Protein Supply in Organic Broiler Production Using Fast-Growing Hybrids

Welfare and Performance Aspects

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Cover: Chickens on outdoor pasture during one of the outdoor experiments.
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Abstract

The broiler's requirements of sulphur amino acids (AA) are high; however, currently available organic protein feedstuffs generally contain only low levels. According to national organic standards, fast-growing broilers in organic production are allowed to be slaughtered at 70 d, provided that their average growth rate does not exceed 50 g/day. This means that a growth restriction is necessary and may be done by using a low crude protein (CP) diet. However, such organic diets imply a risk for low dietary AA levels, which may jeopardize poultry welfare.

The aim of this thesis is to evaluate the effect of organic, low CP/AA diets on bird welfare and to evaluate the use of hemp seed cake as a potential future protein feedstuff in organic diets.

Three experiments were conducted over production periods of 70 d both indoors and in organic outdoor productions. The experiments covered evaluations of behaviour, health, physiological indicators and production when feeding fast-growing broilers low CP and AA diets, also with the inclusion of hemp seed cake.

Birds fed low CP/AA diets had lower live weights, increased activity, decreased mortality, and fewer birds had to be culled due to leg problems compared to birds fed higher levels of CP/AA. However, observed behavioural differences indicated that birds fed the low CP/AA diet showed hunger and associated frustration. These birds used their outdoor pasture much more, and had a higher forage intake, indicating a search for other nutrients than the ones provided. Cannibalism occurred in birds fed the low CP/AA diets, which is a welfare risk with such diets. Even though mortality was lowered for these birds, overall it was high and culling due to leg problems occurred during the last three weeks.

The results indicate that low CP/AA diets used to maintain a maximum growth of 50 g/day are not optimal regarding bird welfare. The use of female broilers in organic production could be advantageous, to further reduce growth rates and culling due to leg problems. Another solution may be to shorten the rearing periods by approximately three weeks. The use of hybrids with lower growth rates may be an alternative in Swedish organic production and needs to be evaluated.

Keywords: Amino acids, fast-growing broilers, organic production, welfare, health, hemp seed cake, biochemical markers.

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List of Publications

This thesis is based on the following papers, referred to by Roman numerals in the text:

I Eriksson, M., Waldenstedt, L., Engström, B. and Elwinger, K. (2009). Protein supply in organic broiler diets. *Acta Agriculturae Scandinavica Section A- Animal Science* 59 (4), 211-219.

II Eriksson, M., Waldenstedt, L., Elwinger, K., Engström, B. and Fossum, O. (2010). Behaviour, production and health of organically reared fast-growing broilers fed low crude protein diets including different amino acid contents at start. *Acta Agriculturae Scandinavica Section A - Animal Science* 60 (2), 112-124.

III Eriksson, M. and Wall, H. (2010). Hemp seed cake in organic broiler diets (submitted).

IV Eriksson, M., Waldenstedt, L., Larsson, A., Konstenius, N. and Tauson, R. Effects of amino acid content in organic broiler diets on biochemical markers in female and male chickens (manuscript).

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Abbreviations

AA	Amino acids
ALP	Alkaline phosphatase
C	Control diet
CP	Crude protein
C. perfringens	*Clostridium perfringens*
d	Day
E. coli	*Escherichia coli*
EU	European Union
FCR	Feed conversion ratio
H	Hemp diet
H/L	Heterophil/lymphocyte
HP	High protein diet
IFOAM	The International Federation of Organic Agriculture Movements
LP	Low protein diet
LPA	Low protein + pure AA diet

Introduction

Organic production in Sweden

Organic poultry production has increased rapidly in recent years due to consumer demand and political goals (Blair, 2008), but during 2009 trends towards a decrease were seen (KRAV, 2010a). The Swedish government set a goal for 2010, in which organic broiler production should be at least 1% of the total broiler production (Report from the Swedish Government, 2005/06:88). Today, organic broiler production in Sweden is less than 0.1% of the total broiler production. Even though the goal has not been reached, organic broiler production in Sweden is increasing. Compared with 2008, it has increased by 44% in 2009 and consists of 179 000 chickens on a yearly basis (KRAV, 2010a).

Organic production is regulated and guided by general principles, recommendations and basic standards (IFOAM, 2005). IFOAM is an umbrella organization that designs international standards for organic production and sets the accreditation criteria for the certifying associations in different countries (IFOAM, 2005). KRAV is the main accreditation organization that sets the standards for organic products in Sweden. KRAV follows basic organic standards and accreditation criteria set by IFOAM and regulations set by the European Council (EC, 2008; EC, 2007). In addition, KRAV has set down its own rules in excess of the Swedish Animal Welfare Ordinance, which is the basis for all production in Sweden (KRAV, 2010b). The main organic standards according to KRAV are summarized in Table 1.

Table 1. *A summary of KRAV directives regarding housing, management and feeding*

Issue	KRAV directive
Housing and management	
Stocking density	Maximum of 20 kg/m^2 indoors with:
	Maximum of 10 $animals/m^2$ indoors in a stationary building
	Maximum of 16 $animals/m^2$ indoors in a mobile chicken house
Production period	Purchased conventional slow-growing broilers should be at least 10 weeks of age at slaughter[1]
	For slow-growing broilers, when the broiler parents have been organically reared, no minimum slaughter age is required.
	For broilers not originating from slow-growing strains, the minimum slaughter age is 81 d[2]
Outdoor access	Stocking density on outdoor pasture: 4 $broilers/m^2$ as available during rotation
	Outdoor access should provide pasture, as a food and occupation source for the birds
	Broilers are allowed to be kept without outdoor access up to 1 month
	Broilers should be kept with outdoor access during at least a third of their rearing period
Feeding	
Feed ingredients	No antibiotics, hormones or drugs given as a preventive measure.
	No animal by-products, except milk products and fish meal
	No grain by-products unless produced from certified organic crops
	No chemically extracted feeds, such as solvent-extracted protein meal from oilseeds
	No pure AA
	No genetically modified grain, grain by-products or organisms, either as feed ingredients, feed additives or in the processing of feed ingredients
Roughage	Free access to roughage should be available

[1]The definition of slow-growing broiler hybrids is not yet set by the Swedish Board of Agriculture. Until further notice, KRAV has defined a slow-growing broiler hybrid as a bird with an average growth rate not exceeding 50 g/day (KRAV, 2010b).

[2]Currently, in accordance with KRAV regulations, conventional hybrids are considered slow-growing, and allowed to be slaughtered at 70 days if average daily growth rate does not exceed 50 g/day.

Feeding broilers in organic production

Protein requirements and amino acid supply

Organic broiler production is facing several challenges regarding nutrition, associated with rules laid down by the European Commission and national directives. Dietary requirements of sulphur AA, i.e. methionine and cysteine, for broilers are higher than for any other meat-producing animal, due to their rapid growth, tissue deposition and their plumage being rich in these AA (Vieira *et al.*, 2004). The most important sulphur AA is methionine, which cannot be synthesized by the chickens and is generally the first limiting AA. The level of the first limiting AA in the diet normally determines the utilization of the other essential AA (Blair, 2008). The utilization is also influenced by interactions between AA, such as conversion of one AA into another. This interaction is seen between methionine and cysteine, which means that cysteine, if deficient in the diet, can be synthesized from methionine. However, on the contrary, cysteine cannot be synthesized into methionine and, therefore, methionine has to be added at a sufficient level in the diet (McDonald *et al.*, 1995).

Achieving the correct levels of AA in the broiler diet is commonly accomplished by using high-quality protein sources such as solvent-extracted protein-rich meals from oilseeds, e.g. soya protein meal, and thereafter balancing the AA levels in the diet with synthetic AA, i.e. lysine, methionine and threonine. In this way the diet can be optimized to include specific CP and AA levels. However, solvent-extracted meals and synthetic AA are not allowed in organic diets (KRAV, 2010b; EC, 2007). The AA compositions of most organically approved vegetable feedstuffs are characterized by low levels of sulphur AA. It is therefore difficult to balance and fulfil the sulphur AA patterns according to the requirements of the birds. According to the organic standards, 95% of the feedstuffs in the broiler diets must be organically approved from 2010; this criterion will increase to 100% in 2012 (KRAV, 2010b; EC, 2007). Thus, until then, the use of 5% non-organically approved protein feedstuffs is allowed. Therefore, fishmeal, maize gluten meal and potato protein, which have a higher content of sulphur-rich AA, can be included in organic broiler diets. However, in the future it is unclear whether these protein feedstuffs will be available as organically approved.

