EFFECTS OF DIFFERENT FEEDING STRATEGIES ON FORAGING ABILITY AND NUTRIENT DIGESTIBILITY OF A SLOW GROWING ORGANIC BROILER GENOTYPE

(Internship report)

BY

ADEBOYE OLUWAKEMI RACHEAL

Supervised by:

Sanna Steenfeldt,

Senior Researcher, Department of Animal Science,

Faculty of Science and Technology,

Aarhus University, Denmark.

AARHUS UNIVERSITY

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Abstracts

The organic poultry production in Europe is increasing. The use of organic feed has been seen as one of the major constraint especially the protein sources, and efforts have been made to secure the future of this system. Access to outdoor area by organic chicken has been described as a means to partly compensate for their nutritional needs and the knowledge of feed items selected in the outdoor area can ease 100% transition to organic feed. An experiment was conducted to examine the effects of different feeding strategies on the foraging ability and nutrient digestibility of slow growing organic broilers with or without supplement. A total number of Seven hundred and twenty birds (RedBroja and Hubbard genotypes) have access to three different diets (Control, F1 & F2) as well as forages. The diets used were formulated such that F1 diets had a lower protein and amino acid than the standard control diet. F2 is the mixture of both control and F1 diets. A digestibility trial with 72 broilers selected from the main experiment on the outdoor area, showed that there was significant different ($P<0.05$) in the nitrogen retention between the treatments as higher mean values were recorded for broilers that had access to low protein diet and supplements (grass and chicory leaves). This can probably indicate that these broilers utilize the nutrient in the foraging materials as a means to partially compensate for the lower protein and amino acid contents in their diets. Moreover, broilers with access to low protein diet gained weight comparable to the control birds during the digestibility experiment, which suggest that their health and welfare are not adversely affected during this experiment. Microscopy analysis of excreta were performed to study the feed items selected by broilers and the results indicated a higher intake of plant material by broilers fed diet F1 compare to the Control. Thus, stimulating foraging activity through the use of low protein diet with slow growing genotype could be one of the strategies to achieve or ease the transition to 100% organic feed supply to organic broilers in the future.

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CHAPTER ONE

INTRODUCTION

The organic poultry production has increased in Europe and other part of the world in the last few years but it is still relatively small. This positive tendency is as a result of increased consumer preferences for organic poultry meat that is perceived to be safe under the production system that minimize the use of antibiotics, synthetic fertilizers, pesticides, growth promoters, additives and genetically modified crop, which tend to be associated with potentially adverse health effects. The guidelines for this system were developed in an attempt to elaborate an alternative to conventional production (Sundrum, 2001). This system of poultry production is more integrated, wholesome and environmentally friendly, the production forecast for organic products continue to increase following the trend in consumer demand with willingness to pay more. A key aim of organic farming is environmentally sustainability by enhancing environmental quality and natural resources, make the most efficient use of non-renewable resources and on-farm resource, and integrate where appropriate, natural biological cycles and control. In addition, it promotes biodiversity and soil biological activities (National Organic Standards Board Definition and USA, 1995).

Despite the EU increasing regulation in the production of organic chicken, the potential for its production is expanding. Owing to the leading poultry meat position in the world meat consumption (after pork), it has an important advantage. Poultry represent an important sector in livestock production because of its ability to provide high nutritious protein in terms of eggs and meat; and reach a market weight within a short period of time. It is consumed all over the world and continually receiving an attention on management, welfare and sustainability in order to satisfy both consumers and environmental interest. Apart from supporting quality nutrients in human diets, poultry are often essential for meeting important social and cultural needs and obligations. Furthermore, it aid in reducing poverty and malnutrition in developing country.

The main differences between organic and conventional broiler production relate to housing system, access to outdoor areas, genotype, range of feedstuffs available for dietary use and disease prevention measures. Freedom of movement is an important part of organic system with an access to an outdoor area (at least $4m^2$ per bird). The flock size found in this system is often below the conventional system and the use of mobile house can be the best for smaller flocks which can be moved to a new pasture at regular intervals (Ciczuk & Sjelin 1996, Bassler et al. 1999). Feed, including pasture and forage, must be produced organically and health care treatments must fall within the range of accepted organic practices, the feed is generally more expensive than the conventional feed often resulting in eggs and meat being twice as costly as the conventional product (Blair, 2008). In general, organic food products are typically more expensive than conventional foods, costing at least 10 to 30 percent more (Lohr, 2001). This may be probably due to the fact that more slow growing genotypes live longer, move more and so need more feed, the raw materials used in feed costs more to grow, more labour is needed, and the capital cost per birds are higher because more space is given to each bird. Thus maximum reliance is placed on locally produce feed or farm derived renewable resources to reduce cost.

In conventional system, antimicrobial agents (a general term for drugs, chemicals or other substances which at certain concentration either kill or inhibit the growth of microbe e.g antibacterial and antiviral drug) can be used for treatment, control and prevention of the diseases as well as for improvement of the growth and feed efficiency (Khachatourians, 1998; McEwen and Fedorka-Cray 2002). Organic system on the other hand, has restricted use of antimicrobial substances on the farm (El-Shibiny et al., 2005). Organic poultry production has been subject to a wide range of regulations and code of practices covering production, marketing, health and hygiene, welfare, killing and processing, which are laid down to guide such practice. This usually command high price premium compared to conventional chicken due to high cost of production and standard. The system provide the birds with a number of welfare advantages that birds are denied in the intensive and conventional systems which include sufficient space for exercise; access to daylight and fresh air; opportunity for natural behavior such as foraging, exploration and nesting; reducing the risk of frustration, stress and injuries (e.g foot pad lesions) that can result from a higher bird density in modern stable systems.

The practice in organic farming is guided by rules, principles and aims as formulated in the Basic Standard of the International Federation of Organic Agriculture Movements (IFOAM, 2002) established in 1972, and for the European Union as written in EU Regulation No. 1804/1999 (EC, 1999). It has been focusing on the living condition that permit natural behavior with improved health and welfare, the use of organic feed, selection of suitable genotype that can adapt to local environment. European legislation on organic poultry often undergoes review and necessary update or amendments are carried out in order to maintain its rule. In organic broiler production, the legislation specified that broilers must have access to an outdoor area during part of the growing period (EU, 2007) which is in accordance with high standard animal welfare and the outdoor area made available must be mainly covered with vegetation (EU, 1991). At least 20% of the raw materials used in the feed production must be produced primarily on-farm or in the same region in cooperation with other organic farmers or feed manufacturers. Even though the transition to 100% organic feeding within the EU has been a continuous process since 2005, the time for introduction of 100% organic feeding has been adjourned until January 1, 2015, allowing for 5% non-organic ingredients for another period (EU, 2012).

However, with the aim to increase the production and market share for organic poultry meat by the farmers, the use of organic feed can pose a major constraint and contribute to high cost of production in this system, thus the transition to 100% organic feeding can be difficult due to insufficient supply of organic protein sources since some of the available sources are expensive and most being imported. The increasing reliance on imported protein sources may also expose farmers and feed mills to price fluctuation. As a result of this, owning to the growing demand of organic poultry meat in Europe, there is a risk that organic protein of high quality can be a limited resource within few years, and there is an urgent need to find alternative sources and feeding strategies. In an attempt to rectify this shortfall in the future, efforts are being made to consider alternative protein sources through access to forage in broiler production. Furthermore, with access to nutritious forage and knowledge of different feed items eaten by broilers on attractive outdoor area can be beneficial and increase the level of self-sufficiency in organic broiler production. In a study carried out by Jonsson (2009), it was shown that mussel meal is an excellent alternative protein source in poultry diets comparable with fish meal and more research is still on-going. Besides, the challenge to produce poultry products with a lower global footprint; can ultimately depend on greater integration of poultry production within the whole farm system and making better use of low-impact feed sources such as grasses, insects or worms (Soil Association, 2013).

The use of outdoor run by organic broilers has been described as a mean to partly compensate for imbalances in the feed by foraging (Hermansen et al., 2004). Research studies have been carried out on the use of outdoor area by organic broilers, taking into account their herbage intake with the effects of vegetation type and shelter addition (Rivera-Ferre et al., 2006). Studies have shown that with access to nutritious vegetation in the finishing period, they can increase their forage intake without adversely affecting their health and welfare (Almeida et al., 2012). Moreover, studies on synthetic methionine and feed restriction effects on performance and meat quality of organically reared broiler chickens have been reviewed (Moritz et al., 2005). It is evident that the use of outdoor area and increased foraging by broilers could improve the use of local resources and help organic farmers in 100% transition to organic feed. However, more information regarding the extent at which these birds can utilize the vegetation and insects on an attractive outdoor area is still needed. The objective of this research work is to examine the different feed items selected in excreta from organic broilers on an attractive outdoor area, and to explore possible benefits that foraging can contribute to their nutritional need depending on feeding strategies involving diets differing in protein and amino acid content. Further, a digestibility experiment was performed in order to study the effects of diets different in protein and amino acid, as well as access to different foraging materials, on the retention of nitrogen and digestibility of some nutrients.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Benefits of foraging in organic broilers

Foraging is an important behavioural trait in poultry. When given the opportunity to utilize the outdoor area covered with pasture, organic broilers will learn to forage on young vegetative plant, grasses and live protein sources such as insects and worms; that supply them with additional nutrients which in turn can contribute to improved meat quality (Glatz et al., 2005; Maio et al., 2004). Foraging does not displace a grain-based diet but may aid nutrient balance as it is important for the nutritionist to provide well balanced diets for the chickens. Studies have shown that the inclusion of moderate amounts of different fiber sources in the diet improves the organ development as the gizzard (Gonzalez et al., 2007; Hetland et al., 2005; Hetland et al., 2007), increases HCL, bile acids and enzyme secretions (Shivus, 2011; Hetland et al., 2003). Broilers cannot digest large quantities of fiber because they do not have the enzymes required to digest cellulose and other complex carbohydrates (Sloan and Damron 2003). However, other poultry species such as geese and turkey can obtain added nutrients from forage because they are better able to digest fiber due to larger microbial population in their digestive tracts (Brad et al., 2010).

