IMPROVING UTILIZATION OF POULTRY FEEDSTUFFS WITH SUPPLEMENTAL AMINO ACIDS AND ENZYMES

by

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Abstract

Four studies were conducted to evaluate threonine and tryptophan requirements by poultry. A purified diet based on the ideal amino acid (AA) profile concept for 0-3 week old broilers was established in the first study. Different ratios of threonine and tryptophan to lysine were evaluated in the second study and the results indicated that dietary digestible threonine and tryptophan for 0-3 week old broilers should be targeted at 65% and 16% of digestible lysine, respectively. Studies using reduced protein (RP) diets based on practical ingredients indicated that dietary total threonine and tryptophan for 0-3 week or 3-6 week old broilers should be targeted at 0.74% (or 4.04% of crude protein (CP)) and 0.23% (or 1.22% of CP) or 0.67% (or 3.20% of CP) and 0.17% (or 0.89% of CP), respectively. The corresponding values for 42-50 week-old layers are 448 and 152 mg/hen/d, respectively. Crystalline AA supplementation of the RP diets improved the AA balance in the diet, thus improving protein utilization efficiency and resulting in reduced nitrogen in the excreta. The nutritive value of palm kernel cake (PKC) was investigated in several studies and it was found to contain low levels of metabolizable energy (ME) and moderate quantities of most nutrients. The low AA digestibility and the low ME of PKC were attributed to nutrient entrapment within the cell, lack of appropriate enzymes to break down mannan, as well as processing method. An enzyme mixture (mannanase, α-galactosidase and protease) significantly increased the ME of PKC and improved the performance of both broilers and layers fed PKC-based diets. The results indicated that a 20% PKC diet could be used during the broiler starter or grower phase, but not during both phases unless supplemental enzymes were used. Laying hen performance was not different from controls when a 12.5% or 25% PKC-based diet was used. However, layers consumed more of the PKC diets and had poorer feed efficiency than the controls. Egg yolks were paler in birds fed the 25% PKC diets. A computerized feed formulation study showed that PKC is probably best suited for layer diets under the prevailing price situation in Malaysia.

Key words: ideal amino acid concept, threonine, tryptophan, palm kernel cake, enzymes.

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Contributions to Knowledge

Two different approaches were used to improve the utilization of poultry feedstuffs, first, with the use of supplemental crystalline amino acids. The responses of broilers and layers to different levels of dietary threonine and tryptophan were studied in an attempt to better understand the requirement of birds for threonine and tryptophan. Secondly, supplemental enzymes were used. The nutritive quality of palm kernel cake (an agricultural by-product) and the potential of enzyme supplementation to improve its nutritive value for poultry were investigated in several studies. New information was obtained as follow:

1. The requirements of 0-3 week old broilers for threonine and tryptophan were estimated. The broiler study indicated that dietary threonine and tryptophan for 0-3 week old broilers should be targeted at 0.74% of the diet (equal to 65% of digestible lysine or 4.04% of CP) and 0.23% of the diet (equal to 16% of digestible lysine or 1.22% of CP), respectively.
2. The requirements of 3-6 week old broilers for threonine and tryptophan were estimated. The broiler study indicated that dietary threonine and tryptophan for 3-6 week old broilers should be targeted at 0.67% of the diet (or 3.40% of CP) and 0.17% of the diet (or 0.89% of CP), respectively.
3. As indicated by the layer study, the daily requirements of 42-50 week old laying hens for threonine and tryptophan were estimated to be 448 mg threonine/hen and 152 mg tryptophan/hen, respectively.
4. This new information on the requirements of poultry for threonine and tryptophan is valuable to poultry nutritionists who want to supplement crystalline threonine and tryptophan in poultry diets.
5. The efficiency of nitrogen utilization was improved in the reduced protein diets with improved amino acid balance and resulted in a 25% to 46% reduction in nitrogen content in the excreta. The use of reduced protein diets could be an option for the poultry producer who wants to reduce the nitrogen content in the excreta.
6. A purified diet based on an amino acid profile proposed by Blair *et al.* (1977) could support better growth than diets based on the amino acid profiles proposed by the National Research Council for poultry (1994) and The University of Illinois. This amino acid profile could be

used by other researchers or poultry nutritionists for the development of an ideal amino acid profile for broilers.

7. Nutrient profiles (proximate constituents, metabolizable energy, true amino acid digestibility) of several palm kernel cakes were determined. These profiles are valuable to the Malaysian feed industry since data reported on palm kernel cake are limited.
8. An enzyme mixture with mannanase, protease and α-galactosidase activity was found to be able to break down the non-starch polysaccharides of palm kernel cake resulting in an increase in apparent metabolizable energy and nitrogen-corrected true metabolizable energy.
9. Dietary inclusion of 20% palm kernel cake either during the starter or grower phase did not reduce broiler performance at the 6^{th} week. However, dietary inclusion of 20% palm kernel cake during both the starter and grower phases is not recommended for broilers unless supplemental enzymes are used.
10. When 20% palm kernel cake grower diets without enzyme supplementation were fed, broilers were found to be more susceptible to heat stress under hot tropical climates. Poultry producers should not include high levels of palm kernel cake in the broiler diet during hot summers.
11. Laying performance of birds was not adversely affected when laying hens were fed either 12.5% or 25% palm kernel cake diets without enzyme supplementation. However, layers consumed significantly more palm kernel cake-based diets and had a poorer feed conversion ratio than layers fed the corn-soybean meal control diets.
12. Egg yolk color became significantly paler for layers fed the 25% palm kernel cake diets. Unless supplemented with synthetic pigmenting agents, the dietary level of palm kernel cake should be limited to 12.5% in countries where customers demand a darker color yolk.
13. Enzyme supplementation improved the performance of broilers and layers fed palm kernel cake-based diets. The use of enzymes in palm kernel cake-based diets remains promising.

CHAPTER I

Introduction

It is well known that methionine and lysine are the first limiting amino acids (AA) in corn-soybean meal poultry diets. Supplementation with these AA contributes to increased utilization of dietary protein and improves broiler growth and feed conversion efficiency (Peisker, 1996). Currently, feedstuffs are combined to meet the bird's needs for the most limiting AA. This usually results in a higher than required dietary protein content due to the presence of AA in excess. For example, in a typical layer diet based on corn and soybean meal, the sulfur AA are at about 100% of requirement; however, the level of other AA varies from 125% to over 300% of requirement (Davis and Austic, 1994). Excess dietary AA are utilized inefficiently by animals because the AA are deaminated and the nitrogen is excreted primarily as uric acid in avian species (Murray *et al.* 1993). Furthermore, the excess AA are an expensive source of metabolizable energy, yielding about 4 kcal/kg (Baker, 1997). Hence, minimizing excess AA in a diet makes economic sense.

Experiments with poultry have supported the hypothesis that diets formulated to minimize an excess of AA over the chicks' known requirements improve the efficiency of protein and energy utilization (Waldroup *et al.,* 1976). In addition, research conducted at the University of British Columbia indicated that reduced protein diets significantly improve protein utilization in feedstuffs, leading to a reduction in the daily nitrogen excretion of broilers by more than 20% and of layers by more than 30% (Blair *et al.,* 1999). The reduction in excretion of nitrogen by poultry is one of the most important benefits of improving protein utilization in their feeds, especially in areas with intensive animal production, where pollution of groundwater by nitrogen originating from animal manure is becoming an increasing environmental burden.

With the use of commercial feed-grade lysine, methionine, threonine and tryptophan, diets with a reduced protein content which meets the bird's requirement for essential AA can now be formulated. However, before nutritionists can reduce the protein content of a feed by crystalline AA supplementation, it is necessary to have an adequate knowledge of the AA requirements of the animal and an understanding of the multitude of factors that affect the AA requirements for poultry. Considerable research has been conducted to determine the requirements of poultry for lysine and methionine. Unfortunately, the requirements of poultry for

threonine and tryptophan are not well established, especially for layers and broilers beyond 3 weeks of age. The National Research Council (NRC, 1994) recommended poultry requirement values for these two AA are derived from research conducted more than 10 years ago. It should be noted that the AA requirement of the 1998 broilers is not the same as the AA requirement of the 1978 broilers because the 1998 broilers weigh nearly twice as much as the 1978 broilers of the same age (Dudley-Cash, 1998). Therefore, accurate information on the quantitative requirements for threonine and tryptophan needs to be revised for various ages of poultry in order for nutritionists to make the best economical decisions in feed formulation.

The cost of feeding poultry for production of eggs and meat is between 70% and 80% of the total cost of production. Corn, fish meal and soybean meal, which constitute the major portion of poultry diets in most Asian countries, are imported and are relatively expensive. Several countries in the Asian region, including Malaysia, have expressed concern over their limited feed supplies, the increasing cost of imported feeds, and the need to increase the use of alternative feedstuffs. Inadequate availability of energy and protein-rich ingredients is the major constraint for the growth of the poultry industry in developing countries. Nutritionists are continually searching for alternative, unconventional agro-industrial by-products to include in poultry diets.

In Malaysia alone, large quantities of different usable by-products and residues of varying nutritive value are produced annually. However, overall utilization remains low when compared to the total availability due to limited appreciation of the potential value of by-products, inadequate knowledge or technology in the usage of the by-products, inadequate transfer of technology, problems of quality and consistency of products, as well as their unsuitability for non-ruminant animals due to high fiber and/or low protein content. Since many by-products or wastes have substantial potential value as ingredients in livestock diets, their utilization may be economically worthwhile, especially in developing countries, where traditional and often more expensive imported feedstuffs could be replaced. Recycling, reprocessing, and use of enzymes to increase nutrient digestibility and availability offer the possibility of putting industrial by-products to beneficial use, as opposed to traditional methods of disposal and relocation of the residues. Of the huge quantities of agricultural by-products produced in Malaysia, palm kernel cake (also referred to as palm kernel meal), the by-product of palm oil extraction, has the greatest potential as an animal feed.

The world population has been growing rapidly. It is estimated that 2000 years ago the population of the world was about 300 million. In 1998, the world's population stood at 5.9 billion and it is growing at 1.3 per cent per year - an annual net addition of 78 million people (United Nations, 1998). Based on the conservative estimations by the United Nations (1998), the world population will reach 8.9 billion in 2050. With this increase in population, the same agricultural lands that have been used for over 2000 years will have to produce much more food. However, unlike the ever growing population, the amount of cultivable land on earth will not increase, and further increases in land area devoted to cereal grains, oil seeds and legumes will be difficult to achieve, especially under the justifiable pressures from environmental groups for sustainable agriculture and nature conservation. Recently, in an anniversary lecture presented at the 6th Asian Pacific Poultry Congress, Sheldon (1998) emphasized the importance of developing alternative and cheaper poultry feed ingredients in order to avoid competition with those essential for human food. Coupled with this, we have seen signs of global warming and unexpected climatic events such as *El niño* and *La niña* that might have a negative impact on crop production. Undoubtedly, improving the energy and protein utilization of alternative feedstuffs that do not compete as food for human use and improving utilization of current feedstuffs will conserve feed resources and ease the tension resulting from human-animal food competition.

During the century that is about to end, the livestock industry has faced many challenges that need to be tackled before it moves into the new millennium. Before stepping into the next millennium, livestock producers need to be made aware that animal production in the future has to be friendly to the animals, the consumers and the environment.

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CHAPTER II

Literature Review

Threonine and Tryptophan Requirements of Poultry

In order to maximize the utilization of dietary amino acids (AA), knowledge regarding poultry requirements for these AA is crucial. Considerable amounts of research have been conducted to determine the requirements of poultry for lysine and methionine. After methionine and lysine, threonine, tryptophan, isoleucine and arginine are the next limiting AA in most poultry diets (Elliot, 1995). However, only limited amounts of requirement data have been published for these essential AA. With the increased availability of commercial feed-grade threonine and tryptophan, a better understanding of the requirement of these AA for poultry is essential. Furthermore, the National Research Council (NRC, 1994) threonine requirement value for 0-3 week old broiler chicks was derived from research done in the 1980s whereas published reports dated from 1947 to 1988 were used to derive the tryptophan requirement value. In addition, not many published data were available for use by the NRC (1994) to derive its recommendations for threonine and tryptophan for 3-6 week old broilers and for laying hens. With today's highly productive birds, it is not clear whether poultry nutritionists should still follow the NRC (1994) recommended requirements for threonine and tryptophan. As a result, more studies are required to derive the threonine and tryptophan requirements for poultry.

Threonine requirements of poultry

A limited number of experiments related to threonine requirements for poultry were conducted in the early 1970s. D'Mello and Lewis (1970) looked at the interactions between certain essential AA. The results of their experiment on threonine-tryptophan interaction indicated that excess threonine markedly retarded the growth of broiler chicks, the extent of the inhibition being proportional to the amount of the surplus. These adverse effects, however, were reversed by appropriate supplementation of the diet with tryptophan. At dietary concentrations of 0.80, 1.30, 1.80 or 2.30% threonine, the tryptophan requirement was determined to be 0.17, 0.18, 0.19 and 0.20% of the diet, respectively. It is clear that as the concentration of threonine is increased, there is a concomitant increase in the requirement for tryptophan. The reason for this

kind of interdependent relationship is not clear. Perhaps these two AA share the same AA transport system within the intestinal gut, and excesses of one reduce the absorption of the other. This kind of interaction has also been reported to occur in rats (Morrison and Harper, 1960; Florentino and Pearson, 1962). Using 18% crude protein (CP) corn, soybean meal and AA diets, Hewitt and Lewis (1972) showed that the threonine requirement for 7-21 day old broiler chicks was 0.53% of the diet, while Woodham and Deans (1975) found that 2-4 week old broiler chicks required 0.50 - 0.52% of threonine in their diet. Even though the age of the chicks in these two separate experiments was slightly different, the derived threonine requirements were quite similar.

Thomas *et al.* (1986) conducted two experiments with male broilers ranging from 7 to 21 days of age that were fed graded levels of threonine which were added to a corn, peanut meal, and soybean meal based diet supplemented with crystalline AA. The optimum level of threonine for weight gain and feed efficiency was found to range from 0.73% to 0.77% of the diet. The threonine requirement for the feed efficiency as determined by a regression equation after a second study was 0.73%. A further study by the same researchers (Thomas *et al.*, 1987) confirmed that the threonine requirement for male broilers was 0.72%, while female broilers tended to need less (0.67%). Data presented by Robbins (1987) clearly showed that when expressed as a percent of the diet, the estimated threonine requirements increased as the dietary CP increased. However, when expressed as a percent of protein, the estimated threonine requirements remained constant relative to dietary protein content. Robbins (1987) concluded that the threonine requirement was 3.7% of dietary protein for female chicks during the starter phase. If this value is correct, then a 23% CP starter diet would need to contain 0.851% threonine, which is 6.4% higher than the 0.80% reported by the NRC (1994).

The threonine requirement of starter male broilers was determined by feeding threonine deficient (0.59%) grain sorghum-soybean meal diets either supplemented with or without crystalline threonine (Smith and Waldroup, 1988a). The threonine requirement for maximum weight gain and feed efficiency was 0.68% and 0.79%, respectively. The requirement value for feed efficiency is in agreement with the NRC (1994) recommended value of 0.80% for 0-3 week old broilers. The University of Cornell studies showed that both the source and the level of protein might influence threonine requirements (Austic and Rangel-Lugo, 1989). The threonine requirement of a commercial strain of broilers fed a diet containing 20% CP was 0.67 - 0.74% of the diet. However, when the CP of the diet was increased to 25%, the requirement for threonine

increased by 9 to 19% (i.e. to 0.75 - 0.81% and 0.81 - 0.88%, respectively) in the two experiments based on wheat, peanut meal and crystalline AA diets. The threonine requirement was 0.68 - 0.75% when chicks were fed 25% CP diets based on corn, soybean meal and crystalline AA. Austic and Rangel-Lugo (1989) suggested that the difference in threonine digestibility in the two experimental diets might explain the observed wide variation in threonine requirement. Recently, similar experiments were carried out by the same group of researchers (Rangel-Lugo *et al.* 1994). Broiler chicks were used to evaluate the threonine requirement for weight gain and feed efficiency up to 2 weeks of age using 20 and 25% CP wheat-peanut meal based diets. Threonine requirements for maximal weight gain in the 20 and 25% CP diets were 0.67 and 0.77%, respectively. These findings are in agreement with the conclusion made by Robbins (1987) that as dietary CP is increased the threonine requirement is also increased. In addition to these studies, the threonine requirement for weight gain and feed efficiency in 16 to 28 day old broilers receiving a 20% CP diet was 0.63 and 0.69%, respectively.

Studies from Mexico (Morales-Barrera *et al.*, 1992) indicated that in 21-22% CP starter diets the threonine requirement is between 0.81 and 0.84% of the diet. This is at variance with another report from Mexico (Moreno *et al.* 1993) which stated that 0.70% dietary threonine was required by 0 to 3 week old broilers fed 20-22% CP sorghum-soybean meal diets and feed efficiency was worst when the level reached 0.85%. From two experiments conducted by Holsheimer *et al.* (1994), it was concluded that in 16% CP maize-soybean meal diets supplemented with essential AA and non-essential AA, containing 13.31 MJ ME/kg, an improvement in gain and feed efficiency was observed for both sexes of broiler chicks, when the dietary threonine content was increased to 0.725% of the diet until 3 weeks of age, and to 0.632% of the diet for females to 4 weeks of age. Two experiments were conducted by Koide and Ishibashi (1995) to confirm the effect of age and AA levels on female broiler requirements for threonine. Data from these studies indicated that dietary threonine requirements expressed as a percentage of diet and CP level for maximum body weight gain and feed efficiency decreased with age and had a tendency to increase with increased dietary AA levels. In one experiment containing 19.8% CP, threonine requirements for broilers 5-15 and 25-35 days of age were 0.818% (4.13% CP) and 0.75% (3.79% CP), respectively. In the other experiment, threonine requirements for birds fed a low AA level diet (15.35% CP) were 0.608% (3.96% CP) and 0.586% (3.81%), respectively, for birds 8-18 and 28-35 days of age. On the other hand, threonine requirements for birds fed the high AA level diet (16.19% CP) were 0.667% (4.12% CP) and

0.621% (3.84%), respectively, for birds 8-18 and 28-35 days of age. In another study from Japan Yamazaki *et al.* (1997a) reported that the available threonine requirements for 1-3 week and 4-6 week old broiler chicks were 0.65% (3.46% CP) and 0.54% of diet (3.18% CP), respectively. This was equal to 0.74% (3.94% CP) and 0.60% (3.53% CP) total threonine, respectively. Both values were much lower than those reported by the NRC (1994) for broilers of a similar age.

Even though broilers consume much more feed in the second phase of the production cycle, knowledge of the threonine requirement for birds aged beyond 3 weeks is limited. Thomas *et al.* (1992) conducted two studies to determine the threonine requirement for 3-6 week old broilers. A 20% CP, 3,212 kcal ME/kg corn-peanut meal basal diet with seven graded levels of L-threonine (0.53 - 0.77%) was used. For males, the performance of the birds receiving 0.61% threonine was similar to those fed higher levels of threonine. The data also showed that females required less threonine, that is, 0.57% of the diet from 4-6 weeks of age. Kidd (1996) indicated that dietary CP in 21 to 42 day old broilers may be reduced from 20% to 16.8% provided that methionine, lysine and threonine are added to the low protein diet. Performance of the birds was not significantly different between the two diets (0.66% and 0.78% of total threonine respectively, in the 16.8% and 20% CP diet). The use of a low protein diet would greatly reduce nitrogen (N) output in the manure. However, as reported earlier, the low protein diet (16.8%) also caused the birds to deposit significantly more abdominal fat. In another study, commercial broilers were fed threonine-deficient experimental diets composed of sorghum, peanut meal, corn and poultry meal from 30 to 42 days of age (Kidd and Kerr, 1997). The total threonine requirement was set at 0.70% of the diet (3.66% CP), as maximum weight gain and feed utilization efficiency was obtained at this level. However, it is interesting to note that birds fed a diet containing lower threonine concentrations (0.65% of the diet or 3.40% of CP) had similar growth performance to the control birds (0.76% threonine). Growth and feed/gain ratios were similar for birds fed either the 0.65 or 0.70% threonine diet. Kidd and Kerr (1997) also noted that a higher level of threonine is required to maximize breast meat yield (0.76% of diet).

Four experiments were conducted to determine the digestible threonine requirement of broiler chickens during the periods of 3 to 6 and 6 to 8 weeks posthatching (Webel *et al.* 1996). Basal diets deficient in threonine (0.40% digestible threonine) were used. Threonine supplementation significantly improved the performance of birds in both age groups. Maximum feed efficiency was achieved at 0.61% and 0.52% digestible threonine respectively for 3-6 and 6-8 week old birds. Extrapolating these digestible threonine requirements to total requirements for

chicks consuming corn-soybean meal diets (threonine digestibility = 87%) resulted in estimates of 0.70 and 0.60% of the diets for broiler chicks during these two periods, respectively. The 3-6 week requirement was identical to that calculated by the ideal ratio procedure (70% of 1.0% lysine = 0.70% threonine) which was lower than 0.74% reported by the NRC (1994). More recently, Penz *et al.* (1997) reported that a concentration of 0.70% threonine for males and 0.60% for females appeared adequate for birds fed a diet containing 3,200 kcal ME/kg and 20% CP. This is in good agreement with Webel *et al.* (1996). By multiplying the threonine digestibility coefficient in the NRC (1994) by the analyzed threonine content of each of the basal diet ingredients, the digestible threonine requirement obtained for male and female was 0.59% and 0.51%, respectively.

The data on the threonine requirements for laying hens are very limited. Huyghebaert and Butler (1991) conducted an experiment with medium weight laying hens to determine their threonine requirement between 28-38 weeks of age. The daily threonine requirement of an individual laying hen was estimated by direct methods to be 8.7 mg/g egg output plus 43.49 mg/kg body weight in this experiment. That is, the threonine requirement for a 2.0 kg laying hen producing 50 g egg mass daily was 521.98 mg/d. However, a study from Japan showed that the available threonine requirement for laying hens 32 to 42 weeks of age was 329 mg/hen/day or 385 mg total threonine/hen/day (Yamazaki *et al.*, 1997b). This study also showed that the performance of the laying hens decreased as dietary threonine content increased beyond requirement. Another two recent studies were carried out in Japan using 29 to 30 week old layers (Ishibashi *et al.*, 1998). Experimental diets contained five graded levels of threonine and were fed for 21 and 58 days in Experiments 1 and 2, respectively. Threonine requirements obtained in Experiment 1 were 453, 456, and 458 mg per hen per day for egg mass, feed efficiency, and plasma threonine concentration, respectively. This agreed with the results obtained in Experiment 2 (457, 467, and 462 mg). However, the threonine requirements expressed as percentages of diet in Experiment 1 (0.425, 0.428, and 0.430%) were higher than those in Experiment 2 (0.395, 0.404, and 0.400%) for egg mass, feed efficiency, and plasma threonine concentration, respectively. The authors suggested that differences in feed intake were responsible for this variation. The authors also observed that as the dietary threonine exceeded the requirement level, egg mass and feed efficiency decreased with increasing dietary threonine levels, the reasons for which remain to be clarified. Meanwhile, Coon (1998) also conducted two studies to determine the digestible AA requirements for 33 and 35 week old laying hens. Corn-

soy-meat and bone meal diets were used in the studies. The estimated digestible threonine requirements for laying hens were found to be quite variable ranging from 430 to 560 mg/hen/day.

In conclusion, the estimates of threonine requirement for poultry are very variable. For 0-3 week old broiler chicks, they range from 0.50% to 0.851% of the diet or 2.78% to 3.70% of CP. They range from 0.57% to 0.78% of the diet or 2.85% to 3.90% of CP for older broiler chicks (4-6 weeks old). On the other hand, laying hens were reported to need from 385 mg to 560 mg/hen/day. It is also important to note that most of the research done on threonine requirements was performed on 0-3 week old broiler chicks. Data reported for broiler chicks beyond 3 weeks of age and for pullets and laying hens were not well established. As a result, one of the main objectives of studies reported in this thesis is to determine the responses of broilers and layers to different levels of threonine.

Tryptophan requirements of poultry

There is only a small number of published research papers on the requirement for tryptophan of growing chickens. The reasons for this are uncertain. Perhaps the lack of research is due to the different analytical procedures employed to measure tryptophan. Unlike other AA, the analysis of tryptophan requires alkaline hydrolysis and a different type of separating column. Most laboratories do not analyze for tryptophan. The availability of crystalline tryptophan on the market has generated a lot of interest regarding the requirements of poultry for tryptophan and the beneficial effect of supplementing diets with crystalline tryptophan.