Demand for new protein feedstuffs

Today, the use of marine sources such as fish meal in organic broiler diets is common. However, the use of fish meal in feed for animals may be questioned, since overfishing of waters leads towards extinction of fish species. Recently, studies made by Jönsson (2009) showed that mussel meal is an excellent alternative protein source in poultry diets, comparable with fish meal. However, at this early stage it is difficult to foresee the future availability and costs of this protein source. Therefore, it is also important to find other alternative protein sources that can be used in organic broiler diets.

Hemp seed (*Cannabis sativa* L.) is one example of a home-grown protein source, well adapted for cultivation in northern climates due to its frost tolerance at all stages of growth, drought tolerance and an early maturing (Callaway, 2004). Hemp seed and its derived products are described as rich sources of protein and sulphur AA (Wang *et al.*, 2008; Tang *et al.*, 2006; Callaway, 2004), although the content of sulphur AA may not be high enough to fill the birds' requirements on its own. The cultivation of hemp seed has been restricted in Sweden since 1965. In 2003 industrial hemp with low levels (<0.2%) of the narcotic substance δ-9-tetrahydrocannabinol was once again permitted for cultivation in EU (EU, 2003). The hemp seed and hemp protein isolates contain greater amounts of methionine than the soy bean (Wang *et al.*, 2008; Tang *et al.*, 2006; Callaway, 2004) and have therefore been confirmed as an excellent source of nutrition when fed to laying hens (Silversides *et al.*, 2005) and pigeons (Hullar *et al.*, 1999). It is thus of interest to evaluate hemp seed cake in organic diets for broilers.

Fast-growing broilers in organic production

Import of poultry to Sweden is regulated by bird health controls; imported birds are kept in quarantine for 8 weeks and tested for different pathogens before they are released. Today, only parent stocks of commercial fast-growing hybrids are imported. Organic broiler production in Sweden is still small scale; the demands for new hybrids of slow-growing strains are therefore small, which so far has made import of such parent flocks unprofitable. Therefore, only conventional fast-growing broiler hybrids have been available for organic broiler production in Sweden.

The minimum slaughter age of fast-growing broiler chickens reared in organic production is 81 d in Sweden, as well as in the rest of EU (KRAV, 2010b; EC, 2007). The stipulated long rearing period in organic broiler production is a problem when using fast-growing broilers, since they are not tailored for such long rearing periods. A long rearing period may result in heavy birds, Bokkers & Koene (2003) showed that the major determinant for leg problems at 7 and 12 weeks of age is the weight of the birds. It has therefore been suggested that these birds are unsuitable for growth up to 81 d of age. Conversely, if a slow-growing broiler hybrid is used the minimum slaughter age is 70 d. Also, if the parent stocks (breeders) of slow-growing hybrids have been reared according to organic standards, no minimum age at slaughter is required for the slow-growing chickens, which enables the producers to slaughter these chickens at a lower age (EC, 2008). No such organic broiler breeder production is established in Sweden. However, the definition of a slow-growing broiler is not yet set by The Swedish Board of Agriculture. Until then KRAV has defined slow-growing broilers as birds with an average daily growth of 50 g/day (KRAV, 2010b), thus enabling fast-growing broiler hybrids to be slaughtered at 70 d. This involves a feed restriction, since these birds are tailored to be slaughtered at 5-8 weeks of age, with an approximate average daily growth of 61 g (ROSS, 2009).

Feed restrictions for fast-growing broilers

There are different strategies to consider when composing an organic diet. The dietary AA levels can be maximized according to organic feed legislations. However, this will lead to an excess of CP levels in the diet which is not an option when growth restrictions are required. Instead, a way of achieving growth restriction is to use a qualitative feed restriction, i.e. a less concentrated, low protein diet. Since organically approved feedstuffs rich in sulphur AA are limited, an organic diet with low protein levels also results in suboptimal AA levels. Using a less concentrated and low protein diet to a certain extent may not induce any problems. However, in order to be able to slaughter fast-growing broilers at 70 d, there is a risk that the organic standards require a too severe feed restriction and suboptimal protein intake which may jeopardize bird welfare and health. Moreover, the birds' needs for nutrients may change in an organic production with outdoor rearing due to factors such as environmental temperatures, disease challenges, changes in housing climate and pasture intake. Even though there are numerous studies on nutritional requirements of fast-growing broilers under conventional rearing conditions, there is little information on how these requirements are affected by different factors encountered in an organic rearing situation.

Welfare in organic production

Animal welfare may be assessed by a combination of indicators, such as health, physiological indicators, and behaviour.

Health

One important parameter to consider when evaluating bird health is mortality rates in the flocks. Long rearing periods, risk of predators, disease challenges, and environmental influences are factors possibly contributing to increased mortality in organic production. Opportunistic pathogens such as *E. coli* may contribute to increased bird mortality, depending on housing climate and management. Intestinal diseases such as coccidiosis and necrotic enteritis may form particular threats in organic production (Pedersen *et al.*, 2003). Coccidiosis is caused by *Eimeria* spp., a commonly found protozoan parasite (Collett, 2008). In conventional production coccidiostats are generally used to prevent coccidial infection. In addition, ionophore coccidiostats have an antibacterial effect on gram-positive bacteria, e.g. *C. perfringens* (Elwinger *et al.*, 1998). Intestinal colonization by *C. perfringens* may cause necrotic enteritis which can result in high mortality (Collett, 2008). Coccidiostats are forbidden in the organic production (EC, 2007), but vaccination against coccidiosis is allowed and available. However, anti-coccidial vaccination does not protect against *C. perfringens*. Hence, outdoor access and the prohibition against coccidiostats in feed for organic chickens increase the risk of bacterial challenges in organic production compared to conventional production.

When using fast-growing genotypes in the long organic rearing period, mortality may increase due to the higher culling rate of heavy chickens with leg problems. Skeletal disorders in broilers are associated with their rapid growth (Whitehead, 1997). In a review by Bessei (2006), it was stated that there is a causal interrelationship between fast growth, low locomotor activity and leg problems. Castellini *et al.* (2002) showed that culling due to leg problems is higher for fast-growing than for slow-growing broilers during the last three weeks in the organic production period. However, a reduced growth rate of fast-growing genotypes has been shown to have beneficial effects on the leg health of such birds (Brickett *et al.*, 2007; Leterrier *et al.*, 2006; Bizeray *et al.*, 2002). Therefore, a qualitatively restricted diet fed to fast-growing broilers may reduce culling due to leg problems, and thereby reduce mortality.

Immune and stress-related responses

Optimal production and health depend on a properly functioning immune system which is necessary to defend birds against infections. A diet with a suboptimal AA composition may negatively affect the birds' immune responses and lead to chronic stress (Rubin *et al.*, 2007; Chen *et al.*, 2003), thereby making the birds more susceptible to factors causing increased mortality. Indicators of nutritional stress are lymphoid organ weights and the H/L ratio (Konashi *et al.*, 2000; Kwak *et al.*, 1999; Klasing, 1998). The H/L ratio changes in response to stress, under which the number of lymphocytes decreases, while the number of heterophils increases (Gross & Siegel, 1983).

Biochemical markers

Production performance, such as live weight or mortality represents commonly used markers for nutritional status. However, when feeding broilers restrictive, live weight is reduced as an effect of the feeding regimen. It may, therefore, be difficult to evaluate whether other nutritional factors are affecting bird health and welfare. Hence, it is desirable to also have other markers that respond to nutritional treatments. Biochemical markers can provide a link between diet and health/welfare and serve as an early response to nutritional challenges. During recent decades there has been a rapid development of clinical chemistry markers for various diseases in humans and such methods are commonly used for human diagnostics. A normal hospital clinical chemistry laboratory performs millions of tests per year, and the methods are now highly automated allowing small sample volumes (often 1-10 μL/test) and low test reagent costs. There is now also an increased use of chemistry markers for veterinary use. It seems likely that several of these markers could also be used for chickens and be helpful in interpreting chicken welfare and health.

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Biochemical markers may be influenced by several factors such as nutritional status, husbandry conditions, age, and environmental temperatures (Rajman *et al.*, 2006; Talebi, 2006). Plasma calcium and phosphorus levels are diet-dependent; hypocalcemia may be caused by malnutrition (Harr, 2002). Calcium and phosphorus levels reflect osteoblastic activity and bone mineralization, and the enzyme ALP is closely associated with the metabolism of these two minerals (Rajman *et al.*, 2006). Hurwitz & Griminger (1961) showed that ALP activity increases with decreasing levels of plasma calcium and that decalcification rather than calcification of bone is associated with increased ALP levels.

