gizzard than hens with no access to roughage. Further, the gizzards were larger and the amount of dry matter in the gizzards higher when hens were 53 weeks of age compared with 23 weeks of age, but only when the hens were fed maize and barley/pea silage. Thus, no differences were seen in hens that were fed concentrate exclusively, indicating that the gizzard adapts to more coarse feed, presumably resulting in a higher capacity of the hen to eat more of this kind of feed.

Besides the coarse nature of roughage, the increased size of the gizzard in hens fed roughage is probably caused by the larger amount of fibrous material in this feed. According to Starck (1999), Japanese quails, which were fed a larger proportion of non-digestible fibres in the diet, had twice as large gizzards as those fed the standard diet. Moreover, size response was found to be reversible, since reduced non-digestible fibres were followed by a decrease in the gizzard size.

In an experiment with rotation of hens in different forage vegetations, we found that wheat-fed hens had larger gizzards than concentrate-fed hens at the end of the 130 days experimental period (Paper 4). This could be due to the wheat-fed hens eating more of the available forage vegetation and larger amount of soil (Papers 3 and 4). Thus, the dry matter content, exclusive of insoluble grit stone, in the gizzard was significantly higher in the wheat-fed hens. In addition to the feed intake from forage, the coarse nature of whole wheat probably enhances the development of the gizzard too (Williams et al., 1997; Ferket, 2000). Thus, larger gizzard sizes have been found in layers as well as broilers fed whole wheat than in those fed pelleted feed (Forbes and Covasa, 1995; Preston et al., 2000; Svihus and Hetland, 2001; Hetland et al, 2002; Plavnik et al, 2002).

3.1.3. Capacity of the small intestine and caeca

In relation to the weights and the pH-value of the content in jejunum, ileum and caeca, no distinct pattern between the types of feed was observed when hens were 23 week of age (Steenfeldt et al., 2001). However, at 53 weeks of age the content of feed in the jejunum as well as the pH-value in the gizzard and the caeca was significantly lower in hens fed the maize silage or barley/pea silage than in hens fed only concentrate. The weight and length of the small intestine is apparently not influenced by the inclusion of whole wheat in broiler diets (Wu et al., 2004), though Gabriel et al. (2003) found that the digestive capacity of the intestine decreased with whole grain feeding despite increased chemical and physical capacity of the upper part of the digestive tract. These results suggest that initially it is the crop and the gizzard that responds to a more coarse and fibrous feed, but during a prolonged period with access to roughage other parts of the digestive tract may respond too.

3.2. Estimates of feed intake from forage

Since the crop, the gizzard, and to some extent, the small intestine develop when increased amount of coarse feed is eaten by the hen, it seems plausible that the hen is able to consume a larger amount of forage after a period of adaptation. Thus, after a period of adaptation laying hens will to a lesser degree be dependent on a nutrient-balanced supplementary diet as indicated by the productivity results in Paper 4. It is, however, difficult to give an exact estimation on the amount of feed that the hens actually consume from an open-air run with vegetation. Moreover, the capacity of the hen to consume and utilize forage is presumably dependent on factors besides the above-mentioned adaptation. The quality of the forage, the type of supplementary feed available, genetics and age of the birds may be physical factors affecting the intake of forage from the open-air run.

3.2.1. Sward-based methods

One method of interest to investigate intake of forage from the open-air run is a sward-based method, where herbage mass is estimated before and after grazing a given area. Thus, Hughes and Dun (1983) estimated grass intake by cutting off grass inside and outside some exclusion cages by which the layers were prevented from removing the herbage contained within. Grass intake was assessed to 24.7-48.3g DM per hen per day for hens with their nutrient requirement covered through layers mash. However, opposing factors, such as different growth rates of the herbage in- and outside the cages were not assessed, just as not all grass removed may be eaten. Danielsson et al. (1994) used a similar method. However, instead of exclusion cages, a method was used where some plots were harvested prior to and after the hens' access to a mobile aviary system. Consumption of herbage was estimated to 10-30g DM per hen per day for hens fed an on-farm made concentrate ad lib.

In the experiment described in Paper 1 a method was used where plots were harvested prior to and after the hens' access to fenced forage areas with approximately one m^2 per hen for two or three days. Despite a huge standard deviation between parcels, the mean values were comparable to the above estimates, since removal of grass/clover ranged from 9-31g DM per hen per day. No distinct pattern between types of supplementary feed (concentrate vs wheat) in relation to the removal of grass/clover was observed. However, in parcels with chicory the removal was much higher with estimates of 51 and 73g DM per hen per day for concentrate-fed and wheat-fed hens, respectively. This indicates that type of forage and type of supplementary feed may influence the amount of herbage removed. For all these studies it must be taken into consideration that not all the removed herbage may have been eaten since the uprooting and discarding of herbage by the scrapping of the hens could not be quantified.

3.2.2. Method based on crop content

In addition to the above methods, the crop has been considered as a possible tool to estimate forage intake in hens on an open-air run. Thus, Wood (1956) suggested that crop examination of chickens having access to pasture was a satisfactory method of studying forage consumption when they were slaughtered just before night rest. This may be due to the crop being full at this time of day (Mongin, 1976), reflecting a higher feed intake at the end of the day (Ballard & Biellier, 1969; Mongin & Sauveur, 1974), just as the gizzard is full at this time of day and an accumulation of feed in the crop thus takes place.

In individually caged hens, Antell and Ciszuk (2006) found a positive correlation between intake of grass recorded and amount of grass found in the crop in the evening. They proposed the relation 'y = 0.144x – 0.178', where "y" is the amount of grass in the crop and "x" is grass intake on an air-dried basis the last day before slaughter. Results on herbage intake using this relation on the content of herbage in the crops from Papers 3 and 4 are shown in Table 1.

These data on herbage intake, based on crop content and the above relation, were subject to analysis of variance using the GLM procedure in SAS (SAS Institute Inc., 1990). The experimental designs are described in Papers 3 and 4. For the statistical analysis below, the average of two slaughtered hens from each subplot was used as dependent variable. Statistical analysis was carried out using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} = intake of herbage; μ = mean; α_i = supplementary feed $(i = 1,2)$; β_j = forage vegetation $(j = 1\text{-}8)$; $(\alpha\beta)_{ij}$ = the interaction of supplementary feed × forage vegetation.

In Table 1, different letters (a,b,c and d) indicate significant differences between forage vegetation. An overall significant difference ($P < 0.001$) in herbage intake between types of supplementary feed was found, since wheat-fed hens had a higher intake of herbage than concentrate-fed hens. However, in Paper 3 the statistical analysis only indicated a significantly higher amount of herbage in the crop of wheat-fed hens when they were foraging the chicory. In Paper 4, the statistical analysis revealed an overall higher amount of herbage in the crop when hens were on the wheat-diet compared with the hens on the concentrate-diet.

Table 1. *Estimated daily dry matter intake of herbage based on the amount of herbage found in the crop in the evening (Papers 3 and 4), LS-means and SEM (different letters indicate significant differences)*

	Supplementary feed		
Forage vegetation:	**Concentrate (g DM)**	**Whole wheat (g DM)**	**Average (g DM)**
Grass/clover (*June/July, 2004, Paper 3*)	14 (*5.2*)	21 (*5.2*)	17 (*3.6*)^{ad}
Mixture of forbs (*June/July, 2004, Paper 3*)	14 (*5.2*)	22 (*5.2*)	18 (*3.6*)^{ad}
Grass/clover (*August, 2004, Paper 3*)	41 (*5.2*)	50 (*5.2*)	46 (*3.6*)^{b}
Chicory (*August, 2004, Paper 3*)	42 (*5.2*)	73 (*5.2*)	57 (*3.6*)^{c}
Pea/vetch/oats (*July, 2005, Paper 4*)	12 (*5.2*)	19 (*5.2*)	15 (*3.6*)^{a}
Grass/clover (*August, 2005, Paper 4*)	17 (*5.2*)	26 (*5.2*)	21 (*3.6*)^{ad}
Lupin (*August/September, 2005, Paper 4*)	12 (*5.2*)	29 (*5.2*)	21 (*3.6*)^{ad}
Quinoa (*September/October, 2005, Paper 4*)	23 (*5.2*)	30 (*5.2*)	27 (*3.6*)^{d}
Average	22 (*1.8*)	34 (*1.8*)	$P < 0.001$

The estimated intakes of forage in Table 1 seem fairly reliable compared to the above estimates based of harvested forage, even though the amount of grass/clover in August 2004 (Experiment 2) seems relatively high compared to these estimates. However, differences in the quality of forage and the motivation of the hens to forage might influence the herbage intake. The estimated amounts of chicory determined by the crop content seem to be comparable to the estimated amounts determined by the harvest method, suggesting that hens ate a considerable amount of this species of plant. This is in accordance with Wood (1956), who found that chicory (*Cichorium Intybus*) together with broadleaf plantain (*Plantago major*) were more abundant in the crops in White Rock chickens (22-24 weeks of age) than other species of plants. Wood (1956) concluded that palatability of plant species should be considered before establishing pastures for chickens. Moreover, Sanderson et al. (2003) concluded that chicory and plantain are of a relatively high nutritive value, which could enhance the nutritional profile of mixed species pastures, suggesting that selectivity of plant species by poultry may be related to the nutritive value, too.

The estimates in Table 1 clearly reveal that hens restricted in nutrients through whole wheat and oyster shells have a higher intake of herbage than hens which have their nutrient requirements covered through ad lib concentrate. Thus, the above estimates indicate an approximately 50% higher herbage intake in nutrient-restricted hens. This is in accordance with visual assessments of the plots, indicating that herbage disappeared much faster in the plots foraged by nutrient-restricted hens. Moreover, chicory seems to be an attractive forage crop for laying hens.

4. Feed selection from forage

Experiments with domestic birds have shown that hens and broilers offered a range of different feedstuffs have the ability to choose a diet which provides them with all the nutrients necessary for growth, maintenance and production (reviewed by Rose & Kyriazakis, 1991; Forbes & Shariatmadari, 1994; Henuk & Dingle, 2002). However, Dana & Ogle (2002) found that, compared to choice-fed confined hens, choice-fed scavenging hens in Ethiopia did not change the pattern of diet selection suggesting that scavenging contributed little to the nutrition of the hens. Moreover, choice-fed hens in general were not able to eat sufficiently to meet their requirements for protein. However, other more palatable sources of protein may influence the diet selection (Kiiskinen, 1987). Moreover, Forbes & Covasa (1995) suggest that periods of learning, previous experience and social interactions are needed before becoming proficient when given a choice of feed.

Also hens on pasture have been found to be able to compose a ration when different feed items were supplied ad lib (Fuller, 1962; Ciszuk & Charpentier, 1996). In our investigations, a higher intake of oyster shells were found in hens fed whole wheat than in hens meeting their nutrient requirements through a pelleted concentrate (Papers 1 and 4). However, very little is known on how layers select between feed items from an open-air run when given access to different forage crops, and fed a normal diet covering their need for nutrients or a nutrient-restricted diet.

4.1 Feed selection determined by crop content

One method to determine feed selection from a cultivated open-air run is to examine the content of the crop. Since limited digestion of feed occurs in the crop, it is relatively easy to identify feed items. Thus, Jensen & Korschgen (1947) found that analysis of crop content is a better method of obtaining information on food habits of quails than analyses of droppings and content of gizzards. Limited literature is available on this subject in relation to free-range poultry, although there have been a few studies (Wood et al. 1963; Amaka Lomu et al, 2004; Antell and Ciszuk, 2006). In other works, analysis of crop content has been used as a method of getting information on food habits in scavenging, rural hens (Gunaratne et al., 1993; Ajuyah, 1999; Mwalusanya et al., 2002; Sonaiya, 2004).

As mentioned above the crop is expected to be full in the evening, since the amount of DM in the crop has been shown to follow a cyclic variation influenced by the photoperiod (Mongin, 1976). However, Mongin & Sauveur (1974) found that hens fed a calcium-poor diet, but with access to oyster shells had a higher intake of mash or pelleted feed in the morning and in the afternoon than hens fed a calcium-rich diet, indicating that different feeding strategies influence feed intake in layers.

In experiments with hens on range, hens had significantly more of almost every feed item in the crop in the evening than in the morning irrespectively of type of forage vegetation and type of supplementary feed (Paper 3). However, when hens were slaughtered on the first day after being introduced to new forage plots, no significant effect of times of slaughtering during the day were seen in relation to the amount of weed seeds, earthworms, insoluble grit stone and soil in the crop (Paper 4). This indicates that hens change foraging behaviour when introduced to new forage vegetation, presumably because the amount of earthworms in the ground surface was very high immediately after the hens were introduced to this new forage vegetation. In general, hens had a very high content of earthworms and larvae in the crops on the first day after being introduced to new forage vegetation compared to crops from hens slaughtered after several days (Papers 3 and 4). According to Forbes & Shariatmadari (1994) short periods of access to one food alone are followed by a preference for the opposite food. Since the amount of earthworms, larvae and insects presumably decreased concurrently with the number of days the hens were present in the plots, these feed items were limited at the end of a period in a given forage vegetation. Thus, hens were very motivated to select these feed items when they were moved to new forage vegetations. Nutrient-restricted (whole wheat + oyster shells) hens were particularly motivated to select these feed items. Thus, we found that these hens had significantly more earthworms and insects in the crops than concentrate-fed hens on the first day after moving to new forage. Since wheat-fed hens were in deficit of amino acids, this result indicates that they were able to give priority to these feed items with a high content of amino acids (Sugimura et al., 1984; Pokarzhevskii et al., 1997). The high amount of soil in the crops in nutrient-restricted hens presumably reflects the scavenging of the hens for earthworms and insects in the ground surface even though microorganism, roots and even soil has been found to contain various levels of amino acids (Pokarzhevskii et al., 1997).

As mentioned in Chapter 3 a higher amount of plant material can be found in the crops from nutrient-restricted hens (Papers 3 and 4). It was suggested that the plant material in the crops from these hens appeared to be more fibrous than the plant material in non-restricted hens (Paper 3). Fisher & Weiss (1956) found that bulk stimulate a greater feed consumption, which might explain why nutrient-restricted hens chose to eat more fibrous feed. Moreover fibrous material, as mentioned in Chapter 3, increases the size of the gizzard, resulting in a greater capacity of this organ which in turn may increase the flow of feed through this organ (Starck, 1999).

Nutrient-restricted hens further had a larger amount of oyster shells in the crop than non-restricted hens, indicating a preference for feed items with a high content of calcium. A higher amount of insoluble grit stone was found in the crop from nutrient-restricted hens too (Papers 3 and 4). This is in agreement with Karunajeewa & Tham (1984) finding that pullets given whole grains ate more hard grit than those given crushed grains, suggesting that hens fed a more coarse feed need more grit stone for the gizzard activity. In contrast, we did not find different amounts of insoluble grit stone in the gizzard between hens fed different kind of supplementary feed; neither was the amount affected of time of day (Paper 4), indicating that excess in grit stone passes out of the gizzard when the requirement is fulfilled (Tyler, 1955; Robinson, 1961).

Hens restricted in nutrient supply did not consume seeds of weeds or seeds of cultivated plants to same extent as did non-restricted hens (Papers 3 and 4). Especially when it comes to peas, lupins and quinoa seeds this seems surprising, since these seeds are known to have a higher content of protein than wheat (Andersen & Just, 1983; Hammershøj & Steenfeldt, 2005; Paper 4). However, for birds selecting feed, visual stimulation evidently plays a major role and feed preferences are clearly recognized (Frazer & Broom, 1997). Since nutrient-restricted hens were fed whole wheat, other seeds were probably considered as being of the same nutritional value as the wheat even though the size and shape was not the same. A longer period in the plots with seeds of cultivated plants might have resulted in a changing of feeding strategy in nutrient-restricted hens as a result of adaptation and learning (Forbes & Covasa, 1995), since feed items of animal origin were scarce at the end of the periods, whereas seeds of cultivated plants were abundant in the plots with wheat-fed hens. Unlike the nutrient-restricted hens, the non-restricted hens were found to eat considerable amounts of seeds of cultivated plants; especially quinoa seeds were abundant in the crops with levels of 4g DM per crop analysed (Paper 4).

Hens on range have a high capability to select feed items from the range area. Moreover, it seems obvious that selectivity from this area or from the feed supplied is influenced by the type of supplementary feed offered and changes in feed items available in the range area, i.e. when new forage is offered. However, the type of forage offered influence the selectivity only to a minor degree (Paper 3) even though more differences were seen between different forage vegetations (days of slaughter) in the experiment in Paper 4.

4.2 Selection of species of plants determined by a microhistological analysis of faeces

Based on analyses of crop content, selectivity among species of plants has been emphasized in a study with White Rock chickens, suggesting that some species of plants are more palatable than others (Wood, 1956). Though analysis of the crop seems to be a reliable way to determine the botanical composition of herbage eaten by the poultry (Wood, 1956, Wood et al., 1963), analysis of faeces would allow considerably more samples to be collected, just as slaughtering of birds is avoided. Analysis of faeces has been used to estimate feed habits in wild birds (Green, 1978; Hill, 1985; Steenfeldt et al., 1991). However, the method of microhistology, which is based on cellular characteristics from leaf epidermis (Hansen et al., 1976), has mainly been used to estimate the botanical composition of the diet of grazing herbivores (Gross et al., 1983; Winder et al., 1996; Miller & Thompson, 2005), whereas no publications on this subject were found in relation to free-range, laying hens. This may be due to the fact that in the industrialization era poultry has primarily been produced in systems without range areas. However, poultry on range has become a practice of commercial importance anew.

In the experiment in Paper 2 we used microhistology to determine the botanical composition of faeces from hens in a forage-based system. The estimated botanical compositions were found to differ between hens restricted in nutrients and hens fed a pelleted concentrate. Thus, nutrient-restricted hens had significantly more of the grass species "*Elytriga repens*" and less of white clover "*Trifolium repens*" in the faeces than hens fed a pelleted concentrate. This was surprising since clover is known to have a higher content of crude protein and some minerals than grasses in general. Thus, by investigating the composition of the sward Thomas (1937) observed that Rhode Island Red layers supplemented a low protein ration by consuming clover and dandelions instead of perennial rye-grass. However, the results from Paper 2 seem to fit with the description of the plant material found the crops (Paper 3).

Besides the estimated botanical composition of herbage in poultry faeces in the study in Paper 2, also selectivity indices were calculated. They were calculated on the basis of visual assessments of the proportion of the ground surface covered by different plant species. These selectivity indices revealed differences in selectivity in the way that negative selectivity was found for "*Trifolium repens*" and positive selectivity for "*Elytriga repens*" for both types of supplementary feed when hens were foraging the grass/clover plots. This may be due to that hens give priority to the grasses at the expense of the white clover.

In the plots sown with a mixture of different forbs (*Fagopyrum esculentum, Phacelia tanacetifolia and Linum usitatissimum*) positive selectivity was found for "*Trifolium repens"* as well as for the grasses "*Elytriga repens*" and "*Lolium perenne*". Negative selectivity was found for "*Phacelia tanacetifolia"* and *"Fagopyrum esculentum"*. The common denominator is the fact that low selectivity indices were given for the tall (60-70 cm) species of plants, whereas high priority was given to the plant species nearer the ground (20-30 cm), suggesting that the hens gave priority to these plants. It cannot be excluded that the species of plants nearer the ground might have been underestimated in the visual assessment.

This experiment (Paper 2) indicates that type of supplementary feed influences the selectivity of laying hens for plant species, since nutrient-restricted hens were found to have more grasses in the faeces, whereas non-restricted hens had more clover. Moreover, the results suggest that hens select species of plants nearer the ground surface at the expense of taller species of plants.

5. Contribution of nutrients from forage to meet nutritional requirements of layers

The contribution of forage for laying hens is primarily described in older studies, mainly dated in the period from the 1930s to the 1950s. The methods used are mainly based on indirect measurements, i.e. measurements on consumption of supplementary feed and egg production. Thus the studies give no estimation of the amount of feed that hens have been eaten from the forage, but estimate instead the amount of supplementary feed that have been saved by giving the hens access to a forage area. Weinmiller (1936) reported savings of 40.8% of the protein feed and 34.3% of the total feed costs for Leghorn hens kept on a good, open pasture for six months (summer) with 20 m^2 per hen compared with six months (winter) with no access to an open-air run. In a second experiment Weinmiller (1936) reported that hens on a summer pasture consumed 32.1% less feed than hens with no access to pasture. Buckner et al. (1945) found that spring bluegrass saved about 20% of an all mash ration for White Leghorn yearling hens while mature bluegrass pasture did not influence the consumption of mash. Moreover, the bluegrass range increased their egg production during the growing season. Sipe & Polk (1941) reported reduced feed requirements for egg production from 5.7 to 10.1% for White Leghorn hens foraging Italian rye grass, oats or Japanese tendergreen mustard. The rye grass provided the greatest number of grazing days, however less feed was required with oats for grazing, with mustard second and rye grass third. Fuller (1962) found that access to pasture resulted in 6% savings of total feed consumed when pullets were fed a conventional mash-grain diet, but 13% when pullets were permitted to select the grain, mineral and protein-vitamin components. However, savings of 20% in total feed consumed was realised where pullets were forced to forage by providing them with grain and minerals only.

From a newer study it is known that high producing laying hens are able to consume and utilize roughage when fed in a floor system. Thus, hens fed roughage had a lower intake of concentrate and a lower intake of concentrate per kg egg produced than hens with no access to roughage (Steenfeldt et al., 2001). However, recent research upon the intake of herbage and other feed items from a forage area is scarce, even though Gustafson & Antell (2005) suggest that supplementary feed intake in hens with access to cultivated areas with oilseed, sunflower and wheat was 20% lower than expected at the actual laying rate, while the feed intake from the forage could not be quantified.

In the experiments included in this thesis the supplementary feed varied in composition in the way that high-producing, laying hens were fed whole wheat and oyster shells, or were meeting their nutrient requirements through a pelleted concentrate. This was done to estimate the contribution of forage in nutrient-restricted versus non-restricted hens.

5.1 Contribution of metabolizable energy from forage

Using the requirements of metabolizable energy (ME) for egg production, maintenance and growth given by the NRC (1994), the contribution of ME from range areas was calculated in two experiments with hens kept in a free-range system for 23 days (Paper 1). Hens that were not restricted in nutrients were barely covered or oversupplied with ME through the supplementary feed (pelleted concentrate) for Experiments 1 and 2, respectively, whereas the forage area contributed with approximately 0.13 MJ ME per hen per day in nutrient-restricted hens (whole wheat) in both experiments. However, the formula for ME requirements (NRC, 1994) does not consider specific requirements of hens kept in a free-range system with a high activity level. Hegelund et al. (2006) found an average feed intake of 1.57 MJ ME per hen per day for brown egg layers at seven organic egg producers. This is approximately 10% (0.15 MJ ME per hen per day) higher than the requirements based on the NRC formula for the hens (brown egg layers) in the experiments in Paper 1.

Assuming that feed intake from range area in traditional, organic egg production systems is negligible; this difference in ME might count for the specific requirements for hens kept in traditional, organic egg production systems. Adding this specific requirement to the above estimates based on the NRC requirements suggests that the forage may supply the nutrient-restricted hens in the experiments in Paper 1 with approximately 0.28 MJ ME per hen per day. Moreover, hens in forage-based systems, such as in the experiments in Papers 1-4, presumably had an even higher activity level than hens in traditional, organic egg production systems; especially the nutrient-restricted hens.

Referring to the above estimates on herbage intake from forage (Chapter 3), hens on grass/clover pasture may consume approximately 30g DM of herbage per hen per day. Using the nutrient contents of grass/clover in Experiment 2 (Paper 1), this would correspond to 0.18 MJ ME per hen per day (Chwalibog, 1993). Since hens also consumed other feed items (Papers 3 and 4), this amount seems to fit very well with the above estimates based on the NRC requirements. However, attractive crops such as chicory may have a higher contribution of nutrients. Thus, 70g DM of chicory (Chapter 3) would correspond to approximately 0.39 MJ ME per hen per day.

In Paper 4 calculations were made for hens kept in a crop rotation system for 130 days with four different types of forage vegetation. However, calculations were based on the last 11 weeks in this experiment, since the first period was considered as an adaptation period because hens lost weight and had a decrease in egg production in this period. Based on the above-mentioned formula for ME requirements (NRC, 1994) it was suggested that nutrient-restricted (whole wheat) as well as non-restricted (pelleted concentrate) hens had a minimum 12% of their requirement for ME met through forage material in average for this period. Again without consideration to specific requirements for ME of hens kept in a free-range system. However, significant effects of week in the intake of supplementary feed indicate that the contribution of forage varied considerably during the experimental period, e.g. concentrate-fed hens had a low intake of concentrate when they were foraging the quinoa plots. This is in accordance with the analyses of the crop content (Paper 3) showing that quinoa seeds totalled more than 18% of the total amount of feed DM in the crops, suggesting that quinoa seeds accounted for a considerable part the requirement for ME; especially since quinoa has a very high content of ME (Paper 4) and presumably a low retention time in the crop due to a small particle size.