In 1971, Boomgaardt and Baker conducted an experiment using female broiler chicks to determine the tryptophan requirements of growing chicks (8 to 14 day post-hatching) and whether dietary CP levels affected the requirement. A crystalline AA diet devoid of tryptophan was fed at 8.7, 11.6, 14.5, 17.4 and 20.3% protein. At each of these protein levels L-tryptophan was supplemented to provide total tryptophan levels of 0.414, 0.621, 0.828, 1.034, 1.241 and 1.448% of CP. When the tryptophan requirement values were expressed as a percentage of the diet, the requirement increased with dietary protein level. However, when expressed as a percentage of CP, the requirements remained constant at 0.87% at all five protein levels. These data agreed with those reported earlier by Griminger *et al.* (1956). While Boomgaardt and Baker (1971) used purified diets to derive tryptophan requirement for broiler chicks, the AA

requirements of growing chicks have been determined using a diet based on soybean meal and maize meal (Hewitt and Lewis, 1972). The diet contained 18% protein in which 14% of the protein was contributed by conventional ingredients and 4% was in the form of free AA. The results indicated that male broiler chicks aged 7 to 21 days required no more than 0.17% of tryptophan in the diet. The sex of the birds used might explain the difference in requirement for tryptophan reported for this study and that of Boomgaardt and Baker (1971). A similar study was conducted with broiler chicks between 14 and 28 days of age (Woodham and Deans, 1975) and it was found that chicks required less than 0.14% tryptophan in the diet, or 0.78% of CP.

In experiments described by Freeman (1979), the requirements of male and female broiler chicks for available tryptophan from 0 to 56 days of age were determined by using the diet-dilution technique. Requirements were estimated from the dose-response relationships between dietary tryptophan and growth performance at weekly intervals throughout the growing period. The available tryptophan requirements were 2.4 (males) and 2.2 (females) g/kg of diet from 0-7 days of age, and 1.7 g/kg (both sex) from 7-35 and 35-56 days of age. Freeman (1979) also concluded that the absolute requirement of a chick for tryptophan increased with age and was significantly different for male and female birds. These requirement (available) values were much higher than in earlier reports when converted to total tryptophan basis. The reason for this is not clear. In another experiment conducted by Steinhart and Kirchgessner (1984), birds fed a diet containing 0.22% tryptophan gave the best results with regard to weight gain, feed intake and feed efficiency. However, the differences from the groups fed 0.19% and 0.25% tryptophan were not significant. Perhaps, as the tryptophan level exceeded the requirement, it caused an imbalance of AA in these diets. Three experiments were conducted to determine the response of male broiler chicks to tryptophan supplementation (Smith and Waldroup, 1988b). Chicks from 7 to 18 or 7 to 20 days of age were fed either a L-tryptophan supplemented sorghum-soybean meal test diet or a control 23% CP corn soybean meal diet. No significant changes in body weight gain were observed when dietary tryptophan level was increased beyond 0.16% of the diet. Even though there were no significant differences in body weight gain between birds fed the control and the test diets, birds fed the test diets were less efficient in feed utilization. The lower protein in the test diets (20% CP) might have been deficient in one or more AA, thus causing the birds to eat more. It was concluded that a diet with 0.16% tryptophan could fulfil a growing chick's requirement for tryptophan.

Researchers from the University of Reading were interested in the effects of protein concentration on responses to dietary tryptophan by 4 to 18 day old broiler chicks (Abebe and Morris, 1990). Eight protein concentrations (16% to 30%) were combined with five tryptophan ratios (7.5 to 13.5 g tryptophan/kg CP) to provide 40 mash diets for the study. The amounts of tryptophan required for maximum growth and feed efficiency were each linear functions of dietary protein concentration. It was concluded that a fixed ratio of tryptophan to protein (12 g/kg CP or 1.2% CP) should be used in practical diet formulation, rather than a minimum dietary concentration of tryptophan. Similar experiments conducted by other researchers confirmed that the tryptophan requirement of broiler chicks is proportional to the dietary CP content (Rogers and Pesti, 1990). Various levels of protein and tryptophan were fed to 8-21 day old chicks. Protein in the diets ranged from 16% to 28% while tryptophan ranged from 0.34% to 2.74% of protein. Maximum gain and feed efficiency were attained when the dietary levels of tryptophan were 0.83, 0.77, 0.77, and 0.78 of the protein for 16, 20, 24, and 28% CP, respectively. This confirmed the results reported by Boomgaardt and Baker (1971) that as the CP of the diet increases, the requirement for tryptophan expressed as a percentage of CP remains constant. However, the requirement value reported (0.80% of CP) reported by Rogers and Pesti (1990) was much lower than that of Boomgaardt and Baker (1971). Han *et al.* (1991) carried out studies to determine requirements for available histidine and tryptophan in 8 to 22 day old chicks fed a histidine and tryptophan deficient intact protein diet containing 25% CP and 3,200 kcal ME_n/kg. It was found that the requirement for digestible tryptophan was 0.20% of the diet (or 0.80% of CP) for maximal weight gain and feed efficiency. This indicates that a total tryptophan level of 0.22% in the diet is necessary for chicks fed a 23% CP corn soybean meal diet. Finally, Kim *et al.* (1997) showed that 1-3 week old broilers required only 71.56 mg/day or 0.173% of the diet (0.99% of CP), 69.84 mg/day or 0.168% of the diet (0.97% of CP) based on the weight gain response and N gain response, respectively. This is much lower than that of the NRC (1994) and Han *et al.* (1991).

Hunchar and Thomas (1976) were one of the few research groups that embarked on a study of the requirement of tryptophan for chicks beyond 3 weeks of age. Basal diets containing approximately 25% CP and 3,300 kcal ME/kg were used in the studies. Based on the regression equations for males (females), the calculated tryptophan requirement for maximum growth, optimum feed efficiency, and molted body feather count for the 4-7 week period was 0.179 (0.173), 0.170 (0.163), and 0.172% (0.172%), respectively. Using a computer model, Hurwitz *et*

al. (1978) predicted that tryptophan requirements for broilers of different ages were 0.141% of the diet for 21-28 day old, 0.134% of the diet for 28-35 day old, and 0.118% of the diet for 35-42 day old chicks. Therefore, the average for 21-42 days of age was 0.131% of the diet. These estimated requirement values were much lower than those suggested by the NRC (1994). Furthermore these values (Hurwitz *et al.,* 1978) have not been rigorously examined.

Early research on the requirement of laying hens for tryptophan was conducted by Ingram *et al.* (1951) using a corn-corn gluten meal diet. The study showed that laying hens did not require more that 0.15% tryptophan in their diets. Further studies revealed that 0.142% tryptophan was required to maintain egg size, body weight and egg production (Ingram and Little, 1958). Using a low protein diet, Bray (1969) found that the requirement of laying hens for tryptophan was 0.110% of the diet or 117 mg/day per hen. Two experiments were conducted with laying pullets between 32 and 47 weeks of age (Morris and Wethli, 1978). A high protein summit diet was mixed with a non-protein mix to achieve different concentrations of tryptophan. For a flock of laying hens with a mean body weight of 1.5 kg producing 55 g egg mass/hen/day and consuming 110 g of feed per day, the optimum dietary tryptophan concentration was found to be 0.17%.

According to Tasaki (1983), poultry diets containing maize as the main ingredient are commonly deficient in tryptophan. White Leghorn pullets producing about 85% of eggs were selected and fed 16% CP maize-soybean meal diets with graded levels of tryptophan (0.066% - 0.721% of diet). Laying performance was lower when diets contained less than 0.1% tryptophan. Birds fed high levels of tryptophan did not show any ill effects and were able to maintain their body weight. The author suggested that 0.11% tryptophan as recommended by the Japan Feeding Standard was too low and that the 0.17% level as recommended by Morris and Wethli (1978) should be utilized. In some cases, the author argued that it might be advantageous to raise the tryptophan content in the diet to more than 0.20%. In order to determine the tryptophan requirement of laying hens, Ishibashi (1985) used 15 month old White Leghorn hens to carry out four experiments. The experimental diets were designed to provide the same amount of essential AA except for tryptophan, which ranged from 0.086 to 0.32% of the diets. The hens needed to consume 210, 212 and 212 mg of tryptophan per day in order to reach maximum egg production rate, egg production and feed intake, respectively. This was equivalent to 0.189% of the diet. In contrast to the finding by Tasaki (1983), the performance of the hens was significantly poorer at a higher dietary tryptophan concentration (0.32%). The author did not give any explanation for

this observation. Another report from Japan showed that supplementing L-tryptophan at 250 or 500 mg/kg to a nutritionally complete diet improved egg production and feed utilization in crossbred laying hens (Ohtani *et al.* 1989). Daily tryptophan intakes were 173, 207 and 239 mg by layers fed the basal diet, 250- and 500-mg tryptophan diets, respectively. Although a daily intake of 173 mg tryptophan was considered adequate by Morris and Wethli (1978) and Tasaki (1983), it was not supported by data reported by Ishibashi (1985) and Ohtani *et al.* (1989).

Several experiments were conducted to estimate the tryptophan requirement of laying hens (Jensen *et al.* 1990). In the first two experiments, hens were fed a 14% CP diet supplemented with four levels of L-tryptophan. Five levels of L-tryptophan supplementation and three levels of protein (14, 16 and 18%) were used in two additional experiments. Even though egg production was significantly improved by tryptophan supplementation in the first two experiments, production was inferior to that expected for commercial laying hens. Egg production was only significantly increased by tryptophan supplementation to the 14% CP diet in a third experiment. The derived requirement values were 0.137% (123 mg/day), 0.118% (95 mg/day) and 0.164% (168 mg/day) for Experiments 1, 2 and 3 respectively. In the fourth experiment, the requirement changed with the protein levels, calculation from the data showing that 0.923% of CP was required. Jensen *et al.* (1990) concluded that the requirement values in the first two experiments might have been underestimated and the diets in the last two experiments were formulated differently.

As with the case of threonine, data describing the tryptophan requirement for poultry are very variable. Reported requirement values for 0-3 week old broilers range from 0.14% to 0.276% of the diet or 0.78% to 1.20% of CP. On the other hand, 4-6 week old broilers were found to require 0.163% to 0.179% tryptophan in the diet or 0.65% to 0.716% of CP. Data reported for laying hens ranges from 95 mg to 212 mg/hen/day. The interpretation of past research is quite difficult because of variations in CP levels, energy levels, digestible threonine and tryptophan levels in the basal diets, essential and non-essential AA levels, bird age, duration of study, and environmental conditions. Certainly, more research should be carried out in the future in order to obtain a better understanding of the requirements of poultry for threonine and tryptophan. As a result, it is one of the objectives of my study to measure the responses of broilers and laying hens to different dietary levels of threonine and tryptophan.

Ideal Protein Concept

Ideal protein is defined as a blend of essential AA that meets an animal's requirement for protein accretion and maintenance exactly, with no excesses and no deficiencies. The ideal protein concept was originated and developed in the work of H. H. Mitchell and H. M. Scott at the University of Illinois during the late 1950s and early 1960s.

Amino acid requirements depend on the needs for maintenance and production. As maintenance needs only account for 3 to 6% for young birds (Emmert and Baker, 1997), the major difference between birds growing at different rates and between birds of different breeds and sexes is in the amount of protein they require depending on their genetic potential for lean tissue accretion. However, the relative amounts of different essential AA needed for the deposition of 1 g of lean meat tissue should not be different in every case. Therefore, it should be possible to establish an optimum balance of essential AA for growth which, when supplied with sufficient N for the synthesis of non-essential AA, would constitute the ideal protein.

The ideal protein concept uses lysine as a reference AA, with the requirements for all other essential AA expressed as a percentage of lysine (weight:weight basis). According to the Illinois group (Baker, 1997), lysine was chosen for several reasons: 1) after sulfur AA, lysine is the second most limiting AA in practical poultry diets, 2) data for lysine requirement under a wide range of conditions are readily available, 3) unlike the sulfur AA and tryptophan, the analysis of lysine in feed ingredients is straight-forward and less complicated, 4) unlike the sulfur AA and tryptophan, lysine's sole function in the body is protein accretion and maintenance (only lysine of endogenous origin (trimethyl lysine) is used for carnitine synthesis).

According to Baker *et al.* (1996), three important factors must be considered when using the ideal protein concept in poultry feed formulation. First of all, ideal ratios for poultry were based upon numerous studies done with purified AA diets. All of the ideal AA ratios are based on digestible levels of dietary AA, because this eliminated differences in digestion, absorption, and utilization of protein of various quality and sources. Obviously, digestibility of AA in feed ingredients must be factored into formulation schemes that use the ideal protein concept. Second, the ideal AA profile for the starter phase (0 to 21 days) differs in some respects from the ideal profile for the grower phase (21 to 42 days) of broilers. Specifically, the ideal ratios of sulfur AA, threonine and tryptophan to lysine increase as birds age, because the maintenance requirement ratios for these AA exceed protein accretion requirement ratios. Finally, since lysine

is the reference AA, its requirement value is very important, because it is the basis for setting requirements for all other essential AA. Even though the concept of the ideal protein was first elaborated in the USA, the best ideal AA ratios were not fully developed, and not much research was done in this area. As a result, the concept was not widely used as far as practical animal feeding was concerned. It was not until 1981 that the ideal protein concept was revived and actually put into practice in swine formulation by the Agricultural Research Council (1981).

For over 30 years, work has been done at the University of Illinois on proper AA ratios for broiler chicks. Most of this work, however, was done with chicks between 0 and 21 days of age fed purified AA diets. Based upon calculations, the Illinois group has estimated the ideal AA ratios for both early and late growth of broilers (Baker, 1997). They have also translated these into projected AA requirements for each growth phase (Table 2.1). The digestible lysine requirement for early growth is based upon the work of Han and Baker (1991, 1993). For the grower phase (3 to 6 weeks), the lysine requirement of Ross x Ross broiler chicks was found to be 0.89% and 0.84% of the diet (3,200 kcal AME/kg) for male and female chicks respectively (Han and Baker, 1994). Recently, two chick bioassays with chemically defined AA diets were conducted to compare the Illinois Ideal Chick Protein (IICP) AA profile with that of the NRC (1984) and the NRC (1994) (Baker and Han, 1994). Birds fed the IICP AA profile diets had higher rates of gain per unit of essential AA N intake than those fed the NRC (1994) profiles. Since the diet based upon IICP ratios did not show any response to further increases in AA supplementation, the authors concluded that the ratios in IICP for the 0 to 21 day growth period are not underestimated. The authors also suggested that the NRC (1994) estimate of the total lysine requirement (i.e., 1.10% of the diet) is too low. If the total lysine requirement in the NRC (1994) had been set to 1.20% of the diet, then the modified NRC AA ratios would be very close to the IICP ratios (Table 2.2).

Beside the ideal AA ratio proposed by the University of Illinois, there exist other suggested AA profiles. For example, if one expressed the requirement values of essential AA reported by Blair *et al.* (1977), the NRC (1994), and the Rhone-Poulenc's Rhodimet Nutrition Guide (1993) to lysine, one would have three AA profiles (Table 2.2). From Table 2.2, it is obvious that there are some differences between various AA profiles. The AA ratios proposed by the University of Illinois are quite similar to the NRC (1994), and that of Blair *et al.* (1977) is close to the Rhodimet Nutrition Guide (1993). Blair *et al.* (1977) and the Rhodimet Nutrition Guide (1993) also suggested that the ratios of most AA to lysine should be higher, whereas the

IICP and, to a certain extent the NRC (1994) suggested the opposite. Obviously, more research is necessary to clarify these discrepancies. Instead of using solely purified diets, research should also be extended to the use of practical ingredients so that the results can be applied in practice. Therefore, one of the objectives of studies reported in this thesis is to establish an ideal AA profiles for 0-3 week old broilers and use the ideal AA profile to measure the responses of 0-3 week old broilers to different dietary levels of threonine and tryptophan.

Table 2.1 Ideal amino acid ratios and digestible amino acid requirements of broiler chickens at three growth periods as proposed by the University of Illinois[1].

	0 to 21 days			21 to 42 days			42 to 56 days		
Amino acid	Ideal ratio	Requirement *Male*	*Female*	Ideal ratio	Requirement *Male*	*Female*	Ideal ratio	Requirement *Male*	*Female*
	% of Lys	% of diet		% of Lys	% of diet		% of Lys	% of diet	
Lysine	100	1.12	1.02	100	0.89	0.84	100	0.76	0.73
Methionine	36	0.41	0.37	37	0.33	0.31	37	0.28	0.27
Cystine	36	0.41	0.37	38	0.34	0.32	38	0.29	0.28
TSAA[2]	72	0.81	0.74	75	0.67	0.63	75	0.57	0.55
Threonine	67	0.75	0.68	70	0.62	0.59	70	0.53	0.51
Valine	77	0.86	0.79	80	0.71	0.67	80	0.61	0.58
Arginine	105	1.18	1.07	108	0.96	0.91	108	0.82	0.79
Tryptophan	16	0.18	0.16	17	0.15	0.14	17	0.13	0.12
Isoleucine	67	0.75	0.68	69	0.61	0.58	69	0.52	0.50
Leucine	109	1.22	1.11	109	0.97	0.92	109	0.83	0.80
Histidine	35	0.39	0.36	35	0.31	0.29	35	0.27	0.26
Phe + Tyr[2]	105	1.18	1.07	105	0.93	0.88	105	0.80	0.77

[1]Lysine requirement data for 0 to 21 days of age were taken from Han and Baker (1993); for 21 to 42 days of age from Han and Baker (1994); for 42 to 56 days post-hatching from the NRC (1994). Ideal AA ratios for 0 to 21 days of age were obtained from Baker and Han (1994); for later growth periods, ideal AA ratios were calculated based upon projected maintenance requirements and maintenance contributions to the total requirement. Requirement values are based on air-dry basis; [2]TSAA = methionine + cystine; Phe + Tyr = phenylalanine + tyrosine.

Table 2.2 Comparison among different amino acid profiles for the 0 to 21 days starter phase of broilers.

	NRC[1] 1994	Modified[2] NRC 1994	IICP[3] 1994	Blair[4] 1977	Rhodimet[5] 1993
Lysine	100	100	100	100	100
Methionine + Cystine	82	75	72	69	80
Methionine	46	42	36	55	51
Cystine	36	33	36	14	29
Threonine	73	67	67	70	66
Valine	82	75	77	86	85
Arginine	114	104	105	120	118
Tryptophan	18	17	16	20	19
Isoleucine	73	67	67	80	79
Leucine	109	100	109	140	150
Histidine	32	29	35	40	-
Phenylalanine + Tyrosine	121	112	105	140	-

[1]NRC (1994)-1.10% lysine; [2]NRC (1994)-1.20% lysine; [3]Baker and Han (1994) - Illinois ideal chick protein; [4]Blair *et al.* (1977); [5]Rhodimet Nutrition Guide (1993).

The Use of Amino Acid-Supplemented Reduced Protein Poultry Diets

Traditionally, cereals used for animal feeds are low in natural lysine and have been supplemented with protein sources such as soybean meal or fishmeal. However, high levels of protein concentrates are not always practical and have become more expensive during recent years. The higher protein contents of these diets also over-supply many other AA that are not required by the animals for protein synthesis. Recently, with the advances in biotechnology, feed grade crystalline AA such as lysine, methionine, threonine and tryptophan are readily available for use as feed supplements by the feed industry. Supplementation with AA improves the AA balance and protein utilization of feeds, thus allowing for the level of protein-rich feedstuffs to be reduced. Experiments with poultry have supported the hypothesis that diets formulated to minimize excesses of AA over the chicks' known requirements would improve the efficiency of protein and energy utilization (Waldroup *et al.,* 1976; Blair *et al.,* 1999). The resulting decrease in N excretion is a major contribution to reducing the environmental burden, particularly in regions of intensive animal production. By feeding low protein-AA supplemented diets (with less excess AA), fewer AA have to be deaminated, converted to uric acid and excreted into the environment. As these metabolic processes are energy driven, energy can therefore be spared for other purposes. The reduction of N in the excreta also improves air quality by lowering ammonia levels in farm buildings (Archer, 1993; Jongbloed and Lenis, 1998).

Reduced protein diets - Broiler studies

In 1975, Lipstein *et al.* found that reduced protein diets (17.5% CP sorghum-corn-soybean meal) could be fed to broilers 5 to 9 weeks old without losing performance when compared to birds fed a control diet (20.5% CP sorghum-corn-soybean meal). However, birds fed the reduced protein diets also deposited more fat than the control birds. During further studies these researchers found that when this low protein diet was supplemented with methionine and lysine to the levels in the control diets, carcass fat deposition between the two diets was not different. A year later, Waldroup *et al.* (1976) obtained a similar positive result with chicks grown to 21 days of age. Chicks fed a 19% crude protein (CP) corn-soybean meal diet but meeting minimum essential AA requirements gained equally when compared with chicks fed a standard 23% CP corn-soybean meal diet. Birds fed the low protein diet also use protein more efficiently. In another study with broiler finishers, Uzu (1982) showed that broilers

could be fed a 16% CP corn-soybean meal finishing diet that was supplemented with methionine and lysine to levels present in the 24% CP control corn-soybean meal diet without reducing the weight gain. In another study, Stilborn and Waldroup (1988) fed broilers AA supplemented iso-caloric diets from 21 to 42 days of age and found comparable 42 day body weights and feed efficiency with dietary CP levels as low as 14%.

Holsheimer and Janssen (1991) conducted studies to determine if diets based on corn-soybean meal containing 19%, 18% or 17% CP were sufficient for chicks during the period of 3 to 7 weeks of age when compared to a 20% CP corn-soybean meal diet. All diets were formulated to have equal and sufficient amounts of lysine and total sulfur AA. It was also the interest of the authors to find out whether the addition of combinations of threonine, tryptophan and arginine or threonine, tryptophan, arginine, isoleucine, leucine and valine to the diets low in CP would have any effect on the bird's performance. It was concluded from these studies that when the CP in diets fed during the period of 3 to 7 weeks was decreased from 20% to 17%, weight gain and feed/gain ratios increased. The diets containing 18 and 19% CP supplemented with arginine, threonine and tryptophan to the concentrations found in the 20% CP diets showed no negative effect on growth performance. Data from this study also indicated that 0.77% threonine and 0.22% tryptophan are sufficient in finishing diets fed to broilers between 3 and 7 weeks of age.

A series of experiments was conducted at the University of Guelph to evaluate the performance of 7 to 21 day old broilers fed diets in which excesses of essential AA were minimized (Parr and Summers, 1991). A 23% CP corn-soybean meal diet supplemented with DL-methionine was used as a positive control. Protein in the corn-soybean meal diets was reduced stepwise (9 diets, with CP range from 23% to 16.5%) to the point where all essential AA were at the minimum requirement level based on the 1984 National Research Council (NRC) recommendations. As dietary protein was reduced, crystalline essential AA were used to supplement those essential AA that became deficient. These studies showed that weight gain and feed efficiency of birds fed the reduced protein diets were not significantly different from that of birds fed the control diet. The authors concluded that all essential AA appeared to be adequate at levels recommended by the 1984 NRC except tryptophan, which was required at the level of 0.25% of the diet for optimal performance. Reduced protein corn-soybean meal diets (20% CP from 0-3 weeks of age and 17% CP from 3-6 weeks of age) did not affect live body weights in a 6 week broiler study conducted by Moran *et al.* (1992); however, feed conversion was increased

during the grower (3-6 weeks) period when the CP was reduced. Reducing the dietary CP improved protein utilization and decreased the N content of the litter by 23.8% in the 6th week. The birds fed the reduced CP diet deposited much more abdominal fat leading to lower chilled carcass weights compared to the control birds. The yields of breast pieces were also lower in the low CP group.

Beside methionine and lysine, arginine, valine and threonine were also found to be limiting in the reduced protein (19% CP) corn-soybean meal diet for broiler chicks (Han *et al.* 1992). Weight gain, feed efficiency and fat content were not significantly different between birds fed a positive control diet with 23% CP and birds fed a 19% CP diet supplemented with the five limiting AA and amino N in the form of glutamic acid. From 3 to 6 weeks of age, chicks fed a AA fortified 16% CP diet had a growth performance similar to chicks fed a 20% CP diet. More recently, Deschepper and De Groote (1995) found that birds fed reduced-protein wheat-sorghum-soybean meal diets supplemented with crystalline essential and non-essential AA (20% CP) to the amounts in the control diet (21% CP wheat-sorghum-soybean meal) or based on the AA profile of body protein (18% CP wheat-sorghum-soybean meal) gave similar performance when compared to birds fed the control diet. This was not achieved with reduced protein wheat-sorghum-soybean meal diets (17% CP) supplemented with crystalline AA to the amount recommended by NRC (1994). Birds fed the reduced protein diets were also more efficient in protein utilization and their N excretion was reduced by 26% similar to that reported by Moran *et al.* (1992). The authors concluded that it was possible to obtain the same performance with reduced protein diets supplemented with crystalline AA using an ideal AA balance. However, these diets also led to a higher carcass fat content.