Behaviour

Changes in behaviour are made to adapt to alterations in the physical and/or social environment. Deviations from the behaviour which is considered normal for the species may therefore be important signs of suboptimal welfare (EC, 2000). Feed restrictions or suboptimal protein diets induce bird activity (Brickett *et al.*, 2007; Leterrier *et al.*, 2006); feed search behaviour in broilers increases with decreasing protein to energy ratio in the diet (Savory & Lariviere, 2000; Rovee-Collier *et al.*, 1993). Suboptimal AA levels in diets for laying hens have also been shown to cause welfare problems due to abnormal behaviour, such as feather pecking and cannibalism (Elwinger *et al.*, 2008; Tiller, 2001; Ambrosen & Petersen, 1997; Savory, 1995). Therefore, studies of feeding behaviour and other behavioural aspects associated with feeding play an important role in the interpretation of welfare and production results (Nielsen, 2004).

Access to pasture and forage intake

According to organic standards, chickens should have daily access to an outdoor grass covered pasture for at least a third part of their rearing period (KRAV, 2010b). The outdoor pasture provides exercise, and stimulation in the form of foraging (Blair, 2008). Access to foraging material has been shown to lower the incidence of feather pecking and cannibalism in laying hens (Steenfeldt *et al.*, 2007; Huber-Eicher & Wechsler, 1997; Wechsler & Huber-Eicher, 1997). The outdoor access might therefore contribute to a less stressful situation due to positive effects on bird behaviour. Also, suboptimal protein and energy diets have been shown to increase birds' intake of forage on the outdoor pasture (Nielsen *et al.*, 2003b). Buchanan *et al.* (2007) showed that poultry may obtain small amounts of energy from pasture forage and have the ability to utilize AA, such as methionine, lysine and threonine, found in forage. However, the utilization of nutrients from pasture intake also depends on the quality of the outdoor pasture (Rivera-Ferre *et al.*, 2007). Ponte *et al.* (2008) showed that forage intake itself did not improve FCR for slow-growing birds on pasture, but forage intake promoted intake of cereal-based feed which thereby improved performance. Also, Bassler (2005) showed that outdoor pasture has only marginal effects on the nutrition of broiler chickens. If the chickens are able to utilize forage, the outdoor access may contribute to a less stressful situation by satisfying nutritional needs.

Aim

The aim of this thesis was to evaluate whether it is possible to compose a qualitatively restricted protein diet for fast-growing broilers according to the standards set by KRAV that sufficiently reduces growth rate without negatively affecting animal welfare and health.

The objectives for each study were:

I. To measure effects on health, immune responses, stress and production of fast-growing broilers fed organic diets with different levels of CP and AA, when reared up to 70 d in a controlled indoor environment.

II. To measure effects on behaviour, health, stress, and production of fast-growing broilers fed organic diets, with low CP and AA content in organic production with access to outdoor pasture.

III. To measure effects on behaviour, health, and production of broilers fed organic diets with and without hemp seed cake, in organic production with access to outdoor pasture.

IV. To evaluate biochemical markers as indicators of health and welfare in female and male broilers fed low CP and AA diets.

Material and Methods

This thesis encompasses four studies performed at the Funbo-Lövsta Research Center at the Swedish University of Agricultural Sciences (SLU) in Uppsala. The experiments were approved by the National Ethics Committee for animal research in Uppsala.

The chickens used were of the hybrid Ross 308, and the production period in all three experiments was 70 d (Paper I, II and III). All birds were given free access to water and pelleted feed, each diet comprising a starter and a grower feed. The diets used in each study contained no coccidiostats. Stocking density was planned to a maximum of 20 kg/m^2, in accordance with Swedish organic standards (KRAV, 2010b). Live weight and feed intake were recorded weekly on a pen basis until slaughter and the FCR was calculated and corrected for mortality. Twice daily, chickens were inspected and birds with health problems were culled immediately. Mortality was defined as the sum of culled and dead birds. It was registered daily and calculated as the frequency on a weekly pen basis. If the daily mortality increased, representative samples of birds that died or were culled during the experiment were necropsied at the National Veterinary Institute. Methods and experimental procedures are described in detail in each paper. An overview of the experimental designs are shown in Table 2.

Table 2. *Three experiments were conducted, one indoor experiment and two outdoor experiments, each resulting in a paper (Paper I, II, and III). A fourth study evaluated blood samples taken during the three experiments, resulting in Paper IV.*

	Indoor experiment	Outdoor experiments	
	Paper I	Paper II	Paper III
Chickens (n)	180	1400	1200
Housing	Climate controlled house	Organic chicken house access to outdoor pasture	Climate controlled house (up to 21 d) Organic chicken house (from 21 up to 70 d) access to outdoor pasture
Diets[1]	LP HP LPA	LP LPA	H C
Pen size	0.94 m^2	38 m^2	38 m^2
Pens (n)	45	8	8
Chickens/pen (n)	4	175	150
Replicate pen/diet (n)	15	4	4

[1]The LP diets in Papers I and II were based on the same recipe. The LPA diets in Papers I and II were not based on the same recipe (Paper I, II).

The indoor experiment (Paper I)

Birds and housing

180 day-old chickens were randomly divided between 45 pens with initially four chickens per pen (Table 2; Figure 1). The chicken house temperature, initially 34 ˚C, was gradually decreased to 19 ˚C at the end of the rearing period. The chickens had continuous artificial light during the first three days; thereafter the dark period was increased to 5 h per 24 h at 21 d.

Diets

Three diet concepts were designed (Table 2), two of which were composed without additional synthetic pure AA, i.e. according to organic standards. The first diet (LP) was designed to give a low CP level (16%), resulting in low lysine and methionine contents. The second diet (HP) aimed at AA levels used in conventional production (ROSS Manual, 2009), resulting in a high CP diet (18-20%). The third diet (LPA) consisted of the LP diet supplemented with pure AA, i.e. lysine, methionine and threonine, up to levels comparable with the HP diet. Starter feed was given to the chickens up to 21 d and thereafter grower feed until slaughter.

Figure 1. The climate controlled house and pens used in the indoor experiment. (Photo: K. Elwinger (left) and R. Kalmendal (right))

Vaccination, registrations and analyses

Chickens were vaccinated with an inactivated infectious bursal disease vaccine at 18 d and 42 d in order to study their immune response to a mild immunological challenge. At 34 d and 62 d, blood samples were taken from 90 randomly collected chickens (two in each pen) for evaluation of immune response and H/L ratio. The chickens were weighed; thereafter, 45 chickens were stunned, and killed by cervical dislocation. Individual lymphoid organs, i.e. spleen, bursa of Fabricious and thymus, were collected and the ratio of immune organ weight to live weight was calculated.

Outdoor experiments (Paper II and III)

Birds and housing

The two experiments were performed during separate time periods in two identical organic chicken houses (Figure 2) and included 1400 chickens (Paper II) and 1200 chickens (Paper III). In the second experiment, the chickens were reared in a climate controlled house until 21 d and thereafter moved into the two organic chicken houses (Paper III).

Each organic chicken house was divided into four floor pens, with initially 175 chickens (Paper II) and 150 chickens (Paper III) per floor pen. The houses were placed directly on a pasture, and the earth floor inside was covered with straw. The inside temperature was increased using heat lamps.

The ventilation consisted of natural ventilation via net windows, along the long sides of the house, which were also the only source of light. Thus, the lighting periods followed the outdoor light, which in Sweden during the periods of the trials was between 14 and 19 h light per day. From 21 d (Paper II) and 28 d (Paper III) the chickens had daily access to outdoor runs with pasture (Figure 2).

Figure 2. Housing of the chickens during the two outdoor experiments. The organic chicken house (right) and the outdoor pasture (left) (Paper II and III). The photo to the left shows chickens on the outdoor pasture, the LPA birds (left outdoor run) and the LP birds (right outdoor run) (Paper II). (Photo: Niklas Blom)

Diets

In the first outdoor experiment, two diet concepts (Table 2), with low CP contents were designed, with (LPA) and without (LP) pure AA supplements (Paper II). The LP diet was optimized to give a relatively low CP level (17-18%), resulting in low lysine and methionine contents. The LPA diet consisted of the same basal diet (LP) supplemented with pure AA (lysine, methionine and threonine). Birds were given the starter feed up to 28 d and thereafter grower feed up to slaughter. In the second outdoor experiment, two diet concepts were designed (Table 2), with (H) and without (C) hemp seed cake (Paper III). Both diets were composed according to organic standards and optimized to give a relatively low CP level (17-18%), resulting in low lysine and methionine contents.

Registrations and analyses

The number of birds on the outdoor pasture was recorded daily for each pen, and the percentage of birds on the outdoor pasture was calculated for each pen on a weekly basis. At 64 d, 5 randomly selected birds per pen, in total 40 birds, were individually weighed and blood samples were collected for recording of H/L ratio (Paper II).