These studies indicate that forage may contribute considerably to the ME requirements of free-range, laying hens irrespectively of type of supplementary feed; especially after an adaptation period. However, since intake of supplementary feed varied during the experimental period in Paper 4, the contribution of ME from forage seems to depend on type and value of forage vegetation. Further, the level of activity may be considered. It seems, however, possible that hens in a forage-based system may consume up to 0.25 MJ ME per hen per day from the forage. This is approximately 16% of the ME intake in the above study by Hegelund et al. (2006) where organic hens consumed an average of 1.57 MJ ME per hen per day. Moreover, the intake of ME from forage may be considerably higher in periods with plenty of earthworms in the ground surface, with access to seeds of cultivated plants such as quinoa, or with attractive herbage like chicory.

5.2 Contribution of lysine and methionine from forage

The essential amino acids methionine and lysine is considered as the two first limiting amino acids in commercially prepared poultry rations (Bønsdorf, 1996) for which reason attention has been paid to these amino acids in studies on laying hens (Al Bustany & Elwinger, 1987; Shafer et al., 1998; Kjaer & Sørensen, 2002). The NRC (1994) estimates for lysine and methionine are 0.76 and 0.33g per hen per day, respectively. However, just as the NRC requirements for ME these may be considered, as minimum requirements for hens not kept in a free-range system. Thus, Lohmann Tierzucht (2006) estimates a daily requirement of 0.87g lysine and 0.44g methionine per hen per day for brown egg layers kept in a free-range system and with a daily egg mass of 57.5g.

In the experiments in Paper 1 non-restricted hens were fully covered with lysine and methionine through the intake of concentrate. According to the Lohmann Tierzucht (2006) estimates, lysine was oversupplied by 33% and 61% for Experiments 1 and 2, respectively, whereas the methionine were oversupplied by 64% and 94%, respectively. In contrast, the whole wheat undersupplied the nutrient-restricted hens in both experiments by approximately 66% and 57% for lysine and methionine, respectively. Since nutrient-restricted hens lost weight in the experimental period (23 days), these undersupplies were probably not compensated for through intake of forage, even though a reduced intake of ME might be an explanation too. However, based on the NRC requirements it can be assumed that the requirements for maintenance for lysine and methionine are approximately 0.30g and 0.15g per hen per day, respectively. Based on this assumption the egg production requires about 0.01g lysine and 0.005g methionine per g egg mass according to the requirements of Lohmann Tierzucht (2006). Thus in Paper 1, it was calculated that wheat-fed hens required an average of 0.46g lysine and 0.22g methionine extra for the egg production attained, indicating that these amounts were supplied from the forage and the bodypool. However, hens foraging the chicory did not loose bodyweight, which was the case for hens on grass/clover. This was presumably due to the high consumption of chicory, just as the content of amino acids in this forage crop was favourable (Paper 1).

As pointed out in the above Section 5.2 calculations in Paper 4 were based on the last 11 weeks in this experiment. For nutrient-restricted hens in this experiment the wheat supplied the hens with 0.27g lysine and 0.14g methionine. This indicates an undersupply of lysine and methionine of 69 and 68%, respectively, according to the above requirements of Lohmann Tierzucht (2006). Since nutrient-restricted hens actually gained body weight and egg weight in this period, just as the average egg mass only was a little lower than the presupposed 57.5g by Lohmann Tierzucht (2006), it seems plausible that nutrient-restricted hens had at least 70% of the requirements of lysine and methionine met by forage material. In contrast, the concentrate fulfilled the non-restricted hens with the required amount of lysine and methionine, even though these hens had a lower intake of supplementary feed in this period. Since concentrate-fed hens further had a considerable intake of forage material (Paper 4), an oversupply of these amino acids seems probable.

From above studies it is plausible that good forage may contribute considerably to the lysine and methionine requirements. Thus, it seems possible that hens fed whole wheat and oyster shells as only supplementary feed had about 70% of the requirements for lysine and methionine met through forage material after a period of adaptation. However, for non-restricted hens the forage did not contribute to the requirement of these amino acids, since these hens were fully covered through the concentrate, suggesting that the standards for lysine and methionine in the supplementary feed can be reduced if good forage is offered.

5.3 Contribution of calcium from forage

Calcium for laying hens is of particular interest due to the fact that calcium is important for the eggshell formation (Clunies et al., 1992; Shen & Chen, 2003). According to the NRC (1994) the calcium requirement for brown egg layers is about 4g per hen per day. In Paper 1 intake of calcium of non-restricted hens was calculated to be 5.3 and 6.7g per hen per day for Experiments 1 and 2, respectively, which is higher than required. Moreover, this is without consideration for the contribution from forage. Nevertheless, non-restricted hens consumed oyster shells despite the fact that the requirement for calcium was met through the concentrate.

In nutrient-restricted hens the intake of calcium from wheat and oyster shells (32% calcium) was calculated to be 1.9 and 2.4g per hen per day in Experiments 1 and 2, respectively (Paper 1) and 3.1g per hen per day in the experiment in Paper 4. This is below the above-mentioned calcium requirement. However, no differences in eggshell percentage and eggshell strength were seen in Experiment 2 (Paper 1), suggesting that the calcium requirement were met through oyster shells and foraging material. It may be postulated that hens on a short-term basis are able to compensate by using the skeletal body pool of calcium, since studies on bone breaking strength have proved to decrease with decreasing dietary calcium (Frost & Roland, 1991). However, nutrient-restricted hens clearly revealed a capability to eat more oyster shells than non-restricted hens (Papers 1, 3, and 4), suggesting that they consumed what was necessary to meet their requirements. Moreover, soil, earthworms, insects and herbage may contribute to the calcium supply of the hens. Except for the hens foraging the chicory, the nutrient-restricted hens in the experiments in Paper 1 had a decreasing egg production, indicating a lower requirement for calcium of these hens. Finally, a long-term experiment (130 days) with the same type of supplementary feed revealed no signs of poorer health and welfare of nutrient-restricted hens (Paper 4), suggesting that the calcium requirement were met through intake of calcium from oyster shells and forage.

Despite a higher intake of oyster shells in nutrient-restricted hens they did not fully meet the NRC (1994) requirements. Assuming that the NRC requirements are not overestimated, the above studies indicate that intake of calcium from the forage area may be 1-1.5g per hen per day.

6. Production in forage-based systems

Poultry production in forage-based systems was mainly of interest in the pre-industrial period as suggested in above Chapter 5. However, along with the introduction of organic farming, cultivation of the open-air run has anew become of current interest. Partly to fulfil the consumer demand for increased animal welfare and partly for the purpose of supplying the hens with nutrients from forage. The latter has further potential in the protection of the environment against leaching of nutrients to the ground water.

Recent research on poultry production in forage-based systems is mainly related to systems where poultry is integrated into cropping systems to obtain synergism. Thus, besides the performance of the poultry, these studies have, more or less, investigated the effect of poultry in crops and pastures on e.g. weed, insect pests and/or soil manuring (Men et al., 2002; Glatz et al., 2005; Gustafson & Antell, 2005; Miao et al., 2005). Similar studies have been conducted on poultry integrated into orchards (Clark & Gage, 1996; Hermansen et al. 2004; Pedersen et al., 2004; Horsted et al.; 2005), whereas studies on poultry in mixed grazing systems with other farm animals are limited (Antell et al., 2006). Thus, there is limited knowledge on how the high producing layers of today produce in a system, based on the contribution of nutrients from forage. This is particularly evident, if hens are restricted in nutrients through the supplementary feed in order to increase feed intake from forage.

Moreover, little is known on how this kind of restriction will influence the circulation of nutrients in the forage-based system, and the welfare of the hens.

6.1 Productivity of hens in forage-based systems

6.1.1 Consumption of supplementary feed in forage-based systems

According to Hy-Line® (2006), the estimated intake of concentrate for Hyline Brown layers in a free-range system is within the range of 125-135g feed per hen per day. However, hens in a free-range system have been found to have a higher intake than expected (Keeling et al., 1988; Hegelund et al., 2006). In Paper 1 it was found that hens in a forage-based system consumed an average of 129g pelleted concentrate per hen per day in Experiment 1, whereas the amount in Experiment 2 was 155g per hen per day. The latter seems relatively high and might be due to wastage of feed even though similar results have been found in farm studies on organic layers (Hegelund et al., 2006). In Paper 4 the average intake of pelleted concentrate was made up to 125g per hen per day; huge variations during the 130 days experiment were seen, however, presumably due to varying supply of feed items such as earthworms, insects and seeds (Chapter 4) from the forage vegetation. In contrast, hens fed whole wheat consumed approximately 90g of this feed per hen per day in both experiments in Paper 1. In Paper 4 the average amounts of wheat consumed were slightly higher (94g per hen per day). However, the intake of whole wheat was only about 80g per hen per day in the first 5-6 weeks, whereas a distinct increase to approximately 100g per hen per day was seen after this period. This level continued throughout the remaining part of the experiment, with minor fluctuations.

It can be hypothesized that this distinct increase in wheat intake may be due to the fact that the digestive organs became adapted to more coarse feed concurrently with the number of days in the experiment as pointed out in Chapter 3. However, Al Bustany & Elwinger (1988) found a higher feed conversion ratio in hens fed whole barley or wheat than ground barley and wheat, suggesting that grinding capacity of the gizzard is not a limiting factor for feed utilization (Svihus et al., 1997). This may indicate that an imbalance in nutrient supply, e.g. shortness in amino acids, may have resulted in decreased intake of wheat because of a high content of ME in this feed. On the other hand, reduced feed intake has been observed for diets with whole grain (Hetland et al., 2002), indicating that the passage rate has been slowed down. According to Svihus et al. (2002) reduced feed intake, due to whole grain feeding, may also be caused by a satiety sensation due to high gizzard activity. Nevertheless, analyses of crop content suggest that hens had a very high intake of earthworms after being introduced to new forage vegetation (Paper 4), i.e. they were presumably not lacking amino acids at this stage. On the other hand, it is possible that hens needed some time for learning and adjustment before becoming proficient when given a choice of food (Forbes & Covasa, 1995) and thus have been undersupplied with some nutrients at the beginning of the experiments in Papers 1 and 4. Consequently, it seems probable that adaptation of the digestive tract and adaptation in relation to learning are important factors when high producing layers are exposed to new feeding strategies involving feed items from the forage vegetation.

As pointed out in Chapter 4, it was shown that nutrient-restricted hens had a higher intake of oyster shells than non-restricted hens (Papers 1, 3, and 4). However, no significant effect between type of forage vegetation were seen (Paper 1), even though a tendency to a higher intake of oyster shells in hens foraging the chicory was seen. This was not expected, since chicory leaves apparently have a relatively high content of calcium (Belesky et al., 2001) compared to grass/clover (Andersen & Just, 1983). However, hens with access to chicory had a slightly higher egg production and thus a higher requirement for calcium (Paper 1).

From older studies as well as from the present papers it seems likely that reduced intake of supplementary feed can be obtained when hens are offered attractive forage vegetation. When hens are offered whole wheat instead of pelleted concentrate a lower intake of this supplementary feed has been found though the hens have shown an increase of intake of this feed a few weeks after introduction.

6.1.2 Performance of poultry in forage-based systems

Besides older studies, newer studies on egg production in forage-based systems have been conducted in Australia, where Glatz et al. (2005) found that the egg production of Hyline Brown layers in a free-range system was lower than the standard performance expected in a cage system. However, according to the authors this was expected since the experiment was carried out during the hottest summer in Australia in a century. A similar study at the same campus a year later revealed good performance of Hyline Brown layers foraging wheat stubbles and fed a commercial layer ration, even though slightly lower egg weights were seen compared with the standards. The authors suggest that this might be due to the hens consuming less protein than required, though body weight of these free-range hens were higher than the Hyline standards (Miao et al., 2005). In both of these experiments hens were fed half a layer ration (55g per hen) in the morning and the other half (55g per hen) in the evening.

In a short-term experiment (Paper 1) with two different forage crops (grass/clover versus mixed forbs) it was found that hens restricted in nutrient (whole wheat + oyster shells) had a lower laying rate and egg weight than hens not restricted in nutrients, suggesting that they were not able to eat sufficient forage material to maintain egg production. However, in a second experiment with chicory versus grass/clover laying rate was maintained and only egg weight significantly lower in nutrient-restricted hens. This was primarily due to hens foraging the chicory since a tendency to a higher laying rate could be noticed. Moreover, nutrient-restricted hens did not loose body weight, when they were foraging the chicory plots, whereas hens foraging grass/clover lost body weight. This indicates that the chicory plots supplied the hens with more nutrients than did the grass/clover.

The positive effect of chicory was further reflected in a higher albumen DM in eggs from hens foraging these plots. Since protein is the major constituent of albumen DM, this indicates a higher contribution of amino acids from the chicory plots. Thus, it has been shown that level of methionine intake is reflected in albumen DM (Shafer et al., 1996; Hammershøj & Steenfeldt, 2005). In Paper 1, this was also reflected in that concentrate-fed hens had a higher albumen DM than wheat-fed hens. Analyses of chicory leaves revealed a relatively high content of lysine (12.1g per kg DM) and methionine (4.0g per kg DM) (Paper 1). However, amino acid concentration in chicory varies according to cultivar, the age of the plant and part of the plant analysed (Foster et al., 2002).

In a crop rotation experiment (Paper 4) non-restricted laying hens maintained a laying rate around 85% throughout the whole experiment, despite a shortening of the length of the day. Nutrient-restricted hens had a decline in laying rate of the beginning at the experiment, particularly pronounced after 5-6 weeks. Subsequently, a remarkable and relatively rapid increase in laying rate was achieved to the same level as for the concentrate-fed hens. This increase occurred simultaneously with the above-mentioned increase in wheat intake and the moving of hens to new forage vegetation. The laying rate was kept throughout the rest of the experiment except for a small decline at the end of the experiment while foraging quinoa plots. Egg weight and body weight approximately followed the same course as the laying rate even though these parameters did not reach the same level as the concentrate-fed hens. Moreover, egg weight and body weight did not decrease at the end of the experiment. It was suggested that the different courses for laying rate, egg weight and body weight at the end of the experiment were due to the nutrient-restricted hens being in a phase where they had to change foraging strategy, because earthworms, insects and green fodder were scarce whereas quinoa seeds were abundant. Referring to chapter 4, nutrient-restricted hens did not select seeds to same degree as non-restricted hens.

One on the most important egg quality parameters for the consumer is the yolk colour, which has shown to become darker with herbage intake (Ringrose & Morgan, 1939; Sipe & Polk, 1941; Hammershøj & Steenfeldt, 2005). However, it has been suggested that hens grazing rape (*Brassica napus*) lay eggs with yolks of an olive-green colour Goodman (1948). In Paper 1, the overall effect of hens foraging different forage crops revealed yolks with a darker, redder and less yellow hue at the end of the experiments. This was particularly pronounced in eggs from hens foraging the chicory. In Experiment 2 yolks from wheat-fed hens were redder than yolks from concentrate-fed hens presumably reflecting a higher intake of herbage. However, in Experiment 1 the yolk was lighter, presumably as a consequence of the diminishing value of the forage vegetation at the end of the experiment.

Studies with foraging broilers likewise indicated good productivity though feed consumption was higher than in a traditional broiler production. Thus, the slow growing broiler strain I 657 and chickens of two dual-purpose breeds (New Hampshire and Light Sussex) grew well despite a reduced protein content in the supplementary feed (Horsted et al., 2005).

Housing conditions in the above studies differed considerably from traditional, organic poultry production systems, i.e. the poultry were to a higher degree exposed to hard weather conditions and changing light intensities, temperatures etc. Despite this, the poultry performed very well in these systems and showed a remarkable capability to adapt to different conditions such as low value of supplementary feed and changing values of the forage area. Besides, some egg quality parameters were improved.

6.2 Nutrient balances in forage-based systems

In traditional, organic egg production systems only 25-30% of nitrogen (N) and 12-15% of phosphorus (P) are found again in the eggs (Kristensen, 1998; Hegelund et al., 2006). The remains are deposited as manure in the henhouse or in the open-air run. In 14 organic flocks of laying hens, Kristensen (1998) estimated that 49% of N and 82% of P in the poultry manure was found in the henhouse. The remains were considered lost (gaseous N) or excreted in the open-air run. Since hens in traditional, organic egg production systems predominantly use the area just in front of the henhouse (Keeling et al., 1988; Zeltner & Hirt, 2003; Hegelund et al., 2005), there is a strong risk of nutrient leaching to the ground water exist (Menzi et al., 1997).

Increased foraging of organic layers may be a way to enhance the biological cycles within the farming system as emphasized by IFOAM (2000). Thus, a higher intake of forage material may reduce the import of nutrients to the production system. As pointed out in the introduction this may reduce the feed costs and ease the transition into 100% organic feed supply. Moreover, increased foraging may results in improved distribution of hens in the open-air run, which in turn lead to a better distribution of the manure in the open-air run.

Based on the results on productivity in Papers 1 and 4, nitrogen- and phosphorus-balances for three forage-based experiments are calculated in Tables 2 and 3. Contents of N and P in eggs were estimated according to Poulsen et al. (2001). Contents of nutrients in the supplementary feed are shown in Papers 1 and 4. Since the analyses in Paper 4 were on a wet weight basis, a dry matter percentage of 90 is assumed.

It is clear from Tables 2 and 3 that the average N- and P-surplus is considerably smaller in forage-based system, when hens are fed wheat and oyster shells as the only supplementary feed, compared with systems where hens were not restricted in nutrients through the supplementary feed (concentrate). This confirms that these hens found a considerable part of their nutrient requirements from local resources in the shape of herbage, earthworms, insects etc. In the plots with wheat-fed hens, surpluses of N and P were found to be approximately 0.9 and 0.3g per hen per day, respectively, whereas in plots with concentrate-fed hens the average N-surplus varied from 2.5-3.4g per hen per day and the average P-surplus was around 0.8g per hen per day. The analysis of the concentrate used in the experiment in Paper 4 revealed a higher content of N and P per kg DM, for which reason a higher surplus was found for these hens despite the fact that they had a lower average intake of supplementary feed than concentrate-fed hens in the experiments in Paper 1. However, Hegelund et al. (2006) found similar results in a study with 13 flocks of organic laying hens fed concentrate supplemented with grain and roughage.

Table 2. Nitrogen balances in three experimental forage-based systems (Papers 1 and 4)

	Paper 1				Paper 4	
Experiment:	**Exp. 1, 23 days**		**Exp. 2, 23 days**		**Exp. 1, 130 days**	
Type of supplementary feed	**Concentrate**	**Wheat**	**Concentrate**	**Wheat**	**Concentrate**	**Wheat**
N supplied to the system (g/hen)						
N in hens at introduction	54.4	54.4	56.1	56.1	54.5	54.5
N in supplementary feed	86.0	40.2	103.4	38,9	568.8	232.2
N removed from the system (g/hen)						
N in hens at termination	57.9	51.8	60.1	53.5	62.8	56.8
N in eggs produced	24.2	18.5	21.7	20.9	138.9	111.0
N-balance						
N per hen	58.3	24.3	77.7	20.6	421.6	118.9
N per hen per day	2.5	1.1	3.4	0.9	3.2	0.9

Table 3. Phosphorus balances in three experimental forage-based systems (Papers 1 and 4)

	Paper 1				Paper 4	
Experiment:	**Exp. 1, 23 days**		**Exp. 2, 23 days**		**Exp. 1, 130 days**	
Type of supplementary feed	**Concentrate**	**Wheat**	**Concentrate**	**Wheat**	**Concentrate**	**Wheat**
P supplied to the system (g/hen)						
P in hens at introduction	6.6	6.6	6.8	6.8	6.6	6.6
P in supplementary feed	17.8	8.5	21.4	8.2	130	48.9
P removed from the system (g/hen)						
P in hens at termination	7.0	6.3	7.2	6.5	7.6	6.9
P in eggs produced	2.4	1.8	2.1	2.0	13.6	10.9
P-balance						
P per hen	15.0	7.0	18.9	6.5	115.4	37.7
P per hen per day	0.7	0.3	0.8	0.3	0.9	0.3

The above nutrient balances and discussion suggests that increased foraging in relation to increased circulation of nutrients in the system has greater potential if the standards for N and P is reduced in the supplementary feed.

6.3 Welfare of poultry in forage-based systems

The ancestor of the domestic layers is believed to be the Red Junglefowl (Siegel et al., 1992; Moiseyeva et al., 2003). Under semi-natural conditions the Red Junglefowl spent 60% of all minutes, during the active part of the day, ground pecking and 34% ground scratching even though the birds were fed three times a day. However, it has been considered whether the distinct selection for egg production and feed conversion rate in layers might have influenced the hen's characteristics in such a way that she has lost some of her ability to function in a free-range system (Sørensen, 1996). Thus, Braasted & Katle (1989) found low-efficient hens spending more time feed pecking and were in general more active than high-efficient hens. Schütz et al. (2001) found that the Junglefowl spent more time feeding from a novel food site, whereas a White Leghorn strain chose a site with easily obtainable, familiar food. The authors concluded that Leghorn used a more energy-conserving foraging strategy than the Junglefowl. Thus, it is not surprising that high producing organic layers choose to stay in the henhouse or in the area just outside the henhouse when feed are fed inside the henhouse (Bubier & Bradshaw, 1998). This results in a high density in the henhouse and immediately outside and may adversely affect the welfare of the hens resulting in increased feather pecking that in turn worsen the welfare and may lead to cannibalism (Kjær, 1996; Bestman & Wagenaar, 2003). Moreover, there is risk for parasitic contamination (Bray & Lancaster, 1992; Permin et al., 1998; 1999).

Initiatives such as artificial structuring and planting of trees were taken among seven Danish organic egg producers to improve the attractiveness of the open-air run to increase the number of hens in this area. Even though no comparative study was made, the producers suggested that the initiatives had improved the dispersion of hens in the open-air run, just as plumage condition at the age of 55 weeks were good in general (Hermansen et al., 2005). Zeltner & Hirt (2003) examined the effect of artificial structuring in the open-air run and concluded that it had a positive effect on the dispersion of hens, even though the effect of this structuring at the very end of the open-air run was small. Besides cover of the open-air run, several factors may influence the dispersion of hens, e.g. flock sizes (Hirt et al., 2000; Bestman & Wagenaar, 2003), rearing conditions (Grigor et al., 1995), weather conditions (Keeling et al, 1988), and breeds (Kjær & Isaksen, 1998; Nielsen et al., 2003; Elwinger et al., 2004; Horsted et al., 2005). Even though dispersion of the outdoor run was not recorded in the experiments in Papers 1-4, it was quite clear that nutrient-restricted hens performed a higher foraging activity than non-restricted hens. In addition, hens in general only rarely stayed inside the henhouses.

It is a well-known fact that deficiency in protein and some amino acids may be critical for the plumage condition of the hens due to increased feather pecking (Ambrosen & Petersen, 1997; Elwinger et al., 2002), even though strains can be selected to produce well on a low protein and energy diet without any signs on feather pecking (Al Bustany & Elwinger, 1986). However, clinical indicators on welfare revealed an excellent welfare in forage-based systems whether the experiments lasted for 23 days (Paper 1) or 130 days (Paper 4) and whether the hens were nutrient restricted or not. In the experiment in Paper 4 welfare assessments were carried out for all hens eight times during the experiment using the methods of Tauson et al. (1984) and Gunnarson et al. (1995). Maximum scores were found for plumage condition in four out of five parts of the body. Only the plumage condition on the thorax was given a lesser score concurrently with number of days in the experiment. It was suggested that this could be due to the wearing of the thorax-feathers as a consequence of nesting, since the poorest score was found for a few broody hens. Only three hens died during the experimental period; two were on the concentrate-diet and one was fed whole wheat and oyster shells. Causes of death were unknown.

Slow growing broilers and dual-purpose breeds foraging in orchards were found to have excellent plumage condition and foot health at 120 days of age, despite reduced protein content in the pelleted broiler concentrate. Moreover, no salmonella was found in the cloacae samples. Mortality rate was made up to 5% and cause of death was mainly attacks by sea gulls in the first week after grouping in the orchard (Horsted et al., 2005). Fuller (1962) found no negative consequences on mortality rate in hens on range whether they were fed a conventional diet or restricted in nutrients, and Sipe & Polk (1941) found a lower mortality rate when hens had access to forage vegetation than hens with access to dry lots.

