Evaluation of the effects of reducing crude protein content and supplementing with crystalline amino acids on growth performance and litter quality in turkey

By

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بسم الله الرحمن الرحيم

DECLARATION

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

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DEDICATION

I dedicate this work to all researchers in this field

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ABSTRACT

Dietary factors contribute to ammonia emission and amount of nitrogen in the excreta. The excretion of nitrogen originating from dietary protein is largely responsible for some of the environmental issues associated with poultry production. Protein is essential as the very building block of the animal itself and hence protein nutrition takes a centre stage in poultry feeding. The use of dietary crude protein with crystalline amino acids as a means to decrease the impact on the environment of intensive poultry production is consequently of importance.

Two experiments were carried out to investigate the effect of dietary manipulation using four different concentration of protein with and without amino acid supplementation on turkey performance, litter quality and nutrient utilization. The birds were raised in an environmentally controlled house and lighting procedure followed was the recommendation for the turkey breed.

In the first study, the aim was to investigate the effect of dietary manipulation using four different concentrations of crude protein. One-hundred and twenty 7-day old BUT 10 turkeys were allocated to 4 treatments in a randomised complete block design. Each treatment had 6 replicate pens with 5 birds per replicate pen. The treatments were a diet adequate in protein and amino acids (diet 1) according to the breed specification and three other diets (diets 2, 3 and 4) formulated to have stepwise reduction of at least 1.4% protein from the previous diet such that the last

diet had approximately 4% lower protein level than the first diet. The diets were fed in four phases of four weeks each (except the first phase that lasted 3 weeks).

The highest protein levels were 28.8, 25.9, 21.7, and 18.5% and the lowest protein levels were 24.5, 21.0, 18.2 and 15.0%, respectively for phases 1, 2, 3 and 4. The diets were supplemented with appropriate crystalline amino acids that were present in the diet at lower than the requirement for the specific phase in each of the diets. The diets were formulated on digestible amino acid basis. Growth performance data were collected at the end of each phase. Overall there were no significant effects of diet on daily weight gain, feed intake or gain: feed. The final body weight was not influenced by the dietary treatments. The data from this experiment showed that supplementing a low-protein diet with crystalline amino acid produced weight gain similar to that of birds receiving adequate intact protein in their diets and support a superior efficiency of protein utilisation for weight gain.

The second study was designed to investigate the nutrient utilisation response of turkey to reducing the dietary protein supply by soybean meal and supplementing with amino acids. A total of 96 seven-day old male turkeys (B.U.T.10) were used for the study. On day 7, the birds were allocated to four dietary treatments in a randomized complete block design using initial body weight as the blocking criterion to ensure equal body weight in all the treatments at the start of the experiment. Each treatment had 6 replicate cages with 4 birds per replicate cage. Body weight and feed intake data were collected at the end of week 3 to compute growth performance responses. Ileal digesta were collected on day 21 and excreta were collected the last three days of the study. There were no effects of dietary treatments on ileal nutrient

digestibility of any of the treatments. Although, N excretion was lower ($P < 0.05$) in the lower-CP diets, the excretion as a proportion of intake was not. However, the dietary CP manipulation influenced ($P < 0.05$) energy metabolizability. It can be concluded from both experiments that it is possible to use reduced protein levels in diets formulated to have similar digestible amino acid content without affecting growth performance. The reduction in dietary protein can reduce the moisture content in excreta and consequently improve litter quality.

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INTRODUCTION

1 Introduction

One major concern in poultry production is the wet litter problem because of its negative impact on animal health, welfare and production. Litter condition significantly influences broiler performance because birds are in continuous contact with the litter (Martland, 1985). Improperly managed litter leads to increased weight and volume of manure which makes litter handling, storage and removal more difficult and costly. This can also lead to an explosion in the flies population as well as increase in the rate of ammonia loss into the environment (Francesch and Brufau, 2004). There are also welfare issues related to improperly managed litter. Poor litter can predispose birds to foot pad dermatitis which can ultimately reduce performance and cause loss of carcass value during processing (Meluzzi and Sirri, 2009).

The causes of wet litter in poultry houses are multi-factorial but are mainly due to complex interactions between management, health (Meluzzi and Siri, 2009) environment, (Le *et al.*, 2008) and nutrition. These factors can be broadly divided to external and internal factors (Lacey *et al.*, 2004).

Management and the environmental conditions can be classified as external factors. Management aspects that can influence the litter condition include the house temperature, ventilation system (rate and quality), heating (Jones *et al.*, 2005), and drinker design (Houldcroft *et al.*, 2008). Consequently, management measures to reduce wet litter problems are necessary to ensure optimum production. In addition, the environmental factors can also lead to high moisture content in excreta or litter and these factors include the level of relative humidity, air temperature, season, consistency and amount of faeces.

There are several nutritional factors that impact on wet litter and these include the dietary electrolyte balance, crude protein (CP) level and source, amino acid content, amino acid imbalance and protein and amino acid utilization efficiency (Bregendahl *et al.*, 2002), fat quality, the level of non-starch polysaccharides (NSP)-containing cereals and the use of NSP-hydrolyzing enzymes (Wang *et al.*, 1996, Olukosi *et al.*, 2008, Francesch and Brufau, 2004). In addition, the diet composition may affect water consumption and consequently excretion dry matter content (Mayne *et al.*, 2007b).

Excreta composition is affected by the water retention capacity of the bird and excreta water evaporation both of which alter litter characteristics. The dietary CP content and quality probably have the greatest influence on litter water content than any other nutrient. In addition, protein feedstuffs are among the most expensive ingredients in poultry diets and protein needs of turkeys are much more complex than that of other nutrients.

Consequently, there are incentives to nutritionally manage the protein feeding. Jacob *et al*. (1994) showed that the level of N excreted in poultry waste can be reduced by up to 21% if dietary CP content is lowered by 2.5% and the diet is supplemented with synthetic amino acids. Additional benefits of reducing the dietary CP content of poultry diet include the possibility for reduction of NH_3 production, which in turn can reduce ventilation and heating costs and also improve bird health. In spite of the potential benefit of feeding reduced CP-amino acid fortified diets however (Bregendahl *et al.*, 2002) indicated that such diets may not support growth

performance that is equal to that of birds on adequate-protein diets. This observation points to the importance of a better understanding of the use of reduced CP-amino acid supplemented diets.

1.1 Aim of the study

This study was designed to investigate the impact of reducing crude protein level by reducing the amount of vegetable protein source and supplementing with crystalline amino acids on the growth performance, litter characteristics and incidence of foot pad dermatitis in turkey up to 16 weeks of age as well as the impact of such dietary modifications on nutrient utilization efficiency of three-week old turkeys.

LITERATURE REVIEW

2 Literature Review

There have been tremendous developments in poultry husbandry in recent decades. As a result of improvements in the nutrition, genetics, management, and disease control in birds, along with advances in technology, there have been considerable improvements in meat quality and composition. By far, the vast majority of birds are raised in intensive production systems characterized by provision of adequate housing. Provision of a house for birds has obvious advantages in relation to animal health and welfare and performance and some disadvantages such as environment and air quality issues which relate to poultry production. For instance ammonia volatilisation represents a substantial loss of fertiliser nitrogen value when manures are applied to agricultural land. Moreover, environmental damage may be caused following ammonia deposition through direct toxicity to plants, changes in plant species composition of natural ecosystems, eutrophication and soil acidification (Nicholson *et al.*, 2004). Regional and national governments are beginning to address air quality concerns through policy development and implementation of regulations. Clearly, provision of an adequate housing for birds makes the production very economical and limits sanitary problems, thus improving productivity and health of birds (Lacey *et al.*, 2004).

However, intensive production of birds when raised on deep litter may predispose the birds to problems associated with wet litter and ammonia emission. Wet litter is caused by multiple factors that interact in a very complex manner. Such factors include feed intake, litter quality, litter moisture content and litter type (Huang *et al.*,

2009). Litter quality is affected by factors such as stocking density, air temperature and moisture, season, quantity and consistency of excreta, which in turn are affected by diet (Roberts *et al.,* 2007). Furthermore, the drinker design is an important contributory factor to wet litter problem (Mayne, 2005). Because of the multi-factorial nature of wet litter problem, the solutions proposed suggest that many factors have to be controlled at the same time (Bilgili *et al.,* 2009, Bregendahl *et al.,* 2002, Nahm, 2005).

2.1 Nutrition

Nutrition is a major contributory factor for wet litter problems. Dietary factors contribute to ammonia emission, amount of N in the excreta, and quantity of litter moisture which all lead to conditions such as hock burn, breast burn and Foot Pad Dermatitis (FPD) (Singh *et al.,* 2009).

2.1.1 Protein

A number of studies have examined the dietary protein-associated factors influencing litter wetness in poultry (Applegate *et al.,* 2008, Nagaraj *et al.,* 2007, Waguespack *et al.,* 2009). Increasing the litter nitrogen and, or litter moisture above the normal can raise ammonia levels in the house to dangerous levels. Rather than the crude protein (CP) per se, it has long been recognized that the amount of amino acids in the feed ingredient, is actually the most important factor in feed formulation (Pesti, 2009, Sterling *et al.,* 2003). Consequently, most of the research on influence of dietary

protein on litter quality has focused much more on amino acids supply rather than the crude protein itself.