Table 3. *Ethogram of recorded behaviours. Posture was recorded for behaviour that could be performed with both a sitting and standing posture.*

Behaviour	Description	Posture recorded
Eating	With head above or in the feeder and pecks directed into the feeder	Yes
Drinking	Pecks directed at a water nipple	Yes
Resting	Lying or sitting with hocks resting on ground without identifiable activity	No
Standing	Standing without any other activity	No
Walking	Locomotion with a normal speed or with quick steps	No
Preening	Directing attention with the beak towards own body and feathers	Yes
Dust bathing	While lying on floor, kicking litter onto body and wiggling body in litter dust	No
Scratch litter/ground[a]	Backward strokes with legs as if to dig litter	No
Peck litter/ground[a]	Pecks directed at the litter or anything on the floor bedding	Yes
Peck bird	Pecks directed at the head or body of a companion bird	Yes
Out/in[b]	The chicken is out/in and not present in the pen for the observer to register behaviour	No
Other	All other behaviour not mentioned above	Yes

[a]Scratch litter/ground and peck litter/ground were added together as feed search in the straw bedding/on the pasture before statistical analysis.
[b]For the observations at 6 and 10 weeks (Paper II) and at 4, 6 and 9 weeks (Paper III) simultaneous observations were recorded on the outdoor pens.

Behavioural observations

Behavioural observations were conducted on two subsequent days at 2, 6, and 10 weeks of age (Paper II) and at 2, 4, 6, and 9 weeks of age (Paper III). Individual observations were made on four focal chickens in each pen, which were individually marked with one of four different marking crayons. Each chicken was observed continuously for 15 minutes, and the behavioural states of the marked birds in the pen were recorded by instantaneous sampling at intervals of one minute, according to an ethogram given in Table 3. During the observations at 6, and 10 weeks (Paper II) and at 4, 6 and 9 weeks (Paper III), two observers simultaneously recorded chicken behaviour on the outdoor pasture and indoors in the pen. One additional parameter, 'changes-between-behaviours', was calculated (after the behavioural observations) as the number of changes between different behaviours indicating the intensity of activity.

Gait score and foot pad score

At 49 d and 63 d, gait score and foot-pad lesions were recorded for a randomly selected group of chickens, individually observed in two replicate pens for each diet (Paper II). In Paper III, gait scores and foot-pad lesions were recorded in all pens using the abovementioned procedure, at 49 d and at 70 d, respectively. Recordings of gait score were made according to Kestin *et al.* (1992); each bird was observed and the ability to walk was assigned to different categories of lameness according to a six-point scale, where score 0 is no detectable walking abnormalities and score 5 is a bird incapable of sustained walking on its feet. Thereafter a mean pen gait score per pen was calculated. Foot-pad lesions were recorded according to a three-point scale, where 0 is no lesions, discoloration or scars, and 2 is a severe, deep lesion, ulcers and scabs according to Ekstrand *et al.* (1998) and thereafter calculated following the method used in practice. The calculated scores were then added together, divided by the number of observed chickens in each pen and multiplied by 100, resulting in a foot-pad score ranging from 0-200. A bird given a gait score of 3 or higher was immediately stunned and euthanized after observation.

Evaluation of biochemical markers (Paper IV)

The fourth study evaluated blood samples collected during the three experiments (Table 2). Blood samples were collected, live weight and sex were registered at 62 d on 90 chickens, two in each pen (the indoor experiment). In the outdoor experiment, five randomly selected birds per pen, in total 40 birds, were selected for blood sampling and registration of live weight and sex at 63 or 64 d. Analyses of plasma were made for ALP, calcium, phosphate, cholesterol and triglycerides.

Statistical analyses

Before statistical analyses of behaviours, indoor and outdoor registrations of behaviours were added together. The mean proportions of the various behaviours relative to the total registrations were then calculated for each pen for each observation period.

Statistical analyses were performed on mean values for each pen and based on analyses of variance (ANOVA) using the GLM procedure (Paper I, II, III, IV) in SAS (SAS, 2004). Statistical analyses of behavioural parameters, as well as the percentage of chickens on outdoor pasture (Paper II and III), were based on analyses of variance (ANOVA) using the MIXED procedure in SAS (SAS, 2004). Variables were tested for normal distribution with the UNIVARIATE procedure of SAS (SAS, 2004). Mortality and relative lymphoid organ weights were arcsine-root transformed before statistical analyses (Snedecor & Cochran, 1989).

Main results

Immune and stress-related responses (Paper I and II)

No differences were observed in the H/L ratio between diets (Paper I and II), nor did the differences in CP and AA content in the different diets significantly affect relative lymphoid organ weights, i.e. organ weights as a percentage of live weight, or immune responses due to vaccination against infectious bursal disease (Paper I).

Behaviour (Paper I, II and III)

Feed spillage, eating of lost feathers in the bedding and forage intake on the outdoor pasture were observed during the experiments in the birds fed diets low in CP and AA (Paper I, II and III). Figure 3 shows the observed differences in the amount of lost feathers in the bedding (Paper I).

Figure 3. Amount of feathers in the bedding for chickens fed the LP (left), HP (middle) and LPA (right) diet.

Outdoor pasture registrations

Chickens fed the diet without AA supplements (LP) used their outdoor pasture to a greater extent ($p<0.001$), than the LPA chickens receiving AA supplements, (Figure 2, Paper II). The use of the outdoor pasture decreased with age for the LP ($p<0.001$), H and C birds ($p<0.001$), whereas the percentage of LPA birds on the outdoor pasture was consistently low (Paper II and III). The relative frequency of chickens on the outdoor pasture was higher for the C diet ($p<0.05$) than for the H diet (Paper, III). The lowest use of the outdoor pasture was observed at 9 weeks for all diets (Paper III).

Table 4. *Registrations of birds on the outdoor pasture at 6 and 9 weeks as a percentage of total birds, and behavioural registrations, both indoor and outdoor, as a percentage of total registrations at 2, 6, 9 and 10 weeks for the two outdoor experiments (Paper II and III). Mean gait scores and foot pad scores are also presented.*

		Diet			
	Age, week	LP	LPA	H	C
Birds on the outdoor pasture, %	6	18.8	1.5	18.6	22.0
	9	11.0	0.5	8.5	11.5
Behavioural alobservations[1], %					
Feed searching	2	23	2	17	15
	6	21	2	3	10
	9, 10	9	2	3	2
Preening	2	3	3	5	7
	6	11	3	11	11
	9, 10	13	3	5	3
Changes-between-behaviour	2	41	35	45	41
	6	54	23	42	44
	9, 10	43	18	35	31
Resting	2	43	79	38	48
	6	42	81	64	49
	9, 10	59	87	76	75
Leg and foot health registrations					
Gait scores	49	0.6	1.3	1.8	1.4
	63	1.4	1.8	-	-
Foot pad scores[2]	49	12.7	7.1	-	-
	63, 70	0	4.4	76.5	28.0

[1]The behavioural observations of the LP and LPA birds were registered at 10 weeks (Paper II) and of the H and C birds at 9 weeks (Paper III).
[2]Footpad scores were registered at 63 d for the LP and LPA birds, and at 70 d for the H and C birds (Paper II and III).

Behavioural observations

The LP birds were walking, standing and feed searching more ($p<0.01$; $p<0.05$; $p<0.01$) than the LPA birds (Paper II). Preening increased for the LP birds between 2 and 6 weeks, resulting in a higher frequency of preening

($p<0.001$) compared to the LPA birds, in which frequency of preening was consistently low (Paper II). In the outdoor experiment, the H and C birds showed the highest frequency of preening at 6 wks ($p<0.01$) (Paper III). The LP birds had a higher frequency of changes-between-behaviours ($p<0.01$) compared to the LPA birds (Paper II). The changes-between-behaviours decreased with age for the LPA birds ($p<0.05$). The LPA birds rested more ($p<0.001$) than the LP birds (Paper II). Resting increased with age regardless of diet ($p<0.05$; $p<0.001$) (Paper II and III). Results from the two outdoor experiments are summarized in Table 4.

Health

Gait score and foot-pad score (Paper II and III)

Overall, mean gait score was low. The LP birds had a lower gait score than the LPA birds at 49 d ($p<0.01$), but no differences were seen at 63 d (Paper II). However, this might be due to a lack of birds with gait score over 3, since the culling of chickens with leg problems increased with age. Gait score was higher for the chickens fed the H diet compared to chickens fed the C diet ($p<0.05$) (Paper III). Foot-pad scores were higher for the H birds compared to the C birds ($p<0.001$) (Paper III). No difference in foot-pad scores were seen between the LP and LPA birds (Paper II). Results from the two outdoor experiments are summarized in Table 4.