Poultry access to both grasses and legumes has been proven to be beneficial as they found out that eggs from hen consuming legumes and grasses, contain more omega -3 fatty acids and vitamins than eggs from hens consuming grass alone (Karsten et al., 2003). Also, the use of high protein forages may serve as a means of reducing the feed cost when diets less in protein content are offered to birds without adversely affecting their health and welfare (Almeida et al., 2012). The use of outdoor runs can help the chicken to express a more natural behaviour such as dustbathing and can have positive impact on their health and welfare. The use of pastures may contribute to flavor (Gordon and Charles, 2002) since some forages and herbs may result in distinctive flavour. Thus, increased foraging in organic broilers may be a way to increase the utilization of locally available resources, contributing to nutrient cycling within the system and ease the transition to 100 percent reliance on organic system with reduced environmental impact in terms of nutrient load.

2.1.1 Factors affecting forage consumption

There are several factors that affect a bird's ability to forage including palatability, the plant type/species, the nutritional content, height and stage of growth of the plant, the nutrient content of the diet and the nutrition requirement of the bird. Different genotypes of poultry have different foraging behaviour and consumption rate. Genetics has also been said to play a role in chicken's ability and efficiency in balancing their intake in order to cover their specific nutritional requirement in free choice feeding system (Pousga et al, 2005); and within a flock individuals show a range in their capacity to select their own feed. Poultry are most active during the morning and evening hours particularly before sunset (Dawkins et al., 2003). Danish research has found that laying hen with constant access to forage consumed the most vegetation prior to sunset (Horsted et al., 2007). When birds are not introduced to forage during the growing period, it takes time for a flock of birds to adapt to a new feed on pasture and some producers may give their bird access to chopped forages daily in the rearing period in order to adapt the chickens to more rough materials. Research has shown that feed intake by broilers is positively correlated with age (de Almeida et al., 2012). Moreover, shade/protective cover encourages foraging (Dawkins et al., 2003), most likely from the protective effect of shelters (Riverra-Ferre et al., 2007) and shorter forages are preferred to longer one when given the choice. It is worth considering that forage height usually correlates with palatability, as younger, more succulent plants tend to be shorter and stems are typically much higher in fibre than leaves (Buxton and Redfearn, 1997). Generally, legumes and young, soft grasses are preferred, while forbs and shrub are less attractive. Clovers and alfalfa can be considered forages of high quality due to high protein content (pasture legumes). The specific variety of a plant can affect the amount of grazing a bird does on pasture. For instance, alfalfa varieties high in bitter tannins or saponins are less palatable than varieties with little of these compounds. The tannins can also depress protein digestibility and reduce overall feed intake, which can reduce feed conversion.

2.1.2 Free choice feeding in broilers

Free-choice feeding otherwise known as cafeteria style feeding in poultry offered a selection of different feed ingredients. This method gives the birds the opportunity to select nutrients, particularly protein and energy, according to their physiological demands (Emmans, 1978). The system may offer several advantages associated with feed processing cost such as grinding and mixing by effectively reducing the feed costs (Kiiskinen 1987; Tauson et al., 1991). It also involves the use of whole grain which may be available on-farm, and the system approaches the natural feeding system much more closely than other feeding system which is therefore highly appreciated. The digestive system of birds is capable of processing whole grain and makes it unnecessary to feed a pre-ground diet (Blair et al., 1973), which thus presents energy savings in feed preparation. The principle behind choice feeding is that birds possess some degree of nutritional wisdom which allow selection from the various feed ingredients on offer and construct their own diet according to their actual needs and production capacity.

Rose and Kyriazakis (1991) indicated that when domestic birds are offered a range of different feedstuffs they have the ability to choose a diet which provides them with all the nutrients necessary for growth, maintenance and production. In selection of feed by birds, visual stimulation evidently plays a major role. A choice-feeding system is of particular importance to small poultry producers in developing countries, because it can substantially reduce the cost of feed. The system also offers an effective way of using home-produced grain, such as maize, and by products such as rice bran. When introducing birds to whole grain it is recommended that will it be done gradually over 2-3 weeks to allow development of the gizzard and regular grit should also be provided.

2.2 Genotypes in organic broilers

The use of slow growing genotype instead of fast-growing ones can be beneficial in reducing mortality and health problems. This can be desirable as against the selection for high growth rate and low feed:gain ratio. For instance, the muscular skeletal system of the leg and some other energy supplying organs do not develop in proper relation to perform their function (i.e over-weight), in this case the bird may develop a leg disorder (lameness) which can have negative impact on their health (Decuypere & Verstegen, 1999; Whitehead et al., 2003). Other metabolic disorder associated with fast growing broiler can includes ascites and sudden death syndrome. Although slow growing broilers are less efficient meat producers, they have better livability, are more active and may have differences in meat quality. Some studies have shown that the breast meat of the slow growing broiler type had half the amount of fat compared with fast growing birds, and the outdoor birds had lower fat than the indoors birds due to the additional space provided which increase leanness of meat probably because of the activity (Robertson et al., 1966; Castellini et al., 2002; Lei & Van Beek, 1997). Zollitsch and Baumung (2004) showed that it is easier to raise broilers with adequate essential amino acid in organic farming, because the slowing growing genotypes used have lower requirement for protein than fast growing broilers. 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. Despite this fact, a reduced growth rate of fast growing genotypes has been shown to have beneficial effects on the leg health of those birds, when this genotype is used in organic poultry production. The use of less concentrated, low protein diet and reduced amino acid content, can increase the broilers use of outdoor pasture, their activity and forage intake. In this case, the diet must be formulated in such a way that broiler health and welfare are not jeopardized since skeletal disorders in broilers are associated with their rapid growth (Whitehead, 1997) in order to reduce the culling rate due to leg problems. Also, another alternative way of lowering the growth rate could be the of 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.

2.3 The use of outdoor area by organic broiler

The uses of outdoor area by broilers enable them to roam freely during the day and are usually confined in a shed at night to help protect them from both predators and reduce the risk of environmental hazards (such as heat and cold stress, wet and windy weather, muddy or dirty condition). One advantage in the use of outdoor area lies on the opportunity to select other nutrients through forage intake apart from the feed provided which can also come along with picking live organisms. Early access to pasture can increase the range usage because chicken get familiar with it (Adas, 2002). Feed searching, soil scratching, pecking and dustbathing can be noticed among other behaviors. Pasture birds also have more access to adequate space, fresh air, sunshine, exercise and stimulation in 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). Poultry may obtain small amounts of energy from pasture and have the ability to utilize amino acid, such as methionine, lysine and threonine found in the forage (Buchanan et al., 2007), but the utilization of nutrients from pasture intake also depends on the quality of the outdoor pasture (Rivera-Ferre et al., 2007).

Ruis et al. (2004) concluded from their study that an outdoor run potentially improves the welfare of broilers; they found that natural light might not guarantee a better welfare as such but probably the quality and intensity of lighting is of importance. In terms of genotypes, slow growing breeds have been found to spend more time foraging, walking, perching while fast growing broiler spent time eating, sitting and drinking (Bokkers and Koene, 2003). Chickens in the outdoor area are more vulnerable to predations; hence they need to be protected from both day time and nocturnal predators. To reduce daytime risk from dogs and foxes, electric net fencing can be used around open pasture pen. Other predators control include moving the pen often so predators remain wary, keeping birds close to the house and having larger grazing animals, such as cattle, pigs or sheep close to the pens. Protecting chicken from aerial predators, such as hawks and eagles is more difficult. However, varying degrees of success have been found with fake owls, alarm system, hanging reflective tapes or CDs etc. Their movement may be restricted with fence on range but survival instinct can prevent chicken from going far away from the shelter providing housing. Darwin et al (2003) found that within their paddocks, chickens either stayed close to the house or sought tree cover. Also, addition of shelter can encourage broiler to stay much longer in the outdoor areas than if the plots were unsheltered which can in turn contribute to even distribution of excreta over the plot (Rivera-Ferre et al., 2006).

2.4 Pasture rotation

Chickens with access to pasture ingest many vital nutrients from grazing weeds, weed seeds, legumes, grasses, insects and worms. Unlike ruminants, chickens lack a multi-compact-mented stomach and cannot efficiently digest cellulose therefore chicken cannot live on pasture alone. A consistent rotation maintains clean condition for the pasture; it is the key to keeping forage young and vegetative in pasture poultry production (Fanatico, 2007). One crucial factor to consider is the even distribution of nutrients that result from moving birds on a regular schedule. Rotating pastures allows grass a resting period which will in turn facilitate grass re-growth; this will ensure forage growth throughout the pastures and prevent bare spots from forming that eventually lead to weed and disease problem. Adequate soil fertility is required for good forage production and soil analysis test can be carried out for new pastures.

Planting diverse forages that improve soil quality by fixing nitrogen or adding organic matter is beneficial. Legumes are desirable forages to have in the pastures because they are high in nutritive quality. The symbiotic association between legumes and rhizobia can provide substantial amounts of nitrogen to plant and soils, which reduces the need for industrial fertilizers (Ledgard and Steele 1992; Vance, 1997). Plants such as red or white clover can typically fix about 100kg N/ha per year (Havlin et al, 1999). For best establishment and stand persistence, soil pH and fertility levels should be medium to high before planting pasture legumes.