In general, hens have been found to have excellent welfare in forage-based systems. Moreover, nutrient-restriction does not seem to have any negative consequences on the welfare. However, good forage may be insured when restricting hens, just as concerns on flock sizes, housing condition etc. have to be taken.

7. Conclusion and perspectives

The present thesis has dealt with several subjects within the field of foraging of organic, laying hens. The motivation for this was to provide a holistic view on the potential of utilizing the foraging behaviour of laying hens in relation to alternative production systems for organic poultry production. To do so three experimental setups were conducted, resulting in four papers included in this thesis. These papers have been discussed in relation to feed intake, feed selection, contribution of nutrients from forage, and production in forage-based systems.

From the experiments in this thesis it is concluded that high-producing layer strains are capable of benefiting from attractive forage vegetation and that a huge potential exists provided that the production system otherwise support good welfare of the poultry. Thus, hens were found to produce well under the experimental conditions, even though nutrient-restricted hens needed a period of adaptation. However, after this period of adaptation it was estimated that the forage area had supplied the nutrient restricted hens with an average of approximately 70% of their requirements for lysine and methionine, and approximately 25% of their requirements for calcium. Irrespectively of type of supplementary feed it seems probable that hens, after a period of adaptation, may consume up to 0.25 MJ ME of their requirements of ME from forage; presumably higher for nutrient-restricted hens due to a higher foraging activity. However, these estimates may vary considerably due to type and value of forage vegetation. Thus, analyses of crop content revealed that non-restricted hens had a high intake of seeds from cultivated plants, whereas nutrient-restricted hens gave priority to earthworms, insects, soil, grit stone and oyster shells. Besides, analyses of faeces indicated that nutrient-restricted hens gave priority to grasses and non-restricted hens to clover, when they were foraging grass/clover plots. When foraging a mixture of forbs hens gave priority to plant species nearer the ground rather than to higher plants. The quantitative intake of herbage was found to be approximately 50% higher in nutrient-restricted hens than in non-restricted hens, though the estimated intake of herbage varied considerably. However, intake of grass/clover may be 10-30g per hen per day in non-restricted hens, and 20-40g per hen per day in hens fed whole wheat and oyster shells. Intake of chicory was found to be considerably higher, and the estimated intakes were approximately 40 and 70g per hen per day, respectively. Thus, it was concluded that chicory was a very attractive forage crop for laying hens. This was reflected in the egg quality, since albumen DM was higher and yolk colour darker and redder when hens were foraging chicory compared to grass/clover.

Nutrient-restriction of hens through feeding whole wheat and oyster shells only, as done in the experiments in this thesis, is a rather drastical restriction, which cannot be recommended in a traditional, organic production system. However, for the development of new systems for organic poultry production it was considered important to provide better knowledge about the capacity of the birds. Subsequently, it may be considered a challenge to adjust the production system to fit the poultry instead of adjusting the poultry to fit the production system. To make the most of a forage-based system, a lesser supply of nutrients through the supplementary feed seems to be a viable method. Hens are more motivated to forage when they are not fully met in nutrients through the supplementary feed. In practice though, a less drastically nutrient-restriction must be considered, since larger flock sizes than used in the present experiments are necessary to produce economic, and this may result in greater risk of inferior behaviour. However, integration of poultry production into other branches of production may be a way to produce economically, though flock sizes most likely will be considerably smaller than in a traditional, organic poultry production. Besides, keepers of the increasing numbers of farm shops may benefit from smaller flocks of laying hens; especially since more focus are paid on the development of rural districts by our politicians. It is suggested that the forage-based system should consist of a rotation between forage vegetations during the six months in summer, since constant access to good forage is necessary to maintain productivity, egg quality and welfare of the hens. This is particularly important if the hens are nutrient-restricted in order to increase intake of feed from forage.

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Short-term effects on productivity and egg quality in nutrient-restricted versus non-restricted organic layers with access to different forage crops

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ORIGINAL ARTICLE

Short-term effects on productivity and egg quality in nutrient-restricted versus non-restricted organic layers with access to different forage crops

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Abstract
Two experiments were conducted to study the effects of different forage crops on productivity and some egg quality parameters of small flocks of organic laying hens fed whole wheat or concentrates. Each experiment was carried out in a split plot design with two different forage crops and two types of supplementary feed (typical concentrate for organic layers versus whole wheat) with three replications. In the first experiment the tested forage crops were of a well-established grass/clover and a mixture of forbs (*Fagopyrum esculentum, Phacelia tanacetifolia* and *Linum usitatissimum*). In the second experiment the tested forage crops were a well-established grass/clover and chicory (*Cichorium intybus* cv. Grassland Puna). The results on productivity and egg quality suggest that laying hens consume large amounts of foraging material when accessible. In nutrient restricted hens (wheat-fed) the forage may yield a substantial contribution to the requirements of amino acids and metabolizable energy although productivity parameters and measurements on dry matter in albumen showed that wheat-fed hens, on a short-term basis, were not able to fully compensate for the lack of protein and amino acids by increased foraging. Of the forage crops investigated especially chicory seems to contribute to the nutrition of the hens. Measurements on eggshell parameters showed that oyster shells together with foraging material were sufficient to meet the hens' calcium requirements. Yolk colour clearly revealed that laying hens consume large quantities of green fodder irrespective of the type of supplementary feed. Yolk colour from hens with access to chicory tended to be darker, of a redder and less yellow hue compared with grass/clover fed hens, which is considered a positive quality.

Keywords: *Albumen, body weight, egg production, eggshell, feed intake, foraging, herbage, organic poultry, yolk colour.*

Introduction

According to the EU legislation for organic poultry production the range area for organic laying hens must be at least 4 m^2 per hen (Council Regulation (EC) No, 1804/1999, 1999). It is however, a commonly recognized problem that up to 90% of the hens stays inside the house or predominantly uses the area just in front of the henhouse (Keeling et al., 1988; Zeltner & Hirt, 2003; Elwinger et al., 2004; Hegelund et al., 2005). The reason for the lack of use of the range area is most likely because the hens do not find the large and open areas attractive and because the feed is provided indoors (Keeling et al., 1988; Bubier & Bradshaw, 1998). This results in a high animal density inside the henhouse that in turn may lead to different kinds of health and welfare problems such as feather pecking and sometimes even cannibalism (Kjær, 1996; Bestman & Wagenaar, 2003). The very uneven distribution of hens outside with the majority staying close to the henhouse may enhance the risk of nutrient leaching to the ground water and parasitic infections (Permin et al., 1998; 1999). One way of minimizing these problems could be to make the range area more attractive by offering attractive forage crops as part of the daily ration. This may encourage the hens to use the entire range area, thus obtaining a lower animal density, and an increasing on-farm recycling of nutrients caused by a reduction in feed imports. This might in turn reduce the environmental load and the feed costs for the farmer. Since the feed costs

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account for more than 60% of the total costs in Danish organic egg production systems (Danish Poultry Council, 2004), even a small reduction in feed costs will be noticeable.

Moreover, it seems to be difficult to procure 100% organic raw materials for feeds, which until recently was stipulated for organic pig and poultry producers by the EU (Council Regulation (EC) No, 1804/1999, 1999), but because of difficulties in fulfilling this requirement, the 100% organic feeding requirement has been deferred until the end of 2011. A higher utilization of local resources may be a way of facilitating the transition to 100% organic feed supply for organic egg producers.

Although laying hens are able to consume considerable amounts of roughages (Steenfeldt et al., 2001), information on herbage intake from range areas by high-performance layers is scarce. Some results suggest that layers on range consume 30–35 g DM/day of herbage besides ad libitum feeding of concentrates (Hughes & Dun, 1983). However, different forage crops may vary in relation to nutritional value and attractiveness to laying hens. Moreover, restriction in nutrients supply has shown to increase forage intake in pullets (Fuller, 1962). However, a drastic reduction in protein and some amino acids may have a negative effect on plumage condition due to feather pecking (Ambrosen & Petersen, 1997; Elwinger et al., 2002) for which reason it is essential that plenty of foraging material is available.

It is important that a feeding strategy based on foraging does not compromise the egg quality and thereby reduces the number of outlets for the organic egg producers. It is well-known that one of the most important egg quality parameters for the consumer is the yolk colour, which has been demonstrated to become less green with increasing supplements of foraging material (Hammershøj & Steenfeldt, 2005). Especially in nutrient-restricted poultry production is it also important to evaluate the shell strength and dry matter of egg albumen, since these quality parameters can be affected by changes in supply of calcium and amino acids (Prochaska et al., 1996; Shafer et al., 1996; Summers et al., 1988; Roland, 1980).

It is our hypothesis that different forage crops may support laying hens differently with nutrients depending on the type of supplementary feed, and that such effects are reflected in the production and egg quality parameters. Thus, the aim of this study was to estimate the short-term effects of the inclusion of different forage crops in the range area on production and some egg quality parameters in nutrient-restricted versus non-restricted organic layers. In present study a short experimental period were chosen to meet the possibility of a declining value of the forage crops due to the foraging activity of the hens.

Materials and methods

Experimental design

Two experiments with different forage crops for laying hens were conducted at Research Centre Foulum. Each experiment was carried out in a split-plot design with access to two different forage crops and two types of supplementary feed (typical concentrate for organic layers and whole wheat) with three replications, i.e. a total of 12 groups, each including 20 hens and one cock. In each experiment three plots of each forage crop were alternately established on the experimental field with four metres between the plots to minimize the possible effect of one forage crop on the other, e.g. where one forage crop attracts insects that are a potential feed for hens. Each plot, which totalled 420 m^2, was then subdivided into two subplots according to type of supplementary feed, resulting in subplots of 210 m^2 (12×17.5 metres).

In experiment 1, one forage crop was a well-established (five-year-old) grass/clover and the other was a mixture of forbs (*Fagopyrum esculentum, Phacelia tanacetifolia and Linum usitatissimum*). These forbs are expected to attract a lot of insects in the flowering season when this experiment took place. In experiment 2, one forage crop was a well-established grass/clover and the other was chicory (*Cichorium intybus* cv. Grassland Puna). The latter was grown for the leaves. Nutrient content and dry matter per hectare of the forage crops are given in Table I. At the start of the experiment, hens were randomly distributed to subplots. The duration of the experiment was 23 days for both experiments, mid June to mid July in experiment 1 and August in experiment 2.

Strains and pre-experimental handling of birds

The hens in both experiments were of the strain "Lohmann Silver" and were reared at two different organic poultry breeders. In both cases the pullets were reared in large flocks (4000 and 7000 animals, respectively) with access to a range area in accordance with the Danish legislation for organic poultry breeding (The Danish Plant Directorate, 2005). When the pullets arrived from the breeders they were placed in a house with access to a range area with vegetation (weed), where they were kept until the experiment commenced eight weeks later. The flocks were fed a commercial concentrate diet

Table I. Nutrient content and herbage mass per hectare of the crops at the beginning of the experiments, means (SD) (n = 6).

	DM (%)	Nutrient content (% in DM)					DM per hectare (kg)
		Ash	Crude protein[1]	Crude fat	Starch	Sugars[2]	
Exp. 1							
Grass/clover	13.38 *(0.73)*	9.74 *(0.48)*	19.26 *(1.49)*	3.22 *(0.16)*	6.28 (0.77)	12.42 *(1.05)*	3033 *(237)*
Mixed forbs	7.01 *(0.33)*	25.29 *(2.88)*	26.47 *(1.38)*	2.87 *(0.21)*	4.90 (0.73)	7.56 *(0.98)*	3460 *(547)*
Exp. 2							
Grass/clover	13.13 *(1.50)*	11.79 *(0.96)*	20.77 *(2.13)*	3.01 *(0.15)*	3.67 (0.33)	8.31 *(1.56)*	2760 *(351)*
Chicory	12.92 *(2.42)*	13.92 *(1.76)*	17.81 *(1.46)*	3.40 *(0.77)*	4.91 (1.75)	6.63 *(2.22)*	4660 *(1447)*

[1] = (N × 6.25).
[2] = the sum of glucose, fructose, sucrose and fructan.

(18.4% protein) as well as oyster shells and grit stone during this adaptation period. In addition five kg of whole wheat was spread out in the range area daily to introduce the hens to this feed. Concentrate and water were given ad libitum both inside and outside of the henhouse.

Housing and feeding of experimental birds

Both experiments began when the hens were about 25 weeks of age and well into egg laying stage. In the experiments the hens were housed in 12 henhouses of 4.6 m^2 each with five nesting boxes placed on the outside of the house and perches inside the house. The houses were made of waterproof plywood on an iron frame and windows were placed at each end of the house to allow daylight in the house. On both sides hatches stretched the full length of the house to avoid corners that potentially could function as nesting areas. There was no artificial light in the houses, just as the hatches were open day and night, giving the hens access to forage all day throughout the experiments. The composition and results of the chemical analyses of the supplementary feed are shown in Table II. Feed, water, oyster shells (32% calcium) and grit were all provided ad libitum outdoors.

Recordings

In each of five 0.25 m^2 plots in each subplot, the herbage was harvested approximately 2 cm above ground level. The squares were distributed on a straight diagonal line across the subplots. The harvested biomass was weighed for each square and a representative sample from each subplot taken and stored at −20°C until analysis. The samples were analysed for dry matter (DM), ash, crude protein, crude fat, starch and sugar (Table I). DM content was determined by drying at 105 °C for 8 h. Crude protein was determined by the Kjeldahl method (AOAC, 1990a) and ash according to method 923.03 (AOAC, 1990b). Crude fat 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 & Li, 1991). Starch was analyzed by the enzymatic-colorimetric method (Bach-Knudsen, 1997). Total NSP and their constituent sugars were determined as alditol acetates by gas-liquid chromatography for neutral sugars and by 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.

Eggs were collected daily in the afternoon, weighed, and a daily average egg weight for each subplot was calculated. The feeding silos were filled as required, i.e. about two times a week and feed consumption was calculated simultaneously. Before being introduced to the experiment and at termination all hens were subjected to a welfare assessment including an assessment of the plumage condition, skin damages, foot health, wounds and colour of the combs and body weight using the standardized methods described by Tauson et al. (1984) and Gunnarson et al. (1995).

At the end of experiment 1 and at the beginning of experiment 2 all hens were fenced off in a 24 m^2 plot of each subplot for a period of two and three days, respectively, in order to estimate the average daily removal of the forage crops. Two patches of 0.25 m^2 were harvested to estimate the amount of herbage per m^2 prior to and post the hens' access to this area, i.e. the differences were considered as being removed by the hens. This procedure was, however, not

Table II. Dietary composition and nutrient content of concentrate and wheat.

	Concentrate	Wheat
Dietary composition (%)		
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	
Fishmeal	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		
Dry matter (DM), %	90.3	90.3
% in DM		
Ash	13.1	1.8
Crude fat	6.1	2.4
Crude protein (N ×6.25)	18.4	12.0
Calcium	4.1	< 0.1
Phosphorus	0.6	0.4
NSP:		
Cellulose	3.3	2.0
Soluble NSP	3.3	5.0
Insoluble NSP	11.4	9.1
Total NSP	14.7	14.1
Lignin	3.1	2.1
Fibres (Total NSP + lignin)	17.8	16.2
Amino acids (aa) (g/kg DM)		
Alanine	7.6	4.3
Arginine	11.8	5.8
Asparagine	15.4	6.1
Cystine	3.2	2.6
Glutamine	38.1	32.8
Glycine	8.1	4.9
Histidine	4.7	2.9
Isoleucine	7.5	4.1
Leucine	12.8	7.8
Lysine	8.7	3.4
Methionine	4.6	1.9
Penylalanine	8.5	5.3
Proline	11.6	10.7
Serine	8.9	5.8
Threonine	6.1	3.5
Valine	8.8	5.3
Starch	37.9	64.2
Sugar	2.4	<0.5
ME (MJ/kg DM)	11.6	13.4

applicable in the mixed forbs due to the character of this forage crop (high plants that were easily trampled down).

In each experiment, six randomly chosen eggs were collected twice from each plot for egg quality analysis. First time was immediately after the hens were introduced and the second time was 23 days after introduction in both experiments. The eggs were weighed individually and stored at 4°C for 18–27 days before the following analyses were performed.

Egg Yolk Colour. The eggs were broken and the albumen was removed from the yolk by cutting into the thick part of the albumen before yolk colour measurement. The yolk colour was analysed by a Minolta Chroma Meter CR-300 (Minolta Co. Ltd., Osaka, Japan) using the CIE (Commission Internationale de L'Enclairage) Lab scale with standardised daylight (D65). The $L^\star$, $a^\star$ and $b^\star$ values reflect lightness (0 = black, 100 = white), redness (−100 = green, 100 = red) and yellowness (−100 = blue, 100 = yellow) of the samples, respectively. The instrument was calibrated against a white standard plate with L* 92.3, a* 0.33 and $b^\star$ 0.1.

Albumen Dry Matter (DM) Content. The chalazae-free egg albumen was homogenised by an Ultra-Turrax T25 (IKA®-Labortechnik, Janke & Kunkel GmbH & Co., Staufen, Germany) at 10,000 rpm for 15 seconds. The DM content (%) of the egg albumen was analysed by weighing 3 ml albumen into a porcelain vial. The samples were dried for 18 h at 98°C in an oven and reweighed after equilibration to room temperature (r.t.).

Shell percentage. The eggshells were carefully washed in demineralised water to remove remains of egg albumen, but leaving the shell membrane intact. The washed eggshells were dried at r.t. for a minimum of two days before weighing and the shell percentage of the egg weight was calculated.

Shell strength. Eggs from day 23 in experiment 2 were additionally analysed for shell strength by uniaxial compression at the equator of the egg on a TA-HD*i* Texture Analyzer (Stable Micro Systems Ltd., Surrey, England) with a 100 kg load cell, 0.001 N detection range, 75 mm diameter flat plate probe and a compression speed of 0.01 mm/s. Recordings of force (N) and displacement (m) were obtained until break detection, and the maximum force was used as shell strength value (N).

Statistical methods

The experiments were analysed as a split plot design with forage crops (grass/clover vs. mixture of forbs in experiment 1, and grass/clover vs. chicory in experiment 2) being the main plots and supplementary feed (concentrate vs. wheat) being the subplots. Each treatment was replicated three times. The Mixed Models procedure (SAS Institute Inc., 1990) was used. Statistical analysis on egg quality was based on the average of six eggs from each subplot on eggs collected at the end of the experimental periods. Egg quality as well as intake of supplementary feed and oyster shells were analysed by the following model 1:

Model 1: $Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + A_k + B_{kj} + \varepsilon_{ijkl}$

where Y_{ijkl} = response variable ($L^\star$, $a^\star$ and $b^\star$ colour values, DM in albumen, egg shell percentage, intake of supplementary feed, intake of oyster shells); μ = mean; α_i = supplementary feed $(i = 1,2)$; β_j = forage crop $(j = 1,2)$; $(\alpha\beta)_{ij}$ = interaction supplementary feed × forage crop; A_k = random effect of block $(k = 1,3)$; B_{kj} = random effect of block × forage crop; e_{ijkl} = error. In all cases, data were found to be normally distributed and no outliers were observed.

Statistical analysis on egg production was based on the average for each week by the following model 2:

Model 2: Y_{ijklm}

$$= \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + A_l + B_{lj} + C_{lij} + \varepsilon_{ijklm}$$

where Y_{ijklm} = response variable (number of eggs, egg weight); μ = mean; α_i = supplementary feed $(i = 1,2)$; β_j = forage crop $(j = 1,2)$; $(\alpha\beta)_{ij}$ = interaction supplementary feed × forage crop; γ_k = week (k = 26, 27, 28, 29 in experiment 1 and 32, 33, 34, 35 in experiment 2); $(\alpha\gamma)_{ik}$ = interaction supplementary feed × week; $(\beta\gamma)_{jk}$ = interaction forage crop × week; $(\alpha\beta\gamma)_{ijk}$ = interaction supplementary feed × forage crop × week; A_l = random effect of block $(l = 1,3)$; B_{lj} = random effect of block × forage crop; C_{lij} = random effect of block × supplementary feed × forage crop; e_{ijklm} = error.

The statistical analysis on body weight was based on the average weights of the birds from each subplot and using body weight at introduction as covariate. Final body weight as affected by treatments was analysed by the following model 3:

Model 3: Y_{ijkl}

$$= \mu + \alpha x_{ijkl} + \beta_i + \gamma_j + (\beta\gamma)_{ij} + A_k + B_{kj} + \varepsilon_{ijkl}$$

where Y_{ijkl} = response variable (final weight); μ = mean; αx_{ijkl} = weight at introduction; β_i = supplementary feed $(i = 1,2)$; γ_j = forage crop $(j = 1,2)$; $(\beta\gamma)_{ij}$ = interaction supplementary feed × forage crop; A_k = random effect of block $(k = 1,3)$; B_{kj} = random effect of block × forage crop; e_{ijkl} = error.

Results

The welfare assessment showed no differences among treatments as nearly all hens, at the end of the experiments, were given maximum scores for plumage condition, foot health, colour of the comb and breastbone (data not shown).

Feed consumption and removal of herbage

Estimates for removal of herbage (except for the mixed forbs) are given in Table III. The removal of herbage by the hens was particularly pronounced in the chicory plots. Visual assessment of the chicory plots revealed no signs of chicory leaves left in the plots. The remaining herbage was exclusively weeds.

No significant interactions between supplementary feed and forage crop on consumption of supplementary feed were found. Therefore only

Table III. Estimated removal of herbage DM (g) by laying hens in small experimental plots, mean (SD).

Exp. diet	DM per m^2 at introduction	DM per m^2 at termination	Removed DM per hen per day
Experiment 1			
Grass/clover			
Wheat	323 *(47)*	305 *(13)*	15 *(48)*
Concentrate	365 *(31)*	328 *(35)*	31 *(35)*
Experiment 2			
Grass/clover			
Wheat	269 *(51)*	228 *(29)*	17 *(31)*
Concentrate	252 *(45)*	228 *(14)*	9 *(21)*
Chicory			
Wheat	423 *(75)*	231 *(86)*	73 *(54)*
Concentrate	372 *(226)*	236 *(92)*	51 *(56)*

results of main effects are presented. Feed consumption of the two types of supplementary feed differed significantly in both experiments (Table IV). Hens consumed approximately 90 g wheat daily in both experiments, whereas they consumed considerably more concentrate (129 g and 155 g concentrates in experiment 1 and 2, respectively). No effect of forage crop on supplementary feed intake was found. The hens on wheat diet had a significantly higher intake of oyster shells compared with those fed concentrate in both experiments. Forage crop did not significantly affect intake of oyster shells, however the hens with access to grass/clover in experiment 1 consumed a numerically higher quantity of oyster shells than hens with access to the mixed forbs, and in experiment 2 the hens with access to chicory consumed a numerically higher quantity of oyster shells than hens with access to grass/clover. No differences in the intake of grit stone were seen.

Body weight

Mean body weights at the start of the experiment were 1875 and 1936 g in experiment 1 and 2, respectively. Body weights at postponement are shown in Figure 1. A highly significant difference between diets was found in experiment 1, as hens fed concentrate were 210 g heavier than those fed wheat ($P < 0.01$), just as wheat-fed hens lost weight during the experiment. No significant effects of forage crops were seen.

In experiment 2 an interaction between forage crop and supplementary feed was observed ($P < 0.05$). Body weight was reduced when wheat-fed hens had access to grass/clover, whereas body weight remained constant when wheat-fed hens had access to chicory.

Egg production

No main effects of forage crops were found on egg production (Table IV). In experiment 1, hens fed wheat had a significantly lower egg production per day compared with hens fed concentrate, whereas no difference was observed in experiment 2. In experiment 1, there was an interaction between week and supplementary feed ($P < 0.001$) and a strong effect of week ($P < 0.001$). As illustrated in Figure 2, this was due to a gradual decline in the number of eggs when hens were fed wheat. Furthermore, the decline tended to be more pronounced with forbs than with grass/clover. In experiment 2 (Figure 3), no significant effect of supplementary feed, forage crop or week was found.

A significantly lower egg weight was seen in both experiments when hens were fed wheat; $P < 0.01$ in experiment 1 and $P < 0.05$ in experiment 2 (Table IV). The type of forage crop had no major effect on the egg weight. However, the interaction "week × supplementary feed" was significant in experiment 1 ($P < 0.001$) due to a decline in egg weight for the wheat-fed hens, whereas the concentrate-fed hens showed an increase (Figure 4). In experiment 2 a tendency ($P = 0.05$) to a higher increase in egg weight was observed in the first week for hens fed concentrate. A gradual increase in egg weight was observed for all groups in the last two weeks (Figure 5). In both experiments we found significant

Table IV. Consumption of supplementary feed and egg production parameters in experiments 1 and 2, LS-means and (SEM) for main effects of supplementary feed and forage crops.