Mortality (Paper I, II and III)

Total mortality was high for the two outdoor experiments (Paper II and III). Mortality was higher in the heavier birds given the LPA and HP diets, compared to the LP birds ($p<0.001$), both in the indoor and outdoor experiments (Paper I and II). In the indoor experiment, mortality was mainly due to the culling of chickens with leg problems; 11 out of 14 culled chickens were males (Paper I). Culling due to leg problems accounted for 79.4% of the total culling of LPA birds and 52.9% of the total culling of LP birds during the last three weeks of the rearing period (Paper II). In the first outdoor experiment, mortality due to yolk sac infections was high among the LP (4.2%) and LPA birds (6.6%) during the first week (Paper II). Necropsy showed infections with *E. coli* up to 4 weeks of age (Paper II), and infections with *C. perfringens* and necrotic enteritis was found during the eighth week of rearing (Paper II). At 35 d, an outbreak of cannibalism occurred among birds in the LP pens, and 1.7% of the birds had to be culled due to injuries (Paper II). In the second outdoor experiment, a high mortality was seen at 61 d, due to a heat shock (Paper III). Most of the dead birds were males ($p<0.001$), i.e. 83.2% for the C diet and 89.0% for the H diet (Paper III). Necropsy revealed several pathological lesions in the muscle on the legs and breast, typical for heat stress (Paper III). Results from the three experiments are summarized in Table 5.

Production performance (Paper I, II and III)

Chicken live weight increased with increasing CP and AA content in the diets ($p<0.001$) (Paper I and II). A summary of live weights at 70 d as well as average daily growth rates for the total rearing period are presented in Table 5.

Table 5. *A summary of results from the three experiments (Paper I, II and III); total mortality (dead and culled birds), total culling as a percentage of the total number of chickens, reasons for culling as a percentage of the total number of culled birds, total live weight at slaughter (70 d), and average daily growth rate for the whole rearing period.*

	The indoor experiment			The outdoor experiments			
Diet	LP	LPA	HP	LP	LPA	H	C
Mortality, %	0	8.3	15.0	12.7	28.0	21.0	23.3
Cullings, %	0	8.3	15.0	5.1	13.4	3.5	5.2
Reasons for culling							
Cannibalism, %	0	0	0	29	0	0	0
Leg problems, %	0	80	67	53	79	100	68
Other signs of illness, %	0	20	33	18	21	0	32
Live weights, g	3868	4264	4631	4086	5241	4166	4165
Average growth rate, g/day	55.3	60.9	66.2	58.3	74.8	59.5	59.5

Biochemical markers (Paper IV)

The LP chickens had lower plasma calcium levels than the LPA birds ($p<0.01$). In the two outdoor experiments, the plasma calcium levels were numerically lower than in the indoor experiment. Male chickens given the LP diet had higher plasma ALP levels ($p<0.05$) than the other chickens in the first outdoor experiment. Also a tendency to higher plasma ALP levels ($p=0.09$) was seen for the male chickens fed the C diet in the second outdoor experiment.

General discussion

Since most organically approved protein feedstuff are relatively low in AA content, current organic legislation for broiler production is facing the challenge of composing a diet with optimal levels of AA to meet the birds' requirements. In Sweden, this challenge is exacerbated by the use of commercial fast-growing broiler hybrids. The aim of this thesis was to evaluate whether it is possible to compose a qualitatively restricted protein diet for fast-growing broilers according to the standards set by KRAV that sufficiently reduces growth rate without negatively interfering with animal welfare and health.

One of the experiments (the indoor experiment) was performed in a climate controlled environment to exclusively evaluate the effect of diets differing in protein and AA content on fast-growing broilers kept up to 70 d. However, organic production is quite different from a controlled indoor production and factors such as environmental temperatures, disease challenges and variations in housing climate may affect the birds' need of nutrients. Therefore an outdoor experiment was performed to evaluate the effects of low protein diets varying in AA content on broiler health and welfare when kept in an organic production. Furthermore, a second outdoor experiment was performed to evaluate hemp seed cake as a potentially good protein source in organic production. As a forth study, an evaluation of biochemical markers commonly used in human diagnostics was carried out on blood samples taken during the three experiments. This study was added in order to find possible additional effects on nutritional status in birds fed low CP and AA diets.

Effects of varying CP and AA diets on bird welfare

When discussing effects of diets with varying protein levels on bird welfare, it is important to consider the effects of live weight. As intended, feeding the chickens with diets differing in protein and AA content implied live weight differences (Paper I and II). Lower live weights have been shown to enhance leg health and increase bird activity (Leterrier *et al.*, 2008; Leterrier *et al.*, 2006; Rutten *et al.*, 2002). This was also shown in the present studies (Paper I and II) where resting behaviour increased in the heavier birds fed diets higher in AA (Paper II). Resting also increased with age (Paper II and III), which might be due to decreasing leg health and increased live weight (Bokkers & Koene, 2003; Nielsen *et al.*, 2003a; Weeks *et al.*, 2000). It is difficult to know whether the difference in such parameters is a direct effect of the difference in live weight or an effect of the difference in protein and AA content, since live weight and AA content are highly correlated. Therefore live weight can be regarded as included in effects of diet treatments. However, the increased changes-between-behaviour, indicating the intensity of bird activity, seen for the LP birds may be more related to the effect of AA content in the diet than to live weight differences (Paper II). It has been shown that the degree of lameness affects walking and standing ability and number of visits per day to the feeder, as well as the time spent at the feeder (Weeks *et al.*, 2000). Thus, it could be expected that chickens, regardless of diet would decrease their changes-between-behaviours with increasing age. However, the opposite was seen among the LP chickens and the increase in changes-between-behaviour was seen during the period when cannibalism outbreaks were observed among these birds (Paper II).

Other observations, i.e. feed spillage, feather eating, as well as increased feed search and preening behaviour with age, made among the birds given diets low in protein and AA content further indicated frustration and hunger due to the qualitative feed restriction (Paper I, II and III). Increased feed search and preening behaviour with increasing age have in previous studies been referred to as a reflection of chronic hunger and frustration (Kostal *et al.*, 1992; Duncan & Wood-Gush, 1972). The registration of behaviours which can be perceived as an event, such as preening and pecking other birds, might be underestimated by instantaneous sampling (Martin & Bateson, 1993), used in the present experiments. If continuous sampling had been undertaken the frequency could have been measured and possibly interpreted more accurately. Nevertheless, the use of instantaneous sampling still showed differences in preening behaviour which might strengthen the present results. Also, cannibalism occurred among birds fed low CP and AA diet (Paper II). Incidence of abnormal behaviours, i.e. cannibalism, are rarely seen in conventional broiler production, although it has been reported among slow-growing broiler chickens reared in an outdoor production system (Bassler, 2005; Nielsen *et al.*, 2003b). This suggests that when feeding fast-growing broilers low CP and AA diets, problems are introduced that are not beneficial for bird welfare.

Contradictory to the behavioural results indicating hunger and frustration in the chickens fed low CP and AA diets, no significant differences were seen in stress responses (H/L ratio) and immune responses (antibody titres and relative lymphoid organ weights) between chickens fed either diets (Paper I and II). Unfortunately, the immune responses were not evaluated in the two outdoor experiments where other factors than the qualitatively restricted diets could have further affected immune responses (Paper II and III). H/L ratios have been suggested to be affected by various stressors, such as housing system, environmental temperature, disease challenges, and nutritional stress, such as low AA levels (Klasing, 1998). De Jong *et al.* (2002) showed that the H/L ratio can be questioned as an indicator of stress caused by food restrictions and pointed out the importance of behavioural measurements as well as a wide range of physiological parameters to study such effects. It is unclear from the present experiments whether the lack of differences in H/L ratio means that the chickens were not subjected to any stress or that they were subjected to different stressors.

Outdoor access and access to pasture

The registered difference in the birds' use of the outdoor pastures was clear in Paper II. The chickens given the diet supplemented with pure amino acids used their outdoor runs to a very small extent; the contrary was seen for those birds given diets low in amino acids (Figure 2). The small number of LPA birds on the outdoor pasture was seen throughout the rearing period and was also confirmed by the behavioural study. Interestingly, the resting frequency of the LPA birds increased from 79% to 87% throughout the behavioural study, while the resting frequency of the LP birds increased from 43% to 59%. This indicates that the LPA birds spent much of their time resting from an early age. Other studies have shown that fast-growing broilers exhibit a marked decrease in activity already at 2 to 3 weeks (Nielsen *et al.*, 2003a; Pedersen & Gaardbo Thomsen, 2000). Also, it has been shown that nutritionally satisfied chickens (with a low presence of leg disorders), despite access to ample space (2 chickens/m^2) prefer to stay near drinkers and feeders, thereby minimizing their physical effort (Arnould & Faure, 2004); locomotor activity may have lost some of its adaptive value when feed and water are easily reached (Bizeray *et al.*, 2002). However, chickens tend to increase the use of outdoor pastures with increasing familiarity (Christensen *et al.*, 2003). This is not in agreement with the result from the present experiment, since the LPA birds did not increase the use of the outdoor pasture with age. The result regarding the LPA birds might reflect a general characteristic of fast-growing broilers.