The excretion of N originating from dietary protein is largely responsible for the environmental issues associated with poultry production. In response, dietary CP means to decrease the impact on the environment of intensive livestock production have successfully been implemented, one of which is the partial replacement of intact protein with crystalline amino acids (AA) (Bregendahl *et al*., 2002). This may decrease the disposal problems and pollution potential of the resulting litter.

However the balance of AA is important because birds need AA in certain ratios to ensure optimum performance (Kamran *et al*., 2010). Most N losses through excreta are due to the inability of dietary CP to meet the AA requirements and particularly the imbalances between different AA. Dietary CP also has profound effects on metabolism and endocrine functioning of broiler chickens (Kamran *et al*., 2010).

Additionally, Applegate *et al.*, (2008) reported that reduced dietary CP content would cause a decrease in total N and uric acid excretion, and therefore have less potential for microbial conversion of uric acid to NH_3. Intuitively, the rate of N excretion increases as the level of dietary protein increases and consequently, it seems reasonable that the first strategy to reduce N excretion is by improving amino acid availability or reducing dietary total protein (Collett, 2009).

Other researchers have shown that feeding low CP to broilers will decrease the N content of the excreta, thus reducing the N loss to the environment. Le *et al*., (2008) suggested that dietary CP is the main source of ammonia emission from pig manure,

as a result ammonia emission from pig manure can be reduced considerably by a decrease in dietary CP and an increase in fermentable carbohydrates (Le *et al*., 2008).

Kamran *et al* (2010) examined the effect of low levels of dietary crude protein with constant metabolisable energy on nitrogen excretion, litter composition and blood parameters of broilers. Their data showed that low crude-protein diets significantly reduced the moisture and N contents of the litter. Nitrogen excretion decreased, while N retention and its excretion as percent of N intake were not different among treatments.

Sklan and Plavnik, (2002) studied the interactions between dietary crude protein and essential AA intake on performance in broilers. Their result showed that feed intake decreased and feed efficiency increased with higher dietary energy and interactions between protein and energy were significant. Abdominal fat content and the efficiency of protein retention decreased with increasing dietary protein intake. Using constant essential amino acid: crude protein ratios at increasing crude protein intakes resulted in feed intake, weight gain and feed efficiency all increasing before reaching a plateau.

Abdominal fat decreased with protein intake and the efficiency of protein retention was quadratic, decreasing at the higher protein intakes. It was proposed that broiler performance at the lower protein intakes was limited by either nonessential amino acid intake whereas at high protein intakes the decreased efficiency of amino acid

utilisation after growth requirements are fulfilled resulted in poorer performance (Sklan and Plavnik, 2002).

Foot pad dermatitis (FPD) is a common condition in broiler chickens, broiler breeders, and turkeys. It is also called paw burns or NH_3 burns. FPD is a type of contact dermatitis characterized by lesions on the plantar region of the footpad, occasionally extending to the rear surface of the hock joint (Greene *et al*., 1985). Nagaraj *et al.*,

(2007) studied the influence of dietary protein levels, protein source and sex of birds on the incidence and severity of pod dermatitis in broiler chickens. Their result suggested that both protein level and protein source significantly increased the incidence and severity of footpad lesions. Incidence of FPD was higher for males (61%) than females (55%). Litter total N was significantly affected by protein level and protein source. The litter NH_3-N content, although not significant, except at 29 d of age, showed an increasing trend for each feeding period. The incidence and severity of FPD was significantly affected by protein level, protein source, sex, and age. Hence, it is clear that nutritional factors play a significant role in the aetiology of FPD in broilers.

2.1.2 Biotin and Riboflavin

Another dietary factor influencing the incidence of footpad dermatitis (FPD) is biotin deficiency which is known to be equally prevalent in broiler and turkey flocks (Whitehead, 1977). The relationship between biotin deficiency and FPD is likely

because biotin plays an important role in skin formation (Mayne *et al.*, 2007a). Early investigations showed that supplementation of diets with synthetic biotin was more effective with birds on dry litter while no effect was found in wet litter (Whitehead, 1977). On the other hand, Mayne *et al.*, (2005) showed that biotin supplementation is able to reduce FPD in growing turkeys and broilers.

Mayne, (2005) suggested that biotin deficiency causes FPD, and that commercial rations do not contain enough biotin to prevent these lesions. Consequently, supplementations of biotin have been shown to reduce the severity and incidence of FPD lesions. Biotin supplementation is able to reduce FPD to a certain extent if birds are reared on dry litter, whereas lesions can still occur when birds receiving adequate biotin supplementation are raised on wet litter (Wang *et al.*, 1998).

2.1.3 Non Starch polysaccharides

The use of cereals rich in soluble non-starch polysaccharides (NSP) like rye, barley, triticale, and wheat have been associated with litter problems related to the increase in amount of excreta or excreta stickiness and wateriness (Francesch and Brufau, 2004). The digestibility of high-NSP cereal starch is lower than that of maize starch. The most abundant NSP in animal feed based on cereals are cellulose, 1-3, 1-4-β-D-glucans and pentosans of the arabinoxylan type (Simon, 2000). Birds do not possess endogenous enzymes capable of cleaving and digesting the β (α) linked NSP. The water-insoluble NSP can be considered practically undigested by poultry and only soluble NSP have the potential to be digested by birds.

However, increased digesta viscosity can lead to increased water consumption and excretion in birds fed barley, rye or wheat (Animut, *et al.*, 2002). Exogenous enzymes can be used to hydrolyze specific links in NSPs and this could help alleviate the problems associated with NSP in feedstuffs being used in poultry diets (Simon, 2000). According to Choct *et al*., (1995), diets containing wheat that have increased levels of viscous NSP tend to have lower metabolisable energy (ME) values and higher digesta viscosity than diets based on normal wheat.

Bedford and Morgan (1996) indicated that enzymes are probably used in practice more for their effect on reducing wet litter than for any other reason. Although the negative effect of NSP is more significant in young birds than in older ones, enzyme addition has shown some beneficial effect on reducing excreta viscosity and number of dirty eggs (Francesch and Brufau, 2004). Therefore, the use of enzymes offers both economic benefits in the ability to choose from a greater range of cereal grains for the best value for money cereals and to reduce the wet litter.

2.1.4 Mineral

Supplementary inorganic trace minerals are used to supply birds with sufficient amounts of each mineral to support optimal growth, health, and reproduction (Nollet *et al*., 2008). Sodium and potassium are the principal electrolytes in extracellular and intracellular fluids, respectively. High dietary intakes of these minerals will give large osmotic changes within the intestinal lumen of birds which, in turn, increase the

water content of the faeces (Smith *et al.*, 2000). Moreover, the same author reported that increasing dietary concentrations of Na, K or P resulted in linear increases in water intake and excreta moisture content of laying hens. According to (Enting *et al.*, 2009) an increase in dietary Na level can result in increased litter moisture content and in impaired litter quality. The effect of high Na levels in feed becomes greater with age, as water and feed intake and excreta production per m^2 increase. Although there is evidence that excess dietary minerals can increase excreta moisture, there is little information that quantitatively describes this increase (Smith *et al*., 2000).

In addition, Ca and P also may occur in relatively large concentrations in poultry feeds and dietary phosphorus in excess of the daily requirement is excreted via the kidneys (Smith *et al.*, 2000). Incorrect calcium to phosphorus ratio has a strong negative effect on litter quality, particularly in young broiler chickens (Enting *et al.*, 2009)

The relationship between the chloride levels in the poultry diet and excreta moisture is not obvious. (Enting *et al.*, 2009) demonstrated that the effects of chloride on litter quality seem to be less clear than those of sodium and potassium. To the contrary, (Murakami *et al.*, 2003) reported that Na levels had no significant effect on excreta moisture; while chloride levels had a quadratic effect on the moisture content of excreta. According to Enting *et al.*, (2009) high dietary Mg levels resulted in increased water to feed ratio and subsequently in impaired litter quality in broilers.

The same authors reported that the effect of Mg on water to feed ratio in broilers was comparable to that of potassium.

2.2 Management

Litter management is an important aspect in rearing poultry. Litter serves to provide thermal insulation, moisture absorption and protective barrier from the ground (Shepherd and Fairchild, 2010).

2.2.1 Litter Material and Litter Depth

Bedding availability issues are arising rapidly in the poultry industry that may alter the type and quality of bedding available to growers to rear poultry. Most research agrees that litter quality and type are important predisposing factors in the onset of FPD. Materials used as poultry litter should be absorbent, dust-free, not consumable by the bird, easily handled and shipped, and inexpensive. In addition, the litter material must not retain excessive moisture as this creates a reservoir for disease causing organisms (Nahm, 2005). Bedding material must not be too coarse, as higher incidence of FPD has been found when coarse particleboard were used as bedding material (Hester *et al.*, 1997). The bedding material must also not be toxic to the birds or their caretakers (Nahm, 2005).

Bilgili *et al*. (2009) study examined pine shavings, pine bark, chipped pine, mortar sand, chopped wheat straw, ground hardwood pallets, ground door filler, and cotton-gin trash as possible bedding materials. The authors found that birds on mortar sand

(MS) and the ground door filler (DF) had significantly lower incidence of FPD than birds on other types of bedding materials. The authors theorized that the birds on ground door filler performed better because of the superior moisture-holding capacity of DF whereas birds raised on MS bedding materials performed well because of the ability of the bedding material to release moisture (Bilgili *et al*., 2009). Therefore, the bedding material needs to have the ability to absorb and quickly release moisture and these are probably the most important characteristics. Several works along this line agree that litter quality and type are important factors influencing the onset of FPD.