2.5 Alternative protein sources in organic broiler feed

There has been some interest in the introduction of novel organic sources of protein from both plants and animals. Animal protein such as fly pupae, larvae meal, earthworm meal or mussel meal could be valuable alternatives to fishmeal, which will be a limited source in the future. The current cost of producing them is high so they are not able to compete with typical organic poultry diet. With regard to alternative plant sources, it could include rapeseed, peas and beans, lupins, hemp seed and cottonseed meal etc. However, one of the disadvantages is that some of these have anti-nutritional factors which can limit their use in poultry diet but they represent a very good source of protein.

2.5.1 Animal Protein

Insects

The use of insects as an alternative source of protein in animal feed is becoming more globally appealing. The proposed action was made as a result of high cost of animal protein sources being used presently and partly due to increasing growing human population in order to combat severe challenges on the global capacity to supply enough feed. However, EU law currently prohibits including protein derived from insects in animal feed. The promising species of insect identified are the Black soldier fly (Hermatica illucens), Common housefly (Musca domestica), and Yellow mealworm (Tenebrio molitor), these species receive increasing attention because potentially they can valorize organic waste. Insects contain between 30% and 70% on a dry matter basis (Veldkamp et al., 2012). Table 1 shows protein and fat composition of larvae for three insect species in comparison to fishmeal and soybean meal.

Table 1: Crude protein and fat content (dry matter basis) of larvae of three insect species compared to fish meal and (defatted) soyabean meal.

Protein source	**Crude protein (%)**	**Crude fat (%)**
Hermetia illucens (Black soldier fly)	35-57	35
Musca domestica (Common housefly)	43-68	4-32
Tenebrio molitor (Yellow mealworm)	44-69	23-47
Fishmeal*	61-77	11-17
Soybean meal (defatted)	49-56	3

*CVB (2007)

The larvae of Black soldier fly contain a relatively high amount of protein and fat, which make them a suitable source of feed for livestock. As a component of a complete diet, they have been found to support good growth of chickens, swine (Newton, 1977), and also of several commercial fish species (St-Hilaire et al., 2007; Newton et al, 2005; Sheppard et al., 2008). Apart from this, they can potentially reduce manure pollution up to 50-60%, and reduce harmful bacteria and housefly populations. Housefly larvae (maggots) can also provide an excellent source of animal protein for poultry. Maggots contain high protein content and could alleviate the environmental problem of manure accumulation and reduce harmful bacteria.

A study conducted by Awoniyi et al, (2003) reported an efficient average weekly weight gain and protein efficiency ratio when diets with 25% fishmeal protein were replaced with maggot meal protein. Other research studies have also confirmed successful replacement of fishmeal with maggot meal (Hwangbo et al., 2009). An experiment with mealworm grown on low-nutritive waste products and fed to broiler chickens showed that the mealworms were able to transform the low nutritive waste products to a high protein diet (Ramos-Elordoy et al., 2002). The chitin contained in the hard outer shell of insect is difficult to digest by domestic poultry, although the high chitin content of insect meals does not appear to have detrimental effects on poultry performance (Ravindran and Blair, 1993). The uses of insects need to be further processed in order to get it into a form in which they are usable in the feed industry. There is a need for evaluation of nutrient digestibility of (processed) insects as feed ingredient at different stages of growth; and potential beneficial functional properties of insect protein need to be further investigated in order to create an added value for insect protein.

Earthworm

Numerous macro-organisms can be found on healthy pasture soils out of which earthworms represent an important nutrient cycling and protein source. Earthworms are palatable to poultry, they are very active in converting plants and animal waste into biomass that can be used as a feed ingredient in animal production; and represent a logical source of protein in chicken feed (Fisher, 1988). An earthworm population in a pasture depends on a number of factors (Curry, 1998) and can be raised in large scale (Vermiculture). As a supplement, earthworm has been found to equal or surpass fish meal and meat meal as an animal protein source for protein (Harwood and Sabine, 1978; Toboga, 1980; Mekada et al., 1979; and Jin-you et al., 1982). A study by Prayogi (2011) showed that 10% earthworm meal could replace a large portion of the fish meal in the diet with no adverse effect on body weight gain or feed efficiency, but feed intake was reduced at 15% inclusion level. It can replace fishmeal in chick and layer diets but care must be taken to balance the dietary calcium and phosphorus contents, since these minerals are low in earthworms due to absence of an exoskeleton. Moreover heavy metals and other pollutants are taken up by the worms and can be passed to the birds consuming the earthworms (Sharma et al., 2005), hence there is need to take caution when growing earthworms for use in poultry feed.

Mussel

Mussel meal could be used as a high quality protein source in poultry diets and it could be part of the solution needed to fulfill the protein requirement in organic diet. Mussels have a high content of protein with an amino acid pattern similar to fishmeal (Jonsson & Elwinger, 2009; Berge & Austreng, 1989). Mussels are filter feeders feeding on phytoplankton and organic materials, and under favourable conditions one mussel can filter 2-3 litres of water per hour (Lindahl et al., 2005). This means that mussels during the growth period have the capability to filter large volumes of coastal water. In contrast to fish farms where feed is added, the mussels utilize nutrients in the water through algae and plankton. Studies carried out for both broiler and layer chickens have shown that mussel may replace fishmeal in organic diets, the inclusion of mussels in both broiler and layer diets did not affect production performance and plumage condition was improved (Jonsson, 2009). However, it is important that all sites intended for mussel farms are subject to a thorough risk assessment concerning the hygienic quality of the water (Hernroth et al., 2002). Mussels can accumulate; concentrate different pathogens such as bacteria and viruses and some different algae toxins. Thus the potential risk for consumption of mussels is dependent on the occurrence and composition of these pathogens, and toxins in the areas where the mussel farms are located (Rehnstam-Holm & Hernroth, 2005).

2.5.2 Plant protein

The need to meet protein requirement especially the sulphur amino acids is an important factor in organic poultry production as deficiency of methionine can affect the growth rate in broiler chickens (Bunchasak, 2009). This problem can be partially solved in the conventional system where addition of pure (synthetic) amino acid is allowed but not for organic system. Most plant protein sources available in poultry feed includes soybean meal, lupins, sunflower, rapeseed, peas etc. Currently, soybean meal has been a dominant protein source in poultry feed, it is extensively used because of its high protein and energy content, its high availability of amino acid (AA), quality and composition (Stein et al., 2008). There has been an increasing reliance on imported protein (Merry et al., 2001) in Europe particularly soybean which increases farmers and feed mills exposure to price fluctuations, currency movements and supply shortages. The need for new sources of high quality protein supplement in organic poultry feed is of paramount interest as other conventional protein sources are prohibited in this system. In an attempt to solve the protein feed dilemma in the future and to ease 100% transition to organic feed, some new protein sources with high prospects have been identified.

Hemp seed

The seed of hemp and its derived products are commonly described as rich sources of protein and amino acids important to poultry (Odani & Odani, 1998; Callaway, 2004; Wang *et al.* 2008). Whole hempseeds contain approximately 25% proteins, 31% fats, 34% carbohydrates and 75-80% polyunsaturated fatty acids, in addition to vitamins and minerals (Darshan and Rudolph, 2000; Leizer *et al.,* 2000). A direct comparison of amino acids profile showed that hempseed protein is comparable to those from egg white and soybeans in quality (Callaway, 2004), the fact that trypsin inhibitory substances are absent in hemp protein (Odani and Odani, 1998) partially explains its superiority over soybeans. Rifat Ullah Khan et al., (2009) reported a positive effect on carcass quality of broiler chicks when a hemp seed powder was added to the feed at a rate of 20%.

Lupin, pea and beans

Other legumes such as lupins, peas and beans also provide a good source of home grown protein. Lupins tend to be deficient in sulphur containing amino acids and are also low in lysine (Haq, 1993), this means that it can only be supplements to soybean meal but cannot replace it totally unless valuable animal protein such as mussel meal are included in the diets. The relative importance of dry peas as an alternative to soybeans in animal nutrition has been investigated (Hedley, 2001). Pea seeds are a rich source of protein, carbohydrates, fibre, vitamins and minerals (Hedley, 2001; Paul and Southgate, 1988). However, they contain anti nutritional factors which include α-Galactosides, trypsin inhibitors and phytates which differ widely in concentration among the different varieties of peas (Adsule and Kadam, 1989; Paul and Southgate, 1988).

Sainfoin seed

Sainfoin is a unique forage legume, high yielding and drought resistance which thrive on alkaline soils. The plant is a natural anthelmintic and fodder produced is highly nutritious and bloat free. A feeding trial involving the use of Sainfoin seeds (Onobrychis viciifolia) as a protein source for weaned piglets has been investigated (Baldinger et al., 2012), the results of the experiment showed that neither feed intake and body weight gain nor feed conversion ratio differed between treatments. This suggests that Sainfoin seeds could be a promising protein source for pigs and other monogastrics in the future.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Experimental birds and Housing

The experiment was performed at The Department of Animal Science (Foulum), The Faculty of Science and Technology, Aarhus University, Denmark. A total number of seven hundred and twenty broilers of both sexes were raised from day old consisting of two different slow growing genotypes (RedBroja & Red Hubbard, 1657 genotypes). The I657 is considered to be the more slow growing type. After hatching, the chicks were reared indoor for four weeks, weighed in groups and distributed by wing numbers. The temperature, humidity and lighting program used were standard. An organic starter diet was provided from day-old and when the birds were a week old, corn silage was offered as this is important for the development of gastro-intestinal tract and thereby utilization of nutrients from foraging in the outdoor area. There was continuous allocation of whole wheat to the chickens in a separate silo. At 4 weeks old the chicks were weighed and moved to the outdoor facilities and randomly allocated to 18 plots (9 plots each for both genotypes) with access to pasture on each plot, where the pasture field consisted of grasses, herbs, legumes. A small house was placed on each plot, where feeding troughs and water equipment was placed. Perches were installed inside the house according to the legislation. When the chicks were moved to the cottage at the outdoor area, they were fed directly with the experimental diets. During the experimental period, the weight of the chickens was taken every two weeks including the feed offered as well as the return weight. The experiment lasted until the broilers reached the age of 14 weeks middle September. The experiment was done with compliance with the Danish regulation for organic broilers and there was daily supervision during this period.