More recently, a series of experiments was conducted by Ibrahim (1997) to investigate the effect of reduced protein (wheat-soybean meal) diets on broiler performance and N excretion. In one of the experiments, the growth over a 3-6 week period was unaffected by the reduction of CP levels in the diet from 21% to 18%, and the N output was reduced by 20% in the reduced protein diets. In another experiment by this author, a control diet with a commercial level of CP (24% starter and 20.5% grower) was compared with three reduced-CP diets (20% starter and 17% grower) which differed in the level of limiting AA (90, 100 or 110% of industry standards). The results showed that there was no significant difference in growth performance between birds fed the control or the other dietary treatments. However, reduction in dietary CP led to a 10-27% reduction in excreted N. Broilers fed the reduced protein diets also retained significantly more

dietary N than broilers fed the control diet, thus indicating a better utilization of protein and AA in reduced protein diets.

Conversely, researchers at the University of Georgia (Fancher and Jensen, 1989a,b,c; Pinchasov *et al.*, 1990; Colnago *et al.*, 1991; Jensen and Colnago, 1991) concluded that optimal performance of broilers could not be achieved with reduced protein diets supplemented with crystalline AA. Despite the formulation of the reduced CP corn-soybean meal diet (15 to 16% CP) to be adequate in all NRC (1984) recommended essential AA requirements, body weight gain and feed efficiency were inferior in two out of three experiments to values obtained from female broilers (3-6 week of age) fed a 18% to 19% CP corn-soybean meal diet (Fancher and Jensen, 1989b). Further studies using male chicks indicated that the dietary protein requirement for maximum feed efficiency during the starter period is greater than 18%, and that feed efficiency during the grower phase appeared to decline with dietary CP levels less than 22% (Fancher and Jensen, 1989c). Pinchasov *et al.* (1990) showed that performance of broiler chicks fed reduced protein corn-soybean meal diets supplemented with several essential AA was generally inferior to that of birds fed a higher protein diet in which the protein was mainly intact. These experiments also showed that reducing essential AA in proportion to CP or equalizing amino N by an inclusion of glutamic acid failed to prevent the reduction in performance of broiler chicks fed reduced protein diets supplemented with several crystalline AA.

Similar findings were observed by Edmonds *et al.* (1985) when the protein level of corn and soybean meal diets was reduced from 24% to 16%. Cabel and Waldroup (1991) found that feeding lower levels of CP to broiler chicks had a more pronounced effect on males than on females, with the primary effects being reduced body weight, poorer feed efficiency, and increased carcass fat content. Increased abdominal fat deposition was a major problem with lower protein diets fortified with AA. With each decrease in the dietary CP content, a corresponding decrease in the dietary heat increment should have occurred. One of the primary mechanisms involved in reducing carcass fatness by feeding higher CP diets is the associated increased energy expenditure and increased heat increment involved in degrading excess amino N to uric acid (Bartov, 1979).

Reduced protein diets - Layer studies

Because of the inefficiency in protein utilization, N pollution is also a major issue in the laying hen industry. In 1973, a group of Washington State University researchers reported that a

corn-wheat-soybean meal diet containing 13% protein and supplemented with lysine and methionine was as effective as levels of 15, 17 and 18% CP for supporting egg production and egg size (Fernandez *et al.*, 1973). Layers fed a barley-wheat-corn-soybean meal diet containing 14.1% CP and supplemented with methionine and lysine had a 75% egg production when compared to 73% for layers fed a conventional diet (Blair *et al.*, 1976). Several experiments were undertaken by Summers (1993) to investigate N excretion by laying hens fed corn-soybean meal diets varying in level of dietary protein. In one study, the performance of 24-week-old laying birds was not jeopardized even when the dietary CP in the layer diet was lowered from 17% to 13%. Birds on the 13% protein diet also excreted approximately 34% less N per day than those fed the 17% protein diet. Further study with 45-week-old hens indicated that N balance was reached by feeding between 9-11% dietary protein. However, egg mass started to decline as dietary protein level was reduced below 17%. The author also concluded that there is merit to optimizing rather than maximizing egg mass output because exceptionally high intakes of dietary protein are required to maximize egg size.

An experiment was carried out by Ibrahim (1997) to determine the effects of reduced protein wheat-soybean meal diets on egg production and N output in excreta of laying hens. Reducing the dietary CP level from 17% to 13.5% while maintaining the limiting AA levels at 90, 100, or 110% of industry standards had no significant effect on egg production or egg quality. Layers fed the low protein diets were also more efficient in N utilization and excreted 30% less N. Coon's (1998) research indicated that reduced protein diets with added crystalline AA can replace feeding higher protein diets to layers. The egg composition and performance of layers fed corn-soy-meat meal 14% CP diets with added methionine, lysine, isoleucine, and valine was equal to layers fed 18% CP control diets.

Contrary to this, pullets receiving a 14.5% CP corn-barley-soybean meal diet in the laying period produced significantly fewer and smaller eggs than those fed a 16.5% CP corn-barley-soybean meal diet (Keshavarz, 1984). According to the author, lower intakes of lysine and other AA might have been the reasons for the poor performance of the birds fed the reduced protein diet. Egg weight and sometimes the rate of egg production were significantly reduced when laying hens were fed 13% corn-soybean meal diets supplemented with adequate levels of methionine, lysine, and tryptophan in comparison to hens fed a 16% CP corn-soybean meal diet (Jensen and Colnago, 1991). Keshavarz and Jackson (1992) conducted an experiment to determine the effect of feeding AA-supplemented reduced protein corn-barley-soybean meal

diets during growing and laying periods on the layers' performance. At 18 weeks of age, birds fed the negative control diets (16, 13.5, and 11.5 % CP diets during the growing period and 14, 13, and 12% CP diets during the laying period) supplemented with methionine and lysine or supplemented with methionine, lysine and other deficient essential AA had comparable body weights to those fed the positive control diets (20, 16, and 14% CP diets in the growing period and 18, 16.5, and 15% CP diets in the laying period). There was no significant difference in egg weight and overall egg production between the high protein (positive control) group and the low protein AA-supplemented (negative control) group. However, egg mass output and body weight of laying hens fed the negative control diets were inferior to laying hens fed the positive control diets. As is in the case for broiler studies, there were conflicting results reported in the literature regarding the use of low protein diets in the egg industry. Keshavarz (1984) suggested that difference in strains, management programs, season housed, and the extent and the age of the initiation of protein restriction may be factors that have contributed to the discrepancies.

In conclusion, the above data show that it is possible to reduce the CP of poultry diets without negatively influencing birds performance. However, a number of authors showed that reduced dietary protein would lead to a poorer performance by birds. Edmonds *et al.* (1985) and Fancher and Jensen (1989b) stated that no clear-cut explanation could be provided as to why fortifying the reduced protein diet with all limiting essential AA and nonessential amino N failed to support maximal performance. Pinchasov *et al.* (1990) and Colnago *et al.* (1991) suggested that a minimal level of intact protein is necessary for optimum broiler performance. According to Pinchasov *et al.* (1990), this need may relate to differences in absorption of single AA versus peptides. Matthews (1975) has demonstrated that a majority of the intact protein consumed by monogastric animals enters the absorptive cell as small peptides and that the absorption is independent of the uptake of free AA and is more rapid. It is also possible that different rates of absorption of peptides and free AA may result at times in a less than optimal availability of all essential AA at the site of protein synthesis in the tissues. However, Han *et al.* (1992) stated that in the University of Georgia studies (Fancher and Jensen, 1989a,b,c; Pinchasov *et al.*, 1990; Colnago *et al.*, 1991; Jensen and Colnago, 1991) the reduced protein diets were formulated such that the AA nitrogen was accounted for, and this effectively lowered the soybean meal (at the expense of corn) more than was the case in their current studies. That is, the diets might be deficient in amino N for the synthesis of non-essential AA. Thus, chick performance on reduced protein diets was inferior in these studies. It is very important to note that in addition to the

requirement for essential AA, poultry diets should also supply adequate N for the synthesis of non-essential AA. Otherwise, essential AA nitrogen would be used for the synthesis of non-essential AA and growth and production would be jeopardized. It is important to devote more research to the area of reduced-protein diets, so that the utilization of protein can be further improved resulting in decreased N excretion by animals.

Palm Kernel Cake

The recent escalation in ingredient costs in South East Asia due to the economic crisis has stimulated a lot of interest in re-evaluating the feeding quality of some locally available feed ingredients for use in the poultry industry. Locally grown food such as corn and soybean meal are not sufficient for human consumption in the South East Asia countries, not to mention the need for these ingredients for the animal feed industry. Most of the conventional feed ingredients such as corn and soybean meal are therefore imported from the developed countries. However, most of the South East Asia countries also produce millions of tonnes of agricultural by-products, which are not suitable for human consumption. Agricultural by-products such as coconut meal, palm kernel cake, rice bran, cottonseed meal and others are abundant in many countries. However, their role in the feed industry has been minimized, mainly because insufficient nutritive information is available regarding these potential ingredients. Of these, palm kernel cake (PKC), which is also known as palm kernel meal or palm kernel expeller, has the greatest potential as an animal feed in countries that produce it in large quantities such as Malaysia, Indonesia, South America and Africa.

An introduction to oil palm

The oil palm (*Elaeis guineensis* Jacq.) is a monocotyledonous plant widely believed to be native to West Africa. It belongs to the family Palmae, order Palmales and genus *Elaeis*. The name of the genus *Elaeis* is derived from Greek word "elaion," meaning oil. The specific name *guineensis* indicates its origin in the Guinea Coast. *Elaeis guineensis* is one of the largest palm species. It has a stem that can reach a height of 25-35 m, topped by 35 to 60 pinnate leaves. An oil palm has an economically productive life of 20-30 years, and replanting is usually carried out after about 24 years (Hartley, 1988). Almost 6 months after pollination, a fruit bunch weighing

15 to 20 kg and consisting of approximately 1,000 to 1,500 fruit is produced. The shape and the size of the fruit vary considerably. The fruit is about 2.5-5 cm in length and 2.5 cm in diameter and weigh about 3-30g (Godin and Spensley, 1971; Gascon *et al.,* 1989). The mature fruit is a deep orange-red drupe containing pulp (mesocarp), shell (endocarp) and kernel (endosperm). The fibrous mesocarp is rich in oil and is yellowish-orange in color, due to its high carotene content. The palm fruit produces two types of oil: crude palm oil from the pulp (about 50% oil on a fresh weight basis) and palm kernel oil from the kernel.

Oil palm in Malaysia

The oil palm was first introduced to Malaysia in 1878, while its use as a plantation crop was only developed in the early 20th century. During the last 20 years, oil palm has overtaken rubber as the major plantation crop. Palm oil has become a major vegetable oil during the past few decades. Production has rapidly increased, more than quadrupling from 1970 to 1990. In 1996 the world production of palm oil was reported at 16.11 MT (PORLA, 1997), taking second place after soybean oil. The bulk of palm oil (about 82%) is produced in South East Asia, notably in Malaysia and Indonesia (PORLA, 1997). Concomitant with the production of palm oil, palm kernel oil also became important because of the similarity of its oil composition to that of coconut oil (Gascon *et al.,* 1989). The oil palm considerably out-yields other oil crops in oil per hectare. Malaysia, the world's leading producer with more than 50% of the market share, produced 8.39 MT of it in 1996 (PORLA, 1997).

Nutrient composition of palm kernel cake

Palm kernel meal is the major byproduct of palm kernel oil extraction. It is a useful source of protein and energy for livestock. However, it is highly variable in composition especially in its oil and fiber contents. Such differences in composition may be due to oil palm types that exist in different geographical regions, to the extent of oil extraction from palm kernel and also to the methods of processing used in producing the PKC (Onwudike, 1986a). Palm kernel cake generally contains 17-21% protein, 10-17% crude fiber, 4-5% ash and ether extract values of 0.7-9.0% depending on the efficiency of oil extraction from the kernel (Nwokolo *et al.,* 1977; Devendra, 1978; Hutagalung *et al.,* 1982; Onwudike, 1986a). The apparent metabolizable energy (AME) for PKC has been reported to range from 2,008 to 2,999 kcal/kg (Nwokolo *et al.,*

1977; Onwudike, 1986a; Ngoupayou, 1984; Longe and Tona, 1988). However, values as low as 1,482 kcal AME/kg were reported by Yeong (1985). The protein is of relatively good quality, but its high crude fiber content may affect the AA availability and the digestibility of PKC. Some of the reported AA values for PKC are listed on Table 2.3.

Table 2.3 Crude protein and amino acid composition of palm kernel cake (PKC, % dry matter)

	Nwokolo *et al.* (1976b)	Onwudike (1986a)	Yeong (1983)
Origin of PKC	Nigeria	Nigeria	Malaysia
Crude protein	21.3	19.2	16.06
Amino acids			
Arginine	2.68 (12.58)[1]	2.65 (13.80)	2.18 (13.57)
Histidine	0.41 (1.92)	0.42 (2.19)	0.29 (1.81)
Isoleucine	0.60 (2.82)	0.62 (3.23)	0.62 (3.86)
Leucine	1.23 (5.77)	1.20 (6.25)	1.11 (6.91)
Lysine	0.69 (3.24)	0.68 (3.54)	0.59 (3.67)
Methionine	0.47 (2.21)	0.32 (1.67)	0.30 (1.87)
Cystine	-	-	0.20 (1.25)
Phenylalanine	0.82 (3.85)	0.74 (3.85)	0.73 (4.55)
Tyrosine	0.58 (2.72)	0.53 (2.76)	0.38 (2.37)
Threonine	0.66 (3.10)	0.68 (3.54)	0.55 (3.42)
Valine	0.43 (2.02)	0.88 (4.58)	0.93 (5.79)
Aspartic acid	1.69 (7.93)	1.72 (8.96)	1.55 (9.65)
Glutamic acid	3.62 (17.00)	4.01 (20.89)	3.15 (19.61)
Proline	0.50 (2.35)	0.62 (3.23)	0.63 (3.92)
Serine	0.90 (4.23)	0.92 (4.79)	0.69 (4.30)
Glycine	0.91 (4.27)	0.92 (4.79)	0.83 (5.17)
Tryptophan	-	-	0.17 (1.06)
Alanine	0.81 (3.80)	0.76 (3.96)	0.92 (5.73)

[1]Value in brackets is % of CP.

There seem to be some differences in the AA content among samples from different countries. There is good agreement between samples from the same country (Nigeria). However, the concentration of AA in Malaysian PKC seem to be lower than the concentration of AA in Nigerian PKC. In general, PKC is deficient in lysine and sulfur AA. The data on the digestibility of AA in PKC for poultry are very limited. Only a limited number of researchers (Nwokolo *et al.*, 1976b; Yeong, 1983; Onwudike, 1986a) have reported the digestibility of the AA from PKC (Table 2.4). The digestibility of AA in PKC averaged 83.3% and 84.5% according to Onwudike (1986a) and Nwokolo *et al.* (1976b). However, research conducted in Malaysia (Yeong, 1983)

reported a low value of 64.4%. The reason for the low digestibility of AA in PKC from Malaysia is not clear.

Table 2.4 Digestibility of palm kernel cake (PKC) amino acids (%).

	Nwokolo *et al.* (1976b)	Onwudike (1986a)	Yeong (1983)
Origin of PKC	Nigeria	Nigeria	Malaysia
Amino acids			
Arginine	93.2	92.7	87.0
Histidine	90.1	88.7	66.8
Isoleucine	86.1	87.5	64.9
Leucine	88.5	90.6	66.7
Lysine	90.0	88.9	58.6
Methionine	91.0	92.1	72.1
Phenylalanine	90.5	91.6	70.4
Tyrosine	85.0	89.9	65.7
Threonine	86.5	85.3	60.7
Valine	68.4	66.7	62.8
Aspartic acid	87.6	85.3	64.4
Glutamic acid	90.1	88.6	74.4
Proline	68.0	64.2	55.0
Serine	88.7	85.4	65.0
Glycine	63.3	52.1	25.8
Alanine	85.5	83.0	67.7
Overall mean	84.5	83.3	64.4

Palm kernel cake contains a relatively high amount of minerals, particularly calcium, phosphorus and iron (Nwokolo *et al.*, 1976a). However, the availability of most minerals is poor. The availability of calcium, phosphorus, magnesium, manganese, zinc and copper was reported as 68.6, 70.8, 56.4, 45.7, 13.9, and 44.7, respectively (Nwokolo *et al.*, 1976a). In another study, Nwokolo and Bragg (1977) found that PKC contained 1.42% phytic acid and that half of the total phosphorus was in the form of phytate phosphorus. The study also concluded that phytic acid reduced the availability of calcium, phosphorus, magnesium and zinc, whereas the crude fiber content depressed the availability of all minerals tested. The ratio of calcium to phosphorus in PKC is reported to be more favorable than in other oilseed meals (McDonald *et al.*, 1982).

Non-starch polysaccharides in palm kernel cake

Although starch is the most-researched and economically the most important seed storage polysaccharide, it is by no means the only polymeric carbohydrate stored in seeds. There are

seeds of many plant species that contain little or no starch, but are nevertheless rich in other forms of polysaccharide reserves. The special groups of non-starch polysaccharides are stored outside the plasmalemma, and were categorized collectively as cell wall storage polysaccharides by Meier and Reid (1982). Mannan-type cell wall storage polysaccharides are all based on a linear β-1,4-linked chain or "backbone." They may be subdivided into the "pure" mannans, the glucomannans in which some of the D-mannose residues in the backbone are replaced by D-glucose, and the galactomannans in which the backbone carries α-1,6-D-galactosyl substituents. According to Meier and Reid (1982), the mannan-group polysaccharides of seeds are major reserve substances only in endosperms, as opposed to storage cotyledons. "Pure" mannans may be defined to include those polysaccharides that contain less than 10% non-mannose sugar residues. Pure mannans form the major part of the endosperm (kernel) of many palm seeds. They take the form of massive wall thickenings in the endosperm and are clearly the molecular basis of the palm kernel's characteristic hardness (Meier and Reid, 1982).

A group of researchers at the Agricultural University of Netherlands (Dusterhoft and Voragen, 1991; Dusterhoft *et al.,* 1992) has characterized the polysaccharide components of PKC. Palm kernel cake was found to contain mannans, cellulose and xylans, with the major part of the mannans originating from the endosperm (kernel) and xylans being almost exclusively located in the endocarp (shell). Palm kernel cake was found to contain negligible amounts of starch (1 g/kg) and some of the protein was not digested even after the Pronase treatment, implying that the residual protein was either structurally bound in the cell wall or present as inaccessible cytoplasmic material. There were about 726 g of cell wall materials per kg of PKC containing approximately 7.3% protein, 17.5% lignin, 5% ash and 74.6% non-starch polysaccharides (Dusterhoft and Voragen, 1991). Further analysis showed that mannose (from mannan) made up about 57.1% of the cell wall material, which means that PKC contained about 41.45% mannose in this particular study. Further study confirmed that major polysaccharides in PKC are linear mannans with very low galactose substitution (78% of total non-starch polysaccharides), followed by cellulose (12%) and small amounts of (4-O-methyl)-glucuronoxylans and arabinoxylans (3% each) (Dusterhoft *et al.,* 1992). Daud and Jarvis (1992) also showed linear β(1,4)-D-mannan to be the major component of PKC cell wall non-starch polysaccharides, and that the mannans of PKC appeared to be highly crystalline, in keeping with their insolubility.

Palm kernel cake - Enzyme studies

Mannanase is found only in the endosperm and its activity increases from a negligible value shortly after imbibition to a high value and then decreases (Matheson, 1990). Even though endogenous enzyme activities (especially mannanase) were found in the palm kernel (endosperm), the unfavourable environments (e.g. high temperature) during the oil extraction process would denature and inactivate it. As a result, mannanase and other enzyme activities, if any, are not expected to be found in PKC.

Several *in vitro* enzyme studies involving PKC have been conducted by researchers from the Netherlands (Dusterhoft *et al.*, 1993a, b, c). Enzymes containing mannanase and cellulase activities were used in the studies. It was found that reducing particle-size, thereby increasing the surface area available for enzymes to attack, enhanced the solubilization of PKC (Dusterhoft *et al.*, 1993a). Since PKC's cell walls are highly lignified, their accessibility might be hindered by lignin and low molecular-weight phenolic compounds forming covalent linkages with sugar residues. Mannans in palm kernel cell wall materials were hydrolyzed by 20-50%, depending on enzyme composition, with mannose monomers and dimers as major end products (Dusterhoft *et al.*, 1993b). Results also indicated the preferential solubilization of the endosperm in PKC, and the resistance of the palm kernel endocarp to enzymatic attack. Comparing the release of glucose (cellulose) and mannose (mannan) from PKC, the authors concluded that neither cellulose hydrolysis enhances mannan-degradation, nor mannan hydrolysis enhances cellulose-degradation (Dusterhoft *et al.*, 1993b). However, with further study they found that in the degradation of palm kernel cell wall materials, a synergistic action of mannanases and glucanases was observed (Dusterhoft *et al.*, 1993c). Daud *et al.* (1997) reported a 8.5% (or 230 kcal/kg) increase in the AME content of a PKC-based broiler diet treated with 0.1% mannanase (Alltech Inc., USA). Cellulase supplementation increased the AME value of the PKC-based diet numerically but not significantly. As synergistic action between cellulase and mannanase was not observed, Daud *et al.* (1997) concluded that mannanase is the most effective enzyme to improve the nutritive value of PKC.

Palm kernel cake - Animal studies

A number of studies concerning the use of PKC in poultry diets have been conducted. In one study, three levels of PKC (10, 20, and 30%) were used to evaluate the feeding value of PKC

for broiler chicks in Malaysia (Ahmad, 1982). A corn-soybean meal diet was used as the control. Feed intake was not significantly different between diets. However, weight gains were significantly depressed when the diet contained more than 10% PKC. Feed efficiency was also poorer for those birds fed diets with more than 10% PKC. An economic evaluation found that there was no economic advantage to use PKC in broiler diets. Nevertheless, other studies in Malaysia found that PKC has been utilized as a supplement at up to 20% in diets for broilers and layers and up to 25% for swine, without any adverse effect on their performance (Yeong, 1980; Hutagalung, 1980). Yeong and Mukherjee (1983) showed that supplementation of a broiler diet containing 20% PKC with 9% palm oil resulted in growth and feed efficiency similar to that obtained with the control diet. Osei and Amo (1987) reported that the inclusion of PKC at 12.5% and 15% of the diet reduced growth and feed efficiency. A more recent work by Onifade and Babatunde (1998) showed that broiler performance was depressed even when 10% PKC was included in a diet when compared to a corn-soybean meal control diet. However, the control diet had a higher AME content and the AME levels in the test diets were not adjusted to that of the control. Researchers from Central America indicated that a low level of PKC (10%) could be used in broiler diets (Garcia and Gernat, 1998) and other reports suggested that high levels of inclusion were possible.

Nwokolo *et al.* (1977) incorporated 30% PKC or 30% alkali-treated (3, 5, 7% NaOH) PKC into a starter diet for 2-week-old broiler chicks. There was no significant negative effect on the chicks' performance when 30% PKC or 30% alkali-treated (3% NaOH) PKC-based diets were fed except when birds were fed 30% alkali-treated (5% or 7% NaOH) PKC-based diets. Ngoupayou (1984) demonstrated that PKC allowed good growth of chicks when fed at a level up to 20% in diets. According to the author, this inclusion rate could replace up to 40% cottonseed cake and 25.4% corn in chick diets. In Nigeria, starter broilers were shown to be able to utilize a diet containing 28% PKC without any significant effect on their performance (Onwudike, 1986c). In a recent study, Panigrahi and Powell (1991) found that up to 50% PKC (from Malaysia) can be incorporated into broiler diets without any negative effects on their performance provided that a high level of oil (11.3-11.4% of the diet) is included. However, they also concluded that such diets may be uneconomical in most developing countries and, perhaps, too oily to be considered practical.