However, less attention has been given to the actual depth of litter being used (Shepherd and Fairchild, 2010). Meluzzi *et al*., (2008) reported that broilers raised on deeper litter had a lower occurrence of FPD than those raised on a thin layer of litter material. Haslam *et al*., (2007) reported that with every centimetre increase in final depth, there was a corresponding decrease in hock burn score of 0.015 points. In contrast, Ekstrand *et al.,* (1997) recorded that there was no significant interaction between litter material and litter depth. On the other hand, Meluzzi *et al*., (2008) indicated that bedding material, depth material, bird's weight, and stocking density influenced the occurrence of FPD in treated groups.

2.2.2 Litter Moisture

Litter moisture can be affected by factors such as stocking density, ventilation, and drinker design. Although there are no specific guidelines it is generally accepted that

good litter must have a dry matter content of 65 to 75% and may be described as "wet" when dry matter content falls below 45% (Ekstrand and Carpenter, 1998, Lister, 2009). Increased water content of the excreta causes increased manure moisture which increases the adherence of manure to the footpads of the birds leading eventually to FPD problems (Shepherd and Fairchild, 2010). Wet litter has also been identified as a possible causative agent for FPD because broilers and poults reared on wet litter have an increased incidence and severity of FPD lesions (Martland, 1985).

Mayne (2005) suggested that continual standing of birds on wet litter will cause the footpad to soften and become more prone to damage, predisposing the bird to developing FPD. It was shown that drying the litter and moving birds from wet litter to dry litter helped to reverse the severity of FPD. Footpad dermatitis lesions have been found to be more severe as litter moisture increases, especially when the litter contains high moisture and sticky droppings (Allain *et al*., 2009). In contrast, (Eichner *et al.*, 2007) report that there was no significant correlation between litter moisture and the incidence and severity of FPD.

2.2.3 Drinker Design

Drinker design can have a significant impact on water and feed intake and consequently poultry health (Lister, 2009). The flocks receiving water through small drinker cups were shown to have higher prevalence of FPD than those receiving water through nipple drinkers (Shepherd and Fairchild, 2010). However, (Allain *et*

al., 2009) reported that nipple drinkers can increase the scratches in broiler compared to other drinkers. In turkeys, the use of small water cups produced a lower occurrence of FPD than bell drinkers (Ekstrand and Carpenter, 1998, Whitehead, 1977, Mayne *et al.*, 2006, Hocking *et al.*, 2008).

This may due to the fact that closed nipple systems reduce water splashing onto the litter compared with other systems, especially the traditional open bell drinkers. However, drinker systems of whatever design can cause wet litter if they are badly maintained (excessive leakage) or poorly managed, for example if there is a wrong water pressure setting or if the drinker is set at the wrong height for the growing birds, leading to loss of water onto the litter at drinking (Lister, 2009).

Ambient temperature has a great bearing on water consumption and excretion and consequently litter wetness. Water consumption is reported to be approximately twice the feed intake (i.e. 1.7 to 2.0:1, water: feed) at 20°C whereas at an ambient temperature of 26ºC this increased to 2.5:1 but at ambient temperature of 35ºC water: feed ratio increased to 5:1. This increased water consumption will lead to increased water output and hence higher litter moisture content (Lister, 2009).

2.2.4 Stocking Density

Stocking density in general is a significant factor in broiler performance. A number of studies have reported that higher stocking densities are associated with a greater incidence of FPD than lower stocking density (Martrenchar, *et al.*, 2002; Haslam, *et*

al., 2007; Meluzzi & Sirri 2009.). According to (Shepherd and Fairchild, 2010), flocks stocked at a higher density (≤0.48 ft^2/bird) had 10% more hock lesions and 20% more breast lesions when compared with flocks at a lower stocking density [≥0.49 ft^2/bird. In addition, litter conditions deteriorate rapidly and litter moisture increases as stocking density increases (Bessei, 2006). (Feddes *et al.*, 2002) found that as stocking density increased, water consumption increased per bird. As birds drink more water, their excreta may become more watery and thus contributes to overall wet litter. However, Feddes *et al*. (2002) concluded that, although very high stocking densities do affect chicken welfare, stocking density per se is, within limits, less important than other factors in the birds' environment.

2.3 Health and welfare

Wet litter, and litter quality all have significant effects on bird health, welfare and performance (Lister, 2009). Foot pad dermatitis is a significant welfare issue for the broiler industry and is increasingly being used as an indicator of broiler flock welfare (Pagazaurtundua and Warriss, 2006; Haslam *et al.*, 2007; Shepherd and Fairchild, 2010). Consistent with the Lister (2009) report, litter moisture can have a potent impact on bird health, welfare and performance. These effects are mediated through direct contact FPD from wet litter, through to exacerbation of respiratory disease from poor air quality and food safety issues associated with soiled birds being presented for slaughter.

There are several infectious causes of wet litter. The most important infectious agents contributing to wet litter include parasites infections such as Coccidiosis, Hexmatiasis, Trichomoniasis and Cochlosoma. In addition, bacterial infections such as *Clostridium perfringens*, Dysbacteriosis which can be caused by the imbalance of bacteria within the gut and Spirochaetes e.g. Brachyspira spp. The viral infection such as Gumboro disease, Infectious Bronchitis and Astroviruses is one of important infectious agents contributing to wet litter (Norton *et al* 2000; Lister, 2009).

The direct effects of contact with the litter are in terms of possible development of dermatitis of foot pads, hocks and breast skin, or through the effects of noxious gaseous on the eyes and respiratory tract. Indirect effects relate more generally to air quality, for example, dust levels, air humidity and ammonia levels that can influence the incidence and severity of respiratory tract damage especially when in combination with infectious diseases. Good air quality is essential in reducing the likely impact of infectious diseases such as mycoplasma, bacterial (e.g. *Ornithobacter rhinotracheale, Pasteurella, Haemophilus spp.*) and viral (e.g. avian pneumovirus, infectious bronchitis, Newcastle disease, avian influenza etc) infections (Norton *et al* 2000; Gomis *et al* 2002; Lister, 2009).

2.4 Conclusion

Several factors can affect the excreta/litter moisture content; and some of these relate to diet composition. Diet composition directly affects the excreta moisture produced by poultry and so could contribute to the variation in excreta moisture within a

poultry house. The dietary causes of the variation in excreta moisture are multi-factorial; but dietary protein level and the balance of AA are probably the most important. Other dietary factors such as mineral composition, feed ingredients causing high digesta viscosity or containing high levels of non-digestible fibre fractions all increase litter wetness. Other factors are related to management and housing (amount and type of litter, temperature, ventilation, heating, and drinking system) are also important in wet litter problems. Finally factors that are related to diseases caused by different infectious agents also affect water consumption and excretion. The current research investigated the role of nutrition, in terms of crude protein and amino acids, on growth performance, litter quality and nutrient utilisation of turkeys.

MATERIALS AND METHODS

3 Study design

In this study, two experiments were carried out to investigate the effect of dietary manipulation using four different concentration of protein with and without amino acid supplementation on turkey performance, litter quality and nutrient utilisation. The birds were raised in an environmentally controlled house and lighting procedure followed was the recommendation for the turkey breed.

3.1 Experiment 1

A total of 120 seven-day old male turkeys (BUT10) were used for the experiment. All the birds were brooded together until seven days old. On day 7, the birds were allocated to four dietary treatments in a randomized complete block design using initial body weight as the blocking criterion to ensure equal body weight in all the treatments at the start of the experiment. Each treatment had 6 replicate pens with 5 birds per replicate pen. The animal experimentation procedures were approved by the Animal Experimentation Committee of the Avian Science Research Centre of Scottish Agricultural College.

3.1.1 Diets

The diets had graded levels of crude protein with the adequate-CP diet (control treatment) having 28.8 % crude protein and supplemented only with lysine, methionine and threonine. The three successive diets had 1.4 percentage points less protein than the previous with the lowest CP diet (treatment 4) having 24.5 % CP and being supplemented with methionine, lysine, threonine, arginine, valine, isoleucine

and tryptophan. The two intermediate diets were made by mixing diets 1 and 4 at the ratio of 2:1 and 1:2 to produce 27.4 (treatment 2) and 25.9 (treatment 3) % CP, respectively. All the diets were isocaloric and similar in contents of digestible amino acids, Ca, P and Na with the exception that the contribution of amino acids from plant sources decreased as CP level decreased. The diets were fed in four phases over a 16-week period with each phase lasting four weeks. Each of the three control diets in subsequent three phases were formulated according to breed specification for that age and the treatments maintained the difference in CP as follows: 1.6, 1.2 and 1.2 percentage points for periods 2, 3 and 4, respectively. The diet formulas are presented in Tables 3.1 and 3.2

3.1.2 Husbandry

The birds received the four experimental diets from day 7, and water was provided *ad-libitum* using a rainbow drinker. The lighting regimen followed the breed specification and the temperature was controlled using automated temperature regulation.