	Feed intake g/hen/day	Oyster shell intake g/hen/day	Laying rate %	Egg weight g
Experiment 1:				
Supplementary feed:				
P-value (df = 4)	*P < 0.001*	*P < 0.001*	*P < 0.05*	*P < 0.01*
Wheat	92 *(3.3)*	5.7 *(0.2)*	75 *(3.0)*	55.2 *(0.5)*
Concentrate	129 *(3.3)*	1.7 *(0.2)*	91 *(3.0)*	59.6 *(0.5)*
Forage crop:				
P-value (df = 2)	*ns*	*ns*	*ns*	*ns*
Grass/clover	111 *(3.3)*	4.2 *(0.3)*	83 *(3.2)*	57.6 *(0.5)*
Herb mixture	110 *(3.3)*	3.2 *(0.3)*	83 *(3.2)*	57.2 *(0.5)*
Experiment 2:				
Supplementary feed:				
P-value (df = 4)	*P < 0.01*	*P < 0.001*	*ns*	*P < 0.05*
Wheat	89 *(10.6)*	7.2 *(0.3)*	83 *(2.3)*	56.5 *(0.6)*
Concentrate	155 *(10.6)*	3.1 *(0.3)*	82 *(2.3)*	59.2 *(0.6)*
Forage crop:				
P-value (df = 2)	*ns*	*ns*	*ns*	*ns*
Grass/clover	119 *(10.6)*	4.6 *(0.3)*	80 *(2.3)*	58.0 *(0.7)*
Chicory	126 *(10.6)*	5.8 *(0.3)*	85 *(2.3)*	57.8 *(0.7)*

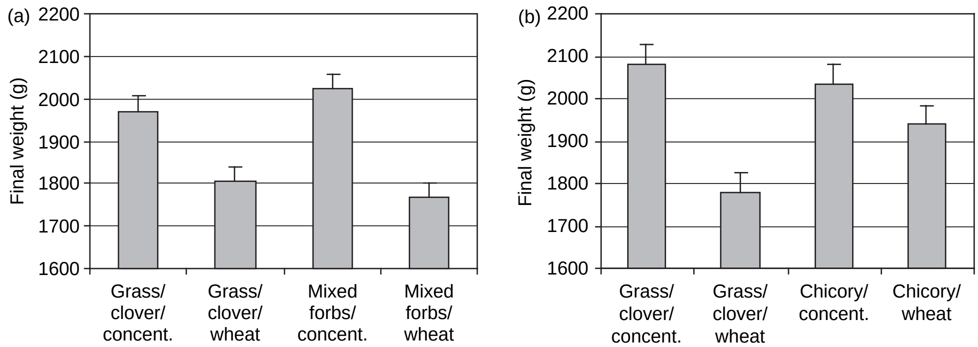

Figure 1. LS-Means of body weights of hens at the end of the experimental period of 23 days as function of crop/diet in a) Experiment 1 and b) Experiment 2. Vertical bars indicate SEM.

differences for the effect of week ($P < 0.05$ and $P < 0.001$ in experiments 1 and 2, respectively).

Egg quality

Table V give mean values across treatments at introduction and after 23 days for the egg quality parameters. In both experiments egg yolk colour had taken on a darker, more reddish and less yellow hue after 23 days, whereas a decrease in DM in albumen and a higher eggshell percentage was seen.

No interactions on egg quality parameters were found. In experiment 1, the egg yolk colour increased significantly in lightness (higher $L^\star$) and albumen DM decreased, when hens were fed the wheat diet compared with the concentrate diet (Table V). In experiment 2, the DM in albumen was also significantly lower when hens were fed wheat. Yolk colour was found to be significantly redder when hens were fed wheat (higher $a^\star$). There were tendencies to effects of forage crops in experiment 2 on yolk colour and DM in albumen. Hens eating the chicory crop produced egg yolks that were darker (lower $L^\star$) and of less yellow hue (Table V). Moreover $a^\star$ was numerically higher from hens on the chicory crop, indicating a redder yolk colour. The chicory treatment also improved the albumen DM content compared with grass/clover.

In experiment 2, we measured the strength of the eggshell and the length (mm) of rupture. No differences among treatments were however observed (data not shown), indicating that all the hens were able to cover the need for calcium through an increased intake of oyster shells and forage material.

Discussion

The typical metabolizable energy (ME) requirements for layers of the present weight and the ambient temperature (14.4 °C in experiment 1 and 17.9 °C in experiment 2) is approximately 1.42 MJ per hen per day (NRC, 1994). The corresponding NRC estimates of daily lysine and methionine requirements are 0.76 and 0.33 g per hen per day, respectively. These may be considered as minimum requirements and do not consider specific requirements of hens kept in a free-range system with a high activity level. Based on a daily egg mass of 57.5 g, Lohmann Tierzucht (2006) estimates a daily requirement of 0.87 g lysine and 0.44 g methionine

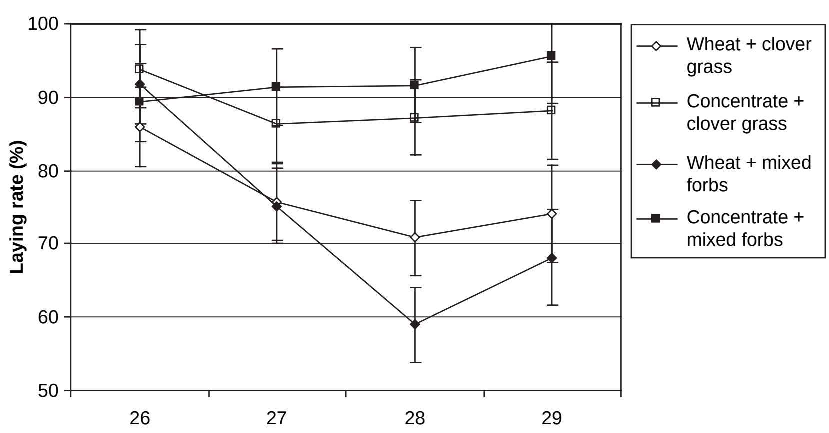

Figure 2. Experiment 1. Egg production given as laying rate (%) as function of time. Vertical bars indicate SEM.

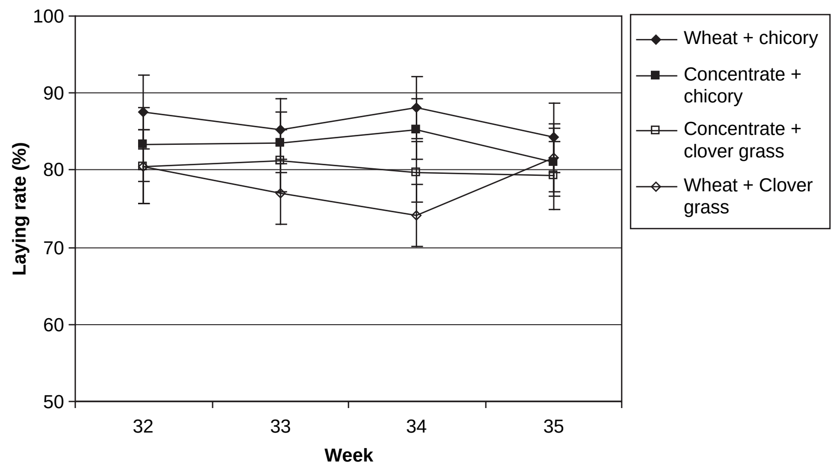

Figure 3. Experiment 2. Egg production given as laying rate (%) as function of time. Vertical bars indicate SEM.

per hen per day for the strains used in the present study and kept in a free-range system. Comparisons of these requirements with the nutrients in the allocated feed shows that concentrate-fed hens barely had their energy requirements covered in experiment 1 (1.35 MJ ME per hen per day) and were oversupplied in energy (1.62 MJ ME per hen per day) in experiment 2. In both experiments the concentrate-fed hens were oversupplied in lysine with 33% in experiment 1 and 61% in experiment 2. For methionine they were oversupplied with 64% and 94%, respectively. It cannot be excluded that the oversupply for concentrate-fed hens in experiment 2 was due to wastage of the concentrate. However, Hegelund et al. (2006) found an average feed intake of 1.57 MJ ME per hen per day at 7 organic egg producers, which is close to the level recorded here.

For both experiments, the wheat diet supplied the hens with an average of 1.10 MJ ME, 0.28 g lysine and 0.16 g methionine per hen per day, indicating an undersupply of 23%, 66% and 57%, respectively, compared to the requirements given above. If we assume a daily requirement of 0.30 g lysine and 0.15 g methionine per hen per day for maintenance, the egg production requires about 0.01 g lysine and 0.005 g methionine per gram egg mass per day according to the requirements of Lohmann Tierzucht (2006). Based on these assumptions the wheat-fed hens required approximately 0.46 g lysine and 0.22 g methionine extra for the attained egg production in both experiments, which indicate that the forage area considerably contributed to the amino acid supply. By including weight loss or gain in the formula given by NRC (1994), the required intake of ME by the wheat-fed hens is calculated to be approximately 1.23 MJ ME per hen per day, i.e. 0.13 MJ ME more than actually supplied by the wheat diet, and without including energy for physical activity. This suggests that hens actually found a considerable part of their nutrient requirement during foraging from the forage area, even though the hens lost weight during the experimental periods.

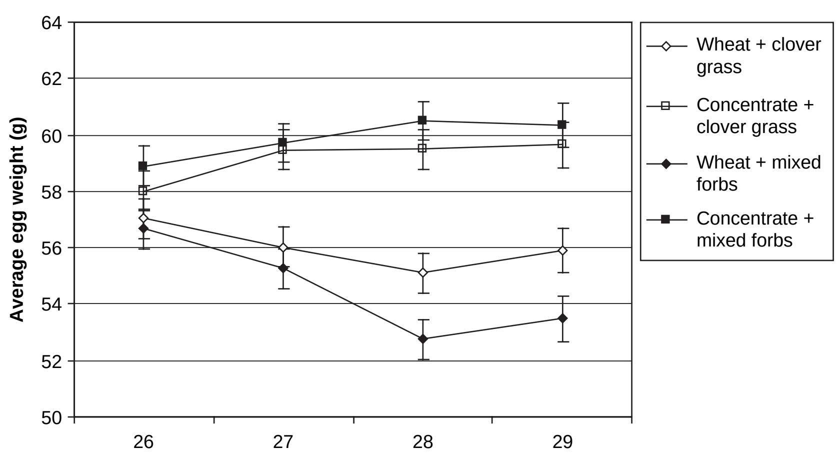

Figure 4. Experiment 1. LS-means of egg weight as function of time. Vertical bars indicate SEM.

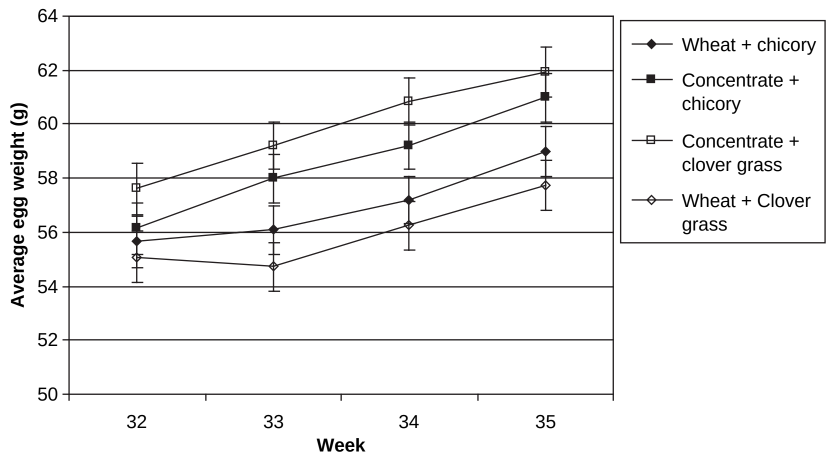

Figure 5. Experiment 2. LS-means of egg weight as function of time. Vertical bars indicate SEM.

Especially the hens with access to chicory showed a relatively high egg production and did not loose weight to same extent as hens with access to grass/clover or the mixed forbs. This is consistent with the amount of herbage removed from the plots (Table III), and the fact that amino acids analysis of the pure chicory leaves revealed a relatively high content of lysine (12.1 g per kg DM) and methionine (4.0 g per kg DM).

The higher albumen DM content in the eggs from hens with access to chicory also reflects the positive contribution to the nutrition of the hens. Hammershøj & Steenfeldt (2005) showed that methionine intake below the recommended intake results in a decreased albumen DM content, whereas increased methionine intake from 325 to 423 mg per hen per day, i.e. beyond the requirement, increases the albumen DM (Shafer et al., 1996). This relation is

Table V. Egg quality parameters in experiments 1 and 2, LS-means and (SEM) for main effects of supplementary feed and forage crops.

	Colour of the yolk				
	L*	a*	b*	DM in albumen (%)	Egg shell percentage
Experiment 1					
Mean values:					
At introduction	61.4	4.7	60.6	12.8	9.6
23 days after introduction	56.3	6.1	52.0	12.5	10.3
Effect of treatments (LS means + SEM for main effects):					
Forage crop:					
P-value (df = 2)	*ns*	*ns*	*ns*	*ns*	*ns*
Grass/clover	56.2 *(0.5)*	6.4 *(0.6)*	51.6 *(0.9)*	12.5 *(0.1)*	10.4 *(0.1)*
Mixed herbs	56.4 *(0.5)*	5.8 *(0.6)*	52.4 *(0.9)*	12.6 *(0.1)*	10.2 *(0.1)*
Supplementary feed:					
P-value (df = 4)	*P < 0.05*	*ns*	*P = 0.08*	*P < 0.01*	*ns*
Concentrate	55.2 *(0.5)*	6.9 *(0.6)*	50.5 *(0.9)*	12.9 *(0.1)*	10.4 *(0.1)*
Wheat	57.4 *(0.5)*	5.4 *(0.6)*	53.5 *(0.9)*	12.2 *(0.1)*	10.2 *(0.1)*
Experiment 2					
Mean values:					
At introduction	62.7	−2.0	58.1	13.4	9.1
23 days after introduction	56.5	7.6	53.4	12.9	10.1
Effect of treatments (LS means + SEM for main effects):					
Forage crop:					
P-value (df = 2)	*P = 0.06*	*ns*	*P = 0.06*	*P = 0.06*	*ns*
Grass/clover	57.8 *(0.7)*	6.6 *(0.8)*	55.2 *(1.5)*	12.7 *(0.1)*	10.1 *(0.1)*
Chicory	55.3 *(0.7)*	8.7 *(0.8)*	51.7 *(1.5)*	13.1 *(0.1)*	10.1 *(0.1)*
Supplementary feed:					
P-value (df = 4)	*ns*	*P < 0.05*	*ns*	*P < 0.01*	*ns*
Concentrate	56.5 *(0.7)*	6.8 *(0.8)*	53.5 *(1.5)*	13.2 *(0.1)*	10.2 *(0.1)*
Wheat	56.6 *(0.7)*	8.5 *(0.8)*	53.3 *(1.5)*	12.6 *(0.1)*	10.0 *(0.1)*

also reflected in the present work in that the concentrate-fed hens had higher albumen DM content than the wheat-fed hens. Protein is the major constituent of albumen DM and the actual protein content of albumen determines e.g. the gel textural properties of heat-treated eggs before consumption (Hammershøj et al., 2001; Ould-Eleya & Gunasekaran, 2002).

In experiment 1, the decline in egg number and egg weight for the wheat-fed hens was more pronounced in the plots with mixed forbs than the grass/clover (Figures 2 and 4). This was surprising since herbage mass dry matter per hectare were a little higher in the mixed forbs than the grass/clover, as well as the chemical analysis showed more protein per DM in the mixed forbs. However, the broadcast mixed forbs (mostly *Phacelia tanacetifolia*) were easily trampled down since they were more delicate than the well-established grass/clover, just as the ground was looser due to ploughing prior to sowing. For that reason the mixed forbs were more vulnerable to the scavenging of the hens. Thus, the plots with wheat/mixed forbs rapidly showed signs of the foraging activity after hens were introduced, and at the end of the experiment hardly any vegetation remained in these subplots. Therefore this forage crop cannot be recommended for nutrient restricted laying hens. In general, a visual assessment of the vegetation at the end of the experiment revealed a much more worn down vegetation in the subplots with the wheat-fed hens. This indicates a higher degree of foraging activity by the nutrient-restricted hens compared with the concentrate-fed hens. A high foraging activity could partly be due to the hens eating more of the forage crops, but also a more intensive search for earthworms and insects that are an important source of protein and essential amino acids (Sugimura et al., 1984; Pokarzhevskii et al., 1997).

In experiment 2, the chicory was much more resistant to the scraping of the hens. The fact that it was drilled and has a taproot is most likely important for the resistance of this forage crop. Moreover, the chicory had a higher yield per hectare than the other forage crops but with a large variation between subplots (Table I). This may be caused by the weed present in some of the chicory plots, and could also explain the large variation in the content of especially fat and sugar (Table I).

Feed consumption of the wheat-fed hens was significantly lower than hens fed concentrate. Whole corn is retained for a longer time in the crop than cracked corn or meal (Heuser, 1945). However, more recent research has shown that passage rate in the upper digestive tract is similar for whole and ground wheat in broilers (Svihus et al., 2002), just as Al Bustany & Elwinger (1988) found a higher food conversion ratios in hens fed whole barley or wheat than ground barley and wheat. This might indicate that hens in our study stopped eating as a result of an imbalance in nutrient supply (overdose of ME and shortness of amino acids). Nevertheless, coarse particles will remain in the gizzard until particle size has been sufficiently reduced, and reduced feed intake has been observed for diets with whole grains (Hetland et al., 2002), and is suggested to be caused by a satiety sensation due to high gizzard activity. Moreover, gizzard sizes have been found to be larger if poultry was fed whole wheat compared to pelleted feed (Forbes & Covasa, 1995; Plavnik et al., 2002; Hetland et al., 2003) indicating that the coarse nature of whole wheat probably, as a result of adaptation, enhance development of the gizzard allowing improved grinding, gut motility and nutrient utilization. The results in present study indicate that the upper limit for the amount of consumed whole grain is lower than for the amount of consumed concentrate, which could explain why the restricted hens were unable to consume the required amount of ME by simply eating more of the wheat. The weight loss especially in experiment 1 for the wheat-fed hens further indicates that high-performing layers still maintain an egg production despite the loss of weight, at least to a certain level.

Laying hens have a distinct capability to select and compose a feed ration that covers their nutrient requirements without compromising the egg production when feed items are allocated separately (Ciszuk & Charpentier, 1996). In our study, hens in all subplots were given oyster shells ad libitum in order to ensure sufficient access to calcium. The results with oyster shells clearly revealed that high-performance laying hens are capable of supplementing a calcium-poor diet, e.g. wheat, with calcium-rich feed, if it is available. Thus, we found a significantly higher intake of oyster shells in the subplots with wheat-fed hens compared with concentrate-fed hens in both experiments. The calcium requirement for brown-egg layers is about 4 g per hen per day (NRC, 1994). In our study hens fed concentrate had their calcium requirements fully covered by the concentrate and the intake of oyster shells. Thus, calcium intake was calculated at 5.3 and 6.7 g per hen per day in experiment 1 and 2, respectively. Conversely, the wheat-fed hens did not get their calcium requirement covered by the wheat and the higher intake of oyster shells with a calcium intake of 1.9 and 2.4 g per hen per day in experiment 1 and 2, respectively. However, no difference in eggshell percentage and eggshell strength was seen between treatments, indicating that the requirement for calcium was filled by foraging material.

One of the naturally occurring pigment groups in plants are the carotenoids. Of these, only the non-pro-vitamin A carotenoids that have a functional group containing oxygen, the xanthophylls, are known to affect the egg yolk colour (Belyavin & Marangos, 1987). Wheat has a low content of xanthophylls, whereas the concentrate diet contains sources of xanthophylls originating mainly from the maize, maize gluten and peas. Maize is reported to contain cryptoxanthin and zeaxanthin (Belyavin & Marangos, 1987), and peas contain lutein, violaxanthin and neoxanthin (Edelenbos et al., 2001). The low intake and content of xanthophylls in wheat indicates a higher intake of green fodder by the wheat-fed hens in experiment 2, since yolk colour was significantly redder in hens on the wheat-diet and no differences between diets were seen in relation to lightness and yellowness. However, in experiment 1 we found the yolk colour to be significantly lighter, more yellowish and to have a tendency to be less red when hens were fed wheat compared with yolks from concentrate-fed hens. This, on the contrary, does not indicate a higher intake of green fodder among the wheat-fed hens, presumably reflecting the diminishing value of the forage crops in the wheat subplots at the end of experiment 1.

The yolk colour parameters indicate in experiment 2 that the chicory was a very attractive crop for the poultry, since the yolks tended to be darker, more red and less yellow in the eggs from the hens with access to chicory compared to those with access to grass/clover. In general, grass pasture contains mainly the xanthophylls lutein ~350 μg/g DM and zeaxanthin ~50 μg/g DM (Prache et al., 2003), whereas chicory leaves contain approximately 55 μg lutein, 25 μg violaxanthin and 20 μg neoxanthin per g wet weight (Kimura & Rodriguez-Amaya, 2003; Niizu & Rodriguez-Amaya, 2005). Based on the DM content of the chicory leaves in Table I, it is estimated that the total xanthophyll content is higher in chicory than in grass. Together with a presumably higher intake of chicory than grass by hens, as indicated by Table III, and a slightly higher fat content in chicory, this may result in differences in yolk colour parameters between the two forage crops.

The mean egg quality values in Table V at the start and end of experimental periods are calculated across treatments to illustrate the overall development in time in the egg quality parameters as a consequence of giving layers unlimited access to foraging material. The improvement in egg shell percentage probably arises from the nutrient-restricted hens needing a few days to discover that the oyster shells could make up for the lack of calcium in the feed. The mean values of yolk colour clearly show an improvement during the experimental period. Thus the yolk colour became darker, redder and less yellow during the experimental periods across treatments and experiments.

We conclude that high-performance laying hens are capable of utilizing attractive forage crops in the range area and that forage crops can provide laying hens with important nutrients. Thus, it seems possible to lower the standards of the amino acid content in the supplementary feed when access to attractive forage crops such as chicory is given. Moreover, access to different forage crops, especially chicory, seems to improve certain egg quality parameters such as yolk colour and DM in albumen. However, on a longer term rotation between different forage crops is necessarily to maintain a valuable range area. A longer experimental period with continuously access to forage crops might even throw light on whether adaptation of the digestive tract to coarse feed will increase the intake of these feed items.

Acknowledgements

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2

Botanical composition of herbage intake of free-range laying hens determined by microhistological analysis of faeces

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Running title: Microhistological analysis of poultry faeces

Botanical composition of herbage intake of free-range laying hens determined by microhistological analysis of faeces

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Introduction

The feeding strategy in Danish organic egg production primarily consists of importing a concentrate feed mixture that fulfils the hens' requirements and feed this in the henhouse. This suggests that roughage is mainly used as a supplement for welfare reasons (WECHSLER & HUBER-EICHER, 1998) and that producers do not include nutrient intake from outdoor areas in their consideration of the nutritional needs of poultry. Cultivation of the hen yard in order to supply poultry with nutrients is therefore rarely seen, despite the requirement that organic hens must have access to an outdoor area of at least 4 m^2 per hen (THE DANISH PLANT DIRECTORATE, 2005). Since feed costs accounts for more than 60% of the total costs in Danish organic egg production systems (DANISH POULTRY COUNCIL, 2004) even a small reduction in feed costs will be noticeable. This reduction could be caused by higher feed intake from the outdoor area. Moreover, a higher utilization of local resources may be a way to facilitate the transition to 100% organic feed supply for organic egg producers.

Information about feed intake of high performing layers from outdoor areas is extremely scarce, even though HUGHES & DUN (1983) suggest that layers on pasture may consume up to 30-35 g DM/day of herbage when their nutrient requirements are met through ad libitum concentrate. Thus, there is a need for more information about herbage intake and botanical composition of intake from outdoor areas by free-range poultry in order to understand the contribution by the forage. A reliable technique for estimating herbage intake for poultry has however proven difficult to obtain.

Microhistological analysis of faeces has been used to estimate the botanical composition of the diet of grazing herbivores (GROSS ET AL., 1983; WINDER ET AL., 1996; MILLER & THOMPSON, 2005). The method is based on the fact that the cuticle and epidermis of plants are identifiable after passing through the digestive tract of herbivores (STORR, 1960). Cellular characteristics from leaf epidermis are used for species or genus identification. These microscopically visible characteristics include cell walls, stomata, trichomes, glands, druses, crystals, and silica formations (HANSEN ET AL., 1976).