On the other hand, finishing broilers were found to be able to utilize up to 35% PKC without any significant effect on their performance (Onwudike, 1986c). Based on these results,

Onwudike (1986c) concluded that the use of up to 35% PKC in the diet of finishing broilers would reduce the cost of production, improve feed efficiency and also reduce the fat content of finished broilers. For layers, Onwudike (1986b) demonstrated that diets containing up to 34% PKC could be fed to starter pullets without any adverse effects on performance. He also showed that diets containing up to 38% PKC could be fed to grower pullets without affecting the rate of egg production, egg weight, weight of first egg dropped and feed intake. In addition, cost of feed for one kg of gain decreased as the level of PKC increased. With laying birds, Longe (1984) using PKC from Nigeria found that layers fed a 20% PKC diet ate significantly more feed and produced less eggs than control birds. Layers were also less efficient in utilizing feed containing PKC. However, there were no negative effects on egg weight, shell thickness or cholesterol levels. Interestingly, another report from the same country found that 40% PKC could be fed without any adverse effect on laying performance and egg quality (Onwudike, 1988). As the proportion of PKC increased beyond the 40% level, there was a significant drop in egg production, egg weight, feed intake and feed efficiency. Furthermore, birds produced very watery droppings, for a reason that has not been fully explained. This is in agreement with another report that used up to 40% PKC in layer diets (Panigrahi and Waite, 1998). West African PKC containing 17.6% CP, 14.2% crude fiber and 10.2% fat was used in a 6 week layer study. There were no significant differences in laying performance between control and PKC based diets. However, the body weight of the hens fed 40% PKC was depressed. This effect was overcome by increasing the energy value of the diet. It was also observed that the colour of the yolks became paler as the amount of PKC in the diets increased.

Beside PKC, palm kernel was found to be tolerated by broilers at a 5% inclusion rate, and the kernels were reported to contain 10% CP, 8% fiber, 0.21% calcium, 0.16% phosphorus, 0.44% lysine and 0.29% methionine (Oruwari *et al.,* 1995). Fifty-six percent of the kernel is made up of oil and this results in the high TME value of 6,400 kcal/kg. In pigs, dietary PKC tends to produce firm pork of good quality (Gohl, 1975). Because of low lysine availability, a higher concentration of PKC in the diet of pigs requires balancing with supplemental lysine (Hutagalung *et al.*, 1983). Ruminants are capable of utilizing PKC more efficiently when compared to non-ruminant. Apparent digestibility of organic dry matter (DM), CP and neutral detergent fiber of PKC for sheep was reported to be 75%, 75%, and 73%, respectively (Moss and Givens, 1994). A ME of 3,131 kcal/kg DM was also reported in this study. Palm kernel cake

tends to produce a firm butter when fed to dairy cattle (Gohl, 1975). Palm kernel cake also has a tendency to increase the fat content of bovine milk (McDonald *et al.,* 1982).

From the above discussion, it seems that the maximum amount of PKC that can be included in diets ranges from 10% to 50% for broilers and from 20% to 40% for layers. Most of the research reported in the literature concerns PKC originating from Africa. From several studies (Yeong, 1980; Hutagalung, 1980; Yeong and Mukherjee, 1983) that used PKC originating from Malaysia, 20% PKC seems to be the maximum level that can be used in poultry diets without jeopardizing the performance of the birds. The only exception is the study by Panigrahi and Powell (1991) which is not practical due to the high level of oil inclusion. The major limitations of using PKC could be attributed to its high fiber content (especially high non-starch polysaccharides), low energy content and to low digestibility of nutrients like protein, AA, minerals, and vitamins.

Palm kernel cake could be a valuable raw ingredient for the animal feed industry in countries that produce it in large quantities. Due to the differences in the methods of processing palm kernel oil, the duration of the studies, the sources of PKC and the age of the birds, the data obtained from the literature on the utilization of PKC in poultry diets are quite contradictory. Even though PKC is less digestible for poultry than for other animals, the application of new biotechnology such as enzyme supplementation might offer hope for its use in the poultry industry. Therefore, the current studies were conducted with the objectives of evaluating the potential of PKC as a poultry feedstuff and the potential of enzyme supplementation in PKC-based poultry diets.

In conclusion, the above review of the literature clearly shows that further research is necessary to determine the validity of the NRC (1994) recommended poultry requirements for threonine and tryptophan so that protein utilization of conventional feed ingredients could be improved and to evaluate the nutritive quality and to improve the nutrient utilization of non-conventional feed ingredient such as PKC for poultry diets.

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CHAPTER III

Use of the Ideal Protein Concept to Investigate the Response of 0-3 Week Old Broiler Chicks to Different Levels of Threonine and Tryptophan in Chemically Defined Diets

Summary

Two starter broiler experiments were conducted to compare the efficacy of diets based on essential amino acid (AA) profiles proposed by Blair *et al.* (1977), NRC (1994) and Illinois Ideal Chick Protein (IICP) (Exp. 1) and to evaluate the responses of broilers to three levels of threonine and three levels of tryptophan (Exp. 2) by using the AA profile derived in Exp. 1. During a study of broilers 0-2 weeks old (Exp. 1), three semi-purified diets (Diet 1 = NRC (1994); Diet 2 = IICP; Diet 3 = Blair *et al.* (1977)) were formulated by using isolated soy protein and crystalline AA. The body weights of the birds fed Blair's diet were significantly higher ($P < 0.05$) at the end of weeks 1 and 2 when compared with the other two diets. It was concluded that the diet based on AA profile proposed by Blair *et al.* (1977) could support better growth and it was appropriate for a follow-up study to examine the responses of broiler chicks to threonine and tryptophan in a purified diet based on crystalline AA. In Exp. 2, three levels (90%, 100% and 110% of NRC (1994)) of threonine and tryptophan were arranged in a 3 x 3 factorial manner to give nine chemically defined diets containing crystalline AA as the only source of AA. The amounts of the other AA in the diets were based on the Blair *et al.* (1977) AA profile. Broiler chicks were fed a commercial diet during the first week of age and experimental diets were fed from 1 to 3 weeks of age. The interactions between threonine and tryptophan for body weight (week 3), weight gain (week 2-3) and overall weight gain (week 1-3) were significant ($P < 0.05$). The differences of both body weight and body weight gain increased as the levels of threonine and tryptophan increased. Weight gain of the birds was significantly lower ($P < 0.05$) at the highest levels of both AA (110% threonine and 110% tryptophan). There were indications that the AA in the diets became imbalanced at high levels of both threonine and tryptophan. The results of Exp. 2 indicate that when formulating diets for 0-3 week old broilers, dietary levels of threonine and tryptophan should be targeted at 65% of lysine (equivalent to 0.63% of digestible threonine) and 16% of lysine (equivalent to 0.16% of digestible tryptophan), respectively. These

data and other recent data indicate that NRC (1994) has overestimated the threonine requirements and underestimated the tryptophan requirements for 0-3 week old broiler chicks.

Key words: ideal protein, broiler, threonine, tryptophan.

Introduction

The requirements of broilers and layers for commonly limiting amino acids (AA) such as lysine and methionine are well established as a result of numerous investigations. However, the recent availability of feed grade threonine and tryptophan has increased the need for accurate estimates of the requirements for these two potentially limiting AA. The published literature on threonine and tryptophan provide widely varying estimates of the requirements that cannot be readily explained. The requirement values of 0-3 week old broilers for threonine range from 0.50 to 0.85% of the diet (or 2.8 to 3.7% of CP) (Woodham and Deans, 1975; Robbins, 1987). On the other hand, tryptophan should be formulated at 0.14 to 0.28% of the diet (or 0.78 to 1.2% of CP) (Woodham and Deans, 1975; Abebe and Morris, 1990).

The National Research Council (NRC, 1994) recommended requirement values (0-3 weeks old broiler chicks) are 0.80% of the diet (3.48% of dietary crude protein) for threonine and 0.20% of the diet (0.87% of dietary crude protein) for tryptophan. The NRC (1994) threonine requirement values for 0-3 week old broiler chicks was derived from research done in the 1980s, whereas published reports dated from 1947 to 1988 were used to derive tryptophan requirements for animals of markedly different productive potential than the one existing today. Therefore, it is not certain whether poultry nutritionists in the feed industry can still use the requirement values for threonine and tryptophan recommended by the NRC (1994) in feed formulation.

D'Mello and Lewis (1970c) concluded that it is impossible to determine the chick's actual requirement for essential AA unless the diet is in good AA balance, since the AA pattern of the diet will affect the chick's response to supplementation. The usual method of determining the AA requirements involves the technique of adding graded concentrations of a single AA until a maximum response is obtained. According to D'Mello and Lewis (1970c), this method is inadequate in providing an accurate assessment of AA requirements, because the requirements for AA are interdependent. The interdependence in AA requirements have been reported between lysine and arginine (D'Mello and Lewis, 1970a), leucine, isoleucine and valine

(D'Mello and Lewis, 1970b) and threonine and tryptophan (D'Mello and Lewis, 1970c). Furthermore, AA requirements of poultry are influenced by a multitude of dietary, environmental and genetic factors. All of these factors have led to the development of the ideal protein concept. Ideal protein is defined as the perfect AA profile or balance in terms of dietary concentrations among the essential AA to meet all AA requirements for a particular species or age without any excesses or deficiencies. In addition, formulating diets according to the ideal protein concept allows for the most efficient and economical use of dietary protein by maximizing nitrogen utilization and minimizing nitrogen excretion (Mack *et al.*, 1999).

Therefore, the objectives of the present experiments were: 1) to compare the efficacy of the essential AA profiles present in the Blair *et al.* (1977), NRC (1994) and Illinois Ideal Chick Protein (IICP) (Baker and Han, 1994) recommended diets and; 2) using the AA profiles that support better growth, to evaluate the responses of 0-3 week old broiler chicks to different dietary levels of threonine and tryptophan.

Materials & Methods

Bird Management, Diet and Data Collection

Experiment 1

A total of 108 day old male (Peterson x Arbor Acres) broiler chicks was obtained from a local commercial hatchery. All birds were vaccinated against Marek's disease and housed in battery brooding units (Petersime Incubators Co., Gettysburg, OH 45328) with 23 h of light daily (7:00 a.m. to 6:00 a.m.). Group feed intake and individual body weights were recorded at the start of the experiment and then weekly for the two week study. Three semi-purified diets (Diet 1 = NRC 1994; Diet 2 = IICP and Diet 3 = Blair *et al.* (1977)) with different AA profiles (Table 3.1) were used and the compositions of the diets are shown in Table 3.2. There were four replications (nine chicks per replication) for each dietary treatment. Cornstarch and corn oil served as the main energy source, whereas isolated soy protein (NURISH® 1500) and crystalline AA were used as the only source of AA. Hardwood sawdust was used in the original study by Blair *et al.* (1977). Therefore, it was also included in this current study and the sawdust used in Diet 3 was obtained from a local lumberyard and ground to pass through a 2 mm screen size

(Tyler Standard Screen Scale, Ohio, USA). It was later extracted with dichloromethane for 24 h to remove any resin or toxin.

Table 3.1 Experiment 1: Amino acid ratio proposed by different researchers

	NRC [1]	IICP [2]	Blair *et al.* [3]
Lysine	100	100	100
Arginine	114	105	120
Histidine	32	32	40
Methionine	46	36	55
Cystine	36	36	14
Phenylalanine	66	55	70
Tyrosine	56	50	70
Threonine	73	67	70
Leucine	109	109	140
Isoleucine	73	67	80
Valine	82	77	86
Tryptophan	18	16	20
Glycine	114	65	100
Proline	55	44	70

[1]National research council for Poultry, 1994
[2]Illinois ideal chick protein. Baker and Han, 1994.
[3]Blair *et al.* 1977

Table 3.2 Experiment 1: Composition of diets (% of diet).

Ingredient	NRC 1994	IICP (1994)	Blair *et al.* (1977)
Corn starch	54.44	52.71	38.16
Isolated soy protein[1]	15.71	12.57	16.80
Amino acid mixtures[2]	3.69	4.00	3.86
L-glutamic acid	10.64	14.97	9.26
Corn oil	5.85	6.08	12.25
Cellulose	3.00	3.00	3.00
Mineral-vitamin mixtures[3]	6.67	6.67	6.67
Extracted saw dust[4]	0.00	0.00	10.00

[1]NURISH® 1500, a gift from Protein Technologies International™. Checkerboard Square, St. Louis, MO, USA.
[2]All diets were made isonitrogenous (3.68% N) by adjusting the level of L-glutamic acid. Dietary percentages of all amino acids can be calculated by multiplying the ratios in Table 3.1 by 1.2%.
[3]Supplied per kg of diet: 2 g choline chloride (100%), 20 mg thiamin HCL, 10 mg riboflavin, 30 mg calcium pantothenate, 50 mg niacin, 6 mg pyridoxine HCL, 4 mg folacin, 0.6 mg biotin, 0.04 mg vitamin B12, 100 mg inositol, 2 mg para-aminobenzoic acid, 5,200 IU vitamin A, 600 ICU vitamin D_3, 20 IU vitamin E, 2 mg vitamin K, 125 mg antioxidant (ethoxyquin), 5 g NaCI, 33 g $Ca_3(PO_4)_2$, 0.6 g MgO, 4.5 g K_2CO_3, 5 g $NaHCO_3$, 5 g $Al(OH)_3$, 650 mg $MnSO_4.H_2O$, 50 mg ZnO, 567 mg $FeSO_4.7H_2O$, 20 mg $CuSO_4.5H_2O$, 0.4 mg Na_2SeO_3, 40 mg KI, 1 mg $CoSO_4.7H_2O$, 9 mg H_3BO_3, 9 mg $Na_2MoO_4.2H_2O$.
[4]Extracted with dichloromethane for 24 h.

Isolated protein was used at such levels that the need for supplementation with crystalline AA was minimized. The isolated soy protein contained 83.2% protein and the following AA

content (% of product): alanine (3.5%), arginine (6.2%), aspartic acid (9.6%), cysteine (1.0%), glutamic acid (15.7%), glycine (3.4%), histidine (2.2%), isoleucine (4.0%), leucine (6.7%), lysine (5.2%), methionine (3.0%), phenylalanine (4.3%), proline (4.2%), serine (4.3%), threonine (3.1%), tryptophan (1.1%), tyrosine (3.1%), valine (4.2%).

All AA were supplied as L-isomers except methionine, which was supplied as the DL-isomer. Free-base forms of AA were used with the exception of lysine which was provided as the hydrochloride. Feed-grade sources were used for lysine HCl (78.8%), threonine (98.5%), and methionine (98%); the remaining AA were pharmaceutical grade. The true digestibilities of AA in the isolated soy protein and free AA were assumed to be 100% (Chung and Baker, 1992). All diets were made isonitrogenous (3.68% N) by adjusting the level of L-glutamic acid. Apparent metabolizable energy and digestible lysine were set at 3,500 kcal/kg and 1.2% (of diet), respectively. Feed and water were offered *ad libitum* during the 14-day experiment.

Experiment 2

A total of 180 male day old broiler chicks (Peterson x Arbor Acres) was used in the study. All birds were vaccinated against Mareks disease and housed in battery brooding units (Petersime Incubators Co., Gettysburg, OH 45328) with 23 h of light daily (7:00 a.m. to 6:00 a.m.). All of the chicks were wing-banded at day old and fed a commercial broiler starter diet for the first week of life. On the morning of Day 8, all chicks were individually weighed. The chicks of approximately same weight were randomly assigned to battery pens. Individual body weight and group feed intake were measured weekly. There were a total of nine diets fed to birds from 1-3 weeks of age (Table 3.4), with four replications of five birds per diet.

Since locally available feed ingredients were used to formulate a practical broiler diet, it was not possible to formulate a diet with less than 90% of the National Research Council (NRC, 1994) broiler recommendations for threonine and tryptophan. As a result, levels of 90%, 100% and 110% of the NRC (1994) recommendations for threonine and tryptophan were used. In the previous ideal protein study (experiment 1), we found that the Blair *et al.* (1977) AA profile gave the best growth response. Therefore, the Blair *et al.* (1977) AA profile was used in this experiment but the amounts of threonine and tryptophan in the diets were adjusted to 90%, 100% and 110% of the NRC (1994) recommendations. Three levels of threonine and three levels of tryptophan were arranged in a factorial manner as shown in Table 3.3.

Table 3.3 Experiment 2: Arrangement of diets [1].

	THR: 90% NRC or 0.72% of the diet	**THR: 100% NRC or 0.80% of the diet**	**THR: 110% NRC or 0.88% of the diet**
TRP: 90% NRC or 0.18% of the diet	0.72%THR /0.18% TRP Diet 1	0.80%THR /0.18% TRP Diet 2	0.88%THR /0.18% TRP Diet 3
TRP: 100% NRC or 0.20% of the diet	0.72%THR /0.20% TRP Diet 4	0.80%THR /0.20% TRP Diet 5	0.88%THR /0.20% TRP Diet 6
THR: 110% NRC or 0.22% of the diet	0.72%THR /0.22% TRP Diet 7	0.80%THR /0.22% TRP Diet 8	0.88%THR /0.22% TRP Diet 9

[1]Threonine = Thr; Tryptophan = Trp; NRC (1994).

Nutrient composition of the diets is shown in Table 3.4. Cornstarch and oil served as the main energy sources, whereas crystalline AA were used as the only source of AA. Contrary to the previous study, the diets were based on crystalline AA rather than partly on isolated soy protein. The intention was to provide more exact details of the AA contents of the diets, and to aid with the interpretation of the results. All AA were supplied as L-isomers except methionine, which was supplied as the DL-isomer. Free-base forms of AA were used with the exception of lysine which was provided as the hydrochloride. Feed-grade sources were used for lysine HCl (78.8%), threonine (98.5%), and methionine (98%); the remaining AA were pharmaceutical grade. The true digestibility of free AA was assumed to be 100% (Izquierdo *et al.*, 1988; Chung and Baker, 1992; Zhang and Parsons, 1993). All diets were made isonitrogenous (2.83% N) by adjusting the level of L-glutamic acid. Apparent metabolizable energy and digestible lysine were set at 3,644 kcal/kg and 1.10% (of diet), respectively. The digestible lysine level was reduced from 1.20% to 1.10% of the diet, because several recent publications indicated that 1.10% lysine is required by starter broilers (Han and Baker, 1991, 1993; Rhodimet Nutrition Guide, 1993; Vazquez and Pesti, 1997). Feed and water were offered *ad libitum* during the experiment.

The guidelines of the Canadian Council on Animal Care were followed and protocols for the two experiments were approved by The University of British Columbia Animal Care Committee.

Table 3.4 Experiment 2: Composition and calculated analysis (%) of diets[1]

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Ingredients (% of diet)									
Corn starch	52.46	52.46	52.46	52.46	52.46	52.46	52.46	52.46	52.46
Amino acid mixtures [2]	11.54	11.65	11.75	11.58	11.68	11.78	11.61	11.72	11.81
L-glutamic acid	11.98	11.87	11.77	11.94	11.84	11.74	11.91	11.80	11.71
Corn oil	10	10	10	10	10	10	10	10	10
Cellulose	3	3	3	3	3	3	3	3	3
Min-vit mixtures [3]	6.02	6.02	6.02	6.02	6.02	6.02	6.02	6.02	6.02
Extracted saw dust [4]	5	5	5	5	5	5	5	5	5
Amino Acid Ratios									
Lysine	100	100	100	100	100	100	100	100	100
Arginine	120	120	120	120	120	120	120	120	120
Histidine	40	40	40	40	40	40	40	40	40
Methionine	55	55	55	55	55	55	55	55	55
Cystine	14	14	14	14	14	14	14	14	14
Phenylalanine	70	70	70	70	70	70	70	70	70
Tyrosine	70	70	70	70	70	70	70	70	70
Threonine	***65***	***73***	***80***	***65***	***73***	***80***	***65***	***73***	***80***
Tryptophan	***16***	***16***	***16***	***18***	***18***	***18***	***20***	***20***	***20***
Leucine	140	140	140	140	140	140	140	140	140
Isoleucine	80	80	80	80	80	80	80	80	80
Valine	86	86	86	86	86	86	86	86	86
Glycine	100	100	100	100	100	100	100	100	100
Proline	70	70	70	70	70	70	70	70	70

[1] All diets were made isonitrogenous (2.83% N) by adjusting the level of L-glutamic acid. Dietary percentages of all amino acids can be calculated by multiplying the ratios above by 1.1%.

[2] Amount of amino acids except threonine and tryptophan remained the same across diets.

[3] Supplied per kg of diet: 2 g choline chloride (100%), 20 mg thiamin HCL, 10 mg riboflavin, 30 mg calcium pantothenate, 50 mg niacin, 6 mg pyridoxine HCL, 4 mg folacin, 0.6 mg biotin, 0.04 mg vitamin B12, 100 mg inositol, 2 mg para-aminobenzoic acid, 5,200 IU vitamin A, 600 ICU vitamin D_3, 20 IU vitamin E, 2 mg vitamin K, 125 mg antioxidant (Ethoxyquin), 5 g NaCI, 33 g $Ca_3(PO_4)_2$, 0.6 g MgO, 4.5 g K_2CO_3, 5 g $NaHCO_3$, 5 g $Al(OH)_3$, 650 mg $MnSO_4.H_2O$, 50 mg ZnO, 567 mg $FeSO_4.7H_2O$, 20 mg $CuSO_4.5H_2O$, 0.4 mg Na_2SeO_3, 40 mg KI, 1 mg $CoSO_4.7H_2O$, 9 mg H_3BO_3, 9 mg $Na_2MoO_4.2H_2O$.

[4] Extracted with dichloromethane for 24 hours.

Statistical Analyses

Experiment 1

This was a completely randomized design experiment. Data were subjected to analysis of variance (ANOVA) procedures appropriate for completely randomized design by using the General Linear Models (GLM) procedure of SAS® software (SAS Institute, 1996). If treatments were found to be significantly different, Tukey's multiple range test (Snedecor and Cochran, 1980) was used to determine the significant differences between treatment least-square means.

Experiment 2

This was a completely randomized design with a factorial arrangement of treatments. Data were subjected to analysis of variance (ANOVA) procedures using the General Linear Models (GLM) procedure of SAS® software (SAS Institute, 1996). If treatments were found to be significantly different, Tukey's multiple range test (Snedecor and Cochran, 1980) was used to determine the differences between treatment least-square means.

Results

Experiment 1

There were no significant differences in body weight among day-old chicks in the different treatment groups (Table 3.5). Live body weight of the birds fed Diet 3 (Blair *et al.*, 1977) was significantly higher ($P < 0.05$) at days 7 and 14 when compared with the birds fed the other two diets (Table 3.5). No significant differences in body weight were found between Diets 1 (NRC, 1994) and 2 (IICP). Body weight gain and feed consumption followed the same trend (Tables 3.5 and 3.6). Feed conversion efficiencies were not significant among birds fed Diets 1-3. Overall, during the 2 week study (Table 3.7) birds fed diet based on the AA profile suggested by Blair *et al.* (1977) gained significantly more weight ($P < 0.05$), and ate significantly more ($P < 0.05$) than those fed diets based on the NRC (1994) and IICP AA profiles, while the differences in feed conversion efficiency were small.

Table 3.5 Experiment 1: The effect of different amino acid profiles on live body weight and body weight gain by 0-1 and 1-2 weeks old chicks.

	Live body weight (g)			Body weight gain (g)	
	Day 1	Day 7	Day 14	Week 0-1	Week 1-2
NRC (1994)	41.8	86.3 [b]	175 [b]	44.3 [b]	88.4 [b]
IICP (1994) [1]	40.3	82.6 [b]	185 [b]	41.7 [b]	103.3 [b]
Blair *et al.* (1977)	40.7	103.5 [a]	233 [a]	61.2 [a]	131.5 [a]
Overall mean	40.9	90.8	198	49.1	107.7
Overall SEM[2]	0.4	2.0	9.7	2.2	7.2

[1]Illinois ideal chick protein (Baker and Han, 1994)

[2]Standard error of the mean; data represent mean of four replications of nine chicks.

[a,b]Treatment means with different superscripts within a column are significantly different at P<0.05.

Table 3.6 Experiment 1: The effect of different amino acid profiles on feed consumption and feed conversion efficiency by 0-1 and 1-2 week old chicks.