3.1.3 Sample collection

Samples of each of the 16 feeds (500 g) were collected and stored in a refrigerator until chemically analysed. Samples of the litter (500 g) were collected at the end of weeks 8 and 16 and analysed for dry matter content. Litter samples from the 2 periods were used for litter DM determination. Collection of litter samples for analysis was done as follows. Two hundred grams of litter were sampled from five locations in each pen. Four samples were collected from the pen corners and one

Table 3.1 Ingredient composition (g/kg) of the experimental diets (0 to 4 weeks)

NUTRIENTS	Phase 1 (1 - 4 week)				Phase 2 (4 - 8 week)			
Diets	1	2	3	4	1	2	3	4
Crude protein	28.8	27.37	25.93	24.5	25.9	24.27	22.63	21
Dig. Lysine	1.6	1.60	1.60	1.6	1.4	1.40	1.40	1.4
Dig. Methionine	0.71	0.73	0.74	0.76	0.66	0.68	0.70	0.72
Dig. Met + Cys	1.1	1.10	1.10	1.1	1.02	1.02	1.02	1.02
Dig Tryptophane	0.32	0.30	0.29	0.27	0.29	0.27	0.26	0.24
Dig. Threonine	1.03	1.03	1.03	1.03	0.89	0.89	0.89	0.89
Dig.Arginine	1.77	1.76	1.75	1.74	1.56	1.54	1.53	1.51
Calcium	1.22	1.22	1.22	1.22	1.12	1.12	1.12	1.12
Av. Phos.	0.65	0.65	0.65	0.65	0.56	0.56	0.56	0.56
Sodium	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16
Salt	0.4	0.40	0.40	0.4	0.4	0.40	0.40	0.4
ME MJ/kg	11.8	11.80	11.80	11.8	12	12.00	12.00	12
D. Isoleucine	1.1	1.10	1.10	1.1	0.97	0.97	0.96	0.96
D. Valine	1.17	1.17	1.17	1.17	1.05	1.05	1.05	1.05
D. Leucine	1.89	1.77	1.65	1.53	1.69	1.55	1.41	1.27
INGREDIENTS								
Wheat	41.94	44.62	47.32	50	36.09	40.72	45.37	50
Barley	0	1.39	2.77	4.16	13	12.00	11.00	10
Wheat feed	0	0.00	0.00	0	0	1.21	2.43	3.64
Soya 48%	49.53	45.15	40.75	36.37	41.77	36.70	31.60	26.53
Soya oil	2.95	2.70	2.45	2.2	3.92	3.56	3.19	2.83
DL-Methionine	0.36	0.40	0.43	0.47	0.35	0.39	0.43	0.47
Lysine HCl	0.24	0.37	0.51	0.64	0.22	0.37	0.52	0.67
Threonine	0.09	0.15	0.22	0.28	0.06	0.13	0.21	0.28
Salt	0.19	0.15	0.10	0.06	0.14	0.11	0.07	0.04
Sodium Bicarb.	0.23	0.30	0.36	0.43	0.41	0.44	0.46	0.49
Limestone	1.67	1.69	1.70	1.72	1.64	1.66	1.69	1.71
Mono-Cal.Phos	2.4	2.43	2.45	2.48	2	2.02	2.03	2.05
Arginine	0	0.11	0.23	0.34	0	0.12	0.25	0.37
Valine	0	0.08	0.15	0.23	0	0.09	0.17	0.26
Isoleucine	0	0.07	0.15	0.22	0	0.08	0.16	0.24
Tryptophane	0	0.00	0.00	0	0	0.01	0.01	0.02
Vitamin-mineral premix*	0.4	0.40	0.40	0.4	0.4	0.40	0.40	0.4
Total	100	100	100	100	100	100	100	100

*The vitamin-mineral premix provided (units per kg diets): retinol, 548 µg; cholecalciferol, 66 µg; DL α-tocopherols, 3.34 mg ; thiamine, 3 mg; riboflavin, 10 mg; vitamin B6, 3 mg; cobalamin, 15 µg; phyloquinone, 5 mg; Nicotinic acid, 60 mg; Pantothenic acid, 14.5 mg; Folic acid, 1.5 mg; Biotin, 275 µg; Choline chloride, 250 mg; Fe (as ferrous sulphate), 20 mg; Cur (as copper sulphate), 10 mg; Mn (as manganese oxide), 100 mg; Co 1 mg; Zn (as zinc oxide), 82 mg; I (as calcium iodate), 1 mg; Se (as sodium selenite), 0.2 mg; Molybdenum, 0.5 mg.

Table3.2 Feed composition table (for phase 3 and 4)

NUTRIENTS	Phase 3 (8 -12 week)				Phase 4 (12 – 16 week)			
	Diet 1	Diet 2	Diet 3	Diet 4	Diet1	Diet 2	Diet 3	Diet 4
Crude protein	21.65	20.50	19.35	18.2	18.5	17.33	16.17	15
Dig. Lysine	1.2	1.20	1.20	1.2	0.9	0.90	0.90	0.9
Dig.methionine	0.57	0.59	0.61	0.63	0.45	0.46	0.48	0.49
Dig. Met + Cys	0.9	0.90	0.90	0.9	0.73	0.73	0.73	0.73
DigTryptophane	0.25	0.23	0.22	0.2	0.2	0.19	0.17	0.16
Dig. Threonine	0.73	0.73	0.73	0.73	0.59	0.59	0.59	0.59
Dig.Arginine	1.31	1.29	1.28	1.26	1.01	1.01	1.01	1.01
Calcium	1.02	1.02	1.02	1.02	0.81	0.81	0.81	0.81
Av. Phos.	0.5	0.50	0.50	0.5	0.38	0.38	0.38	0.38
Sodium	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Salt	0.4	0.40	0.40	0.4	0.4	0.40	0.40	0.4
ME MJ/kg	12.2	12.20	12.20	12.2	12.2	12.20	12.20	12.2
Dig. Isoleucine	0.83	0.82	0.81	0.8	0.64	0.64	0.64	0.64
Dig. Valine	0.9	0.90	0.90	0.9	0.72	0.72	0.72	0.72
Dig. Leucine	1.45	1.33	1.20	1.08	1.14	1.04	0.95	0.85
INGREDIENTS								
Wheat	50	50.00	50.00	50	50	50.00	50.00	50
Barley	9.84	13.22	16.62	20	18	20.33	22.67	25
Wheatfeed	0	0.56	1.12	1.68	4.92	5.63	6.34	7.05
Soya 48%	32.62	28.17	23.72	19.27	20.7	17.19	13.68	10.17
Soya oil	2.83	2.78	2.73	2.68	2.57	2.58	2.59	2.6
DL-Methionine	0.29	0.33	0.37	0.41	0.22	0.25	0.28	0.31
Lysine HCl	0.23	0.36	0.50	0.63	0.19	0.29	0.40	0.5
Threonine	0.03	0.09	0.16	0.22	0.05	0.10	0.15	0.2
Salt	0.18	0.14	0.09	0.05	0.19	0.15	0.12	0.08
Sodium Bicarb.	0.29	0.36	0.42	0.49	0.29	0.34	0.40	0.45
Limestone	1.57	1.59	1.60	1.62	1.35	1.37	1.38	1.4
Mono-Cal.Phos	1.72	1.74	1.77	1.79	1.12	1.14	1.16	1.18
Arginine	0	0.11	0.21	0.32	0	0.10	0.19	0.29
Valine	0	0.08	0.15	0.23	0	0.06	0.12	0.18
Isoleucine	0	0.07	0.13	0.2	0	0.06	0.12	0.18
Tryptophane	0	0.00	0.01	0.01	0	0.00	0.01	0.01
Vitamin-mineral premix*	0.4	0.40	0.40	0.4	0.4	0.40	0.40	0.4
Total	100	100	100	100	100	100	100	100

*The vitamin-mineral premix provided (units per kg diets): retinol, 548 µg; cholecalciferol, 66 µg; DL α-tocopherols, 3.34 mg ; thiamine, 3 mg; riboflavin, 10 mg; vitamin B6, 3 mg; cobalamin, 15 µg; phyloquinone, 5 mg; Nicotinic acid, 60 mg; Pantothenic acid, 14.5 mg; Folic acid, 1.5 mg; Biotin, 275 µg; Choline chloride, 250 mg; Fe (as ferrous sulphate), 20 mg; Cur (as copper sulphate), 10 mg; Mn (as manganese oxide), 100 mg; Co 1 mg; Zn (as zinc oxide), 82 mg; I (as calcium iodate), 1 mg; Se (as sodium selenite), 0.2 mg; Molybdenum, 0.5 mg.

from the centre to have a total of 1kg. The collected sample were mixed well and then subsampled to obtain samples for the subsequent analyses. Each labelled sample were duplicated and placed in a polythene bag and stored in 4°C prior to determination of dry matter.

3.1.1 Calculations

Data on body weight, feed and water intake were collected at weeks 4, 8, 12 and 16 of the experiment. The growth performance at each phase and over the entire period of the experiment was then computed. In addition, protein and energy efficiency ratios were determined at each phase and throughout the length of the experiment. At the end of 12 weeks, the feet of each bird were individually examined and scored for foot pad dermatitis using the scale of 0 to 7 following Mayne *et al.* (2008) description.