3.2 Experimental diets

Six experimental treatments were allocated at random to the 18 floor pens to provide 3 replicates using 3 different feeding strategies i.e for each genotype given a total of 6 treatments. The treatments includes the control diet (C) consisting of standard organic broiler feed diluted with 10% whole wheat, the F1 test feed diluted with 10% whole wheat and F2 diet (mixture of control and F1 well mixed in the bin and diluted with 10% whole wheat). Diet F1 was formulated to have a much lower content of protein and amino acids as contrast to the control. The composition of the control and F1 diet used in the experiment is shown in table 2. It can be seen that the protein and amino acid content of the two diets differed as the protein content in diet C was formulated to be 19.8% and in F1 only 13.9%, which is reflected in the content of amino acids.

In addition to the experimental diets, the chicks had access to forage as grass, herbs and other plant material and insects on the outdoor area. Sample of manure were collected during four days in the last 2 weeks of the experiment from the pasture from both genotypes and stored in a freezer at -20^{o}C for further analysis.

Table 2 showing composition of the diet C and F1 in percentage

	C:Control	F1
Ingredients	Diet (%)	Diets (%)
Wheat	30,39	44,13
Soya cake	21	
Oats	15	15
Corn	13	18
Peas	12	14
Toasted Soyabean	4	4
Calcium carbonate	1,22	1,3
Monocalcium Phosphate	1,22	1,35
Rape cake hea	1	1
DLG feathers Vit	0,4	0,4
Raw danrapsolie	0,3	0,3
Sodium bicarbonate	0,22	0,27
Rock salt	0,2	0,2
Choline chloride	0,04	0,04
Roxazyme G2G	0,01	0,01
Calculated constituents		
Crude protein	19,8	13,9
Crude fat	5,8	3,7
Fiber	4,6	3,8
Crude ash	5,7	4,7
Lysine (g/kg)	10	6,3
methionine (g/kg)	2,9	2,1
Cystine (g/kg)	3,6	3
Calicum (g/kg)	9,5	9,5
Phosphorus (g7kg)	6,7	6,4
Sodium (g/kg)	1,5	1,7

*The vitamin and premix added per kg of diet: Cu 15mg, Zn 80mg, Fe 60mg, Calcium iodate 0.45mg, Mn 80mg, Na 0.2mg, vitamin A 12000mg, vitamin E 25mg, vitamin B1 4mg, vitamin B12 0.01mg, vitamin B2 5mg, choline chloride 200mg, vitamin D3 3000 I.E, (Control: Endo-1,4-betaglucanase 2600 enh & Endo-1,4-beta-xylanase

3.3 Digestibility experiment

At the age of 14 weeks, where the main experiment ended, a digestibility experiment was performed indoor in battery cages with raised floors, each of the three batteries contained 12 cages (50cm x 50cm x 50cm) with feeding troughs outside and two water cups inside. Two feeding troughs were placed in front of each cage for both the compound feed and forage materials. Pelleted feed and supplements were weighed separately. A total number of seventy-two broilers of the 1657 (Hubbard) genotype were used and two hens each were allocated to cages from the control C and

F1 treatments. The birds were selected at random from the whole group at the different plots at the outdoor area representing the two treatments. Six experimental treatments were included in the digestibility trial (F1, F1+ grass, F1+chicory, C, C + grass, C +chicory) with six replicates, which were assigned at random, each treatment being represented in each battery and on each tier. The diet was a combination of pelleted feed and whole wheat (10%), the same as used on the outdoor experiment. Feed consumption was recorded as well as the initial and final weight of the feed leftovers. Feed and water were supplied ad libitum; the initial and final weight of the birds was recorded. The forage materials (grass and chicory) were given fresh in small portions twice daily with adaptation period of 7days, after which excreta samples were collected. Excreta were collected on three consecutive days and stored in the freezer immediately to prevent microbial degradation.

3.4 Selection of food items and estimation of the relative area distribution of food items in excreta

3.4.1. Excreta samples from the outdoor area.

A large number of excreta samples (separate droppings) were collected from all 18 plots on the outdoor area in order to provide information about the different feed items selected by the birds from the 6 treatments. Due to time constraint, samples collected during two days (between 10 and 15 hours) from the 9 plots with the genotype I657, was selected for microscopy analyses and presented in this report. Each dropping was studied separately to estimate the percentage composition by fragment area of food items eaten by the birds. Each dropping was placed on a filter paper (in a funnel) and broken up in water to make the sample more homogenous. When the water had sieved through the filter the material settled evenly on the surface of the filter. Each sample (dropping) was divided in two identical subsamples before microscopy in order to estimate the relative areas as correct as possible. An average was taken of the two subsamples. The filter paper was placed upon an 80-85 mm diameter transparent plastic Petri dish. The bottom of the Petri dish was divided in a number of squares in order to estimate the relative area in percentage of each food type. Any remnants of food items were identified and the relative areas (%) of fragments of feed, wheat, leaves, insect parts (mandibles, scales etc) were estimated under a binocular microscope at 12 x 25 X magnification. The different kind of food items were divided in 5 groups: 1) Diet 2) grass (monocotyledonous), 3) chicory (dicotyledonous) 4) clover (Monocotyledons), 5) others. Plant reference material was collected in order to identify different plant cell structure characteristic for mono- and dicotyledonous, respectively.

3.4.2. Preference study

After the digestibility experiment in the battery cages, a second experiment was performed with the same birds over two days in order to study the effect of giving the birds either grass (representative of monocotyledonous), chicory leaves (representative of dicotyledonous) and meal worm larvae (representative of insects) together with diet F1. The aim of the study was to measure the amount of feed selected by the birds (diet F1 and the supplements) and collect excreta for microscopy analysis in order to estimate the relative area distribution of food items, when the intake is known. The hens fed diet F1 without forage supplement in the digestibility study were given the meal worm larvae. The control diet C was not included in this study. Any remnants of food items were identified and the relative areas (%) of fragments of feed, wheat, leaves, insect parts (mandibles, scales etc) were estimated under a binocular microscope at 12 x 25 X magnification using the same method as described under 3.4.1.Diet F1 was considered as the reference food.

3.5 Statistical Analysis

The experimental layout was Completely Randomized Design arrangement. Data obtained was subject to analysis of variance using SAS (1999) and the significance means among variables were separated using Duncan multiple range test (Duncan, 1995) at confidence level of 5%.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. Digestibility experiment.

In the present study the digestibility of different nutrients were determined by accurately measuring the feed and forage intake and excreta output by the total collection method. Apparent digestibility coefficients were calculated according to the analysed contents of nutrients (% DM) in feed (diet + forage, analysed separately) and in excreta, taking into account the amount of feed eaten and excreta voided on a dry matter basis, using the general formula given by Scott et al. (2001). Digestibility is the proportion of a dietary component (X) in a given feed that is digested and absorbed in the gastro-intestinal tract.

Apparent digestibility coefficient DC is calculated as (in percentage), example. x = fat:

$$DC = \frac{((\text{Feed intake X \% diet fat})-(\text{Excreta output X \% excreta fat}))}{(\text{Feed intake X \% diet fat})} \text{ X } 100$$

The results from the digestibility experiment are presented in Table 3. Variation was observed between dietary treatments analyzed as higher nitrogen retention was recorded for all F1 diets with access to supplement (Chicory and grass) which is significantly different (P<0.05) from the control diets. This may probably indicate that the birds with access to low protein diets have opportunity to utilize the nutrients found in the forage as a means to partly compensate for the lower protein content in their diets. These data also suggest that organic broilers may obtain small amount of protein and amino acids from forage when low protein diets are fed. This result was in accordance with other studies which found that broilers can have up to 5-8g of forage intake per day (De Almedia et al., 2012) with restricted supplementary feeding in the finishing period. It can be considered if the protein content in the control diets were above the requirement. The amino acid digestibility contents measured showed no significant difference except for cysteine which differs significantly (P<0.05) between some of the treatments and where the highest values were seen with the F1 diets. Variation was observed in the mean values between the two diets for organic matter but not within the treatments as control diets showed more significant difference than the F1 diets.