In organic production it is desirable that birds use their outdoor areas for exercise and stimulation in the form of foraging. In this sense the LPA birds were not suitable. This is in accordance with Nielsen *et al.* (2003b), who showed that it is preferable to use slow-growing broilers in the organic production system since they are more active and more likely to use the outdoor pasture than fast-growing broilers. However, feeding fast-growing broilers with a low protein and low AA diet increased their use of the outdoor pasture and it also increased bird activity and forage intake (Paper II) in accordance with the organic purpose. On the other hand, this can be discussed from a welfare point of view, since these birds also showed behaviour related to frustration and hunger (Paper II). According to Rivera-Ferre *et al.* (2007) survival instincts prevents poultry from going far away from their shelter-providing indoor areas. The LP birds used the outdoor pasture more at younger ages when the outdoor pasture was unfamiliar than at higher ages. Feed searching behaviour was the most frequently registered behaviour on the outdoor pasture, indicating that the LP birds were not satisfied with the feed available indoors. This may also indicate that the need for finding nutrients was greater than the need for using shelter. Broilers have been reported to have the ability to select feed that result in a balanced intake of protein when presented with a variety of feeds (Forbes & Shariatmadari, 1994). In the present experiments the pasture was not analysed for nutritional content neither was the amount of forage intake registered. It is therefore difficult to conclude as to what extent birds' nutritional needs were satisfied by forage intake besides the feed provided indoors.

Calcium is strictly regulated and is normally at a constant level in the blood plasma (Sjaastad *et al.*, 2003). Comparing the plasma calcium levels in the indoor and the two outdoor experiments indicated that chickens in the two outdoor experiments had lower levels of plasma calcium, than the chickens in the indoor experiment (Paper IV). Chickens in an outdoor organic environment are more subject to disease challenges than chickens held in a controlled indoor environment. Parasitic and bacterial infections can affect the intestinal epithelium and thereby decrease nutrient absorption (Yegani & Korver, 2008). However, the specific effects in the present experiments are unclear since there were several factors that differed between the indoor and outdoor experiments. Also, the LP birds had lower levels of calcium in the plasma compared to the LPA birds, which was not seen in the indoor experiment for the chickens fed similar low protein and AA diets (Paper IV). In the outdoor experiment, the lower plasma calcium may be an effect of additional diet dilution due to a high pasture intake seen among the LP birds (Paper II). Normally recommended calcium levels in feed for broilers are based on chickens in a controlled indoor environment, and the present results may indicate that these recommended calcium levels are not sufficient when the birds are given low CP and AA diets and reared in an outdoor environment. Further studies on the amount of pasture intake are necessary in order to calculate the possible level of diet dilution.

The organic rearing period

Positive effects of the qualitative feed restriction other than a reduced growth rate were found in lower mortality, gait score, and culling due to leg problems (Paper I and II). As described in the introduction the combination of long rearing periods and heavy birds may result in increased mortality due to high levels of leg problems during the latter part of the rearing period. Other studies have shown that slow-growing broilers have less leg problems than fast-growing broilers and are in this sense better suited for long rearing periods (Bessei, 2006; Bokkers & Koene, 2003; Nielsen *et al.*, 2003b). Even though mortality was decreased for the restrictively fed birds in the outdoor experiment, leg problems were still one of the main reasons for culling during the last three weeks of the rearing period regardless of diet (Paper II, and III). This indicates that even though the growth rate is reduced, leg problems at the end of the rearing period still remain an issue. Leg problems form a complex concept and are influenced by many factors. Bacterial infections with *Staphylococcus aureus* and *E.coli* can result in osteomyelitis, an infection of the proximal part of the tibia or femur (McNamee & Smyth, 2000; Butterworth, 1999). In the present experiment, necropsy revealed osteomyelitis in to the eighth week of the rearing period (Paper II). Also insufficient calcium intake can affect bird leg health (Skinner & Waldroup, 1992). The lower plasma calcium levels seen in chickens in the outdoor experiments (Paper IV) might therefore be a factor involved in the presence of leg problems. In the present study it was difficult to draw conclusions about the reasons for differences in mortality, since not all birds that died or were culled were sent for necropsy (Paper II). However, it is important to point out that even though the mortality decreased for the restrictively fed birds, the total mortality in these groups was still high. The increased culling due to leg problems at higher ages indicates that a rearing period of 70 days is too long for fast-growing birds. Thus, a shorter rearing period seems to be a better way to attain the goal of good bird welfare. Conversely, if the broiler chicken is defined as slow-growing and originate from organically reared broiler parents, the chickens are allowed to be slaughtered at an optional age, provided that they have spent on third of their lives with access to outdoor pasture (KRAV, 2010b; EC, 2007). Since the definition of a slow-growing chicken is not yet set, it is unclear whether the type of birds used in the present experiments, could be defined as slow-growing and used for organic production in the future.

Environmental temperature and rearing conditions

According to KRAV broilers in Sweden are allowed to be kept without outdoor access for up to one month. This allows the producers to raise broilers in a controlled indoor environment during the first weeks, to give them a good start in life. According to the ROSS Manual (2009) it is recommended that chickens have continuous artificial light during the first three days, in order to find feed and water properly. Their environmental temperature should be between 30-33°C depending on the humidity. Among the chickens that were kept in the organic chicken house from day-old, an infection with *E. coli* (yolk sac infection) occurred and mortality became high during the first weeks (Paper II). The infection continued up to 4 weeks of age. As mentioned in the introduction, a low AA diet may impair the immune system. However, mortality did not differ between the LP and LPA birds during this time which might indicate no difference in immune responses. Yolk sac infection is usually established during hatching but infection of unhealed navels with *E. coli* from the floor bedding may occur during the first week (Barns *et al.*, 2008). Young chickens are sensitive to temperature variations and are especially prone to becoming chilled; they usually require an external heat source (Blair, 2008). In both outdoor experiments, chickens were provided with sufficient heat beneath heat lamps (Paper II and III). These lamps also provided some artificial light. Except for beneath the heat lamps, housing temperature followed the outdoor temperature which, during the first two weeks, varied from 14 to 28 °C (Paper II). In the second outdoor experiment, the mortality was low even up to 56 d (Paper III). These chickens were held in a controlled indoor environment during the first three wks, and no signs of unhealthy day-old chickens were observed on arrival (Paper III). Comparisons between the two experiments are difficult to make since the birds did not come from the same parents and hatching. However, good management of chickens with yolk sac infections may lower the mortality. Perhaps this would have been achieved if the chickens were held indoors in a climate controlled environment during the first weeks in the first outdoor experiment.

The thermo-neutral zone, also referred to as the comfort zone of an adult chicken is an environmental temperature between 18-24 °C (Blair, 2008). High surrounding temperatures may give rise to heat stress, depending on the hybrid, feathering, nutrition and production system (Lin *et al.*, 2006). When environmental temperatures increase above 29 °C, older chickens start panting to help dissipate heat (Blair, 2008). However, during the first week the chickens need higher temperatures, as discussed above. A much more severe effect due to heat stress occurred in one of the outdoor experiments (Paper III). At 61 d, the surrounding temperature reached a sudden peak of 35°C and resulted in a high peak mortality of heavy, mostly male, chickens regardless of diet. Excluding this particular day, the indoor temperature ranged between 13°C and 32.9°C during week 9 (56-63 d) (Paper III). In a study of fast-growing male broilers, Cooper & Washburn (1998) showed no difference in mortality at 49 d due to a surrounding temperature of 32°C in a controlled environment. Ventilation is of great importance as a means to reduce mortality during hot weather conditions. The use of a conventional controlled environment or a tunnel-ventilation at mean temperature of 36°C showed no severe mortality for broilers with a mean weight of 2.4 kg up to 53 d as shown by Lacy & Czarick (1992). Unfortunately, the ventilation capacity of the organic chicken houses, with natural ventilation via net windows, was not sufficient for this sudden and quite extreme temperature for Swedish conditions. Furthermore, it has been shown that birds with lower live weight can withstand the effect of heat stress better than heavier birds at the same age (Berrong & Washburn, 1998). This agrees with the results of the present study in which mainly heavy male birds died. Feed restriction has been suggested as a strategy for enhancing the heat resistance in broilers (Lin *et al.*, 2006). Regardless of diet, the chickens had a mean growth rate throughout the production period of 59.5 g per day (Paper III). Therefore, it can be speculated as to what extent the mortality due to heat stress would have been altered if further measures had been taken to reduce the growth rate of fast-growing broilers to the standards of KRAV. In conclusion, additional ventilation is needed in these kinds of mobile chicken houses with only natural ventilation. According to the Swedish Animal Welfare Ordinance (SJVFS 2010:15), no temperature alarms are required in a poultry facility with less than 2000 birds, i.e. applicable to the production size in the present experiment. However, with regard to the results of heat stress registered in the present experiment, temperature alarms are needed even in a production with less than 2000 birds.