	Feed intake (g/bird)		Feed conversion efficiency (gain/feed)	
	Week 0-1	Week 1-2	Week 0-1	Week 1-2
NRC (1994)	53.9 [b]	115.5 [b]	0.82	0.74
IICP (1994) [1]	48.2 [b]	125.6 [b]	0.85	0.82
Blair *et al.* (1977)	64.2 [a]	176.2 [a]	0.95	0.74
Overall mean	55.4	139.1	0.87	0.77
Overall SEM[2]	2.4	9.2	0.03	0.02

[1]Illinois ideal chick protein (Baker and Han, 1994)

[2]Standard error of the mean; data represent mean of four replications of nine chicks.

[a,b]Treatment means with different superscripts within a column are significantly different at P<0.05.

Table 3.7 Experiment 1: Performance of chicks fed different amino acid profile diets during the first two weeks of age[1].

	Weight gain (g)	Feed intake (g/bird)	Feed conversion efficiency (gain/feed)
NRC (1994)	132.7 [b]	169.4 [b]	0.77
IICP (1994) [2]	145.0 [b]	173.9 [b]	0.83
Blair *et al.* (1977)	192.8 [a]	240.4 [a]	0.79
Overall mean	156.8	194.6	0.80
Overall SEM[3]	9.9	10.7	0.03

[1]Data represent mean of four replications of nine chicks.

[2]Illinois ideal chick protein (Baker and Han, 1994)

[3]Standard error of the mean

[a,b]Treatment means with different superscripts within a column are significantly different at P<0.05.

Experiment 2

The level of threonine and tryptophan did not significantly ($P > 0.05$) affect body weight of birds at 2 weeks of age, weight gain during weeks 1-2, daily feed intake or overall feed intake (week 1-3) (Tables 3.8 and 3.9). When the birds reached 3 weeks of age, their body weight was not affected by threonine levels. However, body weight was significantly lower ($P < 0.05$) when tryptophan in the diet reached 110% of the NRC recommendation. The interaction between threonine and tryptophan for body weight (week 3) was also significant ($P < 0.05$). Weight gain during weeks 2-3 was significantly lower ($P < 0.05$) at the 110% threonine and 110% tryptophan levels. Overall weight gain (weeks 1-3) was not significantly affected ($P > 0.05$) by the threonine level as shown in Table 3.8, but it decreased significantly ($P < 0.05$) as the level of tryptophan increased. The gain/feed ratio was not significantly affected ($P > 0.05$) by the threonine level but it was significantly decreased ($P < 0.05$) with the increased tryptophan levels (Table 3.9). The interactions between threonine and tryptophan for body weight (week 3), weight gain (week 2-3) and overall weight gain (week 1-3) were significant ($P < 0.05$). As a result, each situation was interpreted separately by using Figures 3.1 and 3.2 for body weight (week 3), Figures 3.3 and 3.4 for weight gain (week 2-3) and Figures 3.5 and 3.6 for the overall weight gain (week 1-3).

Body weight at 3 weeks of age was significantly lower ($P < 0.05$) in birds fed diets containing 110% tryptophan when threonine level reached 110% of the NRC (1994) recommendation (Figure 3.1) and it was significantly lower ($P < 0.05$) in birds fed diets containing 110% threonine when tryptophan level reached 110% of the NRC (1994) recommendation (Figure 3.2). Diets based on tryptophan at 100% and 110% of the NRC (1994) recommendations significantly reduced ($P < 0.05$) weight gain (week 2-3) of birds when threonine was set at 100% and 110% of NRC (1994) recommendation (Figure 3.3). On the other hand, only birds fed diets containing the highest level of both threonine and tryptophan (110% NRC) had significantly lower ($P < 0.05$) weight gain during weeks 2-3 (Figure 3.4). Overall weight gain (weeks 1-3) was significantly reduced ($P < 0.05$) when the diets contained 100% of threonine and 110% of tryptophan or 110% of threonine and 110% of tryptophan (Figure 3.5). For birds fed diets containing 110% of tryptophan, overall weight gain was significantly reduced ($P < 0.05$) at the highest level of threonine (Figure 3.6).

Table 3.8 Experiment 2: Effect of different levels of threonine and tryptophan on body weight and weight gain by broilers during weeks 1-3 post-hatch [1].

Treatment	Body weight (g) Week 2	Body weight (g) Week 3	Weight gain (g) Week 1-2	Weight gain (g) Week 2-3	Weight gain (g) Week 1-3
Threonine					
90% NRC	273	451	134	178 [a]	312
100% NRC	268	440	136	172 [a]	308
110% NRC	278	434	142	156 [b]	298
Tryptophan					
90% NRC	280	463 [a]	142	183 [a]	324 [a]
100% NRC	270	450 [a]	138	179 [a]	318 [a]
110% NRC	269	414 [b]	133	145 [b]	278 [b]
Overall mean	273	442	138	169	306
Pooled SEM [2]	2.9	5.6	2.0	3.9	5.1
Factorial effects [3]					
Threonine (Thr)	NS	NS	NS	***	NS
Tryptophan (Trp)	NS	***	NS	***	***
Thr X Trp	NS	***	NS	***	***

[1] Study was conducted from week 1-3, mean body weight at the start of the experiment (Week 1) was 135 g.
[2] Pooled standard error of the mean; data represent mean of four replications of five chicks.
[3] NS = not significant ($P > 0.05$) or *** = significant at $P < 0.05$
[ab] Treatment means with different superscripts within a column and within treatment are significantly different at $P < 0.05$.

Table 3.9 Experiment 2: Effect of different levels of threonine and tryptophan on feed intake and gain/feed ratio by broilers during weeks 1-3 post-hatch [1].

Treatment	Daily feed intake (g)	Feed intake (g) Week 1-3	Gain/feed ratio
Threonine			
90% NRC	29.6	414.7	0.75
100% NRC	28.3	396.8	0.77
110% NRC	26.9	376.6	0.75
Tryptophan			
90% NRC	28.9	405.1	0.79 [b]
100% NRC	29.7	415.1	0.76 [ab]
110% NRC	26.3	367.9	0.72 [a]
Overall mean	28.3	396.0	0.76
Pooled SEM [2]	0.7	9.6	0.02
Factorial effects [3]			
Threonine (Thr)	NS	NS	NS
Tryptophan (Trp)	NS	NS	***
Thr X Trp	NS	NS	NS

[1] Study was conducted from week 1-3, mean body weight at the start of the experiment was 135 g.
[2] Pooled standard error of the mean; data represent mean of four replications of five chicks.
[3] NS = not significant ($P > 0.05$) or *** = significant at $P < 0.05$
[ab] Treatment means with different superscripts within a column and within treatment are significantly different at $P < 0.05$.

Figure 3.1 Body weight of 3 week old broilers (Experiment 2) : The effect of different levels of tryptophan (Trp) when threonine (Thr) was set at 90, 100 and 110% of the NRC (1994) recommendations.

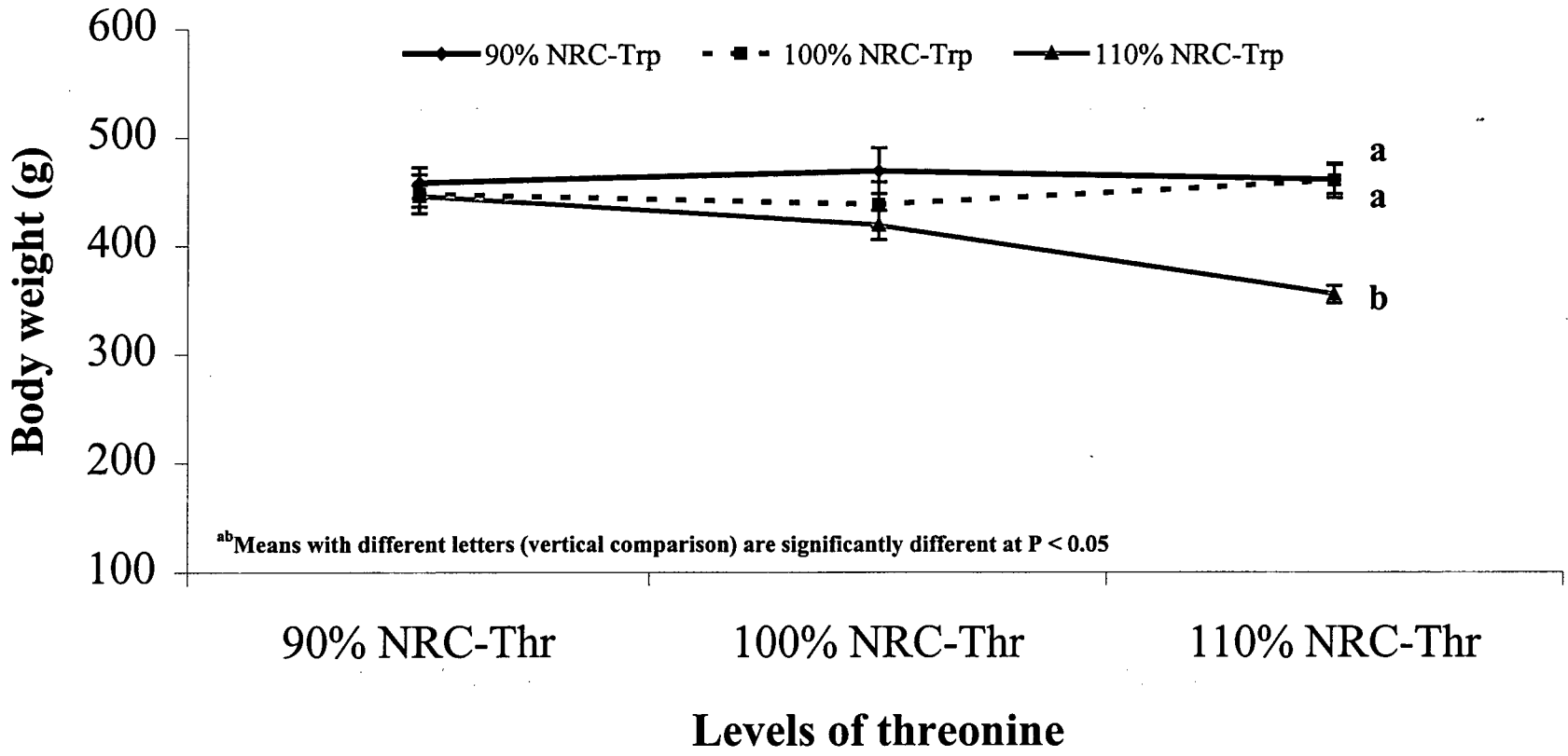


Figure 3.2 Body weight of 3 week old broilers (Experiment 2) : The effect of different levels of threonine (Thr) when tryptophan (Trp) was set at 90, 100 or 110% of the NRC (1994) recommendations.

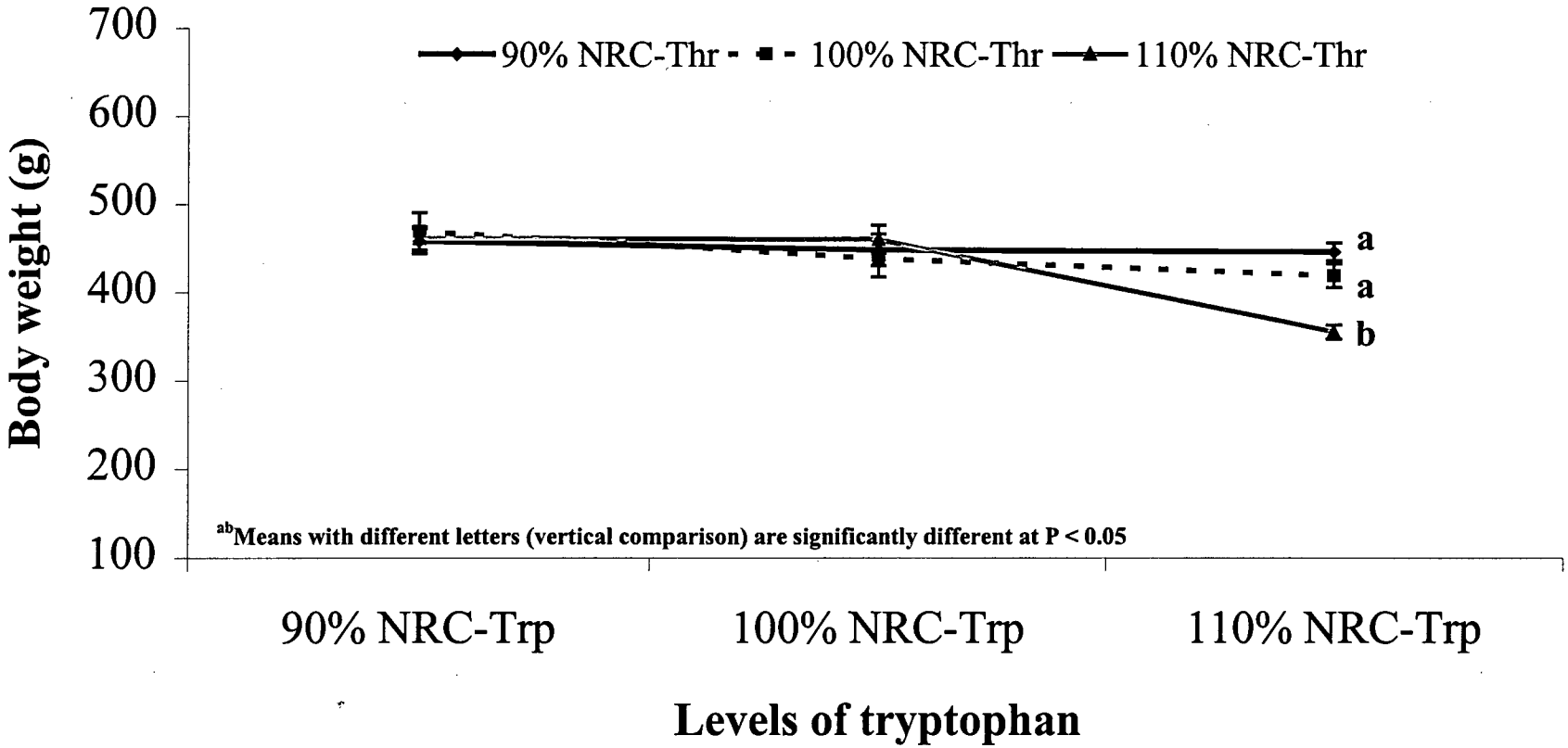


Figure 3.3 Weight gain for week 2-3 (Experiment 2) : The effect of different levels of tryptophan (Trp) when threonine (Thr) was set at 90, 100 or 110% of the NRC (1994) recommendations.

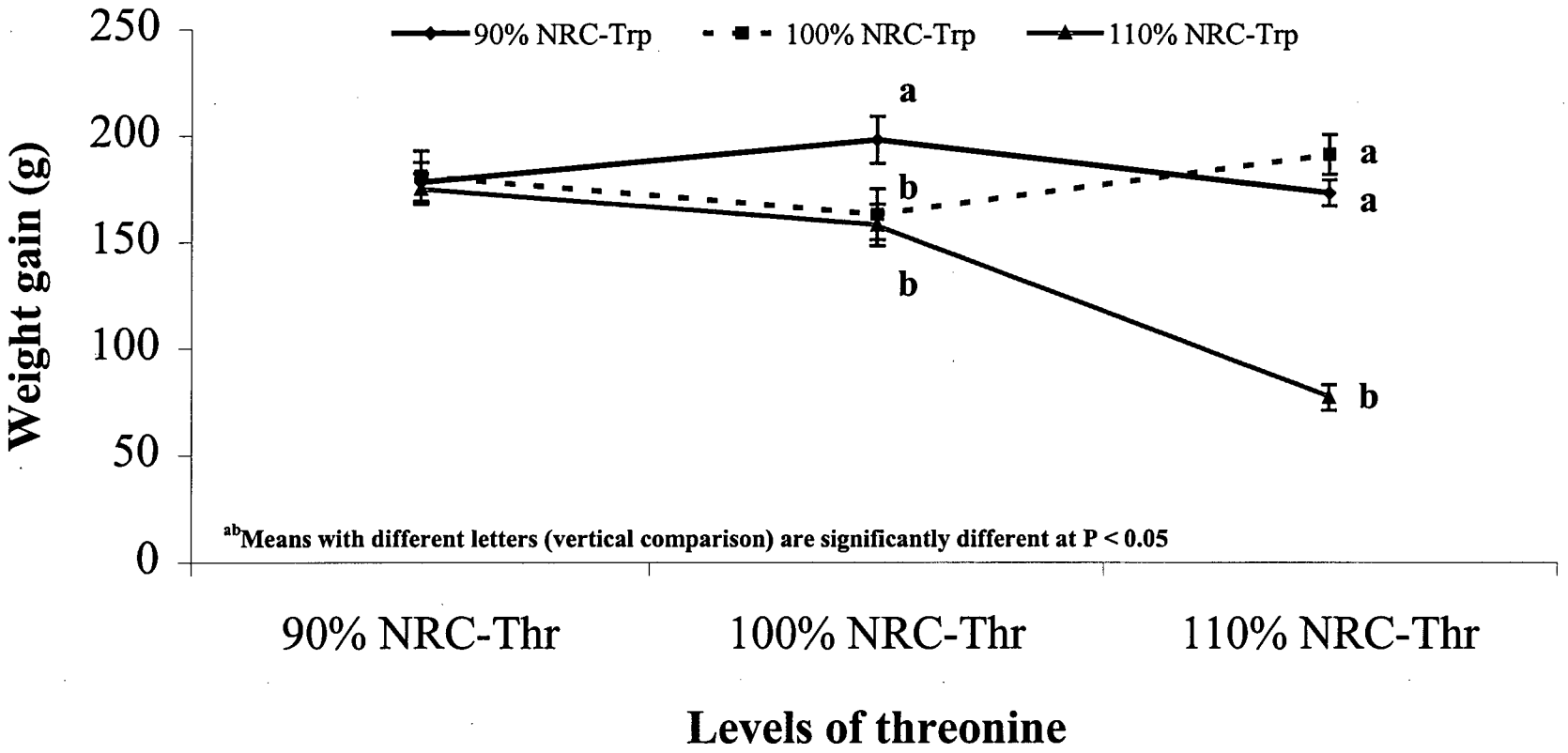


Figure 3.4 Weight gain for week 2-3 (Experiment 2) : The effect of different levels of threonine (Thr) when tryptophan (Trp) was set at 90, 100 or 110% of the NRC (1994) recommendations.

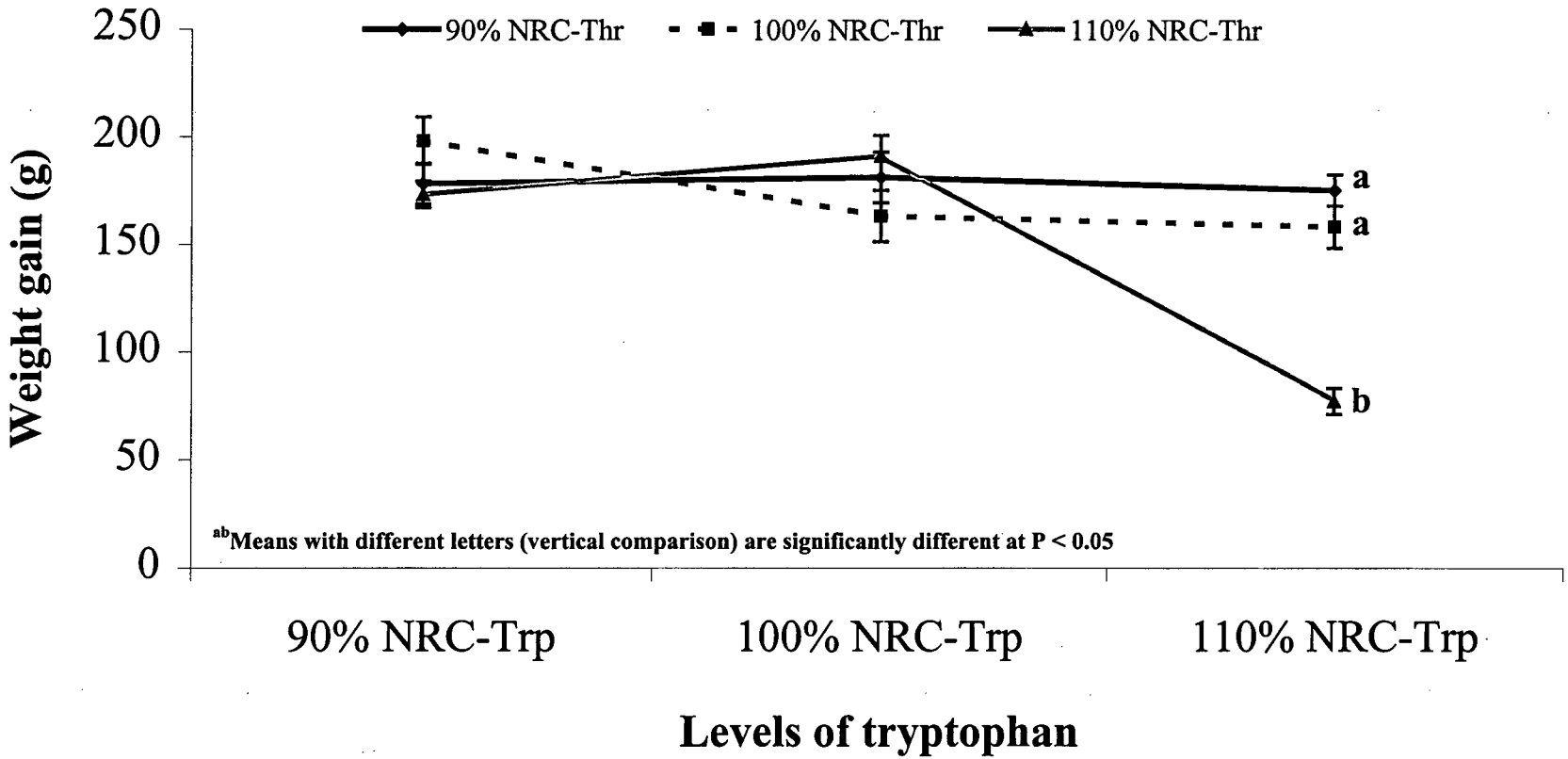


Figure 3.5 Weight gain for week 1-3 (Experiment 2) : The effect of different levels of tryptophan (Trp) when threonine (Thr) was set at 90, 100 or 110% of the NRC (1994) recommendations.