Table 3 Coefficients of total tract apparent digestibility and nitrogen retention in organic broilers fed diets with or without supplements of either grass or chicory leaves

	C	C + grass	C + Chicory	F1	F1 + grass	F1 + Chicory	SEM[2]	P-value
Organic matter	69.54^{bc}	70.25^{b}	68.28^{c}	76.05^{a}	76.13^{a}	76.47^{a}	0.63	0.0001
Fat	85.27^{a}	85.27^{a}	84.37^{a}	84.81^{a}	82.88^{a}	80.34^{b}	0.44	0.0071
<u>Amino acids:</u>								
Methionine	86.40	87.27	85.66	86.89	85.61	85.18	0.34	NS
Cystine	74.98^{abc}	73.85^{bc}	72.11^{c}	77.11^{a}	76.59^{ab}	75.67^{ab}	0.46	0.0094
Lysine	82.37	83.56	81.67	83.45	83.17	83.55	0.34	NS
Threonine	77.85	78.82	75.30	77.63	77.74	77.74	0.42	NS
SUM AA[1]	80.28	80.87	78.69	80.98	80.87	80.60	0.34	NS
N-retention	32.64^{d}	35.62^{cd}	33.95^{cd}	38.78^{bc}	41.90^{ab}	45.48^{a}	1.04	0.0002
DM in excreta	23.56	22.58	22.79	25.03	25.66	23.74	0.43	NS

a,b,c,d means in the same row with different superscripts differ significantly (P<0.05).

[1]SUM met, cys, lys, thr, Standard error of mean

Table 4 shows the chemical composition of the foraging materials (grass and chicory) used in this experiment. It can be seen that the protein content are relatively low compared to the diets, however, on a dry matter basis the methionine content in the forage material has a level, which could contribute to the nutritional needs of the chickens to some extent, dependent on the daily intake of the plant material. The analyses of the two diets showed that the protein content was higher than expected from the calculated content (Table 3), being 20,4% (C) and 14.98% (F1), respectively, on an as is basis. However, the protein content in F1 was still low. The methionine and cysteine content on the other hand was close to the calculated values.

Table 4 Chemical composition (% DM, amino acids: kg DM) of diets F1 and C and foraging material grass and chicory leaves

Constituent	F1	C	Wheat	Grass	Chicory
Dry matter	89.01	89.71	87.82	19.87	13.53
Ash	5.16	5.80	1.56	9.88	13.03
Protein (N*6.25)	16.84	22.75	11.91	20.72	21.81
Fat	4.15	5.01	2.42	4.58	2.88
<u>Amino acids:</u>					
Methionine	2.37	3.06	1.77	3.24	3.87
Cystine	3.27	4.02	2.68	2.11	1.81
Lysine	6.77	9.97	3.29	10.08	11.98
Threonine	5.27	7.30	3.30	8.26	9.29

During this digestibility experiment, there was no record of mortality and the weight gain presented in Table 5 shows that broilers with access to low protein diet are not adversely affected as there was no weight loss during the 7 day experiment.

Table 5: Weights (g) and weight gain (g) (7days) and intake of diets and grass chicory leaves g/bird/day

	C	C + grass	C + Chicory	F1	F1 + grass	F1 + Chicory	SEM^2	P-value
Initial weight	2615	2530	2671	2571	2548	2493	24.4	NS
Final weight	2854^{ab}	2752^{b}	2976^{a}	2778^{b}	2743^{b}	2737^{b}	26.2	0.04
Weight gain	238	222	306	207	195	244	13.5	NS
<u>Feed intake:</u>								
Diet (D)	161	147	161	149	150	148	2.89	NS
Forage (F)	-	25^{b}	42^{a}	-	31^{ab}	45^{a}	2.99	0.05
SUM D+F	161^{cd}	172^{bcd}	203^{a}	149^{d}	181^{abc}	193^{ab}	4.20	0.0002
Forage, % of total intake	-	15^{b}	21^{ab}	-	17^{ab}	23^{a}	1.75	0.02

a,b,c,d means in the same row with different superscripts differ significantly (P<0.05).

The birds gained weight on average 36.5g/b/d for C groups and on average 30.8g/b/d for the F1 groups and the highest gain within each group was seen when giving chicory as supplement. No significant difference was found with regard to feed intake, however, the intake of C and C+ chicory was numerical higher than for the other groups. The positive effect of especially chicory intake on gain, even not significant different, indicates that the chicory contributes with some nutrients to the birds. The grass intake observed from both diets was lower compared to the chicory intake, indicating a preference for chicory being significant within the C treatment.

4.2 Feed selection study.

The results from the microscopy analysis of excreta samples collected on two different days during the last 2 weeks of the main experiment are shown in figure 1 and 2 respectively. The results per treatment are given as percentage by fragment area of the different food items, summed to give a single mean for each of the 5 groups.

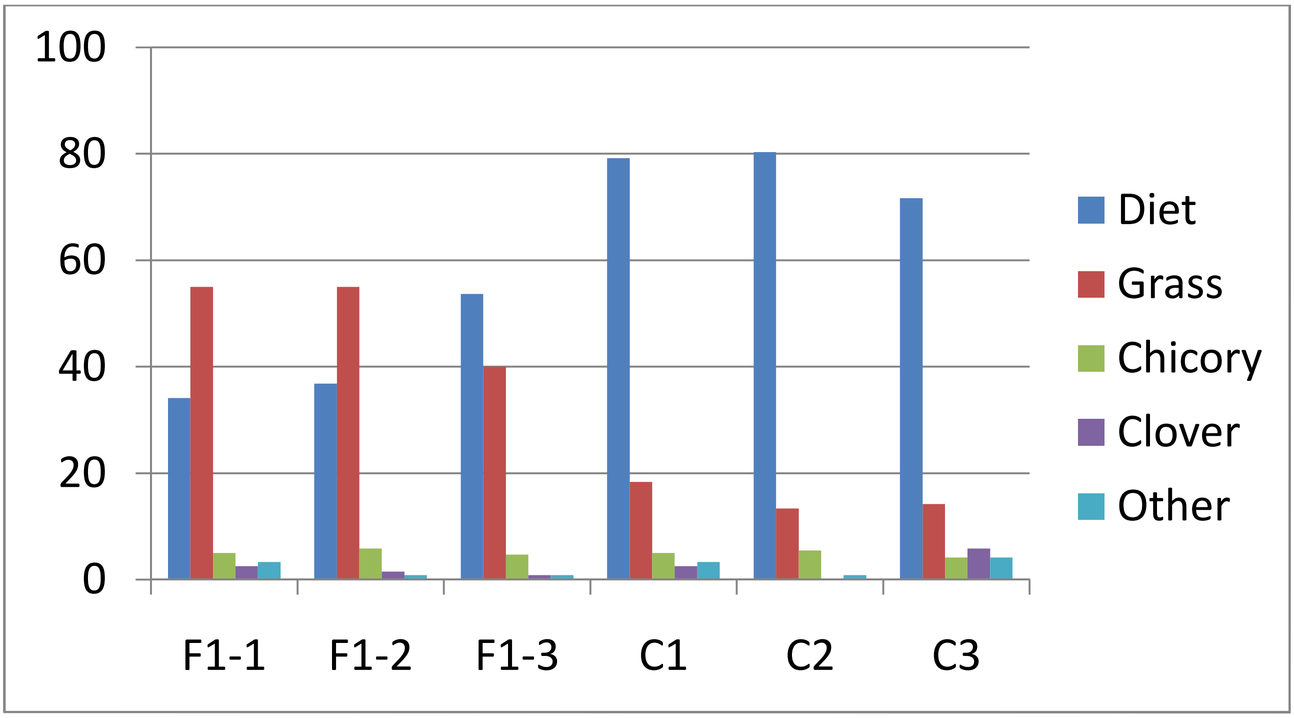

Figure 1. The percentage composition by fragment area of food items in excreta collected from broilers foraging on outdoor area on day 1. F1=diet low in protein, C=control standard organic diet with normal protein.

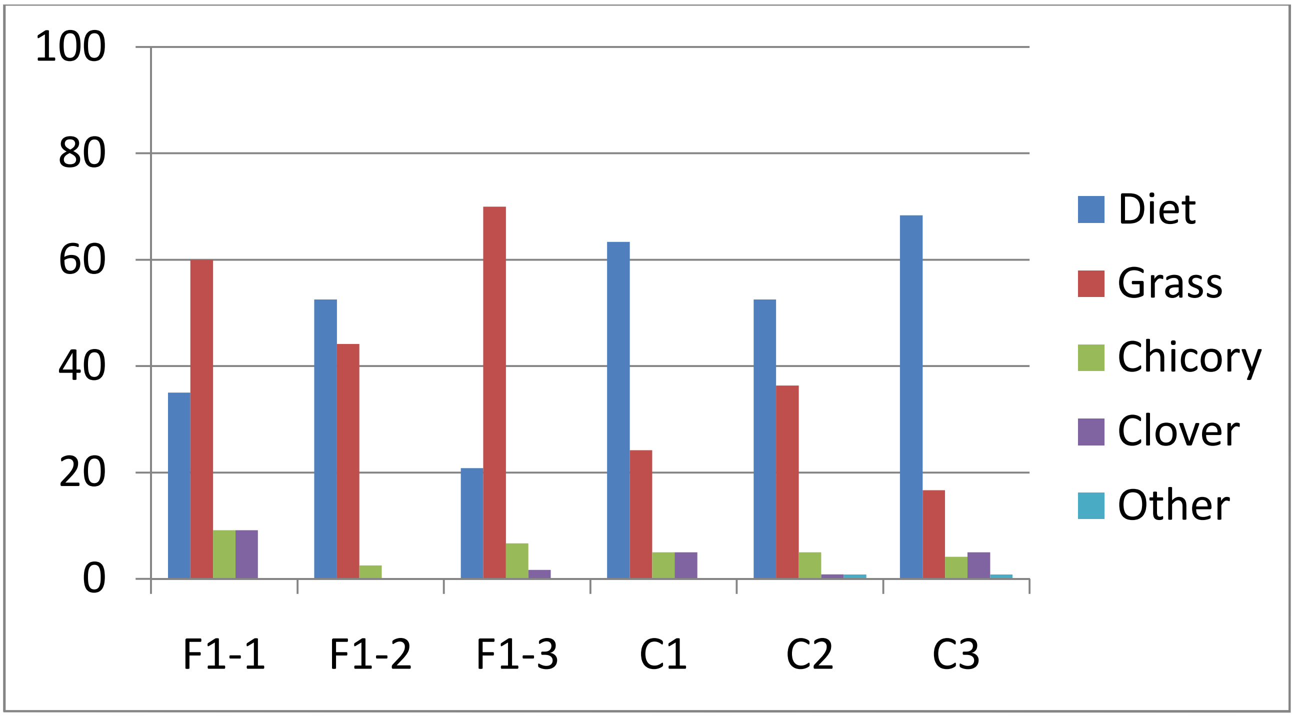

Figure 2. The percentage composition by fragment area of food items in excreta collected from broilers foraging on outdoor area on day 2 F1=diet low in protein, C=control standard organic diet with normal protein.