Identification of herbage intake by poultry is difficult. However, a few studies in this area, using plant identification from the crop of killed birds, have been made (WOOD, 1956; WOOD ET AL., 1963; ANTELL & CISZUK, 2006). No publications were found with the use of the microhistological method to estimate the composition of plant species in faeces from free-range laying hens. This may be due to the fact that poultry in the industrialization era primarily has been produced in systems without range areas. However, poultry on range have anew become a practice of commercial importance. Thus, the microhistological method has potential when developing strategies for increased foraging in organic poultry. Identification of the botanical composition of herbage intake of free ranging poultry, coupled to a knowledge of the nutrient content of the forage would allow formulation of rations with a restricted nutrient allocation to be fed.

The objective of the present study was to evaluate whether type of supplemental feed affect the botanical composition of herbage intake from different forage crops by use of microhistological analysis of faeces from free-range laying hens.

Materials and methods

Experimental design

An experiment with forage crops for organic layers was conducted at Research Centre Foulum in late June 2004. The experiment was carried out in a 2×2 factorial design with two forage crops, two types of feed (typical concentrate for organic layers versus whole wheat) and three replications, i.e. a total of 12 groups, each with 20 hens and one cock. Three plots of each forage crop were alternately planted on the experimental field with four metres between the plots to minimize the possible effects between forage crops. Each plot, which totalled 420 m^2, was subdivided into two subplots according to feed type, resulting in subplots of 210 m^2 (12×17.5 metres). The forage crops consisted of a well-established (five year old) grass/clover pasture (*Lolium perenne* and *Trifolium repens*) and a mixture of forbs that had been sown in spring (*Fagopyrum esculentum, Phacelia tanacetifolia and Linum usitatissimum crepitans*). Although no grass was in the seed mixture, grass cover was identified in the field. Also other species of plants (weeds) in addition to those that have been sown were found in the plots (Table 1).

Table 1. Fractions of different plant species observed in parcels with grass/clover and mixed forbs, average and standard deviation

	Grass/clover		Mixed herbs	
Plant species	**Fraction**	**Standard deviation**	**Fraction**	**Standard deviation**
Capsella bursa-pastoris	0	*0*	0.004	*0.097*
Chamomilla suaveolens	0	*0*	<0.001	*0.002*
Chenopodium album	0	*0*	0.013	*0.034*
Elytriga repens	0.011	*0.038*	0.002	*0.061*
Fagopyrum esculentum	0	*0*	0.130	*0.077*
Galeopsis tetrahit	0	*0*	0.011	*0.027*
Linum usitatissimum crepitans	0	*0*	0.013	*0.009*
Lolium perenne	0.141	*0.142*	<0.001	*0.002*
Phacelia tanacetifolia	0	*0*	0.819	*0.078*
Polygonum convolvulus	0	*0*	<0.001	*0.002*
Taraxacum sp.	0.074	*0.092*	0.004	*0.067*
Thlaspi arvense	0	*0*	0.001	*0.005*
Trifolium repens	0.773	*0.177*	<0.001	*0.002*
Viola arvensis	0	*0*	0.001	*0.003*

Housing and feeding

The hens were of the high producing strain “Lohmann Silver” and after arriving from the breeder at approximately 17 weeks of age the pullets were placed in a house with access to an outdoor area, where they were kept until the experiment commenced eight weeks later. The flock was fed a commercial concentrate diet (18.4% protein) as well as oyster shells and grit stone during this adaptation period. In addition five kg of whole wheat was spread out in the hen yard daily to introduce the hens to this feed. Feed and water were given ad libitum both inside and outside of the henhouse.

The experiment began when the hens were about 25 weeks of age and well into egg laying stage. During the experiment, the hens were housed in hen houses of 4.6 m^2 each with five nest boxes placed on the outside of the house and perches inside of the house. The hens were divided so that there were two groups in each plot (one to each subplot). One group in each plot received the concentrate and the other whole wheat. The composition and results of the chemical analyses of the supplementary feed are shown in Table 2. The concentrate did not contain any roughage. Feed, water, oyster shells and grit stone continued to be provided ad libitum outdoors. Hens fed concentrate consumed on average 129 g of feed per hen per day, whereas hens fed wheat consumed 92 g feed per hen per day. Intakes of oyster shells were 1.7 and 5.7 g per hen per day, respectively (HORSTED ET AL., 2006).

Recordings

Three days prior to introduction of the poultry to the experimental facilities, a visual assessment of the herbage composition in five randomly chosen spots (one m^2 each) in each subplot was conducted. The proportion of the ground surface covered of each plant species was estimated (Table 1) and samples of each species were collected for use as reference material.

Three whole poultry faeces samples were collected from each subplot fresh from the ground three days after introduction in late June. Care was taken that there was no contamination from surrounding plants and/or soil. Faeces from each subplot were pooled resulting in one sample from each subplot. Immediately after collection, the reference plants and faeces were dried in a forced air-drying oven at 55 ºC for approximately 24 hours or until the samples were completely dry.

Table 2. Dietary composition and nutrient content of concentrate and wheat

	Concentrate	**Wheat**
Dietary composition (%):		
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	
Fishmeal	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:		
Dry matter (DM), %	90.3	90.3
<u>% in DM:</u>		
Crude protein (N x 6.25)	18.4	12.0
Calcium	4.1	< 0.1
<u>Amino acids (aa) (g/kg DM):</u>		
Lysine	8.7	3.4
Methionine	4.6	1.9
Starch	37.9	64.2
Sugar	2.4	< 0.5
ME (MJ/kg DM)	11.6	13.4

Preparing samples for microhistological analysis

After the drying process the samples were ground to 1 mm sized fragments using a rotating knife/cutting mill to ensure equally sized fragments. The particles were bleached in household bleach (hypochlorite) for less than 2 minutes in order to remove chlorophyll pigmentation, since this has revealed more accurate estimations (HOLECHEK, 1982). Unidentifiable large and small particles were removed by using a double sieve and particles remaining on a 0.2 mm sieve were mounted on five slides using a metal frame to ensure equal amount of material on each slide. Hoyers solution (BAKER & WHARTON, 1952) was used as mounting media and the slides dried in a forced air drying oven at 60-70ºC for five days.

Identification and quantification of plant particles

A reference collection of the flora found in the study area was made in order to identify the unique characteristics of the plant species, as described by HANSEN ET AL (1976). The species or genus specific characteristics were identified using a phase contrast microscope (Leica DMLS) with 125 and 250 magnification. Drawings of the plant fragments and unique characteristics were made on reference cards.

The identifiable plant particles were quantified by systematically locating 20 non-overlapping fields in which suitable plant fragments were identified on five slides (100 fields per sample). In every field the identifiable plant fragments, which were not smaller than 15-20% of the field, were assessed. A fragment is considered identifiable when at least two separate characteristics are identified. All identifiable plant species were noted by occurrence, i.e. only once in every field they occurred, using the methods described by (HOLECHEK & GROSS, 1982B). If a field did not contain any suitable fragments, the surrounding areas were systematically examined until a field with at least one suitable piece was found.

Statistical methods

The experiment was analysed as a 2×2 factorial design of diet (concentrate vs. wheat) and forage crops (grass/clover vs. mixed forbs) with three replicates. Statistical analysis was based on the total number of binary observations of the multiple plant species in 100 readings from each sample. The following model was used to determine differences in botanical composition of herbage intake:

$E(Y_{ij}) = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij}$

where $Y_{ij} \sim B(1, p_{ij})$; $E(Y_{ij})$ corresponds to the expected value of $log[p_{ij}/(1\text{-}p_{ij})]$ and p_{ij} is the probability for a plant species to be identified in the faeces sample from forage crop i and diet j; μ = mean; α_i = feed $(i = 1,2)$; β_j = forage crop $(j = 1,2)$; $(\alpha\beta)_{ij}$ = interaction feed × forage crop. The data was analysed using the GENMOD procedure in SAS with logit as the link function (KAPS AND LAMBERSON, 2004).

Selectivity indices were used to investigate the effect of forage crop and diet on the consumed plant species. A selectivity index was calculated, using log odds ratio, for plant species that were found as 5% or more of all observed species in either the faeces or the available plots. We defined positive selectivity when the index was greater than 1 and negative selectivity when the index was less than –1, whereas the interval from –1 to 1 was defined as indifferent selectivity. The log odds ratio was used to ensure normal distribution and to compare the probability of finding the different plant species in the faeces with the same species being observed in the available forage crops:

Log odds ratio = $\log(f_f/1\text{-}f_f) - \log(f_s/1\text{-}f_s)$

where f_f is the average relative frequency of plant species calculated from the average occurrences in the faeces, and f_s is the average relative frequency of a species observed in the subplots.

Results

Average counts of occurrence of fragments of the different plant species in 100 fields in the three samples from each treatment are shown in Table 3. In several cases the standard deviation between replicates of treatments amounted to more than 25% of the mean counts. Nevertheless significant effects of forage crop and supplementary feeds were observed in a number of cases.

Table 3. Average counts of occurrence of fragments and standard deviations of the different plant species in 100 readings per parcel, and level of significance of the effect of forage crop and diet

Forage crop:	**Grass/clover**		**Mixed forbs**		**Significance (P)**[+]		
Supplementary feed:	**Concentrate (n = 3)**	**Wheat (n = 3)**	**Concentrate (n = 3)**	**Wheat (n = 3)**	**Forage crop**	**Diet**	**Forage crop x diet**
Plant species:							
Capsella bursa-pastoris	0.7 (1.2)	1.0 (1.0)	2.0 (2.0)	3.7 (3.5)	< 0.05	ns	ns
Chamomilla suaveolens	1.0 (1.7)	0.7 (1.2)	0 (0)	0.3 (0.6)	= 0.05	ns	ns
Chenopodium album	0 (0)	0.3 (0.6)	0.7 (1.2)	1.3 (2.3)	< 0.05	ns	ns
Elytriga repens	22.0 (5.2)	31.3 (24.8)	25.7 (6.1)	28.7 (10.8)	ns	< 0.05	ns
Fagopyrum esculentum	4.3 (2.1)	3.7 (2.9)	2.3 (1.5)	5.0 (4.4)	ns	ns	ns
Galeopsis tetrahit	2.0 (2.6)	2.7 (2.5)	4.7 (3.1)	5.0 (4.6)	< 0.05	ns	ns
Linum usitatissimum crepitans	1.3 (1.2)	0.3 (0.6)	1.0 (1.7)	0 (0)	ns	< 0.05	ns
Lolium perenne	18.3 (10.3)	18.7 (4.7)	19.7 (14.0)	12.0 (8.7)	ns	ns	= 0.05
Phacelia tanacetifolia	2.3 (2.1)	4.0 (3.5)	40.0 (13.7)	39.7 (17.1)	< 0.001	ns	ns
Polygonum convolvulus	1.3 (2.3)	2.7 (4.6)	1.7 (2.9)	1.3 (2.3)	ns	ns	ns
Taraxacum sp.	1.7 (2.1)	8.3 (7.4)	0.3 (0.6)	0 (0)	< 0.001	ns	ns
Thlaspi arvense	3.3 (2.9)	2.0 (2.0)	0.3 (0.6)	0 (0)	< 0.001	ns	ns
Trifolium repens	56.7 (4.7)	38.7 (17.7)	11.3 (12.9)	7.7 (4.0)	< 0.001	< 0.001	ns
Viola arvensis	2.0 (2.0)	0.7 (1.2)	0.7 (2.1)	0.7 (1.2)	ns	= 0.07	ns

[+] ns: P > 0.05

In the visual assessment of the grass/clover plots (Table 1), *Trifolium repens* was observed most frequently and the grass *Lolium perenne* were observed frequently. This was also the case in the faeces collected from these plots. However, as shown by the positive selectivity index in Table 4, *Elytriga repens* was observed more frequently in the faeces than in the plots. The opposite was the case for *Trifolium repens* and *Taraxacum sp.* as indicated by the negative selectivity indices. The selectivity indices close to nil for *Lolium perenne* indicates indifferent selection regarding this species of grass.

Phacelia tanacetifolia was the most frequently observed plant in the plots with the mixed forbs (Table 1). This was also the case in the faeces from the hens foraging these plots (Table 3). However, positive selectivity indices were found for *Elytriga repens*, *Lolium perenne* and

Trifolium repens. Contrary to this, *Fagopyrum esculentum* and *Phacelia tanacetifolia* had negative selectivity indices (Table 4).

Table 4. Calculated selectivity indices for plant species occurring in 5% or more of all observed species in either the faeces or the vegetation

Forage crop:		**Grass/clover**		**Mixed forbs**	
Supplementary feed:		**Concentrate**	**Wheat**	**Concentrate**	**Wheat**
Category:	***Species:***				
Grass	*Elytriga repens*	3.04	3.52	5.01	5.22
Forb	*Fagopyrum esculentum*			-2.00	-1.09
Grass	*Lolium perenne*	0.15	0.15	5.40	4.87
Forb	*Phacelia tanacetifolia*			-2.06	-1.99
Forb	*Taraxacum sp.*	-1.38	0		
Forb	*Trifolium repens*	-1.29	-1.90	4.74	4.38

Table 3 show that Trifolium repens was found significantly more often in samples from hens fed concentrate than in samples from hens fed wheat (P < 0.001). In contrast, *Elytriga repens* was found more often in the samples from the wheat fed hens (P < 0.05). This pattern was also reflected in the selectivity index (Table 4) in that wheat fed hens within the same forage crop had a higher selection for *Elytriga repens* and a lower selection for *Trifolium repens*. No significant interaction between forage crop and feed type were found.

Discussion

Considerable variations among occurrence of identified fragments of plant species in the faeces within treatments were observed. This variation may be related to: 1) a variation in the proportion of plant species actually present in the different subplots within the same forage crop; 2) a variation of intake among hens (from which the faeces were collected) within a given forage crop; 3) and a variation related to the determination of fragments in the faeces. The latter is known to be influenced by the observer (HOLECHEK & GROSS, 1982A; HOLECHEK ET AL., 1982). However, in our study only one observer analyzed the samples and thereby minimizing the risk.

Despite the variation observed, it was possible to detect significant differences in the presence of forage fragments in the faeces from hens fed different supplementary feed. This supports that the microhistological method is applicable for determine the botanical composition of herbage intake in free-range laying hens. However, since poultry are omnivores a lot of different food items of non-vegetable origin were present in the samples, which made the reading of the slides more difficult. Thus, it can be difficult to decide whether certain identification characteristics such as “druses”, “crystals” etc. actually are originally parts of plant tissue or parts of partially digested insects, earthworms etc. Especially *Chenopodium album*, *Fagopyrum esculentum*, *Polygonum convolvulus* and *Viola arvensis* were characterized by having many “druses” and “crystals”. However, constant comparisons of sample fecal material to the reference plant collection were made during identification and quantification.

Among certain plant species a risk of mistake exists since some identification characteristics can be confounded. E.g. among *Trifolium repens* and *Taraxacum sp.* the identification characteristics were very uniform, though a reliable estimation can be made by a trained observer. However, with only a few occurrences of fragments from a certain plant species, a risk for decidedly incorrect estimations exist. This might explain why *Thlaspi arvense* was observed significantly more times in faeces from the grass/clover pasture than in the mixed forbs, even though the visual assessment of the herbage composition (Table 1) indicated that this species only were present in the mixed forbs plots. This could indicate mistaken identification in either the visual assessment of the vegetation, or the plant particles in the faeces. Another possibility is that the hens located a source of the plant that was not identified in the visual assessment of the vegetation. However, the average relative percentage in the faeces was less than 5%.

It is important to notice that the quantitative aspects of microhistology are based on the assumption that there is a relationship between the ingested proportion of each plant species and the proportion of fragments found in the faeces (JOHNSON ET AL., 1983; VAVRA & HOLECHEK, 1980). According to JOHNSON ET AL. (1983) ruminant digestion only rarely alters the relative botanical composition significantly, though the discernibility of some plants can be affected. However, in vitro digestibility has shown to influence particularly the estimation of the relative proportions of grass and forbs content. Thus VAVRA & HOLECHEK (1980) found that the grasses and grass-like species used in their investigation were uniformly overestimated while forbs were uniformly underestimated.

If poultry digestibility is assumed not to alter the proportion of species of plants found in faeces, our studies indicate poultry preference for grasses (20-30 cm's high) compared to forbs, since positive selectivity was found for the grass species *Elytriga repens* and *Lolium perenne* in the plots with the mixed forbs, and *Elytriga repens* in the grass/clover plots. The positive selection may indicate that the hens gave priority to the plants nearer the ground, since we also found a positive selectivity index for *Trifolium repens* (20-30 cm's high) in the plots with the mixed forbs. Moreover we found negative selectivity indices of the much taller *Phacelia tanacetifolia* and *Fagopyrum esculentum* (60-70 cm's high), i.e. a considerable part of the leaves from these species of plants was placed above the head of the hens. The differences of height between the species of plants in the plots with the mixed forbs also involve a risk for underestimation of plants nearer the ground in the visual assessment of the plots.

The highly significant difference between diets in the occurrence of *Trifolium repens*, indicate a preference for clover for concentrate-fed hens compared to wheat-fed hens, which in contrast had significant more fragments of *Elytriga repens* in the faeces. This preference is presumably due to the fact that the nutrient requirements of the concentrate-fed hens were fulfilled by the diet without supplement nutrients obtained during foraging. Therefore, the hens may have been able to eat the species of plants, which they found most attractive. In plots with wheat-fed hens the ground vegetation cover was less consistent. This may be because the hens attempted to cover the protein deficit in the diet by eating earthworms and earth living insects, resulting in a higher foraging activity through scratching. Thus, the higher foraging activity in the nutrient restricted hens might cause the clover to be worn-down, whereas the many underground offshoots of "*Elytriga repens*" may lead to this plant species being more resistant to the scrapping of the hens. However, faeces were collected three days after hens were introduced to the forage area with approximately 10m^2 per hen. Thus, it is not likely that the botanical composition of the forage area have been change that much at this stage. Moreover the passage rate of feed through the digestive tract is several hours, depending on type of feed and the condition of the bird (HEUSER, 1955). This indicates that the herbage have been consumed by the hens several hours before the faeces were collected.

We conclude that the microhistological method is applicable for estimation of botanical composition of herbage intake in poultry. Moreover it seems plausible that free-range laying hens select species of plants nearer the ground at the expense of higher plants. We further found that poultry, that consume their nutrient requirements through concentrate, have a higher preference for clover instead of grass compared to wheat-fed hens. It seems, however, necessary to generate more information on how digestibility effects plant fragment identification for plants commonly consumed by free ranging poultry.

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Summary

To understand the contribution by forage in the nutrition of free-range laying hens, there is a need for more information about the botanical composition of herbage intake from range areas. The effect of different forage crops and feed types on the botanical composition of herbage intake in free-range laying hens was investigated using the microhistological methodology. In this method, cellular characteristics from leaf epidermis found in faeces are used for species or genus identification. The experiment was carried out with 12 flocks of 20 laying hens in a 2×2 factorial design with two different forage crops and two types of supplementary feed (typical concentrate for organic layers versus whole wheat grain) with three replications. The forage crops consisted of a well-established grass/clover (*Lolium perenne and Trifolium repens*) pasture or a mixture of forbs (*Fagopyrum esculentum, Phacelia tanacetifolia and Linum usitatissimum*). On the grass/glover pasture negative selectivity indices were found for *Trifolium repens* and positive selectivity indices for *Elytriga repens* for both types of supplementary feed. In the forage crops sown with the mixed forbs positive selectivity indices were found for the species of plants near the ground such as *Elytriga repens*, *Lolium perenne* and *Trifolium repens.* Contrary to this negative selectivity indices were seen for the taller species of plants *Fagopyrum esculentum* and *Phacelia tanacetifolia*. Our results indicate that hens offered the concentrate diet had a higher preference for *Trifolium repens* than the wheat-fed hens, whereas the wheat-fed hens had a higher preference for *Elytriga repens*.

Keywords: excrete, microhistology, plant species, poultry, selectivity

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- Paper 2 -

Crop content in nutrient restricted versus non-restricted organic laying hens with access to different forage vegetations

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Running title: Crop content in organic layers

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Abstract

Crop content in organic laying hens were studied in two experiments. Each 23-day experiment was arranged with chicken runs in a 2 × 2 factorial design with two types of supplementary feed (concentrate with 184 g/kg crude protein versus whole wheat with 120 g/kg crude protein) and two types of forage vegetation (grass/clover vs. a mixture of forbs in experiment 1 and grass/clover vs. chicory in experiment 2): Each experiment was carried out in three replications. Two times during each experiment two hens from each run were slaughtered in the evening and the following morning. Subsequently the birds' crops were removed. Crop contents were

separated into eight fractions (supplementary feed, plant material, seeds, insects, earthworms and larvae, oyster shells, insoluble grit and soil). Crop content was found to be significantly higher in the evening than in the morning for most feed items. Supplementary feed significantly influenced the content of several feed items in the crop. In both experiments wheat-fed hens had significantly higher amount of soil in the crops and a significantly lower amount of seeds compared to the concentrate-fed hens. Plant material and grit stone were significantly more abundant in wheat-fed hens only in experiment 2 and numerically more abundant in experiment 1, whereas the amount of oyster shells was significantly higher in wheat-fed hens in experiment 1 and numerically higher in experiment 2. Type of forage itself only significantly influenced the amount of soil in the crops in experiment 1 and amount of seeds in experiment 2, since more soil was found in hens foraging the mixed forbs and more seeds were found in the hens foraging the chicory plots, respectively.

INTRODUCTION

In Danish organic egg production systems, hens have access to an outdoor area of minimum 4 m^2 per hen (The Danish Plant Directorate, 2005). However, cultivation of the hen yard for the purpose of supplying the poultry with nutrients from the outdoor area is rarely seen, even though increased foraging in the outdoor area could be a way to enhance self-sufficiency, just as a reduction in the feed costs can be expected. Further, a more attractive hen yard, which in turn may lead to higher utilization of this area by the hens, seems to have a positive effect on the clinical welfare of hens (Bestman and Wagenaar, 2003). Moreover, experiments with supplement of roughage have resulted in reductions in some welfare problems such as feather-pecking (Wechsler and Huber-Eicher, 1998, Steenfeldt et al., 2001).

However, to further develop systems for organic poultry production, more information on actual feed intake from the outdoor area needs to be procured in order to determine the standards of nutrient content in the supplementary feed when access to attractive forage crops is given. It has, however, proven difficult to find accurate methods to determine feed intake from the pasture. One method of interest is analysing the crop content, because limited digestion of feed occurs in the crop, making it easy to identify the feed items. Thus, Jensen and Korschgen (1947) found that analysis of crop content is a better method of obtaining information on food habits of quails compared with analysis of droppings and content of gizzards. The literature on this subject is, however, very scarce in relation to free-range poultry, although there have been a few studies (Wood et al., 1963; Amaka Lomu et al, 2004; Antell and Ciszuk, 2006). In other work, analysis of crop content has been used as a method of getting information on food habits in scavenging rural hens (Gunaratne et al., 1993; Ajuyah, 1999; Mwalusanya et al., 2002; Sonaiya, 2004).

In the study by Antell and Ciszuk (2006), a linear relationship between grass intake and the grass content in the crop at the end of the day was found for confined hens, indicating that green fodder intake during the day can be estimated, if the content of plant material in the crop from hens slaughtered in the evening is known. However, intake of different feed items in the evening or during the day may vary according to the actual conditions offered, e.g. when hens receive a restricted nutrient supply in order to increase their forage intake from the outdoor area (Fuller, 1962), just as different forage vegetations in the outdoor area may be of importance.

The purpose of the present study was to evaluate whether the intake of different feed items, as measured by the crop content, varies according to the time of day (morning vs. evening), and type of forage vegetation available, when hens are fed a normal concentrate for organic layers or a nutrient-restricted diet with whole wheat and oyster shells as the only supplement.

MATERIALS AND METHODS

Experimental design

Two experiments with different forage plants for laying hens (Lohmann Silver) were conducted at Research Centre Foulum. Each experiment were carried out in a 2x2 factorial design with two types of forage vegetations and two types of feed (typically pelleted concentrate for organic layers versus whole wheat) in three replications. Each forage plot, which measured 420 m^2, was subdivided into two subplots according to feed type, resulting in subplots of 210 m^2 (12 x 17.5 metres), i.e. a total of 12 subplots, each with 20 hens and one cock. In experiment 1, forage vegetations consisted of a well-established (five-year-old) grass/clover pasture (*Lolium perenne* and *Trifolium repens*) and a mixture of forbs (*Fagopyrum esculentum, Phacelia tanacetifolia* and *Linum usitatissimum crepitans*). These forbs are expected to attract a lot of insects during the flowering season, when this experiment took place. In experiment 2, forage vegetations consisted of a well-established grass/clover pasture and chicory (*Cichorium intybus* cv. Grassland Puna).