Protein efficiency ratio (PER) was calculated from total protein intake and weight gain as follows:

$$\textbf{Total protein intake } (\textbf{TPI}, \textbf{g}) = \textbf{Total feed intake } (\textbf{g}) \times \textbf{Concentration of CP } (\%)$$

$$\textbf{PER} = \frac{\textbf{Body weight gain } (\textbf{g})}{\textbf{TPI } (\textbf{g})}$$

Energy efficiency ratio (EER) was calculated from total energy intake and weight gain as follows:

$$\textbf{Total energy intake}(\textbf{TEI}, \textbf{kcal}) = \frac{\textbf{Total feed intake}(\textbf{g})}{\textbf{Energy content of feed}\ (\textbf{kcal})}$$

$$\text{EER}\ (\textbf{g/kcal}) = \frac{\textbf{Body weight gain}}{\text{TEI (kcal)}}$$

3.2 Statistical analysis

The data on growth performance, litter dry matter, protein and energy efficiency ratios were analyzed using the generalized linear mixed model of Genstat. The model included block and diets to test for the effect of the diets on the response criteria. The hypothesis was that reducing the level of crude protein and supplementing with crystalline amino acids will have no effect of growth performance but will improve efficiency of nutrient and energy utilization, litter condition and feet scores. When the diet effect was significant, means were separated using orthogonal polynomial contrast to test for linearity or quadratic relationship in the effect of reducing CP on the responses of interest. Significance was declared at 5% probability level.

3.3 Experiment 2

A total of 96 seven-day old male turkeys (BUT10) were used for the study. All the birds were brooded together until seven days old. On day 7, the birds were allocated to four dietary treatments in a Randomised Complete Block Design using initial body

weight as the blocking criterion to ensure equal body weight in all the treatments at the start of the experiment. Each treatment had 6 replicate pens with 5 birds per replicate pen. The animal experimentation procedures were approved by the Animal Experimentation Committee of the Avian Science Research Centre of Scottish Agricultural College.

3.3.1 Diets

The dietary treatments were the same as used for phase 1 of Experiment 1.

3.3.2 Husbandry

The diets were randomly allocated within each block and blocks were randomly placed within the house. The birds were raised in metabolism cages in an environmentally controlled house and lighting procedure followed the recommendation for the turkey breed.

3.3.3 Sample collection

Body weight and feed intake data were collected at the end of week 4 of the study. Ileal digesta were collected on day 28 by gently flushing the ileal content out with distilled water. Excreta were collected on days 19 to 21. The diet, ileal and excreta samples were ground and subsequently analyzed for DM, N, GE, ash, and Ti. Samples of test ingredients and ileal digesta samples used for laboratory analysis were ground to pass through a 0.5 mm using a mill grinder.

Samples were dried at 100°C for 24h in a drying oven for DM determination. Gross energy was determined in bomb calorimeter using benzoic acid as calibration standard. Nitrogen was determined using the combustion method using EDTA as an internal standard. For ash determination, samples (1 g) were ashed at 500°C for 24h in a muffle furnace. Titanium (Ti) was analysed using a method based on the work of Short *et al*. (1996). The amino acids (AA) composition of 8 of the diets (diets 1 and 4 of each phase) were analysed using standard AOAC procedures. For AA analysis, the samples were hydrolyzed in 6 *N* HCl for 24 h at 110°C under N atmosphere. For methionine and cystine, performic acid oxidation was carried out before acid hydrolysis. The AA in the hydrolyzate were determined by HPLC after postcolumn derivatization [AOAC, 2000, method 982.30 E (a,b,c)]. Chemical analyses were performed in duplicate and repeated if individual data differed by >5%.

3.3.4 Calculations

Nutrient digestibility was determined using the index method with titanium as an indigestible marker as follows:

$$\boldsymbol{AND}\ (\%) = \left[\mathbf{1} - \left(\frac{\boldsymbol{Ci}}{\boldsymbol{Co}} \times \frac{\boldsymbol{No}}{\boldsymbol{Ni}}\right)\right] \times \mathbf{100}$$

where: *AND* – apparent nutrient digestibility/retention

Ci and *Co* – concentrations of marker in diet and excreta/digesta, respectively, %

No and *Ni* – concentrations of nutrient in excreta/digesta and diet, respectively, %

3.4 Statistical analysis

The data on growth performance and nutrient utilisation, were analysed using the generalized linear mixed model of GENSTAT. The model included the block and diets to test for the effect of the diets on the response criteria. If the diet effect is significant, means were separated using Tukey. Significance was declared at 5% probability level.

RESULTS

4 Results

4.1 Chemical analysis of the experimental diets

Tables 4.1 and 4.2 show the results of the analysed chemical composition and total amino acids contents, respectively, of the experimental diets. The crude protein contents of the diets especially during the first phase was wider than anticipated and the difference in crude protein content during the fourth phase was narrower than planned. The differences in amino acids composition was similar to variation in crude protein concentrations of the experimental diets.

4.2 Experiment 1

4.2.1 Phase1

The results of the growth performance of turkey receiving graded levels of reduced CP diets supplemented with crystalline AA during phase 1(week 1 to week 4) are summarised in Table 4.3. In the start of the experiment, initial weights of the birds were not statistically different among all treatments. The average of feed intake (77.1 g /b/d) per day per bird in diet1 was higher than all other treatment ($P < 0.001$). Turkeys fed reduced CP diets supplemented with crystalline AA (diet2, diet3 and diet4) had lower ($P < 0.01$) body weight gain compared to turkeys provided the control diet (diet1). Water intake in diet 1 (242.0 g/b/d) was higher than all other treatments ($P < 0.001$). Final body weight at the end of phase 1 decreased with reduced CP ($P < 0.001$). However, reduce dietary CP levels did not affect gain: feed nor water: feed, EER, PER and litter DM content.

Table 4.1 Analysed nutrient composition (%, DM) of experimental diets

	Diet number	CP	Ash	GE, kcal/kg	Ether Extract
Phase 1	1	26.7	7.62	4061	5.39
	2	25.4	7.82	4049	4.81
	3	19.6	5.53	4078	5.75
	4	18.4	5.66	4240	5.46
Phase 2	5	25.0	7.70	4033	6.17
	6	21.4	6.50	4027	5.35
	7	21.4	6.72	3994	5.11
	8	19.0	5.75	3983	5.55
Phase 3	9	21.1	7.68	4002	5.07
	10	19.8	6.56	4017	4.95
	11	18.6	5.01	4068	4.65
	12	17.6	5.80	4030	5.19
Phase 4	13	15.4	5.12	4042	4.86
	14	15.8	5.04	4067	4.32
	15	15.5	5.20	4080	4.50
	16	14.6	4.92	3988	5.03

Table 4.2 Analysed total amino acid compositions (%, DM) of the diets with highest and lowest crude protein contents in each phase

	Phase 1		Phase 2		Phase 3		Phase 4	
	High CP	Low CP	High CP	Low CP	High CP	Low CP	High CP	Low CP
Indispensable amino acids								
Arginine	2.12	1.54	1.92	1.57	1.55	1.38	1.16	1.12
Histidine	0.74	0.48	0.69	0.49	0.57	0.44	0.38	0.35
Isoleucine	1.36	0.99	1.25	1.01	1.02	0.90	0.77	0.74
Leucine	2.19	1.41	2.03	1.43	1.66	1.30	1.12	1.01
Lysine	2.03	1.54	1.86	1.57	1.57	1.42	1.19	1.17
Methionine	0.80	0.66	0.83	0.67	0.74	0.61	0.53	0.52
Phenylalanine	1.45	0.95	1.36	0.96	1.11	0.87	0.76	0.69
Threonine	1.23	0.90	1.10	0.92	0.91	0.81	0.69	0.66
Tryptohan	0.41	0.30	0.39	0.30	0.33	0.27	0.24	0.23
Valine	1.46	1.14	1.35	1.16	1.11	1.04	0.90	0.87
Dispensable amino acids								
Alanine	1.24	0.80	1.17	0.81	0.94	0.73	0.63	0.58
Aspartic acid	3.07	1.77	2.83	1.80	2.20	1.56	1.27	1.10
Cysteine	0.44	0.32	0.43	0.32	0.36	0.30	0.28	0.26
Glutamic acid	5.82	4.14	5.43	4.21	4.66	3.92	3.57	3.33
Glycine	1.25	0.82	1.16	0.84	0.96	0.76	0.66	0.61
Proline	1.69	1.29	1.61	1.32	1.43	1.28	1.19	1.14
Serine	1.45	0.91	1.33	0.93	1.10	0.86	0.73	0.66
Tyrosine	1.04	0.64	0.95	0.65	0.77	0.59	0.51	0.45

Table 4.3 Growth Performance of turkey receiving graded levels of reduced CP with crystalline AA during phase 1 (day 1 to 28)

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	Diet effect
Gain ,g/b/d	49.0[a]	39.3[b]	43.0[b]	40.4[b]	1.47	0.001
FI, g/bird	77.1[a]	63.7[b]	67.5[b]	64.6[b]	1.81	<0.001
Gain: Feed, g/kg	635.7	612.7	636.2	623.5	9.61	0.294
WI[1], g/b/d	242.0[a]	195.3[b]	193.6[b]	162.4[b]	14.4	<0.001
W:F[2]	4.00	4.27	3.95	3.87	0.145	0.271
Litter DM,%	79.9	79.5	81.5	79.8	1.43	0.766
EER, g /Kcal	0.236	0.228	0.236	0.232	0.004	0.294
PER, g/g	2.29	2.25	2.38	2.38	0.036	0.066
Initial Wt, Kg	207.1	207.3	207.2	207.2	0.0002	0.911
Fin. Wt, Kg	1226.8[a]	1040.4[b]	1100.6[b]	1046.3[b]	0.027	< 0.001