Comparing the groups given F1 and C diets, respectively, there is a clear difference as a much higher content of plant material, especially grass was found in excreta from birds on diet F1, whereas the opposite was the case with the C diet, where plant material constituted a much smaller parts of the calculated area. These results indicate that the I657 broilers given the low protein diet were foraging more compared to I657 broilers given the more optimal diet, probably in order to have some contribution of nutrients from plant material. The outdoor plots were covered mainly with grass in September where the samples were taken, whereas only small numbers of chicory was present.   There were differences in results between plots (replicates) with F1, since the contribution of diet was higher than plant material in one plot on both collection days. Only very few insect remnant was found, which could be due to the season being late summer.

The results from the preference trial showed a higher preference for chicory compared to grass confirming the results from the digestibility experiment. The intake of mealworms was very high as seen in table 6. The protein content of mealworms is relatively high and can be considered as a valuable protein sources. According to Veldkamp et al (2012), the protein content can vary from 44-69% DM. The chemical analysis of mealworm has not been finished in time to be included in the report. The meal worm larvae were given as representatives for insects.

Table 6. Intake (g/bird) of diet F1, grass, chicory and mealworm

Treatments	F1+grass	F1+ chicory	F1 + mealworms
Intake, as is basis:			
Diet F1	63.8	65.9	32.2
Grass	14.9	-	-
Chicory	-	23,6	-
Mealworm	-	-	71.1
Supplements, % of total intake	19	26	69
Intake, DM basis:			
Diet F1	56.7	58.6	28.6
Grass	3.0	-	-
Chicory	-	2.0	-
Mealworm	-	-	21.3
Supplements, % of total intake	5	3	43

The results from the microscopy analysis of excreta samples collected in the preference study are shown in figure 3.

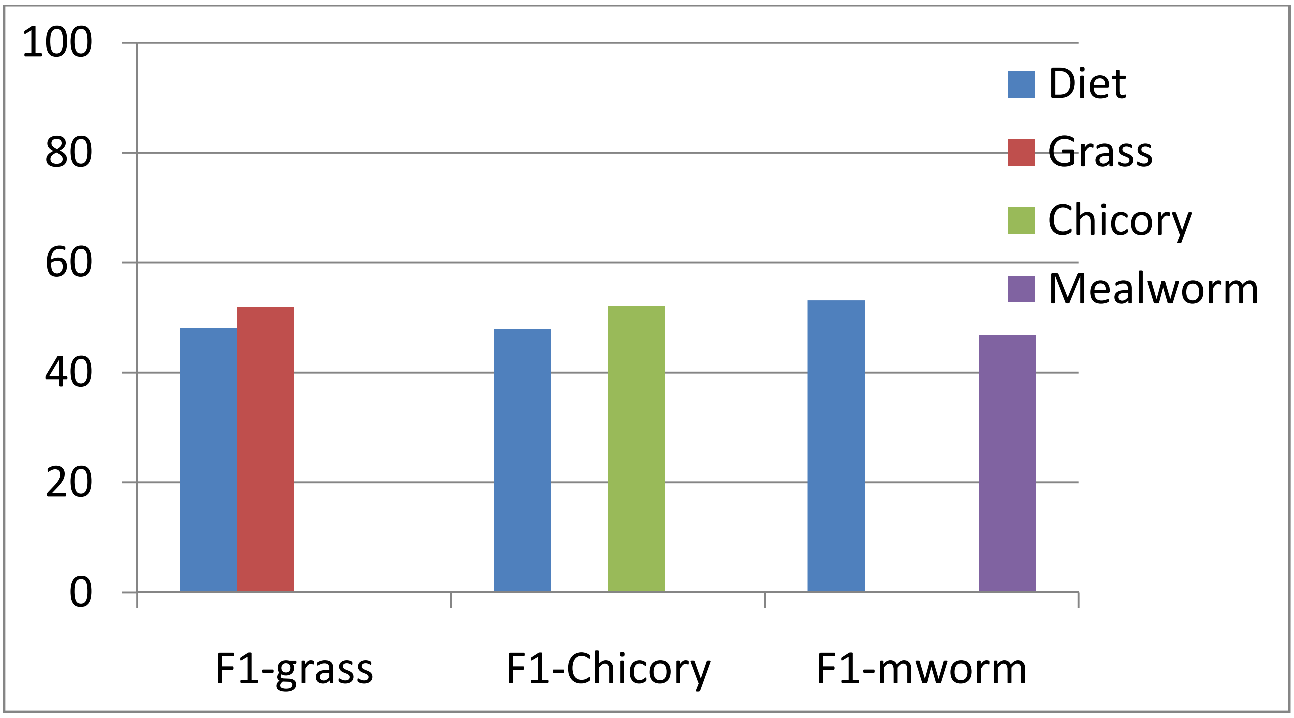

Figure 3. The percentage composition by fragment area of food items in excreta collected from broilers in the preference experiment with diet F1 + supplements.

The results per treatment are given as percentage by fragment area of the different food items, summed to give a single mean for each of the 4 groups. It can be seen that the percentage of both grass and chicory constitute are much larger estimated area in excreta compared to the actual intake, whereas the meal worm remnants identified in the excreta constitute a relatively smaller part compared to the actually intake of diet and meal worm larva, respectively. These results indicate that the plant material is digested to a smaller extent compared to the diet. Comparing with the results obtained with excreta from broilers going on the outdoor area, the relative intake of plant material compared to the diets have been smaller than found from the microscopy analyses of excreta (figure 1 and 2). However, since this is the case with both diets, the results still indicate a different feeding behavior between the two treatment diets F1 and C, where the broilers with F1 have a higher intake of different foraging material.

According to Green (1978) it is possible to find correction factors for each food item, which can be used to calculate the intake of different food items from outdoor area as the digestibility of different plant material and insect are expected to be different.

For each food type a correction factor can be calculated as follows:

$$\mathbf{C_{AB} = W_A FFA_B / W_B FFA_A} \qquad \mathbf{(1)}$$

C_{AB} is the correction factor for food A relative to food B (diet F1). W_A and W_B are the dry weights of the two foods ingested in the experiment. FFA_A and FFA_B their respective faecal fragment area in the resulting excreta. Large values of C indicate food items which leave small areas of identifiable material in excreta relative to the dry weight eaten. The correction factors for grass, chicory and mealworm relative to diet F1 were 0.05, 0.09 and 0.71, respectively in the present study, which is in line with the description given by Green (1978). The correction factors could be used to estimate the actual intake of different feeding items from the outdoor area, but it would be too comprehensive to include in the present report.

5. CONCLUSION

The experiment showed that broilers with less protein in their diets strive to compensate for additional nutrients through the supplement. Hence, the use of low protein diets in organic broilers production can enable the slow growing broiler to explore or utilize the nutrients in the forage on the attractive outdoor area which confirm our hypothesis. This will not only help to secure the future of this system but will prevent excess nitrogen excretion by the chickens, different feed items selected from the outdoor area can contribute to the nutritional need of the broiler especially when given access to a flourish pasture without jeopardizing their health and welfare. Thus, stimulating foraging activity through the use of low protein diet with slow growing genotype could be one of the strategies to achieve or ease the transition to 100% organic feed supply to organic broilers in the future. The estimation of selected feed items from outdoor areas by excreta analysis could be a useful method however, the results should be supplied by analysis of crop content taken the same day as the collection of excreta to evaluate data from excreta analysis.

Appendix: REFERENCES

Adas (2002) Extensive table bird production - health and parasite status monitoring. Final report to Defra on Project AW0221

Adsule RN and Kadam SS, Proteins, in Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization, Vol II, Ed by Salunkhe DK and Kadam SS, CRC Press, Boca Raton, FL, pp 75–97 (1989).

Awoniyi, T. A. M., V. A. Aletor, et al. (2003). "Performance of broiler-chickens fed on maggot meal in place of fishmeal." International Journal of Poultry Science 2(4): 271-274.

Baldinger, L., Werner H., Ulrike S., MAarlene M., Werner Z. (2012). Sainfoin seeds as protein source for weaned piglets – a new utilization of a long-known forage legume. Proceedings of the 2nd OAHC, Hamburg/Trenthorst,

Bassler A, Ciszuk P, Sjelin K. Management of laying hens in mobile houses – a review of experiences. Proceedings NJF Seminar No 303, ‘Ecological animal husbandry in the Nordic countries’, Horsens, Denmark, Sept. 16-17th 1999. Eds Hermansen JE, Lund V & Thuen E, DARCOF Report 2/2000, pp 45-50.

Blair, R. (2008). Nutrition and Feeding in Organic Poultry pp1.

Brad Burbaugh, Elena Toro, and Abel Gernat. (2010). Maximizing Foraging Behaviour. University of Florida, IFAS Extension. Pg 1. http://edis.ifas.ufl.edu/pdffiles/AN/AN23700.pdf.