Two hens randomly chosen from each subplot were slaughtered in the evening (8 - 9 pm) and the following morning (8 – 9 am). Subsequently, these two times of day are considered as the same "day of slaughter" despite the two different dates. Two days of slaughter were carried out in each experiment, with the first slaughter day taking place 9 – 10 days after introduction and the second day of slaughter two days before termination of the experiment. The experimental period and times of slaughter of hens are shown in Table 1. At the end of experiment 1 and at the beginning of experiment 2 the hens were fenced off in a 24 m^2 plot of each subplot for a period of two and three days, respectively, in order to estimate the removal of herbage (data not shown). In experiment 1 the second day of slaughter took place while hens were fenced off in these small plots. This was done since some of the subplots with the mixture of forbs were practically without vegetation at the end of the experiment. Immediately after slaughtering, the hens were cut up and the crop removed and stored in a freezer (-18^{o}C) for later analysis.

Table 1. *Experimental periods, forage vegetations and slaughtering of hens*

Period	Number of days	Forage vegetation	Slaughtering of hens (2 per subplot) Date[a]	Time of day	No. hens per experiment
Eksperiment 1:					
21.06.04 - 14.07.04	23	Grass/clover or mixed forbs	01.07.04 and 12.07.04	Evening and following morning	96
Eksperiment 2:					
03.08.04 - 26.08.04	23	Grass/clover or chicory	12.08.04 and 24.08.04	Evening and following morning	96

[a] Hens slaughtered in the evening on this date and the following morning

Strain, feeding and housing of experimental birds

The hens in both experiments were of the commercial strain "Lohmann Silver". Prior to each experiment, hens arrived from the breeders at approximately 17 weeks of age. The pullets were kept in a house with access to an outdoor area until they were introduced to the experimental units. The flock was fed a commercial concentrate diet and oyster shells and grit stone during this adaptation period. In addition, five kg whole wheat was spread out in the hen yard daily to introduce the hens to this feed.

The experiment began when the hens were about 25 weeks of age and well into the egg-laying stage. During the experiment, the hens were housed in mobile henhouses of 4.6 m^2 each. Pop holes were open day and night, giving the hens access to forage from sunrise to sunset. Concentrate-fed hens received a concentrate containing 184 g/kg DM crude protein, 8.7 g/kg DM lysine, 4.6 g/kg DM methionine and 41 g/kg DM calcium. Whole wheat contained 120 g/kg crude protein, 3.4 g/kg DM lysine, 1.9 g/kg DM methionine and less than 10 g/kg DM calcium. Feed, water, oyster shells (320 g/kg calcium) and insoluble grit stone continued to be provided ad libitum outdoors during the experiments. Hens fed concentrate consumed on average 129 g of supplementary feed per hen per day in experiment 1, and 155 g in experiment 2. Hens fed wheat consumed 92 and 89 g supplementary feed per hen per day, respectively. Wheat-fed hens had significantly higher intakes of oyster shells than the concentrate-fed hens, just as type of forage vegetation influenced intakes of oyster shells. Egg production was significantly lower for wheat-fed hens in experiment 1, whereas no differences were observed in experiment 2 (Horsted et al., 2006).

Determination of crop content

The individual crops were thawed and the contents separated by forceps into the fractions: “Supplementary feed (concentrate or wheat)”, “plant material”, “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 ºC for approximately 24 hours or until the samples were completely dry. Since DM determinations of crop contents in the study by Antell and Ciszuk (2006) did not reveal a wider range of values than 94-97% of those of air-dried crop contents, calculation on crop contents in this study is based on air-dried fractions of crop contents.

Statistical methods

The data were subject to analysis of variance using the MIXED procedure in SAS (SAS Institute Inc., 1990) by the following model:

$$Y_{ijklmn} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \lambda_l + (\alpha\beta\gamma)_{ijk} + (\alpha\beta\lambda)_{ijl} + A_{m(ij)} + \varepsilon_{ijklmn}$$

where Y_{ijklmn} = quantity of each fraction in the individual hen; μ = mean; α_i = supplementary feed $(i = 1,2)$; β_j = forage vegetation $(j = 1,2)$; $(\alpha\beta)_{ij}$ = interaction supplementary feed × forage vegetation; γ_k = time of slaughter ($k = 1,2$); λ_l = day of slaughter ($l = 1,2$); $(\alpha\beta\gamma)_{ijk}$ = interaction supplementary feed × forage vegetation × time of slaughter; $(\alpha\beta\lambda)_{ijl}$ = interaction supplementary feed × forage vegetation × day of slaughter; $A_{m(ij)}$ = the random effect of replication; ε_{ijklmn} = error. In all cases, residuals were approximately normally distributed. One hen was excluded in each of experiment 1 and experiment 2 because of crop impactions. In addition, the analyses were performed separately for morning and evening samples since the crop content levels differed markedly. LS means, standard errors and level of significance in Tables 3 and 4 are based on this model in which time of day factors were not included.

RESULTS

Level of significance of factors influencing crop content is shown in Table 2.

Day and time of slaughter

In experiment 1, there was a significant difference in the amount of plant material found between the first day of slaughter (foraging the large subplots) and the second day of slaughter (foraging the small plot inside each subplot) with 2.0 g and 1.0 g plant material, respectively, but with no interaction with forage vegetation and supplementary feed. Other fractions of crop content did not differ significantly with day in experiment 1.

In experiment 2, apart from the content of plant material, the amount of supplementary feed, earthworms and soil was significantly influenced by the day of slaughter. These effects are presented in Figures 1-4. Figure 1 shows that wheat content in the evening-crops (hens slaughtered in the evening) was significantly higher at the end of the experiment, whereas there were no differences in the concentrate, as indicated by the significant three-sided interaction for supplementary feed (Table 2). In the morning-crops there were no significant differences in supplementary feed levels between days of slaughter.

Table 2. *Level of significance (P-values) of factors influencing crop content, ns = P > 0.10*

	Forage vegetation	Supp. feed	Forage vegetation × supp. feed	Day of slaughter[a]	Time of slaughter	F. vegetation × supp. feed × day of slaughter	F. vegetation × supp. feed × time of slaughter
Experiment 1:							
Supplementary feed	ns	ns	< 0.01	= 0.07	< 0.001	ns	ns
Plant material	ns	= 0.05	ns	< 0.01	< 0.001	ns	ns
Seeds	ns	< 0.01	ns	ns	< 0.001	ns	< 0.01
Insects	ns	= 0.07	ns	ns	< 0.05	ns	ns
Earthworms, larvae, pupae	ns	ns	ns	ns	< 0.05	ns	ns
Oyster shells	ns	< 0.05	ns	ns	< 0.001	< 0.05	ns
Grit	= 0.07	ns	ns	ns	< 0.01	ns	= 0.07
Soil	< 0.05	< 0.01	< 0.05	ns	< 0.01	ns	< 0.001
Experiment 2:							
Supplementary feed	ns	< 0.05	ns	< 0.01	< 0.001	< 0.01	ns
Plant material	ns	< 0.05	ns	< 0.05	< 0.001	ns	ns
Seeds	< 0.05	< 0.05	ns	ns	< 0.001	ns	< 0.05
Insects	ns	ns	ns	ns	< 0.001	ns	ns
Earthworms, larvae, pupae	ns	ns	ns	< 0.001	ns	ns	ns
Oyster shells	ns	= 0.09	ns	= 0.08	< 0.001	ns	ns
Grit	ns	< 0.05	ns	ns	< 0.001	ns	= 0.06
Soil	ns	< 0.05	ns	< 0.01	< 0.01	< 0.05	< 0.05

[a] in experiment 1 the second day of slaughter took place while hens were fenced off in a small plot inside each subplot

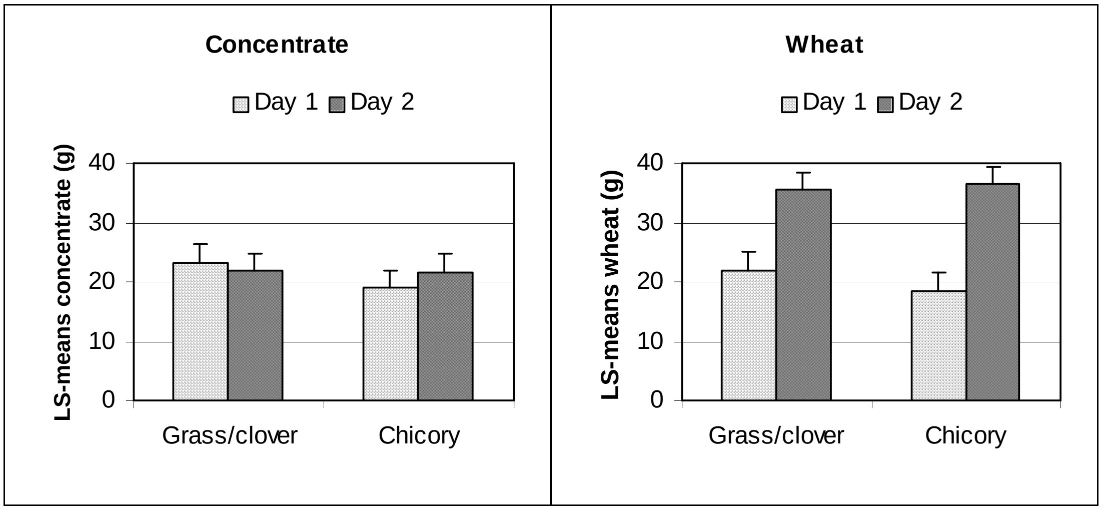

Figure 1. *Content of concentrate and wheat in the crops from hens slaughtered in the evening on two different days of slaughter for hens foraging grass/clover or chicory in experiment 2*

The main effect of day of slaughter was an overall significant decrease in plant material in the crops at the end of experiment 2. This was primarily due to a decrease in the evening-crops as illustrated in Figure 2, since no differences were seen in the morning-crops. However, the significant main effect of day of slaughter on the content of earthworms, etc., in the crops was due to an increase in the evening-crops as well as in the morning-crops (Figure 3).

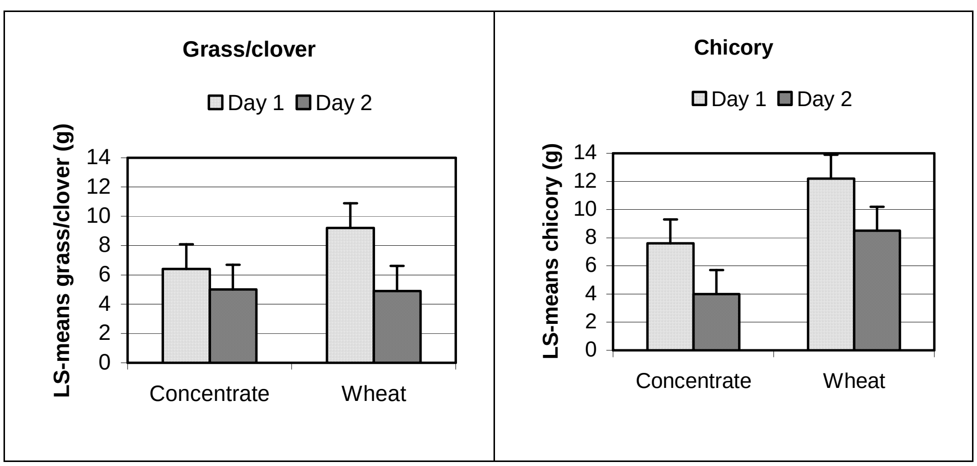

Figure 2. *Content of plant material in the crops from hens fed concentrate or wheat and slaughtered in the evening on two different days of slaughter in experiment 2*

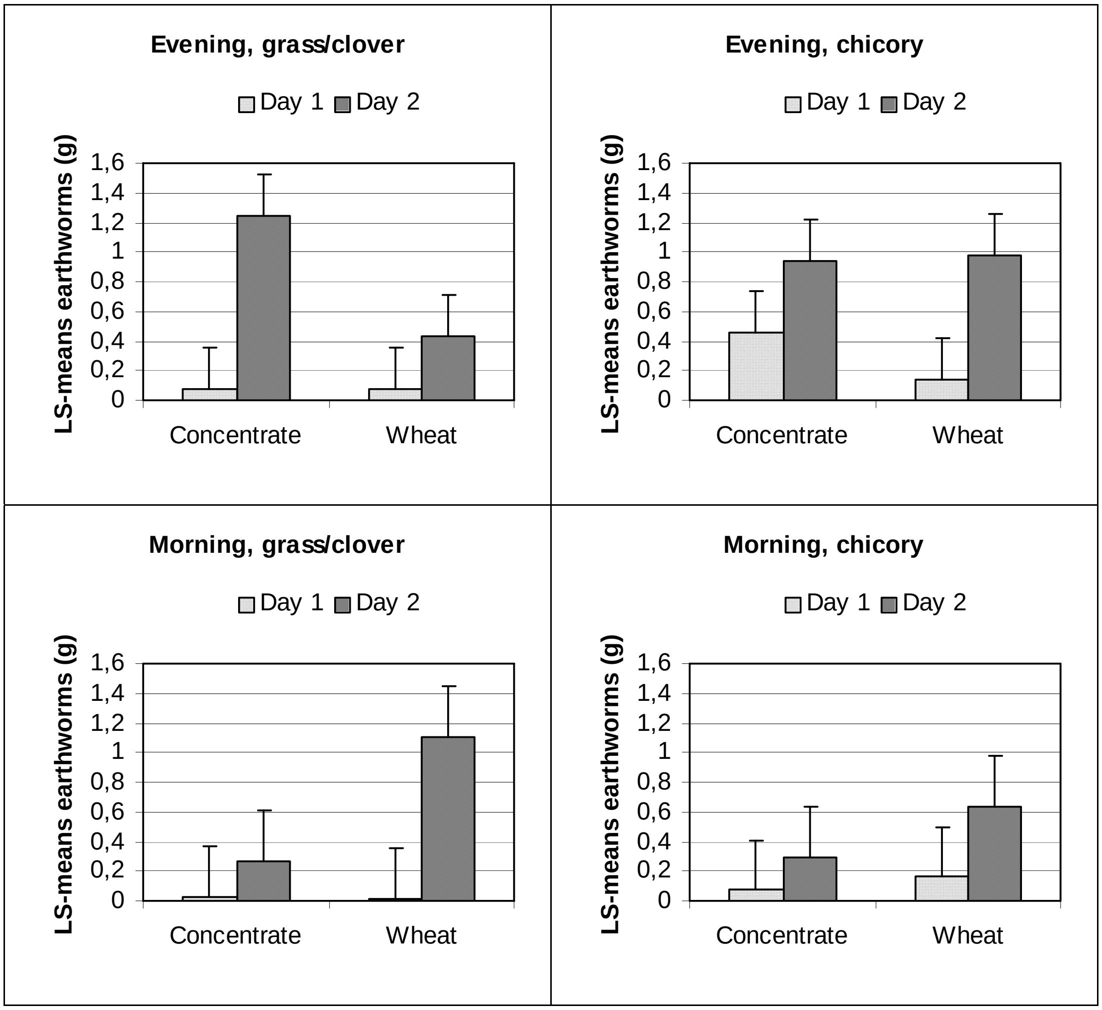

Figure 3. *Content of earthworms, larvae and pupae in the crops from hens slaughtered in the evening or in the morning on two different days of slaughter in experiment 2*

Soil was almost solely observed in the crops from the wheat-fed hens. Thus, the significant effect of day of slaughter in experiment 2 was primarily due to an increase in soil content in the wheat-fed hens slaughtered in the evening (Figure 4).

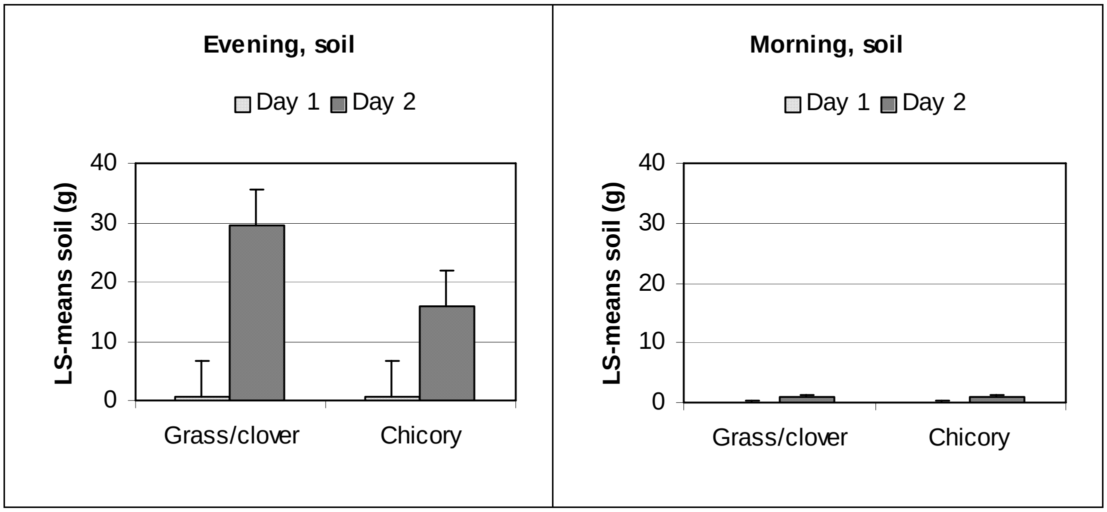

Figure 4. *Content of soil in the crops from wheat-fed hens slaughtered in the evening or in the morning on two different days of slaughter in experiment 2*

Taken the above effects of day of slaughter into consideration, LS means across the day of slaughter for the amount of each feed item in the crops, are presented in Tables 3 and 4. In both experiments we found significantly more of each fraction in the crop when hens were slaughtered in the evening compared with the morning (Table 2). Only the amount of earthworms in experiment 2 was not significantly different, although numerically there were more earthworms in the crops from the evening slaughtering (Table 4).

Supplementary feed

In experiment 2, the amount of wheat in the crop was higher than the amount of concentrate (Table 4). This was also the case in experiment 1 when forage vegetation was grass/clover, but not when hens had access to the mixed forbs as shown by the significant interaction in experiment 1 (Tables 2 and 3).

The supplementary feed consistently influenced several fractions in the crop content, such as plant material, seeds, oyster shells, grit and soil. The amount of plant material in the crops was higher for wheat-fed hens compared with concentrate-fed hens in both experiments. However, in experiment 1, differences within the evening-crop contents were not significant, just as only wheat-fed hens on the grass/clover within the morning crops differed significantly from the other treatments (Table 3). In experiment 2, the content of plant material in the crops from the wheat-fed hens on chicory was significantly higher than the other treatment, for both evening as well as the morning-crops.

Table 3. *Amount of feed items in the crops from hens in experiment 1, LS-means and SEM (a and b indicate significant differences)*

Forage vegetation:			Grass/clover		Mixed forbs		
Feed item:	**Time of slaughtering**	**Unit**	**Concentrate**	**Wheat**	**Concentrate**	**Wheat**	**SEM**
Complementary feed	Evening	g	15.8[a]	23.0[b]	22.8[b]	18.9[ab]	1.9
	Morning	g	5.3[ab]	7.9[a]	7.5[a]	3.9[b]	1.1
Plant material	Evening	g	1.8	2.8	1.9	3.1	0.8
	Morning	g	0.2[a]	1.7[b]	0.2[a]	0.2[a]	0.3
Seeds	Evening	mg	70[a]	17[b]	100[a]	24[b]	16
	Morning	mg	23[a]	8[ab]	14[ab]	1[b]	6
Insects	Evening	mg	23	50	24	44	19
	Morning	mg	2[a]	31[b]	3[a]	13[ab]	8
Earthworms, larvae, pupae	Evening	mg	23	74	18	95	39
	Morning	mg	1	1	13	6	7
Oyster shells	Evening	mg	546	1649	547	1748	400
	Morning	mg	12[a]	144[b]	8[a]	130[b]	36
Grit	Evening	mg	257	383	353	1395	346
	Morning	mg	6	38	153	143	62
Soil	Evening	mg	0[a]	967[a]	37[a]	11025[b]	1595
	Morning	mg	0	1010	0	340	320

Table 4. *Amount of feed items in the crops from hens in experiment 2, LS-means and SEM (a and b indicate significant differences)*

Forage vegetation:			Grass/clover		Chicory		
Feed item:	**Time of slaughtering**	**Unit**	**Concentrate**	**Wheat**	**Concentrate**	**Wheat**	**SEM**
Complementary feed	Evening	g	22.1[ab]	28.7[a]	20.4[b]	27.4[ab]	2.3
	Morning	g	4.0	6.2	6.6	9.5	2.1
Plant material	Evening	g	5.8[a]	7.1[ab]	5.8[a]	10.3[b]	1.2
	Morning	g	0.5[a]	1.5[ab]	0.6[a]	2.3[b]	0.5
Seeds	Evening	mg	105[a]	47[a]	282[b]	121[a]	46
	Morning	mg	13	30	42	14	16
Insects	Evening	mg	48	36	29	48	16
	Morning	mg	6	5	1	18	6
Earthworms, larvae, pupae	Evening	mg	687	254	698	559	200
	Morning	mg	148	562	180	403	295
Oyster shells	Evening	mg	1381	2645	1623	2423	607
	Morning	mg	12[a]	166[ab]	4[a]	304[b]	61
Grit	Evening	mg	235[a]	1056[ab]	692[ab]	1297[b]	252
	Morning	mg	18	58	7	111	40
Soil	Evening	mg	0	15029	0	8168	4940
	Morning	mg	0	555	0	468	202

In both experiments, significantly more seeds were found in the crops from the concentrate-fed hens. The differences were mainly in the evening-slaughtered hens, since no significant differences were found in the morning-slaughtered hens in experiment 2.

In experiment 1, significantly more insects were found in the morning-crops from the wheat-fed hens when access to grass/clover was given. However, no significant differences were found in the evening, just as no significant differences were found in experiment 2. No significant difference in the amount of earthworms, larvae and pupae related to vegetation and supplementary feed was found in any of the experiments.

Oyster shells were observed more frequently in the crops from wheat-fed hens. However, due to a huge standard error, we only found a significant difference in experiment 1, though a tendency was seen in experiment 2 (Table 2). When times of slaughter were analyzed separately, significantly more oyster shells were found in the morning-crops from wheat-fed hens in experiment 1 and wheat-fed hens on chicory in experiment 2.

Soil almost exclusively occurred in the crops from the wheat-fed hens, resulting in a significant main effect of supplementary feed in experiment 1. In experiment 2 the effect were found to be non-significant due to a huge standard error. In experiment 1, soil was more frequent in the mixed forbs, indicated by the significant effect of forage vegetation and the interaction “forage vegetation × supplementary feed”.

Forage vegetation

In experiment 1, forage vegetation only influenced the amount of soil in the crops in that soil was more abundant in crops from hens foraging the mixed forbs (Tables 2 and 3). In experiment 2, the amount of seeds in the crop was higher when chicory was available compared with grass/clover (Tables 2 and 4).

DISCUSSION

Highly significant differences between morning and evening in the amount of the different feed items were found for most feed items. This is in accordance with Mongin (1976) who, in individually caged hens, found that dry matter in the crop follows a cyclic variation, which is influenced by the photo period. Thus, the crop was almost empty 2, 6 and 22 hours after oviposition, but relatively full 10 hours after oviposition. However, Mongin and Sauveur (1974) showed that individually caged hens fed a calcium-poor diet, but with access to oyster shells, had a higher intake of mash or pelleted feed in the morning and in the afternoon, resulting in a higher daily feed intake. In our study, the content of supplementary feed was higher in the evening in the wheat-fed hens in experiment 2 and in wheat-fed hens on grass/clover in experiment 1, compared with the concentrate-fed hens. Despite this, a higher daily intake of supplementary feed was found in concentrate-fed hens, as we calculated average daily intake of supplementary feed to 92 g wheat and 129 g concentrate in experiment 1, and 89 g wheat and 155 g concentrate in experiment 2 (Horsted et al, 2006).

Based on these average daily intakes of supplementary feed and the content in the evening-crops, it seems plausible that daily intake of whole wheat is approximately 3.2 to 4.4 times higher than what is retained in the crop in the evening. For concentrate-fed hens the factor is approximately 6.7 to 7.3. This is in accordance with the work of Heuser (1945), who found that wheat and whole corn is retained for a longer time in the crop than corn meal or mash. Even though Svihus et al. (2002) suggested that passage rates in the upper digestive tract were similar for whole and ground wheat in broilers, reduced feed intake was observed in diets with whole grains (Hetland et al., 2002). This indicates that whole wheat will remain for a longer time in the gizzard compared with pelleted concentrate and thus slow the movement of whole wheat from the crop to the gizzard. However, as indicated by Figure 1, it seems plausible that the crop increases its capability to retain larger amounts of coarse feed when a continuous access to this feed is given. Increased capacity of the crop has been reported in chicks trained to eat rapidly (Lepkovsky et al., 1960), who found that these chicks had larger quantities of feed in the crop per unit time than untrained chicks. Moreover, crops were heavier in trained chicks, presumably reflecting their greater capacity.