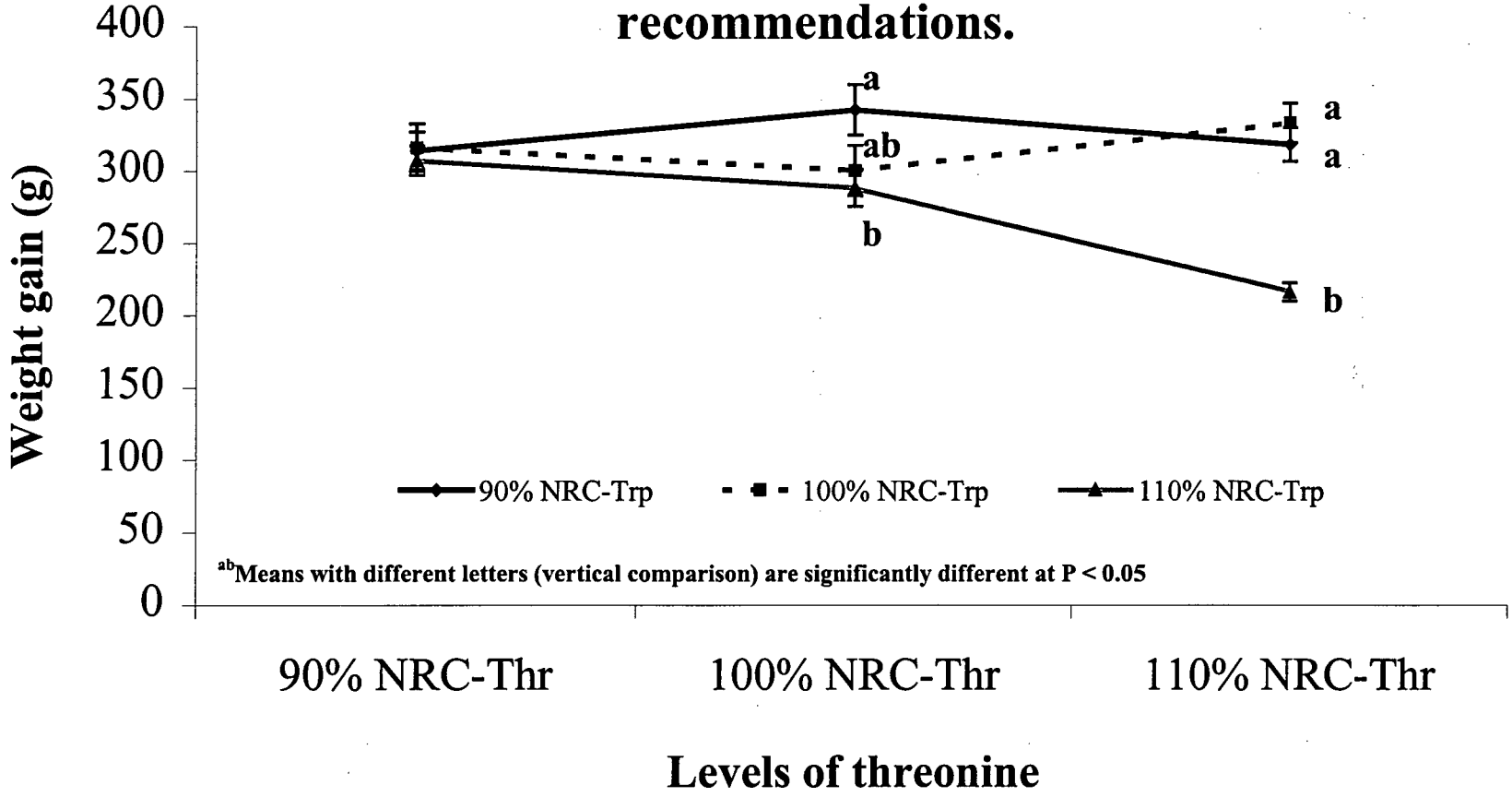
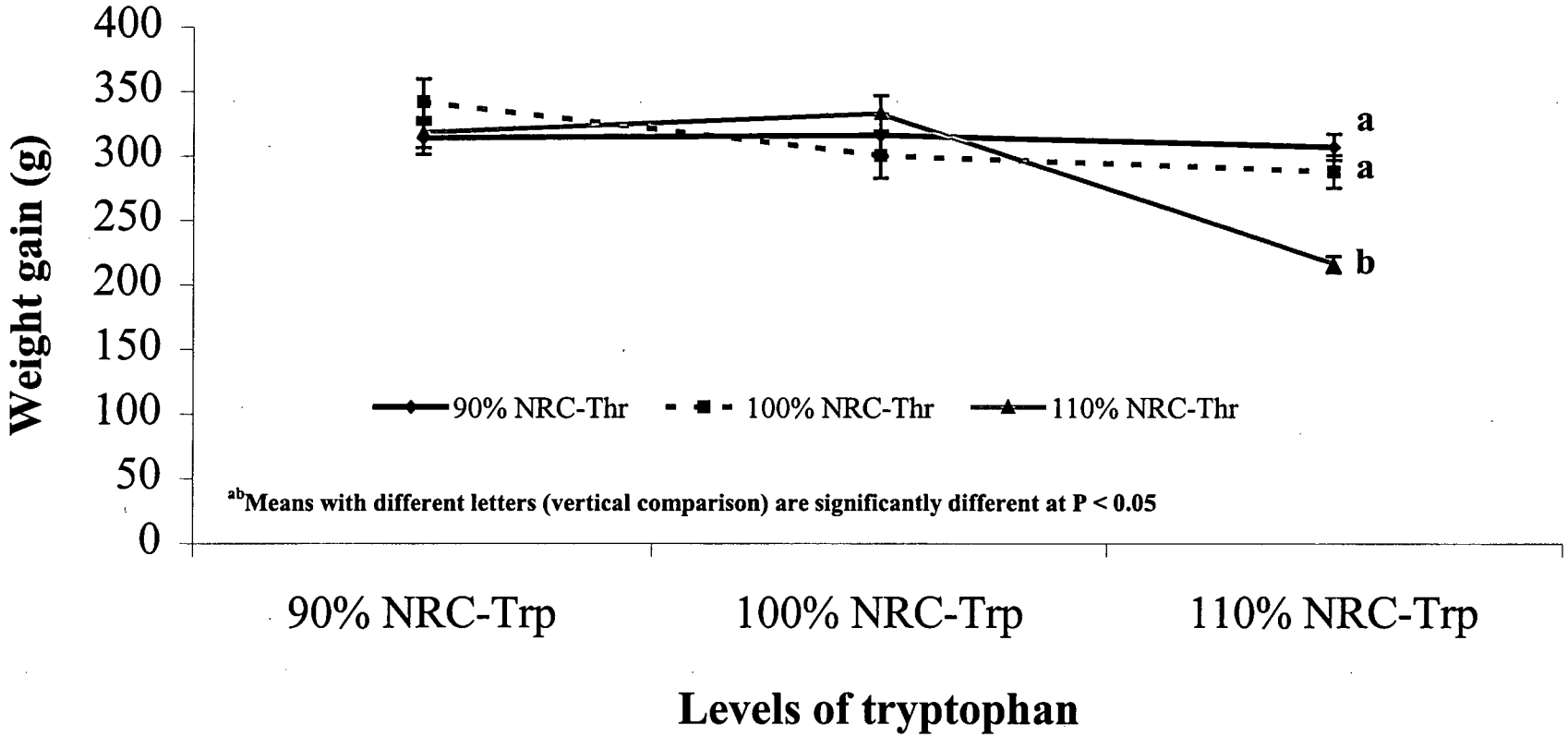


Figure 3.6 Weight gain for week 1-3 (Experiment 2) : The effect of different levels threonine (Thr) when tryptophan (Trp) was set at 90, 100 or 110% of the NRC (1994) recommendations.



Discussion

Even though the NRC (1994) estimates of AA requirements are on a total AA basis, the comparison with the other two amino acid profiles (Blair *et al.*, 1977 and Baker and Han, 1994) that express the requirements on a digestible basis in Experiment 1 is valid because the digestibility of individual AA in corn-soybean meal diets is similar (approximately 88%) (Rhodimet Nutrition Guide, 1993). In fact, Baker and Han (1994) have shown that using digestible AA rather than total AA requirement data for the NRC (1994) did not lower any of the ratios in Table 3.1 below those calculated on a total AA basis. An earlier study by Baker and Han (1994) showed that there were no significant differences in growth performance when birds were fed a diet based on either the NRC (1994) or IICP AA profiles. Since the IICP had also lower AA ratios relative to lysine, the Illinois group concluded that some of the AA ratios set by the NRC (1994) were too high and the ratios set by the IICP were better. However, the results of the present study disagreed with the conclusion of Baker and Han (1994) and showed that birds fed the Baker and Han (1994) diet did not have a better growth performance than those fed the NRC (1994) diet.

Compared to the AA profiles proposed by the NRC (1994) and the Illinois group (IICP), the profile suggested by Blair *et al.* (1977) contained a higher ratio of most essential AA relative to lysine except total sulfur AA, threonine and glycine. The diet based on Blair's AA profile was the diet that gave the best weight gain for the chicks. As shown in Tables 3.6 and 3.7, birds fed the Blair's diet ate significantly more ($P < 0.05$) throughout the 2-week study than birds fed the other two diets. It is obvious that lower feed intake reduced the weight gain of birds fed diets based on the NRC (1994) and IICP AA profiles. The sawdust in the Blair's diet may have improved the texture of the diet, but this does not fully explain the greater feed intake. Moreover, all diets were formulated to have the same energy and nitrogen levels. It is not likely that the differences in feed consumption were due to a deficiency of energy or nitrogen in one of the diets. However, it is well known that the AA balance in general also plays an important role in maximizing the feed consumption of diets. Studies regarding how AA imbalances depress feed intake are well-documented (Knight *et al.*, 1991; D'Mello, 1993; 1994). If the diet is not well balanced in AA, birds are not likely to consume enough of it to grow optimally.

AA imbalances could be a serious problem, especially for birds fed a semi-purified or purified crystalline AA diet. Most AA in a practical type diet are presented as intact proteins, and

excess AA (up to 150% of the requirement) under these conditions do not seem to depress the birds' performance (Baker, 1997). Bach Knudsen and Jørgensen (1986) concluded that the absorption of free crystalline AA is more rapid than that of peptide-bound AA due to the fact that the rate of passage of the free AA from the stomach to the small intestine is faster than that of the peptide-bound AA. As a result, in crystalline AA diets where 100% of all AA are assumed to be immediately available for absorption, small changes in AA balance and AA excesses could have profound effects on the overall efficiency of the diet and particularly on the *ad libitum* feed intake (Dudley-Cash, 1998). The daily live-weight gain of the birds fed Blair's diet was 13.77 g in the present experiment which was close to the gain by birds fed the practical diet (14.4 g) (Blair *et al.,* 1977). On the other hand, birds fed diets based on the AA profiles of the NRC (1994) and IICP gained only 9.55 and 10.36 g per day, respectively, in the present experiment. Some of the AA ratios proposed by Blair *et al.* (1977), however, might have also been too high. At this point, it is difficult to know whether some of the AA ratios could be reduced, however, the ratios are very similar to those of the Rhodimet Nutrition Guide (1993).

The interactions between threonine and tryptophan shown in Figures 3.1 to 3.6 of Experiment 2 clearly show that both body weight and weight gain of the birds decreased as the level of threonine and tryptophan in the diets increased. The differences of both body weights and body weight gains of birds increased as the level of threonine and tryptophan increased. Weight gain was lowest at the highest levels of both AA (110% of threonine and 110% of tryptophan). This finding agrees with that reported by D'Mello and Lewis (1970c), who showed that there were interactions between threonine and tryptophan. Interactions involving other AA have been reported to exist between lysine and arginine (D'Mello and Lewis, 1970a), and leucine, isoleucine and valine (D'Mello and Lewis, 1970b).

D'Mello and Lewis (1970c) also stated that the adverse effects of excess threonine were reversed by appropriate supplementation of the diet with tryptophan. This effect of tryptophan, however, was not observed in our study. The results of the present study indicate that as the level of threonine and tryptophan increase, AA in the diets become imbalanced, leading to a reduction in feed intake as shown in Table 3.9. This agrees with the findings of Knight *et al.* (1991) and D'Mello (1993; 1994) that AA imbalance affects animal performance by depressing feed intake. Excess threonine has been shown by Moreno *et al.* (1993) and Ishibashi *et al.* (1998) to reduce the performance of broilers and layers. Similarly, Tasaki (1983) reported poorer layer performance with excess tryptophan.

Based on the observations of the threonine and tryptophan interaction on weight gain (Figures 3.5 and 3.6) and gain/feed ratio (Table 3.9), the results obtained from the present study (Experiment 2) indicate that the dietary level of threonine and tryptophan in 1-3 week old broiler diets should be targeted at 65% and 16% of lysine, respectively. That is, if the dietary level of digestible lysine was set at 0.95% (equivalent to the requirement value of 1.1% total lysine in the diet recommended by the NRC (1994)) (Table 3.10), then dietary level of digestible threonine and digestible tryptophan should be set at 0.63% and 0.16%, respectively. When converted to total requirement values (Table 3.10), this is equal to 0.73% for threonine and 0.19% for tryptophan in the diet or 4.13% of CP for threonine and 1.07% of CP for tryptophan.

Using a milo-soybean meal-corn gluten meal basal diet, Yamazaki *et al.* (1997) concluded that 1-3 week broiler chicks require 0.65% available threonine in the diet, which is the same as the estimate obtained in this study. Thomas *et al.* (1986, 1987) found that the optimum level of total threonine for feed efficiency was 0.73% (1986), and in a further study Thomas *et al.* (1987) confirmed that the threonine requirement for male broilers was 0.72%. Recently, Thomas *et al.* (1992) reported that male and female broilers aged 0-3 week need 0.77% and 0.71% of dietary threonine (average 0.74%). On the other hand, Holsheimer *et al.* (1994) concluded that in a 16% protein maize-soybean meal diet supplemented with essential AA and non-essential AA, an improvement in gain and feed efficiency was observed for both sexes of broiler chicks, when the dietary threonine content was increased to 0.725% until 3 weeks of age. In another study, Kidd *et al.* (1996) found that threonine levels ranging from 92% (0.736% of diet) to 112% (0.896% of diet) of NRC (1994) recommendations failed to improve weight gain of 1-21 day old broiler chicks. The estimated requirement for total threonine found in the present study also falls into the range of 0.67% to 0.77% reported by Rangel-Lugo *et al.* (1994).

The estimate derived from the present study, however, is higher than those of Hewitt and Lewis (1972) (0.53% of diet), Woodham and Deans (1975) (0.50-0.52% of diet) and Leeson and Summers (1997) (0.70% of diet). On the other hand, Morales-Barrera *et al.* (1992), Rhodimet Nutrition Guide (1993), Austic (1994) and the NRC (1994) reported that about 0.80% dietary threonine was required by starting chicks, which is higher than the estimated threonine requirement obtained in the present study. When expressed as % of CP, the estimated requirement value obtained in the present study is higher than most of the reported values, except that of Holsheimer *et al.* (1994) and Koide and Ishibashi (1995). A value of 4.53% of CP was reported by Holsheimer *et al.* (1994). Recently, data presented by Koide and Ishibashi (1995)

indicate that dietary threonine requirements expressed as a percentage of CP range from 3.96% to 4.13% CP. Based on the findings of the present and other recent studies, there are indications that the NRC (1994) may have overestimated the requirement of 0-3 week old broilers for threonine.

The National Research Council (NRC, 1994) recommendation for tryptophan requirement of 0-3 week old broilers is 0.20% of the diet. However, Hewitt and Lewis (1972) indicated that male broiler chicks aged 7 to 21 days require not more than 0.17% tryptophan in their diet. Woodham and Deans (1975) found that chicks required less than 0.14% tryptophan in their diet and Smith and Waldroup (1988) concluded that a diet containing 0.16% tryptophan could fulfil a growing chick's requirement for tryptophan. On the other hand, several researchers have reported a higher requirement value for tryptophan (Freeman, 1979; Steinhart and Kirchgessner, 1985; Abebe and Morris, 1990; Parr and Summers, 1991; Han *et al.*, 1991). More recently, tryptophan requirement values of 0.225%, 0.23% and 0.24% of diet were reported by Thomas *et al.* (1992), Rhodimet Nutrition Guide (1993) and Austic (1994), respectively.

Boomgaardt and Baker (1971) discovered that when tryptophan requirement was expressed as percent of the diet, the requirement increased with dietary protein level. However, when expressed as a % of CP, the requirement stayed constant at 0.87% at five different protein levels. Smith and Waldroup (1988) reported a value of 0.80% of CP, whereas Hewitt and Lewis (1972) suggested 0.94% of CP. All of these values are lower than the findings obtained from the present study. However, Abebe and Morris (1990) concluded that the amounts of tryptophan required for the maximum growth and feed efficiency were each linear functions of dietary protein concentration (12 g/kg CP or 1.2% CP). Abebe and Morris (1990) also concluded that a fixed ratio of tryptophan to protein should be used in practical diet formulation, rather than a minimum dietary concentration of tryptophan. More recently, Austic (1994) and Leeson and Summers (1997) suggested a value of 1.1% and 0.9% of CP, respectively. Even though the estimated requirement value of 0-3 week old broilers for tryptophan found in the present study agrees with that of the NRC (1994) recommendations, several recent studies indicate that the NRC (1994) may have underestimated the requirement of 0-3 week old broilers for tryptophan. Further study is necessary to explain these disagreements.

Based on the findings of experiment 2, the ratios of threonine and tryptophan relative to lysine were revised from 70% and 20% of lysine to 65% and 16% of lysine, respectively. The ratio of threonine relative to lysine (65% of lysine) obtained in the present study is slightly

higher than 62% reported by Austic (1994), but lower than 67% reported by Baker (1997). The ratio of tryptophan relative to lysine (16% of lysine) obtained in the present study agrees with the finding of Baker (1997), but is lower than the 18% (of lysine) recommended by Austic (1994). The revised AA ratios were used as the base for a deductive approach to estimate the AA requirements (Table 3.10)

Table 3.10 Suggested digestible and total amino acid requirements for 0-3 week old broiler chicks after adjustments were made to Blair *et al.* (1977) [1,2]

Amino acids (AA)	Average Dig. (%)[3]	Blair *et al.* (1977) Ratio of AA to lysine (%)	Blair *et al.* (1977) Dig. req. (% of diet)	Blair *et al.* (1977) Total req. (% of diet)	NRC (1994) Total req. (% of diet)
Lysine	87.8	100	0.97	1.10	1.10
Methionine	91.3	55	0.53	0.58	0.50
TSAA [4]	87.5	69	0.67	0.77	0.90
Threonine	86.7	65	0.63	0.73	0.80
Tryptophan	85.0	16	0.16	0.19	0.20
Arginine	93.8	120	1.16	1.24	1.25
Isoleucine	90.2	80	0.78	0.86	0.80
Leucine	90.9	140	1.36	1.50	1.20
Valine	88.2	86	0.83	0.94	0.90

[1] The amino acid ratios of threonine and tryptophan proposed by Blair *et al.* (1977) were adjusted to reflect the results obtained in this study

[2] Digestible lysine requirement was set at 0.97% of the diet (or 1.10% total Lys) so that comparison with the NRC (1994) is possible. Digestible amino acid requirements for the rest of the amino acids were derived from each profile by multiplying the ratios by 0.97% digestible lysine. In converting from digestible amino acid requirement to total amino acid requirements, it was assumed that a corn-soybean meal diet with 22 to 23% CP would be fed (83% of CP from soybean meal and 17% of CP from corn) (Baker *et al.*, 1993)

[3] Average true amino acid digestibility in a 22 to 23% CP corn-soybean meal diet (Rhodimet Nutrition Guide, 1993)

[4] Total sulfur amino acid (methionine + cystine)

There were some differences between the AA levels recommended by the NRC (1994) and the adjusted Blair *et al.* (1977) estimated requirements (Table 3.10). The proposed requirements for arginine and tryptophan were very close between NRC (1994) and the adjusted Blair AA profiles. Based on the adjusted Blair's AA profile, the estimated requirements for methionine, isoleucine, leucine and valine were higher than those of the NRC (1994) but lower than those of the NRC (1994) for total sulfur AA and threonine. Recently, Beck *et al.* (1998) reported that the cystine requirement recommended by the NRC (1994) is too high. They found that cystine supplementation of a basal diet (0.25% cystine) did not improve the performance of birds. The great discrepancy for the estimated leucine requirement between the two AA profiles indicates that further research in this area is warranted.

Conclusions

Broiler chicks fed diets based on AA profiles proposed by the NRC (1994) and the Illinois group (Baker and Han, 1994) did not grow at the same rate as those fed diets based on the AA profile proposed by Blair *et al.* (1977). The poorer performance of the birds fed diets based on the NRC (1994) and the IICP (Baker and Han, 1994) was likely caused by the significantly lower feed intake that might be due to an imbalance of AA in these diets. The results of experiment 2 indicate that when formulating diets for 0-3 week old broilers, dietary levels of threonine and tryptophan should be targeted at 65% of lysine (equivalent to 0.63% of digestible threonine in the diet or 0.73% of total threonine in the diet or 4.13% of CP) and 16% of lysine (equivalent to 0.16% of digestible tryptophan in the diet or 0.19% of total tryptophan in the diet or 1.07% of CP), respectively. Recent studies conducted here and elsewhere point strongly to the fact the NRC (1994) has overestimated the requirement of threonine for 0-3 week old broilers. On the other hand, even though the results of the present study supported the NRC (1994) tryptophan recommendation for 0-3 week old broilers, several recent studies disagree and indicate that broiler chicks require higher levels of tryptophan in their diets. Since data on threonine and tryptophan requirement for broiler chicks are not well established, more research, especially for broilers beyond 3 weeks of age, should be conducted in the future. Moreover, since the present two experiments were not conducted using practical ingredients, another study should be conducted to evaluate the responses of poultry to different levels of threonine and tryptophan in practical diets. The results from these studies should have implications with regard to the supplementation of normal and reduced protein diets with commercial AA.

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CHAPTER IV

Responses of Broilers and Layers to Threonine and Tryptophan Supplementation in Reduced Protein Diets

Summary

A total of 2,000 mixed sex broilers and 1,020 layers was used in a 6 week broiler study and an 8 week layer study. Each of the two experiments was also composed of two studies that ran concurrently: a growth or production study and a balance study. The objectives of the experiments were to evaluate the responses of broilers and layers to different levels of threonine and tryptophan in reduced protein diets, while maintaining the levels of other essential amino acids (AA) at the NRC (1994) recommendations for growth, egg production and excretion of nitrogen by poultry. The broiler study consisted of a factorial arrangement of treatments in a completely randomized design with 3 levels of threonine (90, 100, and 110% NRC (1994)) and 3 levels of tryptophan (90, 100, and 110% NRC (1994)) to give nine experimental diets (21% CP for starter diets and 18.5% CP for grower diets). Commercial starter (23 % CP) and grower diets (20% CP) were used as control. The layer study used the same design except that the levels of threonine and tryptophan were set at 95, 100, and 105% of the NRC (1994). A commercial layer diet (17% CP) was used as control. The protein contents of the layer experimental diets were reduced to 12.4% CP. Except for threonine and tryptophan, all other AA and nutrients were formulated according to the NRC (1994) recommendations for broilers and layers, respectively. Amino acid analyses revealed that the starter experimental diets contained 83%, 92% and 101% of the NRC (1994) threonine recommendations and 102%, 113% and 125% of the NRC (1994) tryptophan recommendations. The grower experimental diets also contained higher levels of tryptophan than planned (96%, 107% and 118%) of the NRC (1994) recommendation. The levels of threonine in the grower experimental diets and the levels of threonine and tryptophan in the layer experimental diets were as planned. The broiler study indicated that dietary threonine and tryptophan for 0-3 week old broilers should be targeted at 0.74% of the diet (or 4.04% of CP) and 0.23% of the diet (or 1.22% of CP), respectively. For 3-6 week old broilers, dietary threonine and tryptophan should be targeted at 0.67% of the diet (or 3.40% of CP) and 0.17% of the diet (or 0.89% of CP), respectively. Laying hens aged 42-50 weeks, as indicated by the layer

study, should be targeted at a daily intake of 448 mg threonine/hen and 152 mg tryptophan/hen. The results of the balance studies clearly showed that crystalline AA supplementation of the reduced-protein poultry diets improved the AA balance in the diet, hence improving protein utilization efficiency and resulting in a reduction of nitrogen in the excreta.

Key words: reduced protein diets, threonine, tryptophan, requirement, nitrogen excretion

Introduction

When feed ingredients and crystalline amino acids (AA) are combined to supply the essential AA required by poultry, the crude protein (CP) content of the diet is usually high because there are excesses of many AA. For example, in a typical layer diet based on corn and soybean meal, sulfur AA are at about 100% of the requirement. However, the level of other AA varies from 125% to over 300% of the requirement (Davis and Austic, 1994). According to Macleod (1997), the excretion of nitrogen (as uric acid) from AA required six mol of ATP/mol AA for most AA containing one nitrogen atom, but as much as 18 mol of ATP for histidine, which contained three nitrogen atoms. The high energy cost of uric acid synthesis and excretion implies several nutritional advantages of balancing the AA composition of absorbed protein close to the requirement. Experiments with poultry have supported the hypothesis that diets formulated to minimize the excess of AA over the chicks' known requirements would improve the efficiency of protein and energy utilization (Waldroup *et al.,* 1976).

One of the most important factors affecting the utilization of dietary protein is the balance of AA in the feed. The closer the AA composition of the diet matches the requirement for maintenance and growth, the less protein the animal needs and wastes. A better utilization of protein could be achieved with reduced protein diets that were supplemented with crystalline AA such as lysine, methionine, threonine and tryptophan (Blair *et al.,* 1999). Supplementing with crystalline AA to create an AA balanced diet allows the level of dietary protein to be reduced. The performance of the birds was not jeopardized while achieving a 10-27% reduction in the total nitrogen excreted during the six-week broiler production cycle and a 30-35% reduction in daily nitrogen output was achievable for layers (Blair *et al.,* 1999). The growing public concerns about the impact of animal production on the environment have increased the interest of poultry nutritionists in improving protein utilization and the use of reduced protein diets. However,

before the nutritionists can reduce the protein content of a feed, they must have an adequate knowledge of AA requirements of animals.