Berge, G.M. & Austreng, E. (1989). Blue mussels in feed for rainbow trout. *Aquaculture* 81, 79-90.

Bokkers, E.A.M., Koene, P. 2003. Behaviour of fast and slow growing broiler to 12 weeks of age and physical consequences. Applied Animal Behaviour Science 81:59-72

Buchanan, N.P., Hott, J.M., Kimbler, L.B & Moritz, J.S. (2007). Nutrient composition and digestibility of organic broiler diets and pasture forages. *Journal of Applied poultry Research* 16, 13-21.

Buxton, D. and D. Redfearn. 1997. Plant limitations to fiber digestion and limitation. Journal of Nutrition. 127(5):814S-818S.

Bunchasak, C. (2009). Role of Dietary Methionine in Poultry Production. *Journal of Poultry Science* 46, 169-179.

Callaway JC, 2004. Hempseed as a nutritional resource: An overview. Euphytica, 140: 65-72.

Castellini, C., C. Mugnai, and A. Dal Bosco. 2002. Effect of organic production system on broiler carcass and meat quality. Meat Sci. 60:219–225.

Castellini, C., Dal Bosco, A., Mugnai, C. & Bernardini, M. (2002). Performance and behavior of chickens with different growth rate reared according to the organic system. Italian Journal of Animal Science 1(45-54).

Ciszuk P, Sjelin K*:* Mobil hönsskötsel (Mobile henhousing). Ekologiskt lantbruk Nr 20, Uppsala 1996. Proceedings Konferens ekologiskt lantbruk,Uppsala 7-8 November 1995. pp 117-126.

Curry, J.P. (1998) Factors affecting earthworm abundance in soils. In *Earthworm Ecology* (ed. C.A. Edwards). St. Lucie Press: Boca Raton, Florida.'

CVB. 2007. CVB Veevoedertabel 2007. Chemical compositions and nutritional values of feed. Product Board Animal Feed. Den Haag, The Netherlands.

Dawkins, M.S., P.A. Cook, M.J. Whittingham, K.A Mansell & A.E. Harper, 2003. What makes free-range broiler chickens range? In situ measurement of habitat preference. *Animal behaviour* 66: 151–160.

Darshan SK and IL Rudolph, 2000. Effect of fatty acids of w-6 and w-3 type on human immune status and role of eicosanoids. Nutrition, 16: 143–145.

Decuypere, E. & Verstegen, M. 1999. Stoffwisselingsinzichten en voeding bij pluimvee.Tijdschrift voor diergeneeskunde 124(2), 47-51.

De Almeida, G., L. Hinrichsen, K. Horsted, S. Thamsborg and J. Hermansen. 2012. Feed intake and activity level of two broiler genotypes foraging different types of vegetation in the finishing period. Poultry Science. 91(9):2105-2113.

Duncan, D.B. (1995). Multiple Range and Multiple F-Test Biometric. 11:1-42.EC 1999. Council Regulation (EC) No 1804/1999 of 19 July 1999 supplementing Regulation (EEC) No 2092/91 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs to include livestock production. Official Journal of the European Communities 24.98.1999, Brussels. L 222 1-28.

El-Shibiny, A., P. L. Connerton, and I. F. Connerton**.** *2005. Enumeration and diversity of campylobacters and bacteriophages isolated during the rearing cycles of free-range and organic chickens. Appl. Environ. Microbiol. 71**:**1259-1266.*

Emmans G C 1978 Free-choice feeding of laying poultry In "Recent Advances in Animal Nutrition" pp 31-39 (W Haresign & D Lewis eds.) Butterworths London

EU, 1991. Commission regulation N.1538/91 of 15 June 1991 introducing detailed rules for implementing regulation 1906/90 on certain marketing standards for poultry. Off. J. Eur. Communities (L143): 11-22.

EU, 2007. Commission regulation N.834/07 of 28 June 2007 on organic production and labeling of organic products and repealing Regulation (EEC) no 2092/91. Off. J. Eur. Communities (L189): 1-23.

EU, 2012: Commission implementing regulation (EU) No 505/2012 of 14 June 2012 amending and correcting Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council regulation (EC) No 834/2007 on organic production and labeling of organic products with regard to organic production, labeling and control. Official Journal of the European Union. (L154), 12-19.

Fanatico, A. 2007. Specialty poultry production: Impact of alternative genotype, production system, and nutrition on performance, meat quality and sensory attributes of meat chickens for free range and organic markets. PhD diss., University of Arkansas.

Green, R. (1978). Factors affecting the diet of farmland skylarks, *ALAUDA ARVENSIS.* J. Animal Ecology, 47: 913-928.

Fisher, C., 1998. The nutritional value of earthworm meal for poultry. In: Earthworms in Waste and in Environment. SPB Academic Publishing, P.O. Box 97747, 2509 GC The Hague, The Netherlands, pp. 181±192.

Jonsson, L. (2009). Mussel meal in poultry diets: with focus on organic production. Diss. Uppsala: The Swedish University of Agricultural Sciences.

Jönsson, L. & Elwinger, K. (2009). Mussel meal as a replacement for fishmeal in feeds for organic poultry - a pilot short term study. *Acta Agriculturae Scandinavica Section A Animal Science* 59(1), 22-27.

Jin-You, X., Xian Kuan, Z., Zhi- Ren, P., Zhen-Yong, H., Yan-Hua, G., Hong- Bo, T., Xye-Yan, H., and Qiao-Ping, X., (1982). An experimental research on the substitution of earthworm for fish meal n feeding meat chickens. *J.South China Norm*. Coll., 1, 88-94

Glatz, P. C., Y. J. Ru, Z. H. Miao, S. K. Wyatt, and B. J. Rodda. 2005. Integrating poultry into a crop and pasture farming system. *International Journal of Poultry Science* 4(4): 187-191.

González-Alvarado, J. M., E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2007. Effects of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers.Poult. Sci. 86:1705–1715.

Harwood, M. And Sabine, J. (1978) The nutritive value of worm meal. *Cited by:* Edwards, C.A. and Niederer, A. (1988) The production and processing of earthworm protein. *In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 169-180. Academic Publishing, The Hague, The Netherlands.

Havlin, J.L., Beaton, J.D., Tisdale, S.L. & Nelson. W.L. 1999, Soil fertility and fertilisers: an introduction to nutrient management, 6Ih ed. Upper Saddle River, NJ,
USA: Prentis Hall.

Haq, N. (1993). Lupins (*Lupinus* species). pg. 103-130. *In* J.T. Williams, J.T. (ed.) *Underutilized crops: pulses and vegetables*. p. 103-130. London, UK, Chapman & Hall., London, UK.

Hedley CL, Carbohydrates in Grain Legume Seeds. 2001. Improving Nutritional Quality and Agronomic Characteristics. CABI Publishing, Wallingford.

Hermansen, J.E., Strudsholm, K. & Horsted, K. 2004. Integration of organic animal production into land use with special reference to swine and poultry. Livestock Production Science, 90: 11-26.

Hetland, H., B. Svihus, and Å. Krögdahl. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. Br. Poult. Sci. 44:275–282.

Hetland, H. J., B. Svihus, and M. Choct. 2005. Role of insoluble fiber on gizzard activity in layers. J. Appl. Poult. Res. 14:38–46.

Hetland, H., and B. Svihus. 2007. Inclusion of dust bathing materials affects nutrient digestion and gut physiology of layers. J. Appl. Poult. Res. 16:22–26.

Hernroth, B.E., Conden-Hansson, A.C., Rehnstam-Holm, A.S., Girones, R. & Allard, A.K. (2002). Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis: the first Scandinavian report. *Applied and Environmental Microbiology* 68(9), 4523-4533.

Horsted, K., J. Hermansen and H. Ranvig. 2007. Crop content in nutrient-restricted versus non-restricted organic laying hens with access to different forage vegetations. British Poultry Science. 48:177-184.

Huber-Eicher, B & Wechsler, B. (1997). Feather pecking in domestic chicks: Its relation to dustbathing and foraging. *Animal behavior* 54(4), 757-768.

Hwangbo, J., E. C. Hong. (2009). "Utilization of house fly-maggots, a feed supplement in the production of broiler chickens." J Environ Biol. 30(4): 609-614.

IFOAM 2002. IFOAM Basic Standards for Organic Production and Processing. International Federation of Organic Agriculture Movements (IFOAM). Bonn.

Karsten, H. D., G. L. Crews, R. C. Stout, and P. H. Patterson. 2003. The impact of outdoor coop housing and forage based diets vs. cage housing and mash diets on hen performance, egg composition and quality. Paper presented at the International Poultry Scientific Forum, Atlanta.

Khachatourians, G. G. 1998. Agricultural use of antimicrobials and the evolution and transfer of antimicrobial-resistant bacteria. *Can. Med. Assoc. J. 159***:***1129-1136.*

Khan RU, FR Durrani, N Chand, H Anwar, S Naz, MF Farooqi and MN Manzoor, 2009. Effect of *Cannabis sativa* on muscle growth and visceral organs of broiler chicks. Inter J Biol Biotech, 4(1): 79-81.

Kiiskinen, T., .1987. Influence of choice feeding on the performance of growing pullets and laying hens. *Annales Agriculturae Fenniae*, 26: 131-144.

Ledgard S F and Steele K W 1992 Biological nitrogen fixation in mixed legume/grass pastures. Plant Soil 141, 137–153.

Lei, S., and G. van Beek. 1997. Influence of activity and dietary energy on broiler performance, carcass yield and sensory quality. Br. Poult. Sci. 38:183–189.