We found significantly more oyster shells in the crops in the evening than in the morning, particularly pronounced in the wheat-fed hens, though not significantly different from concentrate-fed hens due to the huge standard errors. This is supported by the studies of Mongin and Sauveur (1974), who found that the ingestion of oyster shells, in hens fed a calcium-poor diet (1 % Ca) and housed in individual cages, was very high between 16.00 and 20.00 h, i.e. when egg calcification is in progress (Lavelin et al., 2000). In our study, the average daily intakes of oyster shells were, in experiment 1, up to 5.7 g in the crops from the wheat-fed hens and 1.7 g in the crops from the concentrate-fed hens. In experiment 2, the amounts were 7.2 g and 3.1 g, respectively. In addition, the available forage vegetation influenced the daily intake of oyster shells (Horsted et al., 2006). The daily intake of oyster shells divided by the content of oyster shells in the crop for wheat-fed hens results in factors 3.3 and 2.9 for experiment 1 and 2, respectively. For concentrate-fed hens the factors are 3.1 and 2.1, respectively. This indicates that oyster shells, compared with wheat and concentrate, to a higher degree are ingested during the evening or are retained for a longer time in the crop. The latter would be expected because of the inorganic character of this feed item.

The type of supplementary feed further influenced the amount of plant material found in the crop. Thus, significantly more plant material was found in crops from wheat-fed hens compared with concentrate-fed hens in experiment 2, and a tendency ($P = 0.05$) was seen in experiment 1. This indicates that hens restricted in nutrients increase their intake of plant material from the outdoor area even though wheat has a larger particle size compared with pelleted concentrate. In a recent study by Antell and Ciszuk (2006) the amount of grass found in the crop at 21 h. was found to be significantly correlated to the recorded intake of grass. The authors propose the equation “y = 0.144x – 0.178”, where “y” is the amount of grass in the crop and “x” is grass intake on an air-dried basis the last day before slaughter. Using this equation on the amount of plant material found in crops in our study, following estimates of daily herbage intake on an air-dry basis are revealed: intake of grass/clover in experiment 1; 19.6 and 12.7 g DM for wheat-fed and concentrate-fed hens, respectively; intake of mixed herbs in experiment 1; 21.7 and 13.4 g DM, respectively; intake of grass/clover in experiment 2; 49.5 and 40.4 g DM, respectively, and intake of chicory in experiment 2; 71.2 and 40.4 g DM, respectively. These estimates seem fairly reliable compared with the estimated removal of herbage in our study, using a method where herbage was harvested prior to introduction and after termination in some small plots inside each subplot. Thus, we found removal of grass/clover to be 9-31 g DM per hen per day, whereas removal of chicory was estimated to 73 and 51 g DM per hen per day for wheat-fed and concentrate-fed hens, respectively. The removal of mixed forbs could not be estimated using this method (Horsted et al., 2006). Using the above equation is approximately the same as multiplying plant material in the crop by seven, which seems very high compared to the factors mentioned above for wheat, concentrate and oyster shells. Especially when taken into consideration the slower passage rate for coarse feed from the crop to the proventriculus and the gizzard as indicated by Heuser (1945). This could indicate an overestimation of herbage intake, even though our own estimation of removal of herbage supports the levels estimated. Another explanation might be that hens spread the forage consumption over a longer time span compared to the intake of wheat, concentrate and oyster shells. If so there might be some difference between hens fed different types of supplementary feed, since we observed a higher foraging activity among wheat-fed hens than concentrate-fed hens, though this was not systematically recorded.

In both experiments the type of supplementary feed significantly affected the amount of seeds in the crops, since concentrate-fed hens had a higher intake of seeds than the wheat-fed hens. For hens fed wheat, these weed seeds presumably were of little nutritional value, why priority was given to other feed items, whereas concentrate-fed hens most likely were meeting their behavioural need for foraging. Hens with access to chicory had more seeds in the crops than hens with access to grass/clover ($P < 0.05$). This was presumably due to more weeds being present in the chicory plots, since this variety of chicory (Grassland Puna) only produces seeds the second year after sowing.

The crop content of most feed items, including the plant material, was lower in experiment 1 than in experiment 2. Since the filling of the crop in the evening is related to the time remaining before the night rest, this might be due to the shortening day length. Thus, experiment 1 went on during mid-summer, whereas experiment 2 went on late summer, meaning that day length was shorter in experiment 2. However, the evening slaughtering took place between 8 and 9 pm in both experiments, resulting in a shorter time span between slaughtering and sunset in experiment 2.

An older study, based on the botanical composition of the sward, showed that Rhode Island Red layers supplemented a low protein ration by consuming clover and dandelions instead of perennial rye-grass (Thomas, 1937). However, in our study the plant material in the crops from the wheat-fed hens consisted of a mixture of leaves, stems and roots, while in the crops from the concentrate-fed hens the majority consisted of leaves. Even though Fisher and Weiss (1956) demonstrated that bulk stimulates a greater feed consumption, it seems surprisingly that the wheat-fed hens in our study consumed a more fibre-rich diet compared with the concentrate-fed hens, since whole wheat has a larger particle size than pelleted concentrate. However, this might be due to the nutrient-restricted hens searching and scratching the ground to find earthworms and insects, which are rich in amino acids (Sugimura et al., 1984; Pokarzhevskii et al., 1997). Thereby the roots are laid bare, and to some extent these have been edible to the hens. Moreover, roots, microorganisms and even soils have been shown to contain various amounts of amino acids (Pokarzhevskii et al., 1997). This, together with the intake of earthworms, could explain the huge amount of soil found in the crops from the wheat-fed hens. However, in experiment 2 the intake of soil was primarily seen at the end of the experiment (Figure 4), whereas, in experiment 1, large amounts of soil were found in the crops from hens on the mixed forbs on both days of slaughter. This was probably due to the mixed forbs being more vulnerable to the scavenging of the hens. Thus, the plots with wheat/mixed forbs rapidly showed signs of the foraging activity after hens were introduced, and at the end of the experiment hardly any vegetation remained in these plots. In the other foraging plots, the vegetation was more resistant to the scavenging of the hens, though worn down at the end of the experiments in the wheat plots. This partly explains the large amount of soil in the crops on the second day of slaughter in experiment 2, even though vegetation was still abundant in especially the chicory plots. The decrease in plant material in the crops on the second day of slaughter in experiment 2 (Figure 2) is presumably, not a reflection of this, since a decline in plant material was found in crops from concentrate-fed hens too. It seems more plausible that wet weather produced a larger number of earthworms in surface of the ground (Kaplan et al., 1980), which the hens preferred, as illustrated in Figure 3. A wet and loose soil surface might even explain the wheat-fed hens eating more soil too. The large amounts of soil found in the evening-crops apparently disappeared from the crops during the night, since only relatively small amounts of soil were found in the morning-crops (Figure 4).

Surprisingly, no differences were found in the amount of earthworms and larvae in relation to supplementary feed. The amount of insects was only significantly different between types of supplementary feed for hens slaughtered in the morning in experiment 1, since more insects were found in the crops from wheat-fed hens. Because of the protein deficit in the wheat-fed hens, it was expected that more insects and earthworms would be found in the crops from these hens. However, since the first day of slaughter took place 9-10 days after introduction, it seems plausible that foraging areas had been more or less emptied of these feed items by the hens.

In accordance with Tyler (1955) grit stone was significantly more abundant in the evening-crops. Moreover, we found significantly more grit stone in the crops from the wheat-fed hens in experiment 2 and a tendency in experiment 1. This may reflect the large amount of coarse feed in the crops from these hens, since more grit stone in the gizzard would be expected to grind the feed into smaller particles.

In conclusion, a huge difference in crop content between morning and evening-crops was found, indicating that crops from evening-slaughtered hens give a better estimate of daily feed intake, irrespective of feeding strategy. However, crop content at other times of day were not examined in the present study. The results further indicate that type of forage vegetation used in the present studies only to a minor degree influenced the balance between feed items in the crops. In contrast, the type of supplementary feed clearly affected the intake of several feed items, suggesting that different feeding strategies can be used as a method of increasing foraging from the outdoor area. Still, more knowledge about different levels of nutrient restriction in relation to different types of forage vegetation is needed. Number of days in the experiments before slaughtering had an influence on crop content of different feed items. This could be due to increased capacity of the digestive tract, changing nutritional value of the foraging area, behavioural changes and different weather conditions.

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4

Organic layers' foraging in a sequence of different crops in relation to different supplementary feeds

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Running title: Increased foraging in organic layers

Title: Organic layers' foraging in a sequence of different crops in relation to different supplementary feeds

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Abstract

In many cases health and welfare problems are observed in organic egg production systems, as is also high environmental risks related to nutrient leaching. These disadvantages might be reduced if the layers to a higher degree are able to utilize their ability to forage and 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 merit layers to take advantages of the foraging in covering their nutritional needs, which was the aim of the present work. Six flocks of each 26 hens and one cock, were moved regularly in a rotation between different forage plants for a period of 130 days. Half of the flocks were fed typical concentrate for organic layers and half were fed whole wheat. The forage vegetation consisted of grass/clover, Pea/vetch/oats, lupine and quinoa. At the beginning of the experiment, wheat-fed hens had a lower intake of supplementary feed and a lower laying rate, egg weight and bodyweight. However, after a period at 6–7 weeks, the intake of wheat increased to approximately 100 g per hen per day and the laying rate increased to same level as for the concentrate-fed hens. Egg weight and bodyweight increased during the remaining part of the experiment for both groups of hens. Crop analysis revealed different food preferences for concentrate and wheat-fed hens, respectively. Thus, wheat-fed hens only to a minor degree ate the cultivated seeds, but prioritised earthworms and larvae even though the amount of this feed item seemed to decrease after a few days in a given forage vegetation. The amounts of plant material, oyster shells, insoluble grit stone, soil and to some extent earthworms and insects were larger in the crops from wheat-fed hens, indicating that the hens had an excellent ability to compose a feed ration. Larger gizzard sizes in the wheat-fed hens and an increased content of supplementary feed in the crop as the experiment progressed suggest an adaptation of the digestive organs to coarse feed. Floor eggs were significantly more frequent in the concentrate-fed hens, whereas wheat-fed hens only rarely laid floor eggs. Irrespective of treatment, hens were found to have excellent welfare. We conclude that nutrient-restricted, high-producing organic layers are capable of finding and utilizing considerable amounts of different feed items from a cultivated foraging area without negative effects on the welfare.

Key Words

Crop content, egg production, feed intake, free-range poultry, welfare

Introduction

The potential contribution of vegetation and earthworms, insects, etc. from the outdoor area has to a great extent been overlooked in organic egg production systems, presumably because the production systems of today do not support a proper utilization of the outdoor areas by poultry. Typically, an organic egg production system is characterized by having a high animal density in the henhouse and immediately outside the house, since the hens are not motivated to use the outdoor area (Keeling et al.,1988; Hegelund et al., 2005). This involves a considerable risk of welfare problems (Bestman & Wagenaar, 2003), leaching of nutrients to the ground water and parasitic infections (Permin et al., 1998; 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. This feed is traditionally fed inside the henhouse, leading to the hens being less motivated to leave the house or more motivated to return to the house when they are outside (Bubier & Bradshaw, 1998).

A higher degree of utilization of local resources, in the shape of cultivated forage vegetation in the hen yard, could increase the utilization of the hen yard and thus the welfare of the hens. If hens additionally are capable of finding and utilizing 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 & Gordon, 2003), so even a small reduction in feed costs will be noticeable. Older studies have shown that access to forage vegetation reduces the intake of 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).

Newer studies of the nutritional effect of feed intake from pasture on hens are scarce. However, studies by Gustafson & 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).

We investigated the short-term effect (three weeks) of different forage crops (grass/clover, chicory and a mixture of forbs) and two types of supplementary feed (commercial layer feed vs. whole wheat and oyster shells) on productivity in organic layers (Horsted et al., 2006). The results indicate that the foraging areas contribute to the nutrition of the hens, even though hens fed whole wheat showed a declining egg production during the three weeks. However, hens with access to chicory showed a lower egg weight only compared with concentrate-fed hens, 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 metabolizable energy. 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.

The aim of present study was to evaluate how feed intake, egg production and welfare develop in a system where hens are moved in a rotation between different forage crops and when hens are restricted or unrestricted in nutrient supply.

Materials and methods

Experimental design

Six flocks of each 26 hens and one cock were moved regularly in a rotation between different forage plants. Half of the flocks were fed typical concentrate for organic layers and the other half was fed whole wheat. Three plots of each type of forage plants were alternately distributed on the experimental field with four metres between plots to minimize any inter-plot effects. Each plot measured 420 m^2 and was subdivided into two subplots according to feed type, resulting in subplots of 210 m^2 (12 x 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).

Forage vegetation

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

Table 1. *Periods at different forage plots, and times of slaughtering of hens for crop and gizzard analyses*

Period no.	Period	Number of days	Forage vegetation	Slaughtering of hens (2 per subplot)		
				Date	Time of day	No. 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	Evening	12
3	01.08.05 - 15.08.05	14	Grass/clover	05.08.05	Evening	12
4	15.08.05 - 05.09.05	21	Lupin	18.08.05	Evening	12
5	05.09.05 - 23.09.05	18	Grass/clover			
6	23.09.05 - 27.10.05	34	Quinoa	04.10.05	Evening	12
7	27.10.05 - 31.10.05	4	Grass/clover	28.10.05	Morning, noon, afternoon and evening	4 x 12

Strain and 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 three 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 concentrate diet 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.

Table 2. *Nutrient content of concentrate, wheat and quinoa seed (wet weight)*

	Concentrate	Wheat	Quinoa seed
Crude protein (n x 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
ME MJ/kg	11.0	12.4	13.2
Lysine (g/kg)	8.8	2.6	8.3
Methionine (g/kg)	3.3	1.4	2.6
Cystine (g/kg)	3.0	2.4	2.6
Threonine (g/kg)	6.9	2.8	4.8
Calcium %	3.4	< 0.1	0.1
Phosphorus %	0.7	0.4	0.6

Housing and feeding of experimental birds

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

Recordings

Dry matter (DM), nutrient content and herbage mass per hectare were determined for the forage plants shown in Table 3 prior to introduction of the hens. Herbage was harvested in two randomly chosen patches (0.25 m^2) in each subplot and cut approximately two 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. The harvested biomass was weighed for each square and a representative sample from each plot taken and stored at –20^{o}C until analysis. All samples were analysed for DM and crude ash, and a representative sample from each type of forage vegetation was taken for crude protein, crude fat, starch and sugar determinations. Crude protein was determined by the Kjeldahl method (AOAC, 1990a) and ash according to method 923.03 (AOAC, 1990b). Crude fat was extracted with diethyl ether after acid-hydrolysis (Stoldt, 1952) and the sugars were extracted with 50 % ethanol at 60 ^{o}C and quantified by gas-liquid chromatography (Bach-Knudsen & Li, 1991). Starch was analyzed by the enzymatic-colorimetric method (Bach-Knudsen, 1997).

Tabel 3. *Dry matter (DM) content, nutrient content and herbage mass per hectare of the forage vegetations prior to introduction of the hens, means (SD)*

Period no.	Forage vegetation	DM and nutrient content (% in DM)						DM per hectare (kg)
		DM (%)	Crude ash	Crude protein[1]	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)

[1] N x 6.25

All hens were weighed and an assessment of clinical indicators of welfare was made on the day hens were introduced to the experiment and each time hens were moved between plots, except when hens were moved from the quinoa plots to the grass/clover plots. Instead, this 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 hens continued in these plots for a further nine days. The welfare assessment included an evaluation of plumage condition, foot health, keel bone, pubic bone and colour and wounds of the comb using the standardized methods described by Tauson et al. (1984) and Gunnarson et al. (1995).

Intake of concentrate or wheat was measured Monday and Thursday morning each week. Intake of oyster shells was made up each time hens were moved from a forage vegetation plot to another. Eggs were collected daily in the afternoon and the number of floor eggs recorded. 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). At the end of the experiment, hens were moved from the quinoa plots to the grass/clover plots in the evening. On the following day hens were slaughtered at four different times of the day to estimate the effect of supplementary feed on crop content during the day shortly after being introduced to fresh grass/clover pasture. Immediately after slaughter the hens were cut up and the crop removed and stored in a freezer at -18^{o}C for later analysis.

Analysis of crop and gizzard content

The individual crop was thawed and the contents separated by forceps into the fractions: “supplementary feed (concentrate 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 ºC for approximately 24 hours or until the samples were completely dry. Since DM determinations of crop contents in the study by Antell and Ciszuk (2006) did not reveal a wider range than 94-97% compared with air-dried crop contents, calculation on crop contents in this study is based on air-dried fractions of crop contents.

The gizzards were cut up and their contents removed, after which the gizzards were weighed fresh. Grit stone (>2 mm) was separated from the gizzard contents and weighed on an air-dried basis. The remains were weighed fresh as well as on an air-dried basis and the dry matter percentage determined.

Statistical methods

All data were subject to analysis of variance using the MIXED procedure in SAS (SAS Institute Inc., 1990). Intake of supplementary feed and egg production parameters were based on the following model 1:

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 = 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. The last recordings on laying rate, floor egg rate and intake of concentrate and wheat were considered as outliers and omitted from the statistical analysis. This was primarily due to 48 hens being slaughtered a few days before terminating the experiment, making the last recordings on these parameters uncertain.

The statistical analysis of body weight and welfare parameters was based on model 2:

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 and score of welfare parameters; μ = mean; α_i = supplementary feed $(i = 1,2)$; β_j = periods $(j = 1\text{-}8)$; $(\alpha\beta)_{ij}$ = 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 and gizzard content and the weight of the gizzard were analyzed statistically by the above model 1, where Y_{ijkl} = quantity of each fraction in the crop and gizzard of the individual hen, and the weight of the gizzards; μ = mean; α_i = supplementary feed $(i = 1,2)$; β_j = day of slaughter or time of slaughter $(j = 1\text{-}4)$; $(\alpha\beta)_{ij}$ = interaction supplementary feed × day of slaughter or time of slaughter; $A_{k(i)}$ = random effect of replication; ε_{ijkl} = error. In all cases, residuals were approximately normally distributed.

Results

Overall results on productivity and the welfare assessment, together with the level of significance of variables influencing these scores, are shown in Table 4. A significant interaction between feed type and day of recording of feed intake 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 no significant interaction with oyster shells, although there was a tendency for wheat-fed hens to have a higher intake (Table 4). There was moreover a tendency for the intake of oyster shells to change over time, with the lowest intake at the beginning of the experiment (data not shown).

All egg production parameters as well as bodyweight and measurements on pubic bone were significantly influenced by the interaction between feed and period as illustrated in Figure 1 and Figure 2. The differences in laying rate between hens fed concentrate 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.

Table 4. *Egg production, intake of supplementary feed and clinical welfare characteristics; average of the entire experimental period (LS-means and SE) and level of significance for the effect of feed and period*

	Unit	Supplementary feed: Concentrate	Supplementary feed: Wheat	SE	Significance (P): Feed type	Significance (P): Week/ period	Significance (P): Feed type x 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	%	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
Bodyweight	g	2032	1737	14	< 0.001	<0.001	< 0.001
Pubic Bone	score 1-3	2.7	2.3	0.03	< 0.001	<0.001	< 0.001
Plumage condition							
Neck	score 1-4	4.00	4.00	0	ns	ns	ns
Thorax	score 1-4	3.60	3.52	0.19	= 0.06	< 0.001	< 0.001
Back	score 1-4	4.00	4.00	0	ns	ns	ns
Wings	score 1-4	4.00	4.00	0	ns	ns	ns
Tail	score 1-4	4.00	4.00	0	ns	ns	ns
Foot health							
Boils	score 1-4	4.00	4.00	0	ns	ns	ns
Wounds	score 1-3	3.00	2.99	0	ns	ns	ns
Keel bone	score 1-4	3.96	3.98	0.04	ns	ns	ns
Comb							
Colour	score 1-3	2.96	2.99	0.01	ns	< 0.001	< 0.001
Wounds	score 1-3	2.97	2.94	0.03	ns	< 0.001	< 0.001

[+] Statistical analysis of concentrate and wheat is based on measurements of intake twice each week

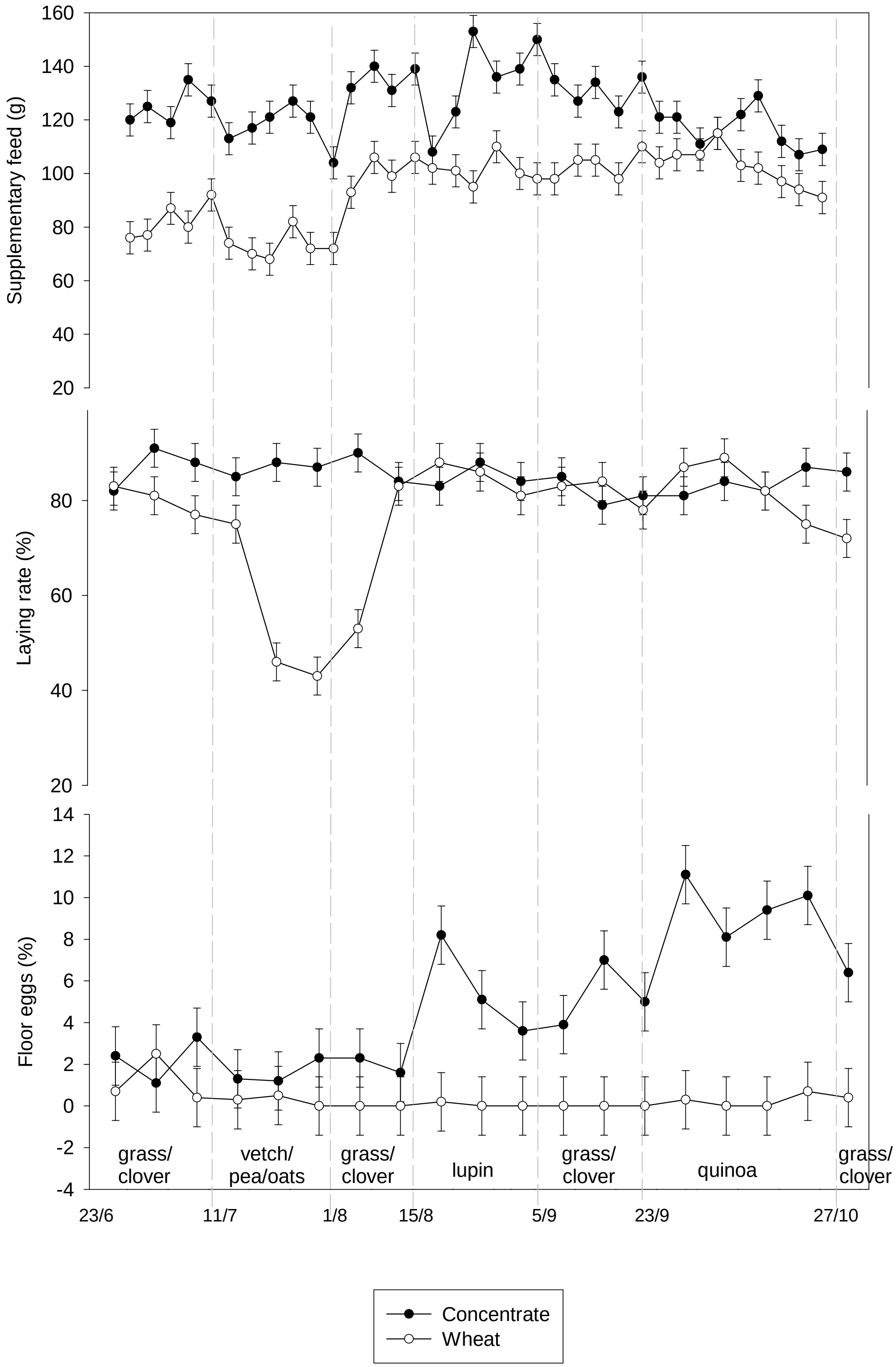

Figure 1. *Intake of supplementary feed (g/hen/day), laying rate (%) and floor eggs (%), respectively, LS-means and SE*