Influences of sex

In the three experiments no separation between male and female chickens were made in the experimental setup. Hence, recordings of sex differences were limited except for blood sampling in Paper IV. However, results on mortality in the three experiments showed that there were some differences between female and male chickens. The chickens culled because of leg problems at higher age (Paper I) and chickens that died due to heat stress (Paper III) were predominantly male. Male chickens have a higher growth rate and are heavier than females and may therefore be more susceptible to heat stress and leg problems. However, even when gait score is adjusted for differences in live weight, male chickens have been reported to have more leg problems than female chickens (Sorensen *et al.*, 2000). Furthermore, in the first outdoor experiments the male birds given the low CP and AA diet had higher plasma ALP levels compared to the female chickens and to the male chickens fed the higher AA diet (Paper II). Similar patterns were seen in the second outdoor experiment for the male birds fed the C diet compared to the other birds (Paper III). Elevated levels of plasma ALP have been associated with decalcification or insufficient calcification of bones (Rao *et al.*, 2003; Hurwitz & Griminger, 1961). Although not statistically confirmed, the plasma calcium levels were numerically lower for the LP and C males compared to the other chickens in the same experiments (Paper II and III). This may indicate that males are more susceptible to negative effects on skeletal metabolism of the outdoor environment compared to females when fed low CP and AA diets. Therefore, it may be preferable to use female chickens in organic production.

Effects of varying CP and AA diets on production performance

None of the low CP/AA diets used in the three experiments were efficient enough to reduce growth rate according to the growth restrictions set by KRAV. Thus, further restrictions are needed in order to lower the growth rate and enable slaughter at 70 d instead of at 81 d. The indoor experiment indicated that the diet low in CP and AA better corresponded to the birds´ requirement at higher ages (Paper I). Therefore a more severe qualitative feed restriction in the grower diet at older ages compared with the levels used in the present experiments might further decrease growth rates and thereby lower final live weights. Another way to reduce growth may be to also impose a quantitative feed restriction. However, it can be speculated as to what extent the stress response associated with hunger and frustration would alter if such measures were taken to lower the growth rate to meet KRAVs standard. Welfare is a dilemma in broiler breeder production, and it has been shown that these birds are subjected to physiological stress, abnormal behaviour and chronic hunger (De Jong *et al.*, 2002; Mench, 2002). Also, the definition of a slow-growing hybrid is not yet established in the organic standards by the Swedish Board of Agriculture. In Denmark the definition is set to an average daily growth of 30 g/day (Pedersen *et al.*, 2003); if the growth rate in Sweden is similarly defined it is questionable whether the broilers used in this thesis ever can be defined as slow-growing broilers.

More severe qualitative or quantitative feed restrictions would not be necessary if a slow-growing hybrid was available for use in the Swedish organic production. It may still remain a challenge to fulfill the birds' AA requirements, and balance AA content when using organic protein feedstuffs. However, since no growth restrictions are necessary for slow-growing birds, severe qualitative feed restriction is not needed and diets can be composed with higher CP levels and thereby AA levels. An excess of CP might, on the other hand, lead to a leakage of nutrients to the environment through the excreta, which is not in accordance with organic aims (Blair, 2008; Bartov, 1985). Excess of protein in broiler diets may also increase water consumption and thereby affect litter moisture and quality (Francesch & Brufau, 2004). Also, future availability of slow-growing hybrids is uncertain in Sweden. It is important to consider the implication of a slow-growing bird for the Swedish organic production system regarding consumer perception, since there are differences in meat quality and characteristics between the genotypes (Branciari *et al.*, 2009; Castellini *et al.*, 2008; Castellini, 2005).

Hemp seed cake as an alternative protein feed source

Inclusion of HSC did not affect total production performance, mortality or bird behaviour (Paper III). This confirms previous research (Silversides *et al.*, 2005) stating that hemp seed can be used as an ingredient in poultry feeds and that performance is retained as would be expected from the nutrient composition of the diet. However, feeding chickens the diet with inclusions of hemp seed cake in the present experiment resulted in wet litter which negatively affected the foot health of the birds. There was also another major variable in the H diet compared to the C diet, i.e. the inclusion of peas instead of soy bean cake. It is difficult to compose diets formulated to evaluate specific ingredients and it could be speculated whether the diet should be optimized to be as equal as possible in ingredient content or nutrient composition. In the present study, the aim was to compose two diet treatments with equal energy levels and AA composition, which resulted in the difference in ingredients (Paper III). Increasing the amount of pea fibre has been shown to decrease the dry matter in droppings and increase excreta output (Jorgensen, 1996). Since the inclusion of both peas and hemp seed differed between diets, the effect of hemp seed on bird foot health needs further investigations.

Practical implications

This thesis has shown health and welfare problems can arise when using fast-growing broilers under current organic standards. Low CP and AA levels in chicken diets resulted in hunger and frustration for the birds. Conversely, feeding chickens diets with higher levels of CP and AA resulted in heavy birds with more leg problems and higher mortality. Neither of the diets in this thesis was efficient enough to reduce growth rate according to the growth restrictions set by KRAV. If the growth restrictions are not reached, the organically reared fast-growing broilers would not be allowed to be slaughtered at 70 d; instead rearing periods would be a minimum of 81 d.

Increasing the rearing periods with another 11 days may further enhance the issue of increased mortality due to leg problems and is not beneficial from a bird welfare point of view. Therefore, further feed restrictions are needed which should preferably be implemented at higher ages when the birds' protein requirements are lower than at younger ages. However, it is unclear how the welfare problems observed among birds used in these experiments would change with more severe feed restrictions. One alternative way of lowering the growth rate could be to use only female chickens in organic production, since they have a lower growth rate compared to the male chickens and are less prone to exhibit leg problems.

According to KRAV, slow-growing chickens originating from organically reared broiler parents are not included in any directives on rearing periods, except for the directive regarding outdoor rearing which states that birds should have access to an outdoor pasture under at least a third part of their lives (KRAV, 2010b). If the production fulfills these requirements, the birds' are allowed to be slaughtered at a lower age and the mortality caused mainly by increased leg problems during the later part of the rearing period can thereby be decreased. However, it is unclear whether the chickens used in the present study can be defined as slow-growing broilers in the future. Other alternatives such as slow-growing broiler hybrids or a change in the regulation regarding the long rearing periods when using fast-growing hybrids might be necessary option for continued organic production in Sweden. The use of slow-growing hybrids instead of fast-growing ones can be beneficial in lessening mortality and health problems. However, abnormal behaviour has also been seen among slow-growing birds (Bassler, 2005; Nielsen *et al.*, 2003b), and the challenge of fulfilling the birds' nutritional requirements when composing a 100% organic diet may still not be solved by using a slow-growing hybrid.

Conclusions and recommendations

- The diets used in this thesis were not efficient enough to reduce growth rate to comply with KRAV's definition of a slow-growing broiler. Further restrictions are needed to lower growth rate at higher ages.
- Feeding low CP and AA diets to fast-growing chickens reduced their live weights and resulted in frequent use of outdoor pastures, increased birds' forage intake and activity while decreasing mortality, gait score and culling due to leg problems. However, there were also indications that these birds experienced frustration and hunger due to the qualitative feed restriction. Outbreaks of cannibalism in feed restricted birds indicated problems not beneficial for the welfare of broilers.
- Using female broilers in the organic system could further reduce the average growth rate. They are less sensitive to heat stress and leg problems at higher age than male chickens.
- Shortening the rearing period by approximately three weeks would be beneficial for bird welfare when using fast-growing broiler in the organic production system.
- The inclusion of slow-growing hybrids in Swedish organic production might be necessary in the future. This depends on the future definition of the growth rate for slow-growing hybrids and if the conventional bird can be defined as slow-growing.
- Hemp seed cake can be used in diets for organic broilers. However, there is a risk of wet litter and decreased foot condition when using 20% inclusion of hemp seed cake.
- A more concentrated diet regarding vitamins and minerals might be needed in organic production, since foraging and feather eating observed among birds dilutes the nutrients in the diet.