[ab] Means in the same row but with different superscripts are different ($P < 0.05$)
[1] water intake data collected for 1 week in week4
[2] water to feed ratio calculated from water and feed intake data for 1 week in week 4, WI- water intake; W:F- water to feed intake, g/g; EER – energy efficiency ratio (g gained per Kcal); PER – protein efficiency ratio (g gained per g protein consumed)

4.2.2 Phase 2

Table 4.4 show the result of growth performance of turkey receiving graded of reduced CP in phase 2. Data of the initial body weight of turkey in this phase were taken into account as a covariate because the bird weight of the start of phase 2 was different between treatments. The average of initial body weight in turkeys fed diet 5 was higher (1.29 kg) compared to other diets ($P < 0.001$). The data of water intake showed that turkeys fed diet 5 (650.8 g/b/d) had higher ($P < 0.01$) water intake compared to diets 7 and 8, whereas diet 6 water intake was similar to diets 5 and 7. Litter dry matter was lower ($P < 0.01$) and similar for turkeys fed diets 5 and 6 compared to those receiving diets 7 and 8 which were higher and similar in litter DM content. Protein efficiency ratio (g/g) increased with decreasing dietary CP as the diet 7 and 8 had higher PER ($P < 0.05$) compared to diet 5 and diet 6. The overall weight gain (up to week 8) in turkeys fed diet 5 was higher ($P < 0.05$) than all other treatments.

4.2.3 Phase 3

Table 4.5 shows the result of growth performance of turkey receiving graded levels of reduced CP- crystalline AA-supplemented diets in Phase 3 (weeks 9 to 12 of age). Initial body weight in this phase statistically was not different for the treatments. Daily body weight gain during phase 3 was the same in all the treatments. There were no effects of diet manipulation in this phase on any of the growth performance responses except PER and final weight ($P \leq 0.05$). Protein efficiency ratio was lowest ($P = 0.05$) in the diet with the highest protein level. Overall significant different ($P =$

0.015) was found in the final body weight for all the treatments. Turkeys fed the diet 11 had higher body weight compared to control diet 9; whereas the lowest body weight was recorded when the turkeys fed the diet 12.

4.2.4 Phase 4

This was the final phase of the experiment and spanned days 84 to 112 of the birds' life (Table 4.6). The initial body weight for this phase was least ($P < 0.05$) for birds receiving diet16 (10.9 kg) and higher but similar for those receiving diets 13, 14 and 15. Decreasing the CP level in the diets had no effect on any of the criteria during phase 4 except litter DM which was lower ($P = 0.01$) in diet 13 than diet 15 whereas diets 14 and 16 were intermediate. Overall feed intake was higher ($P < 0.05$) in diet 13 compared with diet 14 whereas those for diets 15 and 16 were intermediate. Overall gain of the birds was not significantly different between the treatments.

Table 4.4 Growth Performance of turkey receiving graded levels of reduced CP with crystalline AA during phase 2 (day 28 to 56)

	Diet 5	Diet 6	Diet 7	Diet 8	SEM	Diet effect
Gain ,g/b/d	146.2	138.3	138.6	131.5	4.00	0.124
FI, g/bird	259.1	236.9	231.9	227.9	8.66	0.092
Gain : Feed, g/kg	564.5	585.6	598.52	580.4	10.12	0.168
WI[1], g/b/d	650.8[c]	588.4[bc]	563.93[ab]	503.3[a]	20.71	0.001
W:F[2]	1.72	1.65	1.62	1.53	0.052	0.105
Litter DM,%	60.28[a]	60.38[a]	71.40[b]	74.02[b]	2.05	<0.001
EER, g/Kcal	0.197	0.205	0.209	0.203	0.0035	0.168
PER, g/g	2.276[a]	2.400[ab]	2.494[b]	2.459[b]	0.0424	0.013
Ov. Gain, g/b/d	103.4[b]	94.5[ab]	96.5[ab]	91.4[a]	2.78	0.048
Ov. FI, g/b/d	179.0[b]	160.3[ab]	159.5[ab]	156.1[a]	5.85	0.044
Ov. G:F, g/kg	578.0	590.4	605.6	587.8	7.59	0.124
Initial Wt, Kg	1.29[b]	1.10[a]	1.15[a]	1.09[a]	0.0277	<0.001
Fin. Wt, Kg	5.38[b]	4.97[ab]	5.03[ab]	4.78[a]	0.127	0.031

[ab] Means in the same row but with different superscripts are different (P< 0.05)
[1] water intake data collected for 4 week in week 8
[2] water to feed ratio calculated from water and feed intake data for 4 week in week 8, WI- water intake; W:F- water to feed intake, g/g; EER – energy efficiency ratio (g gained per Kcal); PER – protein efficiency ratio (g gained per g protein consumed); Ov gain : gain for week 4 to 8; ov FI – feed intake for week 4 to 8; Ov G: F - ratio gain to feed for week 4 to 8.

Table 4.5 Growth Performance of turkey receiving graded levels of reduced CP with crystalline AA during phase 3 (day 56 to 84)

	Diet 9	Diet 10	Diet 11	Diet 12	SEM	Diet effect
Gain ,g/b/d	188.3	186.0	200.4	185.1	6.19	0.309
FI, g/bird	537.2	499.5	510.7	486.9	15.35	0.166
Gain : Feed, g/kg	350.5	374.0	392.8	381.8	14.17	0.231
WI[1], g/b/d	1072.4	1052.4	994.5	1026.2	40.20	0.564
W:F[2]	1.75	1.81	1.62	1.82	0.055	0.066
Litter DM,%	65.7	68.2	70.1	68.9	1.70	0.352
EER, g/Kcal	0.120	0.128	0.135	0.131	0.0049	0.231
PER, g/g	1.638	1.781	1.907	1.890	0.0684	0.050
Ov. Gain, g/b/d	133.9	125.8	132.7	124.9	3.46	0.194
Ov. FI, g/b/d	233.2	208.1	214.2	208.4	7.34	0.091
Ov. G:F, g/Kg	574.7	607.0	620.7	600.6	14.10	0.177
Initial Wt, Kg	5.38	5.43	5.29	4.83	0.179	0.110
Fin. Wt, Kg	10.65[ab]	10.64[ab]	10.90[b]	10.01[a]	0.174	0.015

[ab] Means in the same row but with different superscripts are different (P< 0.05)

[1] water intake data collected for 8 week in week 12

[2] water to feed ratio calculated from water and feed intake data for 8 week in week 12, WI- water intake; W:F- water to feed intake, g/g; EER – energy efficiency ratio (g gained per Kcal); PER – protein efficiency ratio (g gained per g protein consumed); Ov gain : gain for week 8 to12; ovfI – feed intake for week 8 to 12; Ov G: F - ratio gain to feed for week 8 to 12.

Table 4.6 Growth Performance of turkey receiving graded levels of reduced CP with crystalline AA during phase 4 (day 84 to 112)

	Diet 13	Diet 14	Diet 15	Diet 16	SEM	Diet effect
Gain ,g/b/d	194.4	145.3	162.1	184.5	12.78	0.064
FI, g/bird	721.2	649.1	719.4	686.2	20.57	0.081
Gain : Feed, g/kg	269.7	224.3	225.3	269.7	19.88	0.210
WI[1], g/b/d	1249.8	1108.8	1203.3	1107.5	61.55	0.303
W:F[2]	1.22	1.33	1.27	1.25	0.068	0.702
Litter DM,%	61.9[a]	64.3[ab]	69.8[b]	65.5[ab]	1.43	0.010
EER, Kcal/g	0.060	0.070	0.058	0.067	0.008	0.705
PER, g/g	0.962	1.158	0.990	1.180	0.138	0.590
Ov. Gain, g/b/d	149.9 [b]	130.6 [a]	140.2[ab]	140.6[ab]	4.51	0.062
Ov. FI, g/b/d	416.8[b]	368.3[a]	391.8[ab]	380.5[ab]	9.92	0.022
Ov. G:F, g/kg	359.9	355.5	358.0	370.1	10.81	0.789
Initial Wt, Kg	10.6[ab]	10.6[ab]	10.9[b]	10.0 [a]	0.173	0.015
Fin. Wt, Kg	16.0	14.6	15.3	15.0	0.37	0.096

[ab] Means in the same row but with different superscripts are different (P< 0.05)
[1] water intake data collected for 12week in week 16
[2] water to feed ratio calculated from water and feed intake data for 12 week in week16, WI- water intake; W:F- water to feed intake ,g/g; EER – energy efficiency ratio (g gained per Kcal); PER – protein efficiency ratio (g gained per g protein consumed); Ov gain : gain for week 12 to 16; ov FI – feed intake for week12 to 16; Ov G: F - ratio gain to feed for week 12 to 16.