With the advance in biotechnology, feed grade threonine and tryptophan are now available for use in the feed industry. Unfortunately, the requirements of poultry for threonine and tryptophan are not well studied. More over, there are great variations in reported threonine and tryptophan requirements for both broilers and layers. For instance, estimates of requirement values of 0-3 week old broiler for threonine range from 0.50 to 0.85% of diet (or 2.8 to 3.7% of CP) (Woodham and Deans, 1975; Robbins, 1987). On the other hand, the estimates of requirements for tryptophan ranged from 0.14 to 0.28% of the diet (or 0.78 to 1.2% of CP) (Woodham and Deans, 1975; Abebe and Morris, 1990). The National Research Council (NRC, 1994) recommended requirement value for threonine is 0.80%, 0.74% and 0.43% of the diet, respectively, for 0-3 week old, 3-6 week old broilers and laying hens, respectively. For tryptophan, the NRC (1994) recommended requirement value is 0.20%, 0.18% and 0.15% of the diet, respectively, for 0-3 week old, 3-6 week old broilers and laying hens, respectively.

The NRC (1994) recommendations for threonine and tryptophan were based on research conducted at least ten years ago. Clearly, solid requirement data are not available for most essential AA, particularly during the growth period beyond 21 days of age for broilers and laying hens. It would be in the interests of the nutritionists to question the validity of the NRC (1994) recommended requirement values for essential AA, especially for threonine and tryptophan. Using chemically defined diets containing AA as the sole source of dietary nitrogen, the previous study (Chapter III) clearly showed that the estimated requirement of 0-3 week old broiler chicks for digestible threonine and digestible tryptophan was 0.63% and 0.16% of the diet, respectively. This equates to 0.73% (4.13% of CP) and 0.19% (1.07% of CP) of total threonine and tryptophan in the diet, respectively. Therefore, present studies were conducted to determine the responses of broilers (0-3 and 3-6 week of age) and layers to different levels of threonine and tryptophan in a reduced protein diet based on practical ingredients.

Materials & Methods

A 6 week broiler study and an 8 week layer study were conducted. Each of the two experiments was composed of two studies that ran concurrently: a growth or production study and a balance study.

Broiler Growth Study

A total of 2,000 mixed sex day-old broilers (Peterson x Arbor Acres) was used in the study with two replications of males and two replications of females for each diet. The broiler chicks were sexed and vaccinated against Marek's disease at the hatchery and were randomly distributed, according to sex, among 40 1.5 x 4.0 m floor pens located in the broiler unit at The University of British Columbia Avian Research and Teaching Facility. Chicks were raised to 42 days of age in floor pens in groups of 50 birds. Temperature in the pens at litter level was maintained at 33 °C at the start of the experiment, reducing to 21°C when the birds reached 42 days of age. The lighting program was of increasing photoperiod after 3 days, i.e. 0-3 days, 23 h light (L):1h dark (D); 4-14 days, 6L:18D; 15-21 days, 10L:14D, 22-28 days, 14L:10D; 29-35 days, 18L:6D; 36-42 days, 23L:1D. Light intensity measured 23 cm above the litter was maintained at 20 lx during days 0-6 and 5 lx during days 7-42. At 43 days of age, the birds were marketed and slaughtered at a commercial processing plant.

Maximum and minimum temperatures were recorded daily at two locations in the building. Daily mortalities were recorded and the carcasses saved for post-mortem examinations. Individual body weights of the birds were measured at 1, 21 and 42 days of age. Fresh water and feed were supplied *ad libitum* during the study. Feed conversion ratios were calculated as the ratio of feed consumption in grams to weight gain in grams.

Broiler Dietary Treatment

Using locally available feed ingredients, the level of threonine and tryptophan in a practical broiler diet could not be reduced to less than 90% of the NRC (1994) broiler recommendations. Therefore, levels of 90%, 100% and 110% of the NRC (1994) recommendations were selected for the current study. A 3 x 3 x 2 (threonine x tryptophan x sex) factorial arrangement of treatments in a completely randomized design (Table 4.1) was employed with three levels of threonine (90, 100 and 100% of the NRC) and three levels of tryptophan (90, 100 and 100% of the NRC). Except for threonine and tryptophan, all other AA and nutrients were formulated according to the NRC (1994) recommendations. In addition, commercial broiler starter and grower diets were also brought in and used as the control (Diet 1). Therefore, there was a series of 10 starter diets (fed from 0-3 weeks of age) and a series of 10

grower diets (fed from 3-6 weeks of age). Broilers were assigned to 10 different groups (Table 4.2). The nutrient compositions of broiler diets are presented in Tables 4.3 (Starter) and 4.4 (Grower).

Table 4.1 Factorial arrangement of broiler diets [1]

	90% NRC Threonine (Thr)	100% NRC Threonine (Thr)	110% NRC Threonine (Thr)
90% NRC Tryptophan (Trp)	90% Thr – 90% Trp ***Diet 2***	100% Thr – 90% Trp ***Diet 5***	110% Thr – 90% Trp ***Diet 8***
100% NRC Tryptophan (Trp)	90% Thr – 100% Trp ***Diet 3***	100% Thr – 100% Trp ***Diet 6***	110% Thr – 100% Trp ***Diet 9***
110% NRC Tryptophan (Trp)	90% Thr – 110% Trp ***Diet 4***	100% Thr – 110% Trp ***Diet 7***	110% Thr – 110% Trp ***Diet 10***

[1] Diet 1 = control starter or control grower diets

Table 4.2 Arrangement of different dietary groups in the broiler study.

Dietary group	Starter diet (0-3 week)	Grower diet (3-6 week)
1	Control (diet 1)	Control (diet 1)
2	Diet 2 (90% Thr - 90% Trp)	Diet 2 (90% Thr - 90% Trp)
3	Diet 3 (90% Thr - 100% Trp)	Diet 3 (90% Thr - 100% Trp)
4	Diet 4 (90% Thr - 110% Trp)	Diet 4 (90% Thr - 110% Trp)
5	Diet 5 (100% Thr - 90% Trp)	Diet 5 (100% Thr - 90% Trp)
6	Diet 6 (100% Thr - 100% Trp)	Diet 6 (100% Thr - 100% Trp)
7	Diet 7 (100% Thr - 110% Trp)	Diet 7 (100% Thr - 110% Trp)
8	Diet 8 (110% Thr - 90% Trp)	Diet 8 (110% Thr - 90% Trp)
9	Diet 9 (110% Thr - 100% Trp)	Diet 9 (110% Thr - 100% Trp)
10	Diet 10 (110% Thr - 110% Trp)	Diet 10 (110% Thr - 110% Trp)

Broiler Balance Study

In addition to the growth study, 320 day-old broilers (160 males and 160 females) were used in a balance study. During the first 3 weeks of the balance study, the broilers were housed in battery brooders. A total of 40 pens was used with each pen housing eight broilers (eight males or eight females). There were four replications for each diet (two for each sex). During the second week of the study the total excreta output per pen over a 24-h period was collected. Feed consumption and body weight changes for the same 24-h period were also recorded. At 3 weeks of age, 80 broilers (40 males and 40 females) were individually weighed and moved into individual cages. Four males and four females were assigned to each diet.

Table 4.3 Composition of broiler starter diets (0-3 week)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
	..... kg/tonne									
Corn	350.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Soybean meal	210.0	110.0	110.0	110.0	110.0	110.0	110.0	110.0	110.0	110.0
Corn gluten meal	.....	76.3	76.3	76.3	76.3	76.3	76.3	76.3	76.3	76.3
Feather meal	.....	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Barley	125.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Canola meal	100.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Wheat	89.0	692.6	692.4	692.2	691.8	691.6	691.4	691.0	690.8	690.6
Meat & bone meal	76.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Tallow	30.1	.....	.....	.....	.....	.....	.....	.....	.....	.....
Animal/vegetable fat	.....	48.2	48.2	48.2	48.2	48.2	48.2	48.2	48.2	48.2
Raypell binder	10.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Limestone	.....	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7
Dicalcium phosphate	.....	13.1	13.1	13.1	13.1	13.1	13.1	13.1	13.1	13.1
Vit./Min premix[1]	5.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Salt	2.6	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
Lysine	.....	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
Alimet	2.1	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Threonine	.....	0.9	0.9	0.9	1.7	1.7	1.7	2.5	2.5	2.5
Tryptophan	.....	0.3	0.5	0.7	0.3	0.5	0.7	0.3	0.5	0.7
Coxistac	1.0	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Calculated composition (air dry basis):										
AME (kcal/kg)	2900	3200	3200	3200	3200	3200	3200	3200	3200	3200
Crude protein (%)	22.94	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Lysine (%)	1.23	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Methionine (%)	0.54	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
Methionine + cystine (%)	1.12	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02
Threonine (%)	0.87	0.72	0.72	0.72	0.80	0.80	0.80	0.88	0.88	0.88
Tryptophan (%)	0.28	0.18	0.20	0.22	0.18	0.20	0.22	0.18	0.20	0.22
Crude fat (%)	6.07	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94	6.94
Crude fiber (%)	3.75	2.55	2.55	2.55	2.55	2.55	2.55	2.55	2.55	2.55
Calcium (%)	0.83	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95

Total phosphorus (%)	0.76	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Available phosphorus (%)	0.49	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Determined composition (air dry basis):										
Dry matter (%)	89.42	87.39	87.40	87.44	87.33	88.42	87.77	87.40	88.24	88.92
Crude protein (%)	22.92	18.28	18.40	17.97	18.01	18.58	18.44	18.66	18.44	18.53
Lysine (%)	ND[2]	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Methionine (%)	ND	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Methionine + cystine (%)	ND	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Threonine (%)	ND	0.66	0.66	0.66	0.74	0.74	0.74	0.81	0.81	0.81
Tryptophan (%)	ND	0.20	0.23	0.25	0.20	0.23	0.25	0.20	0.23	0.25
Crude fat (%)	6.36	7.74	7.08	7.37	7.36	7.12	6.86	6.83	7.10	7.08
Ash (%)	6.17	5.37	5.18	5.24	5.47	5.00	5.19	4.96	4.97	5.12
Calcium (%)	0.84	0.98	0.97	1.10	1.01	0.98	0.96	0.89	0.91	0.93
Total phosphorus (%)	0.74	0.68	0.68	0.69	0.69	0.71	0.69	0.66	0.67	0.69

[1]Supplied per kg diet: NaCl 2.0 g; Mn 60 mg; Cu 5 mg; Zn 50 mg; I 0.35 mg; Se 0.1 mg; ethoxyquin 1.25 mg.

Supplied per kg of diet: vitamin A 9,000 IU; cholecalciferol 1,500 IU; vitamin E 10 IU; vitamin K 0.5 mg; cobalamin 0.007 mg; thiamin 0.4 mg; riboflavin 6 mg; folic acid 1 mg; biotin 0.15 mg; pantothenic acid 12 mg; niacin 35 mg; pyridoxine 4 mg; choline chloride 1,000 mg.

[2]Not determined

Table 4.4 Composition of broiler grower diets (3-6 week)

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
	..... kg/tonne									
Barley	125.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
Corn	349.87	162.82	162.82	162.82	162.82	162.82	162.82	162.82	162.82	162.82
Wheat	165.13	599.72	599.52	599.32	599.02	598.82	598.62	598.32	598.12	597.92
Canola meal	125.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
Soybean meal	111.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00
Corn gluten meal	.....	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Meat meal	71.00	41.72	41.72	41.72	41.72	41.72	41.72	41.72	41.72	41.72
Feather meal	.....	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Perma Pel	10.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
Tallow	32.30	.....	.....	.....	.....	.....	.....	.....	.....	.....
Animal/vegetable fat	.....	37.30	37.30	37.30	37.30	37.30	37.30	37.30	37.30	37.30
Salt	2.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10
Limestone	2.00	10.60	10.60	10.60	10.60	10.60	10.60	10.60	10.60	10.60
Dicalcium phosphate	.....	5.90	5.90	5.90	5.90	5.90	5.90	5.90	5.90	5.90
Lysine	0.01	6.01	6.01	6.01	6.01	6.01	6.01	6.01	6.01	6.01
Alimet	0.72	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Threonine	.....	0.92	0.92	0.92	1.62	1.62	1.62	2.32	2.32	2.32
Tryptophan	.....	0.19	0.39	0.59	0.19	0.39	0.59	0.19	0.39	0.59
Liquid choline	0.875	.....	.....	.....	.....	.....	.....	.....	.....	.....
Vit./min premix[1]	4.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Coxistac	1.00	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Calculated composition (air dry basis):										
AME (kcal/kg)	2,949	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
Crude protein (%)	19.98	18.51	18.51	18.51	18.51	18.51	18.51	18.51	18.51	18.51
Lysine (%)	0.99	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Methionine (%)	0.39	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.4
Methionine + cystine (%)	0.83	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Threonine (%)	0.74	0.67	0.67	0.67	0.74	0.74	0.74	0.81	0.81	0.81
Tryptophan (%)	0.24	0.16	0.18	0.198	0.16	0.18	0.198	0.16	0.18	0.198
Crude fat (%)	6.35	6.47	6.47	6.47	6.47	6.47	6.47	6.47	6.47	6.47
Crude fiber (%)	3.99	2.47	2.47	2.47	2.47	2.47	2.47	2.47	2.47	2.47

Calcium (%)	0.86	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Total phosphorus (%)	0.73	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Available phosphorus (%)	0.46	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Determined composition (air dry basis):										
Dry matter (%)	88.14	88.03	88.38	88.83	88.53	88.27	88.67	88.95	89.02	89.07
Crude protein (%)	21.05	19.33	19.71	19.48	19.59	19.18	19.56	19.51	19.83	19.66
Lysine (%)	ND[2]	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Methionine (%)	ND	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43
Methionine + cystine (%)	ND	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Threonine (%)	ND	0.66	0.66	0.66	0.74	0.74	0.74	0.81	0.81	0.81
Tryptophan (%)	ND	0.17	0.19	0.21	0.17	0.19	0.21	0.17	0.19	0.21
Crude fat (%)	7.86	6.08	6.67	5.54	6.42	6.98	6.47	6.64	6.49	6.44
Ash (%)	5.38	5.33	5.24	5.02	5.18	4.83	5.05	4.91	4.46	4.81
Calcium (%)	0.87	0.98	1.00	1.05	0.97	0.91	1.02	1.04	0.95	1.02
Total phosphorus (%)	0.69	0.71	0.73	0.72	0.69	0.67	0.72	0.71	0.71	0.71

[1]Supplied per kg diet: NaCl 2.0 g; Mn 60 mg; Cu 5 mg; Zn 50 mg; I 0.35 mg; Se 0.1 mg; ethoxyquin 1.25 mg.

Supplied per kg of diet: vitamin A 9,000 IU; cholecalciferol 1,500 IU; vitamin E 10 IU; vitamin K 0.5 mg; cobalamin 0.007 mg; thiamin 0.4 mg; riboflavin 6 mg; folic acid 1 mg; biotin 0.15 mg; pantothenic acid 12 mg; niacin 35 mg; pyridoxine 4 mg; choline chloride 1,000 mg.

[2]Not determined

During the fifth week of the balance study the total excreta output for each broiler was collected over a 48-h period. Feed consumption and overall body weight changes for the same 48-h period were also recorded. For both excreta collection periods (during the second and fifth weeks) excreta were collected every 12 h and were frozen until analyzed.

Layer Production Study

A total of 1,020 42-week-old layers (510 Hyline and 510 H & N) was used. The trial was composed of two parts: a production study and a balance study. Both studies ran concurrently for a period of 8 weeks. For the production study, 960 layers (480 Hyline and 480 H & N) were used. Twenty rows of battery cages were used with each row representing a replicate. There were two replications (one row of 44 Hyline layers and one row of 44 H & N layers) per diet. The layers were housed in battery cages (0.3 m wide x 0.4 m deep) with two layers per cage (0.67 ft^2 or 0.06 m^2 per bird). The layers received 15½ h of light each day throughout the trial period. The individual body weight of each layer was recorded at the start and the end of the trial. Daily mortalities were removed and recorded. Maximum and minimum temperatures were recorded daily in at least two locations within the building. Feed intake per row was determined weekly by weighing back unconsumed feed. Eggs were collected once daily and egg production recorded by row. Eggs collected were classified as normal, cracked, deformed shell, soft-shell or no shell. Every two weeks, all eggs produced during a 2-day period were individually weighed.

Layer Dietary Treatment

As in the broiler study, the study was intended to evaluate the effects of reduced dietary protein with 3 levels of threonine (90, 100 and 110% of the NRC (1994) estimated requirement) and 3 levels of tryptophan (90, 100 and 110% of the NRC (1994) estimated requirement) while maintaining the levels of other essential AA at the NRC (1994) recommendations on growth and excretion of nitrogen by layers. It proved to be impossible to formulate a layer diet with 90% of the NRC (1994) estimated requirements for threonine and tryptophan using the ingredients available locally. Thus, the treatment levels were adjusted to 95, 100 and 105% of the NRC estimated requirements. Similar to the broiler study, a 3 x 3 x 2 (threonine x tryptophan x strain) factorial arrangement of treatments in a completely randomized design (Table 4.5) was

employed with three levels of threonine (95, 100 and 105% of the NRC) and three levels of tryptophan (95, 100 and 105% of the NRC). Except for threonine and tryptophan, all other AA and nutrients were formulated according to the NRC (1994) layer recommendations. The nutrient compositions of layer diets were presented in Table 4.6. In addition, a commercial layer diet was also brought in and used as the control (Diet 1). Therefore, there was a series of ten layer diets.

Table 4.5 Factorial arrangement of layer diets [1]

	95% NRC Threonine (Thr)	100% NRC Threonine (Thr)	105% NRC Threonine (Thr)
95% NRC Tryptophan (Trp)	95% Thr – 95% Trp ***Diet 2***	100% Thr – 95% Trp ***Diet 5***	105% Thr – 95% Trp ***Diet 8***
100% NRC Tryptophan (Trp)	95% Thr – 100% Trp ***Diet 3***	100% Thr – 100% Trp ***Diet 6***	105% Thr – 100% Trp ***Diet 9***
105% NRC Tryptophan (Trp)	95% Thr – 105% Trp ***Diet 4***	100% Thr – 105% Trp ***Diet 7***	105% Thr – 105% Trp ***Diet 10***

[1] Diet 1 = commercial control layer diets

Layer Balance Study

In addition to the production study, 80 layers (four Hyline layers and four H & N layers per diet) were used in the balance study. The layers were housed individually in layer cages. Five weeks after the introduction of the test diets, individual measurement of feed intake and excreta output over a 48-h period were recorded. The layers were also individually weighed at the start and at the end of the 48-h collection period. All excreta were frozen until analyzed.

The guidelines of the Canadian Council on Animal Care were followed and the protocol for this experiment was approved by The University of British Columbia Animal Care Committee.

Calculation

Nutrient Retention (%) = [(a-b) / a] x 100 where,

a = diet intake (DM) x % nutrient in diet (DM)

b = excreta voided (DM) x % nutrient in excreta (DM)

Table 4.6 Composition of layer diets

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
	..... kg/tonne									
Wheat	586.3	31.0	31.0	31.0	31.0	31.0	31.0	31.0	31.0	31.0
Barley	75.0	599.3	599.2	599.2	599.2	599.1	599.0	598.9	598.9	598.8
Corn	50.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Meat meal	75.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Feather meal	.....	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Soybean meal	45.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Corn gluten meal	.....	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0
Canola meal	40.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Suncured alfalfa	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Tallow	18.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Animal/vegetable fat	.....	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Limestone(coarse)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Limestone(ground)	33.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0
Dicalcium phosphate	2.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Salt	3.0	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Lysine	2.3	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Alimet	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Tryptophan	.....	0.0	0.1	0.2	0.0	0.1	0.2	0.0	0.1	0.2
Threonine	.....	.....	.....	.....	0.2	0.2	0.2	0.4	0.4	0.4
Avizyme 2300	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Liquid choline	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Calculated composition (air dry basis):										
AME (kcal/kg)	2700	2823	2823	2823	2823	2823	2823	2823	2823	2823
Crude protein (%)	17.00	12.40	12.40	12.40	12.40	12.40	12.40	12.40	12.40	12.40
Lysine (%)	0.82	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Methionine + cystine (%)	0.61	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64
Threonine (%)	0.560	0.455	0.455	0.455	0.470	0.470	0.470	0.494	0.494	0.494
Tryptophan (%)	0.180	0.152	0.160	0.168	0.152	0.160	0.168	0.152	0.160	0.168
Crude fat (%)	4.00	8.30	8.30	8.30	8.30	8.30	8.30	8.30	8.30	8.30

Crude fiber (%)	3.70	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Calcium (%)	3.60	3.70	3.70	3.70	3.70	3.70	3.70	3.70	3.70	3.70
Total phosphorus (%)	0.73	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Available phosphorus (%)	0.41	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Determined composition (air dry basis):										
Dry matter (%)	90.52	90.47	90.72	90.63	90.77	89.81	90.71	90.60	90.74	89.41
Crude protein (%)	17.02	12.34	12.27	12.62	12.29	12.50	12.37	12.31	12.30	12.16
Lysine (%)	ND[2]	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Methionine (%)	ND	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Methionine + cystine (%)	ND	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Threonine (%)	ND	0.40	0.40	0.40	0.43	0.43	0.43	0.45	0.45	0.45
Tryptophan (%)	ND	0.13	0.14	0.15	0.13	0.14	0.15	0.13	0.14	0.15
Crude fat (%)	3.98	7.22	7.63	8.06	7.18	7.79	7.69	7.51	7.59	7.53
Ash (%)	10.03	8.95	8.58	9.64	9.48	9.12	9.53	8.59	9.47	9.07
Calcium (%)	4.17	3.80	4.03	4.24	4.09	3.77	3.97	3.96	3.97	4.18
Total phosphorus (%)	0.79	0.56	0.58	0.61	0.62	0.57	0.60	0.58	0.56	0.61

[1]Supplied per kg diet: NaCl 2.0 g; Mn 60 mg; Cu 5 mg; Zn 50 mg; I 0.35 mg; Se 0.1 mg; ethoxyquin 1.25 mg.

Supplied per kg of diet: vitamin A 9,000 IU; cholecalciferol 1,500 IU; vitamin E 10 IU; vitamin K 0.5 mg; cobalamin 0.007 mg; thiamin 0.4 mg; riboflavin 6 mg; folic acid 1 mg; biotin 0.15 mg; pantothenic acid 12 mg; niacin 35 mg; pyridoxine 4 mg; choline chloride 1,000 mg.

[2]Not determined

Analyses of Samples

Before the start of the experiment, main feed ingredients were collected from the feedmill, ground to pass through a 1 mm mesh screen and then analyzed for CP (N x 6.25), AA composition, and other proximate constituents prior to formulation of the diets. Proximate analysis for CP, crude fiber, ether extract, ash and calcium were carried out following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 1984). Phosphorus was measured using a spectrophotometric method (Estrin and Brammell, 1968). Amino acids were analyzed commercially (Heartland Lysine Inc, Chicago, Illinois 60631, USA), using methods outlined by Spackman *et al.* (1958). Hydrolysates for the determination of methionine as methionine sulfone and cysteine/cystine as cysteic acid were prepared by performic oxidation of the protein followed by 6 N HCl hydrolysis (Moore, 1963), whereas feed ingredients were subjected to alkaline hydrolysis and high performance liquid chromatography for tryptophan determination (Jones *et al.*, 1981).

During the entire experimental period feed samples (approximately 250 g) from each experimental broiler and layer diets were collected every week and stored in a freezer until analyzed. These were then pooled, mixed and sub-sampled for proximate analysis as described above. The excreta samples obtained from the broiler and layer balance studies were dried at 60 °C, re-weighed (dry weight) and ground to pass through a 1 mm mesh screen. Total N content in dry excreta was analyzed according to AOAC (1984) procedures.

Statistical Analyses

Both the broiler and layer studies were factorial experiments in a completely randomized design. All data generated from the experimental diets were subjected to analysis of variance (ANOVA) procedures. If treatments were found to be significantly different, Tukey's multiple range test (Snedecor and Cochran, 1980) was used to determine the statistical significance among treatment least-square means.

The results obtained for birds fed the control commercial diet were compared to those from birds fed the experimental diets using a one-way ANOVA by using the General Linear Models (GLM) procedure of SAS® software (SAS Institute, 1996). If treatments were found to be significantly different, the Bonferroni (Dunn) T test (SAS Institute, 1996) was used to

determine the statistical significance among treatment least-square means. The Bonferonni (Dunn) T test was used because it will avoid detecting random differences when comparing a large group of treatments (ten dietary treatments in both the broiler and layer study).