Leizer C, D Ribnicky, A Poulev, S Dushenkov and I Raskin, 2000. The composition of hempseed oil and its potential as an important source of nutrition. J Nutr Fun Med Food, 2: 35–53.

Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.O., Olrog, L., Rehnstam-Holm, A.S., Svensson, J., Svensson, S. & Syversen, U. (2005). Improving Marine Water Quality by Mussel Farming: A Profitable Solution for Swedish Society. *Ambio* 34(2), 131-138.

Lohr, L. 2001. “Factors Affecting International Demand and Trade in Organic Food Products,” *Changing Structure of Global Food Consumption and Trade*, Agriculture and Trade Report WRS-01-1, U.S. Department of Agriculture, Economic Research Service, www.ers.usda.gov/publications/wrs011/wrs011j.pdf

Mekada, H., Hayashi, N., Yokota, H and Okumura, J. (1979). Performance of growing and laying chickens fed diets containing earthworms (Eisenia foetida). *Jap. Poultry Sci*., 16, 293-7.

Merry, R.J., Jones, R. & Theordorou, M.K**.** (2001). Alternative forages - back to the future. *Biologist*, 48:, 30-34.

McEwen, S. A., and P. J. Fedorka-Cray. 2002. *Antimicrobial use and resistance in animals. Clin. Infect. Dis. 34***:***S93-S106.*

Miao, Z. H., P. C. Glatz, and Y. J. Ru. 2004. Free-range poultry production: A review. *Asian-Aust. J. Anim. Sci.* 18(1): 1-20.

Moritz, J.S., Parsons, A.S., Buchanan, N.P., Baker, N.J., Jaczynski, J., Gekara, O.J. and Bryan W.B. 2005. Synthetic Methionine and Feed Restriction Effects on performance and Meat Quality of Organically Reared Broiler Chickens. 2005 J. Appl. Poult. Res. 14:521-535.

National Organic Standards Board Definition, USA, 1995. Organic Trade Association USA. http://www.ota.com/organic/definition.html.

Newton, G. L., D. C. Sheppard, D.W. Watson, G.J. Burtle, C.R. Dove, J.K. Tomberlin, E.E. Thelen. 2005. The black soldier fly, *Hermetia illucens*, as a manure management / resource recovery tool." State of the Science, Animal Manure and Waste Management. Jan. 5-7, San Antonio, TX.

Newton, G. L., C. V. Booram, R. W. Barker, O. M. Hale. 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. J. Anim. Sci. 44: 395-400.

Odani S and S Odani, 1998. Isolation and primary structure of a methionine and cystine-rich seed protein of *Cannabis sativa* L. Biosci Biotechnol. Biochem, 62: 650–654.

Paul AA and Southgate DAT, The Composition of Foods, 4th edn, Ed by McCance RA and Widdwson EM, Elsevier Applied Science, Amsterdam, pp 175–177 (1988).

Prayogi, H. S. 2011. The effect of earthworm meal supplementation in the diet on quail's growth performance in attempt to replace the usage of fish meal. International Journal of Poultry Science 10:804-806. (Available online at: http://agris.fao.org/agris-search/search/display.do?f=2012/DJ/DJ2012071400407.xml;DJ2012071461 (verified 29 September 2013)

Pousga, S., H. Boly and B. Ogle. 2005. Choice feeding of poultry: a review. Livestock Research for Rural Development. 17:45.

Ravindran, V. and Blair, R. (1993). Feed resources for poultry production in Asia and the Pacific. III. Animal protein sources. World's Poultry Science Journal 49, 219-235.

Ramos-Elorduy, J., E. A. Gonzalez. (2002). "Use of *Tenebrio molitor* (Coleoptera : Tenebrionidae) to recycle organic wastes and as feed for broiler chickens." Journal of Economic Entomology 95(1):214-220.

Rose, S.P. and Kyriazakis, I. (1991) Diet selection of pigs and poultry. *Proceedings of the Nutrition Society* 50, 87–98.

Scott, M. J., Nesheim, M. C. and Young, R. J. (2001). Nutrition of the chicken, 4th edn (University books, Guelph, Ontario, Canada)

Sharma, S., Pradhan, K., S. Satya, and P. Vasudevan. 2005. Potentiality of earthworms for waste management and in other uses—A review. The Journal of American Science 1:4-16. (Available online at: http://www.jofamericanscience.org/journals/am-sci/0101/02-sharma.pdf)(pdf) (verified 29 September 2013)

Sloan, D.R., and B.L. Damron (2003). Small poultry flock nutrition. PS29. Gainesville: University of Florida Institute of Food & Agricultural Sciences. http://edis.ifas.ufl.edu/ps033 (Accessed October 20, 2009).

Sheppard, D.C., G.L. Newton, G. Burtle, 2008. Black Soldier Fly Prepupae. A Compelling Alternative to Fish Meal and Fish Oil. A Public Comment Prepared in Response to a Request by The National Marine Fisheries Service Nov. 15, 2007 to gather information for the

NOAA-USDA Alternative Feeds Initiative. Public Comment on Alternative Feeds for Aquaculture Comments Received by NOAA Nov. 15, 2007 through February 29, 2008.

Statistical Analysis System Institute Inc.,(1999). SASSTAT Programme, Carry, NC: SAS Institute Inc.

Steenfeldt, S., Kjaer, J.B. & Engberg, R.M. (2007). Effects of feeding silages or carrots as supplements to laying hens on production performance, nutrient digestibility, gut structure, gut microflora, and feather pecking behaviour. British Poultry Science (48)4, 454-468.

Stein, H. H., L. L. Berger, J. K. Drackley, G. C. Fahey Jr., D. C. Hernot, and C. M. Parsons. 2008. Nutritional properties and feeding values of soybeans and their co-products. Pages 613–660 in Soybeans, Chemistry, Production, Processing, and Utilization. L. A. Johnson, P. J. White, and R. Galloway, ed. AOCS Press, Urbana, IL.

St-Hilaire, S., K. Cranfill, M.A. Mcguire, E.E. Mosley, J.K. Tomberlin, L. Newton, W. Sealey, C. Sheppard, S. Irving, 2007. Fish Offal Recycling by the Black Soldier Fly Produces a Foodstuff High in Omega-3 Fatty Acids. Journal of the World Aquaculture Society, 38(2): 309-313.

Soil Association, 2013. Action plan for Organic poultry. Pg 5.

Sundrum, 2001. A. Sundrum Organic livestock farming: a critical review Livest. Prod. Sci., 67 (2001), pp. 207–215.

Svihus, B. 2011. The gizzard: Function, influence of diet structure and effects on nutrient availability. World's Poult. Sci. J. 67:207–224.

Tauson R, Jansson L and Elwinger K 1991**:** Whole grain/crushed peas and concentrate in echanized choice feeding for caged laying hens. *Acta Agriculture Scandinavia* 41: 75-83.

Rehnstam-Holm, A.S. & Hernroth, B. (2005). Shellfish and public health: A Swedish perspective. *Ambio* 34(2), 139-144.

Rivera-Ferre, M.G., Lantinga, E.A. and Kwakkel, R.P. 2006. Herbage intake and the use of outdoor area by organic broilers: effects of vegetation type and shelter addition. Wageningen University, The Netherlands. Pp 279-291.

Rivera-Ferre, M.G., Lantiga, E.A. & Kwakkel, R.P. (2007). Herbage intake and use of outdoor area by organic broilers: effects of vegetation type and shelter addition. *NJAS- Wageningen Journal of Life Sciences* 54(3), 279-291.

Robertson, J., M. S. Vipond, D. Tapsfield, and J. P. Greaves. 1966. Studies on the composition of feed. 1. Some differences in the composition of broiler and free range chickens. Br. J. Nutr. 20:675–687.

Ruis, M.A.W., Coenen, E., Van Harn, J., Lenskens, P. and Rodenburg, T.B. (2004). Effect of an outdoor run and natural light on welfare of fast growing broilers. In: Hänninen, L., Valros, A. (Eds), Proceedings of the 38th international congress of the ISAE, Helsinki, p.255.

Vance C P 1997 Enhanced agricultural sustainability through biological nitrogen fixation *In* Biological Fixation of Nitrogen for Ecology and Sustainable Agriculture. Eds. Legocki A, Bothe H and Pühler A. pp. 179–186. Springer-Verlag, Berlin.

Veldkamp, T., Van Duinkerken, G., Van Huis A., Lakemond C. M. M., Ottevanger, E., Bosch, G., Van Boekel, M. A.J.S. 2012. Insects as a sustainable feed ingredient in pig and poultry diets - a feasibility study. Wageningen UR, Livestock Research. ISSN 1570 – 8616.

Wang, X-S., Tang, C-H., Yang, X-Q. & Gao, W-R. 2008. Characterization, amino acid composition and in vitro digestibility of hemp (Cannabis sativa L.) proteins. *Food Chemistry* 107:11-18

Whitehead, C.C. 1997. Dyschondroplasia in poultry. Proceedings of the Nutrition Society 56(03), 957-966.

Whitehead, C.C., Fleming, R.H. & Julian, R.J. 2003. Skeletal problems associated with selection for increased production. In: Muir, W.M. & Aggrey, S.E. (Eds.) Poultry genetics, breeding and biotechnology. CABI Publishing. 29-52.

Zollitsch, W. and Baumung, R. (2004). Protein supply for organic poultry: options and shortcomings. In Hovi, S., Sundrum, A. and Padel, S. Diversity of Livestock Systems and Definition of Animal Welfare, Proceedings of the 2nd SAFO Workshop 25-27 March 2004, Witzenhausen, Germany. University of Reading.