4.3 Litter Score

Table 4.7 shows the result of the litter score of the litters for the experimental diets. Data on litter score were collected on the last day of the study. In comparison to diet 1, only diet 3 had a lower ($P < 0.05$) litter score indicating a better litter condition for diet 3.

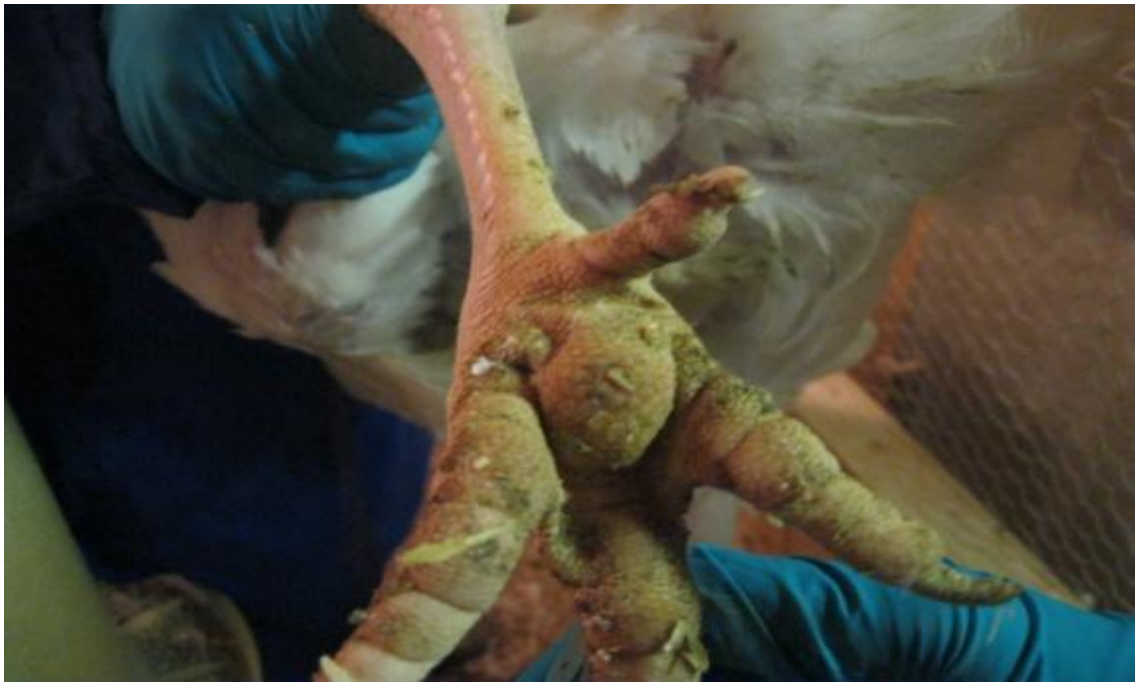
4.4 Foot Pad Score

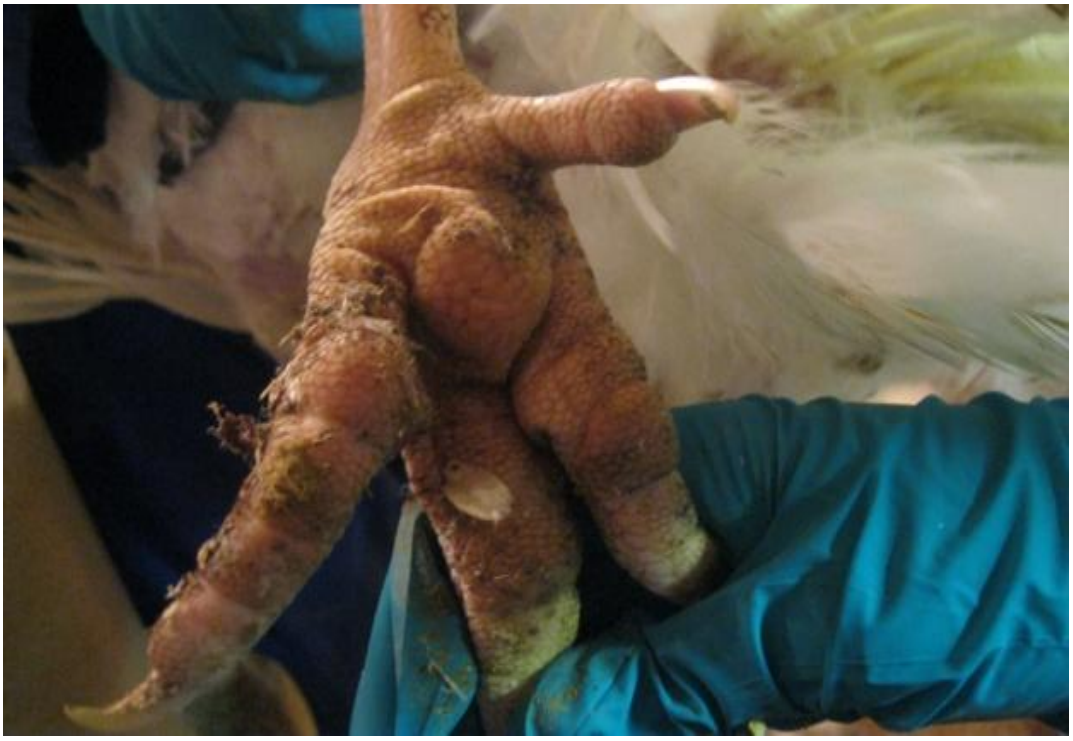
None of the birds showed signs of FPD as the average FP score was about zero. The foot pads were scored at the end of weeks 12 and 16 but there were hardly any signs of foot pad damage. The pictures of the foot pads are shown below (figure 4.1).

Table 4.7 Litter Score for the litters of the birds receiving the experimental diets

	Litter Score
Diet 1	.53
Diet 2	2.22
Diet 3	1.71
Diet 4	2.15
SEM	0.20
P-values for contrasts	
1 vs. 2	0.3261
1 vs. 3	0.0120
1 vs. 4	0.1971

Figure 4.1- Footpad score diagrams





4.5 Experiment 2

The growth performance data for the turkeys receiving graded levels of reduced CP with crystalline AA for 14 days in Experiment 2 are shown in Table 4.8. The final body weight for the turkeys fed diet 1was higher ($P < 0.01$) than for those fed diet 2 and diet 4 whereas diet 3 was intermediate. Crude protein levels had no effect on feed intake; however body weight gain was higher ($P < 0.01$) in diet 1 compared to diet 2 whereas diets 3 and 4 were intermediate. Gain: feed was higher ($P < 0.05$) for diets 1 compared to diet 2 and 4, whereas diet 3 was intermediate. The CP level did not show any significant differences between different diets on energy efficiency ratio ($P>0.05$).

Table 4.9 shows ileal nutrient digestibility of turkey receiving graded levels of dietary CP and supplemental AA at day 21. There were no effects of dietary treatments on the digestibility of any of the treatments. Total tract nutrient retention results are presented in Table 4.10. There were no effects of dietary treatments on dry matter retention. Nitrogen retention was higher ($P < 0.05$) in diet 1 compared to diet 4 but diets 2 and 3 were intermediate. The diet manipulation influenced energy metabolisability. This was higher ($P < 0.01$) and similar for diets 1 and 3 compared to diet 4 whereas diet 2 had intermediate values. Metabolisable energy was higher ($P < 0.01$) in diet 3 compared to diets 2 and 4.

Table 4.8 Growth performance of turkey receiving graded levels of reduced CP with crystalline AA for 14 days (expt. 2)

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	Diet effect
Fin. Wt, g	796.9[b]	671.2[a]	724.2[ab]	671.7[a]	22.92	0.004
Initial Wt, g	200.2	200.4	200.2	200.2	0.14	0.569
EER, g/kcal	0.228	0.201	0.212	0.214	0.005	0.039
FI, g/b/d	928.3	832.2	875.5	783.7	36.31	0.071
Gain :Feed, g/kg	643.4[b]	566.7[a]	597.8[ab]	602.2[ab]	16.67	0.039
Gain, g/b/d	596.8[b]	470.8[a]	524.0[ab]	471.5[a]	22.88	0.004
PER, g/g	2.21	1.98	2.13	2.19	0.059	0.066

[ab] Means in the same row but with different superscripts are different ($P \leq 0.05$)
[1] water to feed ratio calculated from water and feed intake data for 14 day, EER – energy efficiency ratio (g gained per Kcal); PER – protein efficiency ratio (g gained per g protein consumed).

Table 4.9 Ileal nutrient digestibility of turkey receiving graded levels of decreasing dietary CP and supplemental AA at day 21.