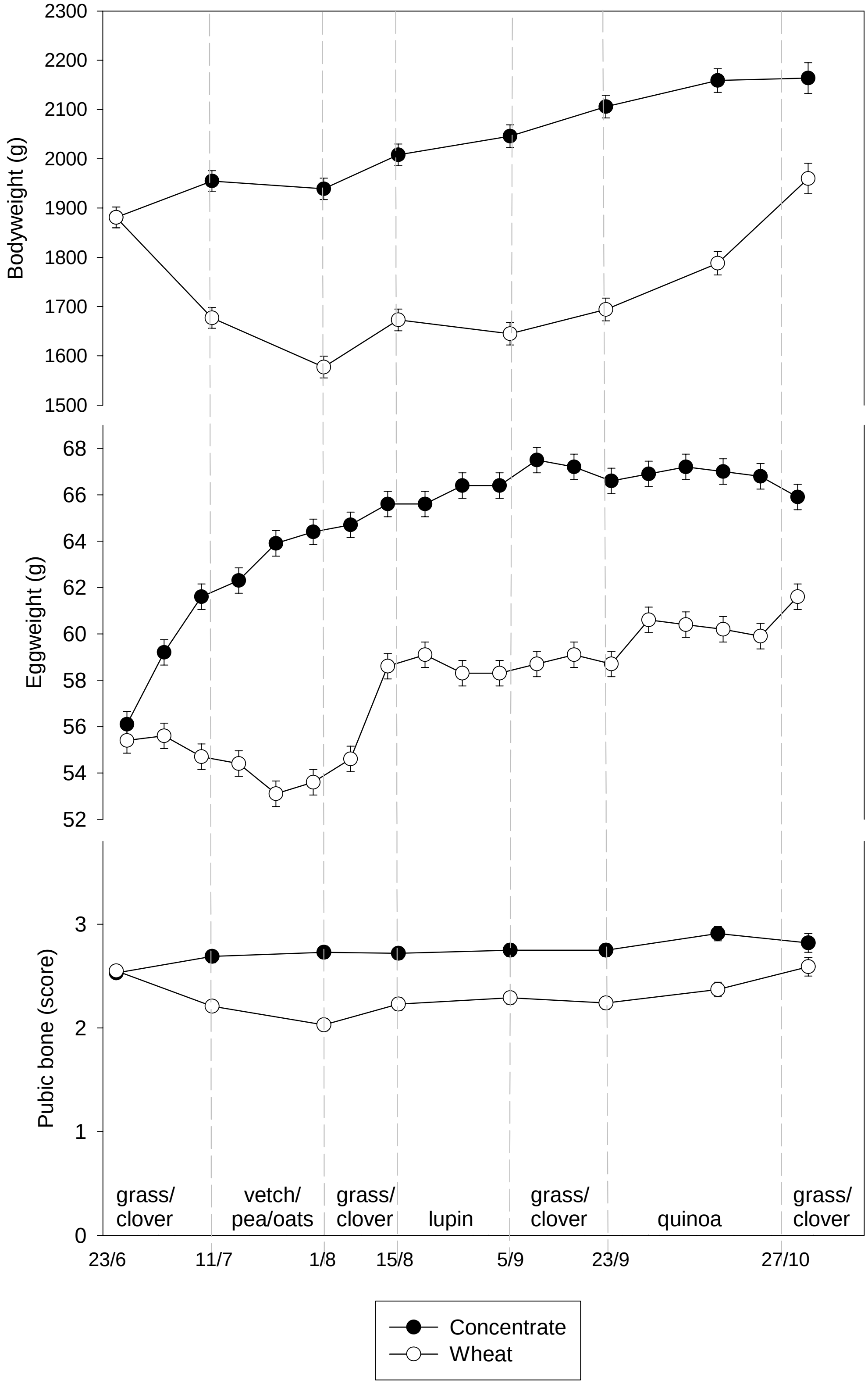

Figure 2. *Body weight (g/hen), egg weight (g/egg) and pubic bone (score), respectively, LS-means and SE*

During the whole experiment, egg weights for the wheat-fed hens were lower than for the concentrate-fed hens, 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 stabilized at approx. 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, with the lowest average body weights when hens were moved from the pea/vetch/oats plots to the grass/clover plots. As was the case with laying rate and egg weight, body weight increased for the wheat-fed hens after this decline, indicating that hens at this stage were capable of gaining body weight along with the increase in egg weight and laying rate. During the remaining part of the experiment, average body weights increased slowly with wheat-fed hens reaching just less than two kilos at the end of the experiment. At the end of the experiment, the weather became colder and a few nights had temperatures below 0^{o} C. Therefore, wheat-fed hens presumably prioritised weight gain at the expense of egg-laying.

All hens received maximum scores for plumage condition for four out of five body parts, irrespective of supplementary feed type and period. Only plumage condition on the thorax was significantly influenced by the period and the interaction “supplementary feed × periods”. Wheat-fed hens had a tendency towards a slightly lower score (Figure 3). Maximum scores were even found for foot health, whereas colour and wounds of the comb were significantly influenced by the period and the interaction “supplementary feed × periods”. For the characteristic “colour of the comb”, this was primarily due to a slightly lower score at the beginning of the experiment, whereas no distinct pattern was seen in relation to the characteristic “wounds to the comb” (data not shown).

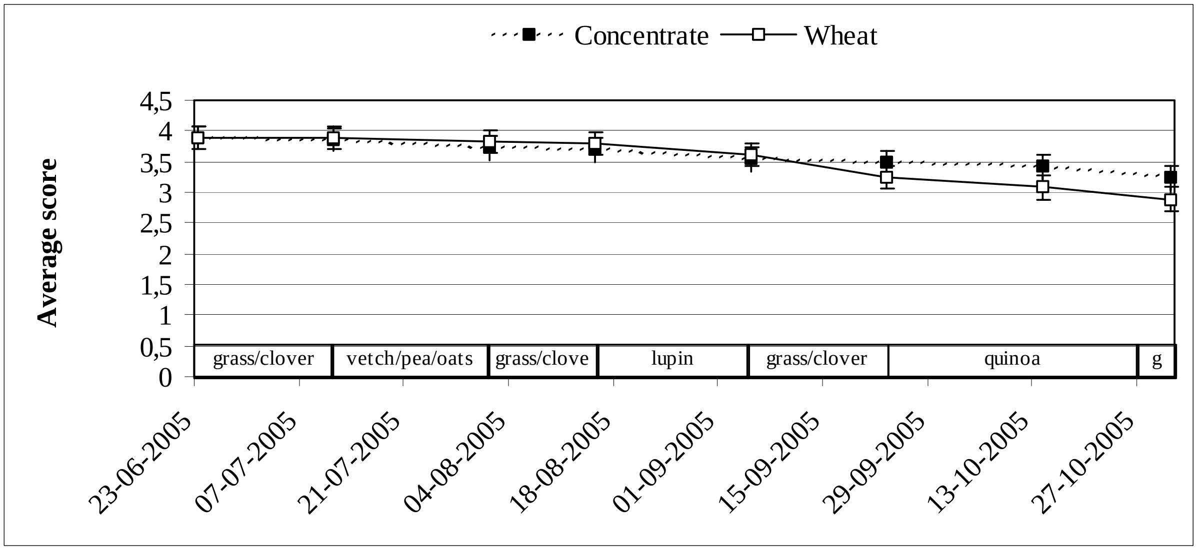

Figure 3. *Plumage condition on the thorax, LS-means and SE*

Table 5 shows the content of different feed items in the crop at four days of slaughter together with the level of significance in relation to supplementary feed, day of slaughter and the interaction. Only one significant interaction was found, with the amount of supplementary feed in the crop interacting with day of slaughter. Thus, the amount of concentrate gradually increased with the number of days in the experiment, whereas wheat-fed hens had more wheat in the crops at the second day of slaughter (grass/clover) compared with the third day of slaughter (lupin).

Wheat-fed hens had significantly more wheat in the crop than concentrate-fed hens had concentrate in the crop. 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 concentrate-fed hens.

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, SE and significance (P) of effect of supplementary feed and day of slaughter*

Supplementary feed:		**Concentrate**				**Wheat**					**Significance (P)**		
Feed item:	**Unit**	**Pea/ vetch/ oats**	**Grass/ clover**	**Lupin**	**Quinoa**	**Pea/ vetch/ oats**	**Grass/ clover**	**Lupin**	**Quinoa**	**SE**	**Supp. feed**	**Day of slaughter**	**Supp. feed x day of slaug.**
Supplementary feed	g	8.0	9.6	9.9	12.7	11.1	19.6	17.7	32.4	2.4	< 0.001	< 0.001	< 0.05
Plant material	g	1.5	2.2	1.6	3.2	2.5	3.5	4.1	4.1	0.9	< 0.05	ns	ns
Seeds of cultivated plants	mg	1140	0	1705	3993	70	0	8	1348	577	< 0.01	< 0.01	ns
Weed seeds	mg	7	15	88	40	7	28	65	25	27	ns	ns	ns
Insects	mg	0	3	585	198	30	12	595	195	155	ns	< 0.01	ns
Earthworms etc.	mg	0	798	62	135	5	368	52	532	153	ns	< 0.01	ns
Oyster shells	mg	305	818	643	1318	2088	4417	3462	3470	1060	< 0.01	ns	ns
Grit	mg	118	333	1358	382	1628	2302	1383	3205	706	< 0.01	ns	ns
Soil	mg	0	0	0	0	16747	30025	13445	8105	3946	< 0.001	ns	ns

The day of slaughter significantly influenced the overall amount of supplementary feed, since the amount of feed in the crops increased with the number of days hens spent in the experiment, except for the above interaction. The day of slaughter 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 when hens were foraging the lupin and quinoa plots. Finally, day of slaughter significantly influenced the amount of earthworms, larvae and pupae in the crops, and were particularly abundant in hens foraging grass/clover or the quinoa.

Table 6 shows crop content and level of significance of different feed items at four different times of slaughter during the day on the first day after they have been moved to the grass/clover plots at the end of the experiment. Wheat-fed hens had significantly more wheat in the crop than concentrate-fed hens had concentrate in the crop. Moreover, the amount of supplementary feed was significantly affected by the time of slaughter, since crops contained more in the evening than in the morning for both types of supplementary feed. A tendency for more plant material in the crops from the wheat-fed hens was noted, just as plant material was more abundant in the crop at the end of the day than in the morning. Hens fed wheat had significantly more insects, earthworms, larvae and pupae, oyster shells and soil in the crops. A tendency towards more insects in the crops during the day was seen, just as the amount of oyster shells significantly increased during the day.

The types of supplementary feed significantly influenced wet weight, air-dry weight and air-dry matter percentage of gizzard content without grit, since these were higher for wheat-fed hens (Table 7). Wet weight and air-dry weight of the gizzard contents were lowest in the morning, indicated by the significant effect of time of slaughter. The amount of grit stone in the gizzard did not differ significantly for any of the treatments, but the emptied gizzards were found to be significantly heavier for hens on the wheat diet (Figure 4).

Table 6. *Amount of feed items (air-dry weight) in the crops from hens slaughtered at four different times of day, on the first day after they have been moved to the grass/clover plots at the end of the experiment, LS-means, SE and significance (P) of effects of supplementary feed and time of slaughter*

Supplementary feed:		**Concentrate**				**Wheat**					**Significance (P)**		
Feed item:	**Unit**	**Mor-ning**	**noon**	**after-noon**	**eve-ning**	**Mor-ning**	**noon**	**after-noon**	**eve-ning**	**SE**	**Supp. feed**	**Time of slaughter**	**Supp. feed x time of slaug.**
Supplementary feed	g	6.4	16.7	16.0	31.5	18.0	25.9	24.4	35.5	4.2	< 0.05	< 0.01	ns
Plant material	g	1.7	2.2	2.1	6.1	2.9	3.3	5.8	6.0	1.0	= 0.06	< 0.01	ns
Weed seeds	mg	2	2	10	3	32	10	12	17	11	ns	ns	ns
Insects	mg	0	3	3	22	25	98	250	335	60	< 0.01	= 0.06	ns
Earthworms etc.	mg	593	370	343	2968	9433	2910	2658	3315	1692	< 0.01	ns	ns
Oyster shells	mg	12	5	365	997	732	1113	1267	4423	554	< 0.01	< 0.01	ns
Grit	mg	92	7	17	95	147	242	1143	418	373	ns	ns	ns
Soil	mg	655	0	0	7	6777	4940	6558	1845	1690	< 0.001	ns	ns

Table 7. *Wet weight, air-dry weight and air-DM percentage of gizzard content excl. insoluble grit, and air-dry weight of insoluble grit, LS-means, SE and significance (P) of effect of supplementary feed and time of slaughter*

Supplementary feed:	**Concentrate**				**Wheat**					**Significance (P)**		
Gizzard content:	**Mor-ning**	**noon**	**after-noon**	**eve-ning**	**Mor-ning**	**noon**	**after-noon**	**eve-ning**	**SE**	**Supp. feed**	**Time of slaughter**	**Supp. feed x time of slaug.**
Content excl. grit												
Wet weight	16.2	16.6	19.2	21.3	18.9	27.7	24.0	26.6	1.7	< 0.001	< 0.05	ns
Dry weight	7.1	6.9	7.9	10.0	11.7	17.3	15.0	16.5	1.2	< 0.001	< 0.05	ns
DM percentage	43.8	41.5	41.4	46.8	62.4	62.0	62.1	61.8	2.2	< 0.001	ns	ns
Insoluble grit	13.4	10.3	12.1	11.5	13.1	13.2	13.7	13.4	1.5	ns	ns	ns

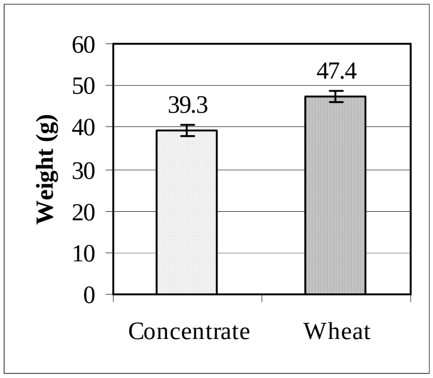

Figure 4. *Fresh-weight of the emptied gizzards at the end of the experiment; LS-means and SE*

Discussion

Feed intake

Average daily consumption of concentrate was 125 g per hen per day (Table 4), which is within the estimated range (125-135 g feed per hen per day) for the strain 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 three weeks (Horsted et al., 2006). However, during the first two periods of the present experiment (grass/clover and pea/vetch/oats), the average intake was only about 80 g per hen per day, whereas six to seven weeks after introduction to the experiment, there was a distinct increase in the intake of this feed to approximately 100 g wheat per hen per day. This level of intake continued throughout the remaining part of the experiment, with minor fluctuations.

Al Bustany & Elwinger (1988) found a higher food conversion ratio in hens fed whole barley or wheat than ground barley and wheat. This might indicate that hens in our study stopped eating as a result of an imbalance in nutrient supply (overdose of ME and shortage of amino acids). However, it can be hypothesized that hens were not able to eat sufficient forage material and whole wheat at the beginning of the experiment, whereas the digestive organs adapted to coarser feed during the experiment. 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 utilization. Moreover, it has been shown that chicks trained to eat rapidly had larger amounts of feed in the crops than untrained chicks, just as crop weight were found to be heavier in trained chicks. This probably reflects the higher capacity of the crop in trained chicks (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 concentrate (Heuser, 1945), the contents of these feed items do not reflect the differences in feed intake as indicated by Table 4 and Figure 1. The different amounts of the other feed items in the crop presumably indicate differences in feed intake for hens on either the concentrate or the wheat diet. 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 microorganisms 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 concentrate-fed hens compared with wheat-fed hens, although peas, lupins and quinoa are rich in protein, which the wheat-fed hens were lacking. According to Forbes and Covasa (1995), growing and laying birds need a period of learning or adjustment before becoming proficient when given a choice of food. Moreover previous experience and social interactions influence how they adapt to choice feeding. Therefore it seems plausible that wheat-fed hens in our study needed more time to change their feeding strategy, which was directed towards the plant material, the soil and the soil fauna (Tables 5 and 6). This might explain the decline in laying rate at the end of the “quinoa period” in the wheat-fed hens, since green fodder, earthworms and insects might have been scarce at this stage. In contrast, the concentrate-fed hens found the quinoa seeds very attractive, since large amounts were found in the crops. Moreover, the intake of concentrate seemed relatively low when hens were foraging the quinoa plots. This also seemed to be the case when these hens were foraging the pea/vetch/oats plots and partly the lupin plots. Visual assessment of the plots indicated that the lupin plots foraged by the concentrate-fed hens were practically without lupin seeds after few days, whereas plenty of lupin seeds were available in the lupin plots foraged by the wheat-fed hens. This could probably explain the increase in the intake of supplementary feed in the middle of the “lupin-period” in the concentrate-fed hens (Figure 1).

Table 6 indicates that especially the wheat-fed hens gave priority to feed items of animal origin, when they were moved to a new foraging area. The amount of earthworms in the crops was much higher immediately after hens had been moved to a new foraging area (Table 6) compared with crops from hens slaughtered after several days on a given foraging area (Table 5). This indicates that hens fed whole wheat and oyster shells as only supplementary feed prioritize earthworms. Moreover, this indicates that the amount of earthworms at the soil surface diminishes with the time spent foraging the area. This explains why there were no significant differences in earthworms and insects in the crops from wheat- and concentrate hens when these were slaughtered after several days in the foraging area. However, since we used the same grass/clover plots throughout the whole experiment, the results in Table 6 suggest that the amount of earthworms will recover if the foraging area is kept without hens for a few weeks. The large number of earthworms in the crops from wheat-fed hens in the morning further indicates that foraging activity at different times of day may vary between hens fed wheat or concentrate. Since hens have been found to be able to compound a feed ration (Kiiskinen, 1987; Ciszuk & Charpentier, 1996), it seems reasonable that hens, primarily restricted in amino acids, have a higher intake of especially insects, earthworms, larvae and pupae, since these have shown to be rich in amino acids (Sugimura et al., 1984; Pokarzhevskii et al., 1997).

In contrast to the amount of earthworms in the crop, the amounts of supplementary feed, plant material, oyster shells and insects were affected by the time of day the hens were slaughtered. Thus, the largest amount of these feed items was seen in the crops from hens slaughtered in the evening and the smallest amount in the morning (Table 6). However, wheat-fed hens had a larger amount of these feed items in the crops all four times of the day compared with concentrate-fed hens. Only the amount of plant material in the “evening crops” was similar for wheat and concentrate-fed hens. According to Mongin (1976), dry matter in the crops follows a cyclic variation with the largest amount in the crop in the evening. However, the results in our study further indicate that feed intake during the day may be influenced by the feed items available (Table 6).

Size and content of the gizzard

The content of insoluble grit stones in the gizzard was not affected by type of supplementary feed or the time of slaughtering (Table 7). Since grit stones are assumed to function as grinding material in the gizzard, the retention time in the gizzard may be several days or weeks (Robinson, 1961), indicating that a larger amount of grit stone would have been expected in the gizzards from the wheat-fed hens, because of a higher intake (Table 5). However, any surplus grit stones will pass out of the gizzard (Robinson, 1961). Thus, Tyler (1955) found most of the insoluble grit stones in the crop, the gizzard and the third segment of the small intestine, while very little was found in the first two segments of the intestine. According to the author, this may indicate a rhythmical output of flint grit from the gizzard.

While the amount of grit stone in the gizzard did not differ in relation to type of supplementary feed and time of slaughter, the weight of the remaining material was significantly affected by these variables. Thus, the wet weight as well as the air-dry weight of the content excl. grit stones was higher in the gizzards from the wheat-fed hens (Table 7). Also the time of slaughter affected the amount of feed in the gizzard with less found in the morning, which was particularly pronounced in the gizzards from the wheat-fed hens. Otherwise no distinct pattern was discernible. The air-dry matter percentage was significantly higher in the gizzard content from the wheat-fed hens, presumably reflecting the differences in feed composition. Thus, gizzard content from the concentrate-fed hens primarily consisted of fibrous material, whereas gizzard content from the wheat-fed hens generally consisted of fibrous material together with soil and sand. The air-dry matter percentage was not affected by time of slaughter, indicating that easily digested feed items pass through the gizzard to the small intestine more rapidly than coarse feed, whereas the latter is accumulated in the crop and the gizzard. The capacity of the gizzard presumably reflects the amount of coarse feed in the crop and the time this is retained in the crop. According to Heuser (1945), there is food in the gizzard as long as there is food in the crop. However, the capacity of the gizzard seems to increase with increased amount of coarse feed since we found the gizzards from wheat-fed hens were larger than those from the concentrate-fed hens (Figure 4). This is in accordance with Hetland et al. (2003), who found larger gizzards in layers after the inclusion of whole wheat and wood shavings. Moreover, gizzard sizes have been found to be larger if poultry are fed whole wheat compared with pelleted feed (Forbes & Covasa, 1995; Hetland et al., 2003). Further, Starck (1999) showed that the gizzard was more than twice as large in adult Japanese quails fed a larger proportion of non-digestible fibres in the diet compared with a control group. The author further showed that the size response was reversible and a reduction in non-digestible fibres was followed by a decrease in gizzard size. Although wheat does not contain more fibre than the concentrate, the wheat-fed hens in our study apparently consumed a larger amount of fibrous plant materiel (Tables 5 and 6).

In our study, the concentrate-fed hens had heavier gizzards than hens without access to forage material (Figure 4). Thus, Steenfeldt et al. (2001) found average gizzard weights ranging from 16.3-33 g in 53-week-old ISA Brown hens, with the lowest weights in hens fed concentrate without access to roughage or with access to carrots. Together with the results in present study, this indicates that the gizzard increases its capacity for coarse feed over time, when continuous access to forage material is given.

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 concentrate-fed hens 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 metabolizable energy rather than protein in the period with the lowest laying rate. This seems paradoxical, since wheat contains more metabolizable energy and less protein than concentrate (Table 2). As pointed out above, 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. On the other hand, a higher amount of earthworms in the soil surface after hens were introduced to the new forage (Table 6) may have increased the amount of amino acids consumed by the hens, leading to a higher intake of wheat to balance the nutrient supply. However, the level of wheat consumed was maintained during the last part of the experiments, whereas the amount of earthworms apparently decreased after a few days.

When the laying rate for the wheat-fed hens reached the level of the concentrate-fed hens, this was maintained for the rest of the experiment, except for a small decline at the end of the study, probably reflecting the diminishing value of the quinoa plots at this stage. Thus, the plots with the wheat-fed hens were by and large without green fodder, just as the number of earthworms and insects most likely diminished during the time hens spent in the plots. This may lead to the assumption that hens at this stage were lacking protein and amino acids rather than metabolizable energy. We kept hens in the plots since a huge amount of quinoa seeds was still available. However, referring to the above discussion on crop content, wheat-fed hens did not seem to eat as many quinoa seeds as the concentrate-fed hens.

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 concentrate 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 hens had been introduced to the lupins, due to some of the concentrate-fed hens laying their eggs in the vegetation. However, in the subsequently 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 concentrate-fed hens 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.

Welfare assessments

Traditionally, deficiency in protein and some amino acids is considered to have a negative effect on plumage condition due to feather-pecking (Ambrosen & 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 (Table 4 and Figure 3). 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. There was a tendency for wheat-fed hens to have a slightly lower score, probably due to these hens 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 appears to be related to an organic diet low in protein and amino acids. Overall, 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 only very few had wounds or keel bone deviations.

Even though strains can be selected to produce well on a low protein and low energy diet without any signs on feather-pecking (Al Bustany and Elwinger, 1986), this was not the case for the strain used in the present study. The good welfare of hens in our study is more likely related to hens 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 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 & Huber-Eicher, 1998).

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 NRC (1994), the requirements for metabolizable energy were 1.42 and 1.58 MJ for wheat- and concentrate-fed hens, respectively. The ambient temperature was on average 12.6^{o}C for the period. Since particularly the wheat-fed hens showed a very high foraging activity irrespective of weather conditions, it is important to realize that the above formula does not include energy requirement for this kind of activity. In the period considered, the wheat and the concentrate 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 concentrate-fed hens, 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 44-52 g DM lupine biomass and 26-31 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 concentrate-fed hens 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 concentrate-fed hens received the required amounts of lysine and methionine in the concentrate 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 (Tables 5 and 6).

We conclude that high-producing layers have a huge capacity for finding and utilizing 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 for development of 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 concentrate-fed hens. Moreover, there was an increase in egg weight and body weight for both groups of hens during this period. The welfare was found to be good during the entire experiment and was not affected by supplementary feed and the 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 and gizzard analyses revealed different food preferences for concentrate and wheat-fed hens, respectively. Further, different genotypes, animal densities, foraging vegetations and housing systems need to be evaluated when developing new strategies for increased foraging in organic layers.

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