- Mechanical ventilation and temperature alarm are required when rearing broilers during the summer period in organic chicken houses with only natural ventilation.
- Rearing organic chickens in an indoor controlled environment during the first three wks of age is preferable.

Future research

As shown in this thesis, the use of fast-growing broilers in organic production may imply a welfare risk. Therefore, it is important to find other models for organic broiler production than the ones used in this thesis.

Suggestions for future research:

- As indicated in this thesis, it may be preferable to use only female broilers in organic production. Further evaluation of differences between male and female chickens in the organic production with regard to welfare, health, production and carcass quality are necessary.
- Another more long term solution may be the use of slow-growing hybrids in the Swedish organic production system. An evaluation of the introduction of a slow-growing bird into the Swedish organic production system regarding consumer perception is important.
- Further studies are needed of the levels of hemp seed cake inclusions in organic diets and the effect on litter quality and bird foot health.
- Since the study on biochemical markers in this thesis was made as a first step in evaluating such markers for welfare and nutritional status, more studies are necessary in this area to find reference intervals for broiler chickens. Other interesting parameters to evaluate may be plasma proteins, i.e. albumin and gamma globulins, where the ratio is used as an indication of infections in human diagnostics.

Populärvetenskaplig sammanfattning

Den ekologiska produktionen har ökat under de senaste åren, men under 2009 sågs en viss nedåtgående trend i EU. Regeringen har satt som mål att Sveriges ekologiska kycklingproduktion under 2010 ska uppgå till minst 1 % av den totala kycklingproduktionen. Produktionen idag av ca 179 000 ekologiska kycklingar per år uppfyller inte målet, även om det finns en ökande efterfrågan från konsumenterna.

Bakgrund

Det finns flera faktorer som hindrar en ökning av den ekologiska produktionen. Ett hinder är svårigheten att tillgodose kycklingarnas proteinbehov med de regler som rör ekologiska råvaror och fodersammansättning. Kycklingar har ett högre behov av aminosyror, framförallt metionin, jämfört med andra djur som används i köttproduktion. Metionin kan inte syntetiseras av kycklingen själv utan måste tillföras i fodret. I den konventionella produktionen kan metionin ökas i fodret genom att använda högvärdiga proteinråvaror, till exempel sojamjöl vilket framställs genom hexanextrahering, och sedan slutligen balanseras till rätt nivå med hjälp av syntetiska aminosyror. Sådana råvaror och syntetiska aminosyror är dock inte tillåtna i ekologiska foder. De flesta ekologiska proteinråvaror innehåller låga nivåer av metionin och därför är det svårt att tillverka fullgoda ekologiska foder till slaktkyckling. Idag får 5 % av fodret innehålla konventionellt producerade råvaror, detta för att kunna tillgodose djurens proteinbehov bättre. År 2012 skärps dock regeln ytterligare och endast ekologiska råvaror tillåts, därmed försvåras den redan påtagliga foderproblematiken ytterligare. Det är därför viktigt att försöka hitta fler alternativa proteinråvaror som kan produceras ekologiskt. Hampan kan vara ett alternativ som även är lämplig att odla i Sverige.

Ett annat hinder kan vara användandet av snabbväxande slaktkycklinghybrider i den ekologiska produktionen. Dessa hybrider har en snabb tillväxt och slaktas vanligtvis efter ca 5-6 veckor. I den ekologiska produktionen är uppfödningstiden dubbelt så lång, dvs. 81 dagar. Den långa uppfödningstiden och den snabba tillväxten hos snabbväxande hybrider kan leda till tunga djur med benproblem i slutet av uppfödningsperioden. Flera studier har visat att sådant djurmaterial inte är anpassat att hållas upp till 81 dagar, men eftersom det länge inte funnits något annat alternativ så har man försökt att hitta lösningar för att använda dessa hybrider. Om kycklingen till exempel definieras som långsamväxande får den slaktas vid 70 dagar. Eftersom det inte finns någon officiell definition av vad som får kallas en långsamväxande kyckling har KRAV tillsvidare definierat den som; en kyckling som i genomsnitt växer max 50 g/dag. Denna definition möjliggör att snabbväxande kycklinghybrider kan slaktas tidigare, förutsatt att tillväxten hålls tillbaka.

Ett sätt att hålla ner tillväxten kan vara att sänka råproteinnivån i fodret. Detta innebär dock att även metioninnivån sänks, vilket ytterligare försvårar balanseringen av fodret. Ett sådant foder kan ha negativ effekt på djurens välfärd. Syftet med studierna i denna avhandling var undersöka om det är möjligt att optimera ett ekologiskt foder som håller tillbaka tillväxten utan att påverka kycklingarnas välfärd negativt.

Sammanfattning av studierna

Tre försök utfördes vid Sveriges lantbruksuniversitets (SLU) forskningscentrum, Funbo-Lövsta. I försöken studerades hur ekologiska foder med låga nivåer av protein och aminosyror påverkade kycklingarnas beteende, hälsa, immunförsvar, stress och produktion. I alla tre försöken var uppfödningsperioden 70 dagar. I ett av försöken hölls kycklingarna inomhus i ett kontrollerat kycklingstall under hela uppfödningsperioden. I de andra två försöken hölls kycklingarna i ekologiska kycklingstall där de efter tre veckor fick tillgång till utevistelse dagtid. Miljön i dessa stallar kontrollerades genom naturlig ventilation via öppningar längs stallets långsida och värme tillfördes med hjälp av värmelampor. I ett av utomhusförsöken användes hampa som proteinråvara i syfte att utvärdera dess potentiella fördelar.

Resultat

Resultaten visar både för- och nackdelar med att sänka råprotein- och aminosyranivåerna i foder till snabbväxande slaktkycklingar, och på så vis hålla ner tillväxten. Fördelarna var att dödligheten minskade och att färre kycklingar behövde avlivas för benfel under de sista tre veckorna innan slakt. Kycklingarna som fick foder med låga nivåer av råprotein och aminosyror utnyttjade rasthagarna i mycket högre utsträckning jämfört med de kycklingar som fått högre nivåer. En stor nackdel var att det förekom kannibalism i ett av försöken där kycklingarna fick låga nivåer av protein och aminosyror. Dessutom sågs andra beteendeförändringar såsom högt foderspill, intag av fjädrar från ströbädden, ökad fjäderputsning och ett generellt ökat födosöksbeteende. Dessa beteenden indikerar att kycklingarna var hungriga och sökte efter annan eller kompletterande föda än det foder som erbjöds. Inget utav fodren i försöken innehöll tillräckligt låga nivåer av protein och aminosyror för att sänka tillväxten till 50 g/dag. Detta innebär att ytterligare foderrestriktioner behövs, antingen genom att sänka proteinet ytterligare eller genom att införa måltidsutfodring, för att kycklingen ska kunna definieras som långsamväxande.

Trots att dödligheten sänktes med restriktiv utfodring så var den totala dödligheten hög. I ett av utomhusförsöken var dödligheten hög pga av en plötslig värmebölja, där främst tunga tuppkycklingar dog. Det förekom även en förhöjd dödlighet pga gulsäcksinflammationer under första veckan i det andra utomhusförsöket. Dessa två incidenter poängterar vikten av god ventilation och miljö under uppfödningsperioden.

Det ekologiska försöksfodret med inblandning av hampa resulterade i en blöt ströbädd och sämre fothälsa för kycklingarna. Det var dock svårt att avgöra om dessa resultat helt berodde på hampans egenskaper, eftersom det även fanns en hög inblandning av ärtor i försöksfodret.

Slutsatser

Avhandlingen visar att det finns en välfärdsrisk med att hålla ner tillväxten för snabbväxande slaktkycklingar i en uppfödningsperiod på 70 dagar. En kortsiktig lösning kan vara att uteslutande använda hönkycklingar i produktionen eller att öka foderrestriktionerna ytterligare. Men det måste till mer långsiktiga lösningar för att undvika välfärdsproblemen. Ett alternativ kan vara att korta ner uppfödningstiden och därmed sänka dödligheten och avlivningen pga benfel de sista tre veckorna i produktionsperioden. En annan lösning kan vara att etablera långsamväxande hybrider i Sverige. Detta beror dock på hur begreppet långsamväxande kyckling kommer att definieras och om de hybrider som finns tillgängliga i Sverige idag kan definieras som långsamväxande i framtiden.

Foderproblematiken återstår dock. Om långsamväxande hybrider används, kan andra strategier för att optimera foder tas till. Detta betyder att det ekologiska fodret kan optimeras genom att höja aminosyranivån och därmed höja proteinnivån i fodret. Å andra sidan, en överutfodring av protein kan leda till ökat kväveläckage till naturen vilket strider mot de ekologiska målen. Fler studier behövs för att lösa frågan kring ett 100 % ekologiskt foder 2012.

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