	Dry Matter%	**Energy %**	**IDE %**	**Nitrogen%**
Diet 1	62.36	63.84	2865	84.37
Diet 2	63.95	65.19	2864	80.64
Diet 3	65.01	67.26	3064	67.62
Diet 4	64.39	66.72	3020	75.37
SEM	4.62	4.58	205.8	4.65
Diet effect	0.922	0.776	0.683	0.372

[ab] Means in the same column but with different superscripts are different ($P \leq 0.05$)

Table 4.10 Total tract retention

	Dry Matter%	Nitrogen%	Energy %	AME kcal/kg
Diet 1	71.02	72.07[a]	74.28[a]	3357[ab]
Diet 2	68.91	57.78[ab]	71.94[ab]	3183[bc]
Diet 3	71.68	61.54[ab]	76.22[a]	3453[a]
Diet 4	65.91	44.86[b]	68.48[b]	3041[c]
SEM	0.184	4.76	1.275	57.8
Diet effect	0.184	0.020	0.003	<0.001

[ab] Means in the same column but with different superscripts are different (P≤ 0.05)

DISCUSSION

5 Discussion

Nutrition is a major contributory factor for wet litter problems. Protein is an important nutrient in poultry but if not properly balanced for amino acids (AA) it can impact negatively on turkey performance, gut health and welfare. There are metabolic and pollution costs associated with providing excess AA, thus it is beneficial to accurately meet the animal`s AA need with a reduced protein diet. In addition, poorly digested protein can cause wet litter which can lead to hock burn and foot pad dermatitis. Therefore reducing the dietary protein level is one of the recognized strategies to address the environmental issues arising from intensive livestock production (Bregendahl *et al.*, 2002). Supplementing low-protein diet with crystalline AA reduces the need for feeding intact protein and may decrease the impact of protein feeding on the environment.

5.1 Experiment 1

Several studies have shown that growth performance and carcass composition become inferior to those of broiler chicks fed standard high-CP diets when the dietary protein is lowered by more than three to four percentage points (Bregendahl *et al.*, 2002, Kamran *et al.*, 2008, Fancher and Jensen, 1989, Pesti, 2009). In the current study, the experimental diets were formulated to be have lower crude protein level while supplementing with crystalline AA to ensure optimum performance and reduce incidence of wet litter.

Over the 4 phases, sixteen diets were designed to have successively reduced crude protein levels. The chemical analyses indicated that the crude protein contents of the diets especially during the first phase were wider than anticipated and the difference in crude protein content during the fourth phase was narrower than planned. Bregendahl *et al.*, (2002) reported that lowering CP level by 1 % relative to the control (23 % CP) produced a reduction in N excretion although it did not support equal growth performance in comparison to birds receiving the high protein control diet. In a similar experiment, Kamran *et al.*, (2010) indicated that lowering dietary CP by 1% relative to the control diet (23 % CP) resulted in reduced N excretion.

Results from phase 1 in the current study showed that reducing the level of protein decreased the weight gain over the first 3 weeks but did not affect the gain: feed ratio or the water: feed ratio. The differences in daily weight gain produced a significant difference in the treatments for the final body weight at the end of phase one chicks fed low protein diets did not gain much weight as those receiving chicks fed the high protein diets. Similarly, Pesti, (2009) observed that lowering CP did not result in maximum growth performance. The data for growth performance in this study shows that during phases 2, 3 and 4 weight gain and feed intake were not influenced by lowering CP compared to those control as opposed to the significant effect observed during phase 1**.**

The differences in the growth performance responses at different growth phases may be an indication of the lower nutrient requirement as the bird's age or ability of the birds at older age to better tolerate reduced dietary intact protein. Therefore, the

depression in growth performance that may result from insufficient nutrient supply at early age may be partly compensated for at later ages if the disparity in nutrient supply is corrected.

In a review Sterling *et al.*, (2003) reported that the amino acid requirements of broilers are in constant proportion of CP levels at least in the range of CP levels commonly fed. In contrast, Sklan and Plavnik, (2002) reported that increasing CP level resulted in a linear decrease in feed intake while weight gain and feed efficiency changed quadratically with a smaller positive effect at the highest crude protein intakes. In this study, decreasing CP level resulted in a decrease in all responses with exception of litter dry matter which decreased in a quadratic fashion and PER which increased in a linear manner.

The effect of decreasing dietary crude protein level and supplementing with crystalline amino acids on litter dry matter can be partly explained by the effect of crude protein on water intake and hence water excretion. Dietary protein fed to birds must be catabolized and excreted via the kidneys in the form of uric acid. The excretion of uric acid requires water and hence increased intake of intact protein is accompanied by higher water consumption.

Furlan *et al.*, (2004) indicated that broilers fed diets with low crude protein content (16%) reduced their water intake. Furthermore, an increase of 1 % point in protein level increased water consumption by 3% (Francesch and Brufau, 2004). In addition, Vieira (2005) reported that water intake may be affected by several factors, including

changes in levels of some nutrients. In the current experiment, water intake in birds fed high CP in diet 1 was higher than those fed lower CP diets 2, 3 and 4. This observation was made in phases 1 and 2, but not in phase 3 and 4. A possible explanation for the disparity in effect of dietary protein on water intake at the different phases may be found by a closer examination of the dietary ingredients. Each diet had soybean meal at different levels within and between phases and it is likely that the soybean meal levels may affect the water intake.

According to Vieira (2005), soybean meal diets can have three times more potassium level than the requirement for the animal. Potassium is an electrolyte known to induce water consumption. Interestingly, the diets with lower crude protein (i.e. lower soybean meal levels) have lower water intake. It may be that the lower levels of soybean meal in the diets fed at phases 3 and 4 and the narrow differences in soybean meal levels in the diets fed at these phases were responsible for the lack of difference in water consumption. Nevertheless, the low level of soybean meal in the diets is coincident with lower dietary intact protein levels. Dietary protein levels in excess of requirements causes an increased heat increment and water intake, which results in an elevated litter moisture content (Kamran *et al.*, 2008).

Swennen *et al.* (2005) showed that birds reared on diets with a high protein level may increase their amino acid (AA) oxidation rate. Therefore, birds receiving high protein diets will usually have higher water intake, possibly because of an ionic imbalance, amino acid imbalance which ultimately will require additional water for uric acid excretion.

Another point to consider in current experiment was the different results for litter dry matter during the growth phases. In the first phase, reduction in dietary CP levels did not affect the moisture content of the litter; similar observation was made in phase 3. In contrast, the reduction in dietary CP level reduced the litter moisture content in phases 2 and 4. It has been reported that birds receiving an excess of dietary protein have reduced protein digestibility, increased heat production and also increased water consumption, which ultimately produce increased moisture content of the litter, and consequently it had been observed that litter moisture linearly decreased with a reduction in dietary CP content (Ferguson *et al.*, 1998, Kamran *et al.*, 2010).

It stands to reason that the effect of dietary protein on litter moisture will be more pronounced the longer the birds stayed on their litter. Apparently, the lack of difference in litter DM in phase 1 in the current study may be because the birds had only been on the litter for 3 weeks, whereas the lack of difference in phase 3 may be because of substantial pen to pen differences in litter dry matter content, as indicated by the relatively large standard error of the means.

Results from all the phases indicated that energy efficiency ratio was not affected by CP reduction ($P > 0.05$). Protein efficiency ratio was lowest ($P < 0.05$) in the diet with the highest protein level. The birds receiving lower intact dietary protein had similar weight gain with those consuming higher CP and so had a more efficient utilization of CP compared with the control. Information of the effect of reducing

dietary protein on PER and EER is scanty but it seems intuitive that birds gaining weight at the same rate with those consuming greater level of crude protein will have a more superior PER.

5.2 Experiment 2

The objective of the Experiment 2 was to investigate the influence of reducing dietary CP level on nutrient utilisation in the turkeys at day 21. In the current study there were no effects of dietary treatments on ileal nutrient digestibility of any of the treatments. In addition, there were no effects of dietary treatments on dry matter retention but nitrogen retention was higher ($P < 0.05$) in diet 1 compared to diet 4 whereas diets 2 and 3 were intermediate. It is expected that diets with lower CP and more crystalline amino acids will have a more efficient nitrogen utilisation. In the current study though, all the diets were formulated to have similar levels of digestible amino acids, and hence the possible negative effect of a likely amino acids imbalance had been removed.

It may be that the lower N retention in the diets with lower CP compared with the adequate-CP diet was due to the wider-than-expected difference in CP level between the highest- and lowest-CP diets. It is likely that the low-CP diets did not support optimum nitrogen and amino acids needs and hence elicited greater N excretion as a proportion of N intake thus bringing down N retention values. Data on effect of reduced CP level and nutrient digestibility are scanty, but the current study is in agreement with (Kamran *et al.*, 2010) observation who observed that lowering dietary CP content resulted in reduced N excretion. It should be noted that although

N excretion was lower in the lower-CP diets, the excretion as a proportion of intake was not.

The dietary CP manipulation influenced energy metabolizability, which were higher in diets 1 and 3 compared with diet 4. Metabolizable energy was also higher in diet 3 compared to diet4 whereas diets 1 and 2 had intermediate values. It would be expected that diets with better amino acid balance will have a more optimal energy utilisation, but such effect is probably nullified in the current experiment because all the diets were formulated to be similar in digestible amino acid content basis. Nevertheless, the high AME in diet 3 may be as a result of its lower ash and higher ether extract contents.

5.3 Conclusions

It can be concluded from the both experiments that it is possible to use reduced protein levels in diets formulated to have similar digestible amino acid content without jeopardizing growth performance. The reduction in dietary protein can reduce the moisture content in excreta and consequently improve litter quality.

5.4 - Future work

- Investigate the effect of reduction of CP on the carcass quality in turkeys.
- Use wider crude protein differences in the diets of turkeys
- Examine the influence of reduction of CP on carcass nutrients accretion in turkeys.

- Study the effects of fortifying low crude protein diet with crystalline amino acids on blood ammonia level in turkeys as an index of protein utilisation.

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