Results

Broiler Growth Study

Experimental diets for the broiler study were prepared at the Agriculture and Agri-Food Canada feed-mill at Agassiz, British Columbia. The broiler starter (0-3 week) diets did not prove to be of the planned standard since a mould infestation was observed in them shortly after the broiler experiment was initiated. In addition, the determined N contents of the broiler starter (0-3 week) and broiler grower (3-6 week) diets did not compare favorably with the planned protein contents (Tables 4.3 and 4.4), even though care was taken to analyze the ingredients for protein and AA prior to the diets being formulated. Experimental diets for the layer study were prepared by a commercial feedmill (Pro-form, Inc, Chilliwack, Canada) and the N contents of the layer diets were of planned standard. In order to confirm the amount of AA in the diets, feed samples from the broiler and layer studies were sent to the University of Manitoba for another AA analysis. Feed samples were analyzed for AA content with a LKB 4151 Alpha Plus AA Analyzer. Feed samples were prepared by acid hydrolysis using the method of Andrews and Baldar (1985). Acid hydrolysis involves digestion in 6N hydrochloric acid for 24 h at 110 °C. Samples for methionine and cystine determination were subjected to performic acid pretreatment and later analyzed by the method of Andrews and Baldar (1985). Tryptophan in the feeds was analyzed following alkaline hydrolysis (Hugli and Moore, 1972).

The CP content of the control commercial broiler diets and all of the layer diets were as planned. However, CP was lower than planned in the starter experimental diets whereas it was higher in the grower diets. The results of the AA analysis from the University of Manitoba indicated that the amount of threonine in the broiler starter diets was lower than planned, whereas tryptophan was higher than planned in both the broiler starter and grower diets. The levels of threonine in the broiler grower diets and the levels of threonine and tryptophan in the layer diets were as planned. Because of the variations in calculated and analyzed values, the levels of threonine and tryptophan in the starter diets were changed from 90%, 100% and 110%

of the NRC (1994) to 83%, 92% and 101% of the NRC (1994), respectively, for threonine, and to 102%, 113% and 125% of the NRC (1994) respectively for tryptophan. Likewise, the levels of tryptophan in the grower diets were changed from 90%, 100% and 110% of the NRC (1994) to 96%, 107% and 118% of the NRC (1994), respectively.

Growth performance, feed intake and feed/gain ratios of broilers during the starter and grower periods are presented in Tables 4.7 and 4.8, respectively. Even though feed intake was not significantly different ($P > 0.05$) among starter diets, broilers fed the control starter diet gained significantly more ($P < 0.05$) weight than broilers fed the experimental starter diets. Significant differences ($P < 0.05$) in weight gain were found among broilers fed different experimental starter diets. Broilers fed the control starter diet were also more efficient in utilizing the starter feed than broilers fed the experimental starter diets (Table 4.7). The male broilers consumed significantly more ($P < 0.05$) feed and gained significantly more ($P < 0.05$) weight than the female broilers from 0-3 weeks of age. Growth performance by broilers during the grower period (3-6 week of age) followed the same trends as that of the broilers during the starter period (Table 4.8). Broilers fed the control grower diet, however, gained significantly more ($P < 0.05$) weight and consumed significantly more ($P < 0.05$) feed than the broilers fed the experimental grower diets. Contrary to the starter period, no significant differences in weight gain was detected among broilers fed the different experimental grower diets. Feed/gain ratios among broilers fed different grower diets were not significantly different ($P > 0.05$) during the grower period. Male broilers were superior to female broilers in performance from 3-6 weeks of age.

For the overall performance (0-6 week) of the birds, birds in the dietary control group gained significantly more ($P < 0.05$) weight than the birds in the dietary groups 2-10. However, no significant differences in feed intake were found among birds assigned to dietary groups 1 (control), 4 (83% threonine and 125% tryptophan in starter diet and 90% threonine and 118% tryptophan in grower diet), 6 (92% threonine and 113% tryptophan in starter diet and 100% threonine and 107% tryptophan in grower diet) or 8 (101% threonine and 102% tryptophan in starter diet and 110% threonine and 96% tryptophan in grower diet). Birds in the dietary control group were significantly more ($P < 0.05$) efficient in converting feed into body tissues (Table 4.9) than birds in dietary groups 2 (83% threonine and 102% tryptophan in starter diet and 90% threonine and 96% tryptophan in grower diet), 4 and 6.

Table 4.7 The effect of different dietary treatments on the performance (body weight gain, feed intake and feed/gain ratio (F/G)) of 0-3 week old broilers.

Starter diet	Threonine [1]	Tryptophan [1]	Weight gain Wk 0-3 (g)	Feed intake Wk 0-3 (g)	F/G Wk 0-3
1	Control	Control	536 [a]	747	1.39 [b]
2	83%	102%	478 [cd]	767	1.60 [a]
3	83%	113%	491 [bcd]	773	1.56 [a]
4	83%	125%	494 [bcd]	800	1.61 [a]
5	92%	102%	476 [d]	742	1.55 [a]
6	92%	113%	505 [b]	792	1.56 [a]
7	92%	125%	501 [bc]	768	1.52 [a]
8	101%	102%	494 [bcd]	774	1.56 [a]
9	101%	113%	489 [bcd]	750	1.52 [a]
10	101%	125%	498 [bcd]	772	1.53 [a]
		Male	507 [a]	787 [a]	1.54
		Female	485 [b]	750 [b]	1.54
		Overall mean	496	768	1.54
		Pooled SEM [2]	2.6	5.7	0.01

[1] % of the NRC (1994) recommendation; [2] Pooled standard error of the mean; data represent mean of four replicates (two replicates of males and two replicates of females).

[abcd] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 4.8 The effect of different dietary treatments on the performance (body weight gain, feed intake and feed/gain ratio (F/G)) of 3-6 week broilers.

Grower diet	Threonine [1]	Tryptophan [1]	Weight gain Wk 3-6 (g)	Feed intake Wk 3-6 (g)	F/G Wk 3-6
1	Control	Control	1718 [a]	3086 [a]	1.82
2	90%	96%	1559 [b]	2840 [b]	1.84
3	90%	107%	1537 [b]	2819 [b]	1.85
4	90%	118%	1556 [b]	2869 [b]	1.86
5	100%	96%	1544 [b]	2794 [b]	1.83
6	100%	107%	1575 [b]	2891 [b]	1.86
7	100%	118%	1534 [b]	2798 [b]	1.84
8	110%	96%	1589 [b]	2858 [b]	1.82
9	110%	107%	1563 [b]	2849 [b]	1.85
10	110%	118%	1532 [b]	2791 [b]	1.84
		Male	1715 [a]	3054 [a]	1.80 [b]
		Female	1427 [b]	2665 [b]	1.88 [a]
		Overall mean	1571	2860	1.84
		Pooled SEM [2]	17.7	34.9	0.01

[1] % of the NRC (1994) recommendations; [2] Pooled standard error of the mean; data represent mean of four replicates (two replicates of males and two replicates of females).

[ab] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 4.9 The effect of different dietary groups on the overall performance (body weight gain, feed intake and feed/gain ratio (F/G)) of broilers.

Dietary group	Starter diet [1]	Grower diet	Weight gain Wk 0-6 (g)	Feed intake Wk 0-6 (g)	F/G Wk 0-6
1	Control	Control	2254 [a]	3834 [a]	1.71 [b]
2	83% Thr - 102% Trp	90% Thr - 96% Trp	2037 [b]	3607 [b]	1.78 [a]
3	83% Thr - 113% Trp	90% Thr - 107% Trp	2027 [b]	3592 [b]	1.78 [ab]
4	83% Thr - 125% Trp	90% Thr -118% Trp	2049 [b]	3669 [ab]	1.80 [a]
5	92% Thr - 102% Trp	100% Thr - 96% Trp	2019 [b]	3537 [b]	1.76 [ab]
6	92% Thr - 113% Trp	100% Thr - 107% Trp	2080 [b]	3683 [ab]	1.79 [a]
7	92% Thr - 125% Trp	100% Thr - 118% Trp	2034 [b]	3566 [b]	1.76 [ab]
8	101% Thr - 102% Trp	110% Thr - 96% Trp	2083 [b]	3632 [ab]	1.75 [ab]
9	101% Thr - 113% Trp	110% Thr - 107% Trp	2053 [b]	3598 [b]	1.77 [ab]
10	101% Thr - 125% Trp	110% Thr - 118% Trp	2030 [b]	3563 [b]	1.76 [ab]
		Male	2222 [a]	3841 [a]	1.74 [b]
		Female	1911 [b]	3415 [b]	1.80 [a]
		Overall mean	2067	3628	1.77
		Pooled SEM [2]	19.4	38.3	0.01

[1] % of the NRC (1994) recommendations for threonine (Thr) and tryptophan (Trp).
[2] Pooled standard error of the mean; data represent mean of four replicates (two replicates of males and two replicates of females).
[ab] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

No significant differences in weight gain, feed intake and feed/gain ratio were observed among birds assigned to dietary groups 2-10 for the overall 0-6 week period. Overall performance of the male broilers was superior to the performance by the female broilers, as expected.

When the results obtained from the experimental starter diets (diets 2-10) were compared in a factorial manner, no significant differences ($P > 0.05$) in weight gain and feed intake were observed with birds (0-3 weeks of age) fed diets containing the three levels of threonine (Table 4.10). However, feed/gain ratios were significantly lower ($P < 0.05$) for birds fed starter diets containing 92% or 101% of the NRC (1994) recommendations for threonine. Young broiler chicks (0-3 week of age) gained significantly more ($P < 0.05$) weight when they were fed diets containing 113% or 125% of the NRC (1994) recommendations for tryptophan (Table 4.10). There was also a significant threonine and tryptophan interaction for 0-3 week weight gain (Table 4.10). When the dietary level of threonine was set at 92% of the NRC (1994) recommendation, birds fed diets containing 102% of the NRC (1994) recommended tryptophan level gained significantly less ($P < 0.05$) weight than birds fed diets containing 113% of the NRC (1994) tryptophan level (Figure 4.1). However, there were no significant differences in weight gain among birds fed diets containing different levels of threonine when dietary tryptophan was set at 102%, 113% or 125% of the NRC (1994) recommendations (Figure 4.2). Feed intake was similar among experimental diets during the 0-3 week period. During the starter period, male broilers performed significantly better ($P < 0.05$) in all of the parameters measured except for the feed/gain ratio, in which there was no difference between the males and the females.

When the results obtained from the experimental grower diets (diets 2-10) during the grower period were compared in a factorial manner, no differences were observed in any of the parameters measured (weight gain, feed intake and feed/gain ratio), but the male broilers did perform significantly better ($P < 0.05$) than the female broilers (Table 4.10).

Broiler Balance Study

Broiler chicks (2-week-old) fed the control starter diet excreted significantly more ($P < 0.05$) excreta and N than chicks fed the experimental starter diets (Table 4.11). The percentage of N in the excreta excreted by birds fed the control starter diet was significantly higher ($P < 0.05$) than that excreted by birds fed the experimental starter diets 2-10.

Table 4.10 Factorial comparison: The effect of different levels of threonine and tryptophan and sex on 0-3 week and 3-6 week old broilers performance (body weight gain, feed intake, feed/gain ratio (F/G)).

0-3 week	Weight gain Wk 0-3 (g)	Feed intake Wk 0-3 (g)	F/G Wk 0-3	3-6 week	Weight gain Wk 3-6 (g)	Feed intake Wk 3-6 (g)	F/G Wk 3-6
Threonine [1]				***Threonine*** [1]			
83% NRC	487	780	1.59 [a]	90% NRC	1551	2843	1.85
92% NRC	494	767	1.54 [b]	100% NRC	1551	2833	1.84
101% NRC	494	765	1.54 [b]	110% NRC	1561	2828	1.84
Tryptophan [1]				***Tryptophan*** [1]			
102% NRC	483 [b]	780	1.57	96% NRC	1564	2853	1.83
113% NRC	495 [a]	772	1.55	107% NRC	1558	2831	1.85
125% NRC	497 [a]	761	1.56	118% NRC	1541	2819	1.85
Male	503 [a]	790 [a]	1.56	Male	1694 [a]	3027 [a]	1.80 [b]
Female	480 [b]	752 [b]	1.56	Female	1414 [b]	2642 [b]	1.88 [a]
Overall mean	492	771	1.56	Overall mean	1554	2834	1.84
Pooled SEM [2]	2.4	6.1	0.01	Pooled SEM [2]	17.3	34.4	0.01
Source of variation [3]							
Threonine (Thr)	*NS*	*NS*	***	*Threonine (Thr)*	*NS*	*NS*	*NS*
Tryptophan (Trp)	***	*NS*	*NS*	*Tryptophan (Trp)*	*NS*	*NS*	*NS*
Sex	***	***	*NS*	*Sex*	***	***	***
Thr X Trp	***	*NS*	*NS*	*Thr X Trp*	*NS*	*NS*	*NS*
Thr X Trp X Sex	*NS*	*NS*	*NS*	*Thr X Trp X Sex*	*NS*	*NS*	*NS*

[1] % of the NRC (1994) recommendations

[2] Pooled standard error of the mean; data represent mean of four replicates (two replicates of males and two replicates of females).

[3] NS = not significant, $P > 0.05$ or *** = significant, $P < 0.05$

[ab] Treatment means with different superscripts within a column for threonine or tryptophan are significantly different at $P < 0.05$.

Figure 4.1 Broiler weight gain for 0-3 weeks : The effect of different levels of tryptophan (Trp) when threonine (Thr) was set at 83, 92 and 101% of the NRC (1994) recommendations.

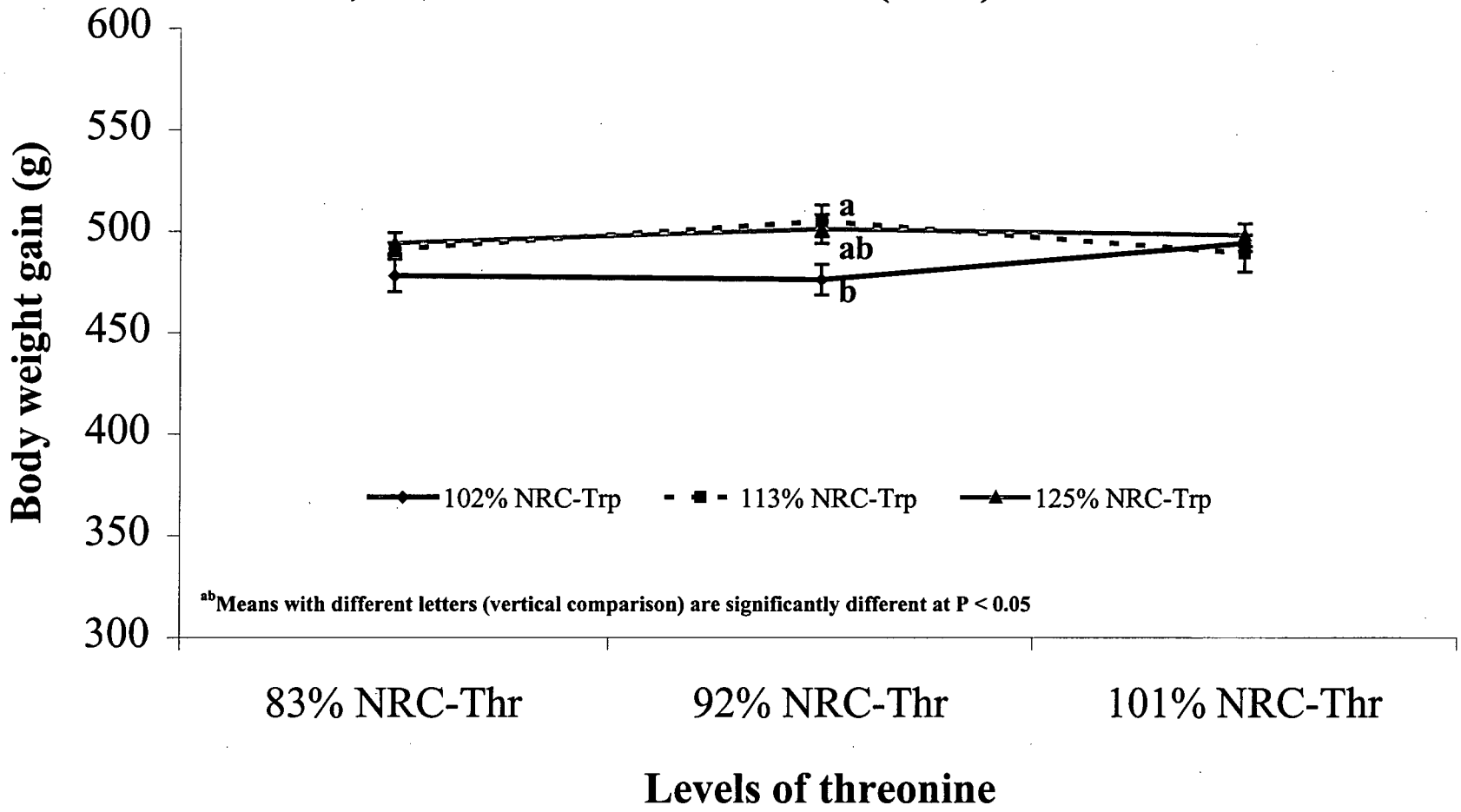

Figure 4.2 Broiler weight gain for 0-3 weeks : The effect of different levels of threonine (Thr) when tryptophan (Trp) was set at 102, 113 or 125% of the NRC (1994) recommendations.

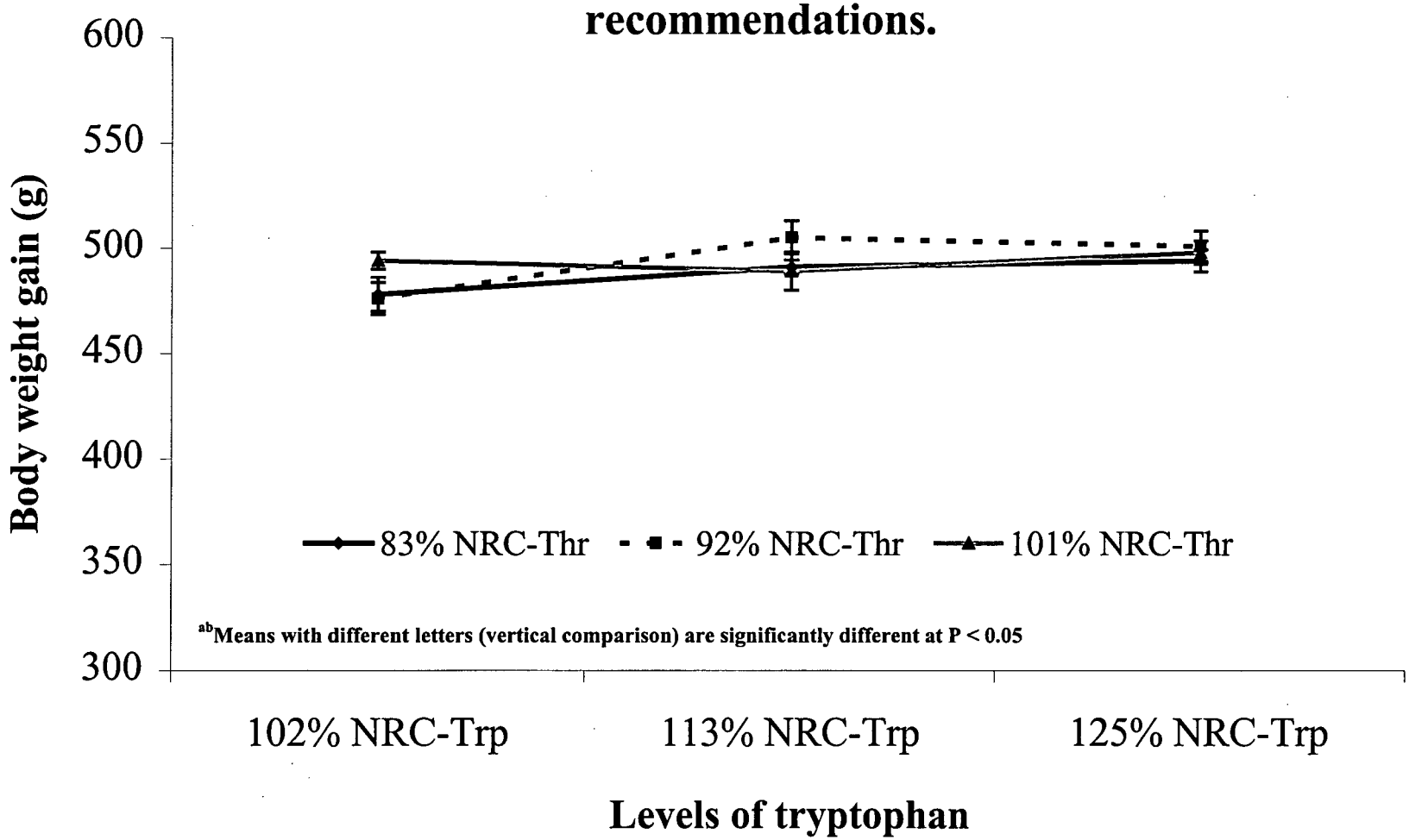

Table 4.11 The effect of different dietary treatments on nitrogen (N) excretion and retention by 2-week-old broiler chicks.

Diet	*Threonine* [1]	*Tryptophan* [1]	Excreta output [2] (g DM/24h/pen)	N output [2] (g DM/24h/pen)	N in excreta (% DM)	N retention (%)
1	Control	Control	163 [a]	8.33 [a]	5.12 [a]	54.6 [b]
2	83% NRC	102% NRC	131 [b]	5.78 [b]	4.40 [b]	59.9 [ab]
3	83% NRC	113% NRC	113 [b]	4.88 [b]	4.31 [b]	66.9 [a]
4	83% NRC	125% NRC	120 [b]	5.34 [b]	4.46 [b]	62.9 [ab]
5	92% NRC	102% NRC	119 [b]	5.38 [b]	4.57 [b]	62.6 [ab]
6	92% NRC	113% NRC	111 [b]	4.90 [b]	4.42 [b]	67.1 [a]
7	92% NRC	125% NRC	109 [b]	5.07 [b]	4.64 [b]	65.6 [a]
8	101% NRC	102% NRC	120 [b]	5.55 [b]	4.61 [b]	62.8 [ab]
9	101% NRC	113% NRC	124 [b]	5.50 [b]	4.46 [b]	62.7 [ab]
10	101% NRC	125% NRC	127 [b]	5.59 [b]	4.40 [b]	62.3 [ab]
		Male	126	5.56	4.38 [b]	63.2
		Female	121	5.70	4.70 [a]	62.2
		Overall mean	124	5.63	4.54	62.7
		Pooled SEM [3]	3.0	0.17	0.05	0.8

[1] % of the NRC (1994) recommendations
[2] Data represent mean of four pens (two pens of each sex) with eight birds per pen.
[3] Pooled standard error of the mean
[ab] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Broilers fed the control starter diet also retained significantly less ($P < 0.05$) N when compared to the birds fed experimental starter diets 3 (83% threonine and 113% tryptophan), 6 (92% threonine and 113% tryptophan) and 7 (92% threonine and 125% tryptophan) (Table 4.11). No significant differences were observed in any of the parameters measured between the birds fed experimental starter diets 2-10. The N content in the excreta excreted by the female chicks was significantly higher ($P < 0.05$) than that by the male chicks.

When data (starter diets 2-10) were analyzed in a factorial manner, no significant differences were observed in any of the parameters measured and calculated, except in the percentage of N retained by 2-week-old broiler chicks (Table 4.12). The broiler chicks fed starter diets containing 113% of the NRC (1994) recommended dietary tryptophan level retained significantly more ($P < 0.05$) N than the chicks fed diets containing 102% of the NRC (1994) recommendation for tryptophan. No significant difference in excreta output, N output and N retained was detected between the sexes except that the percentage of N in the excreta from the female birds was significantly higher ($P < 0.05$) than that from the male birds.

In the second balance study, broilers (5 weeks old) fed the control grower diet excreted significantly more ($P < 0.05$) excreta (except birds fed diet containing 100% threonine and 96% tryptophan) and N than broilers fed the experimental grower diets (Table 4.13). However, there was no significant difference ($P > 0.05$) in the percentage of N in the excreta excreted by birds fed different grower diets. When compared with the birds fed diets 2 (90% threonine and 96% tryptophan), 5 (100% threonine and 96% tryptophan), 7 (100% threonine and 118% tryptophan), 9 (110% threonine and 107% tryptophan) and 10 (110% threonine and 118% tryptophan), the proportion of N retained was also significantly ($P < 0.05$) lower by the birds fed the control grower diet. There were significant differences ($P < 0.05$) between sexes in all of the parameters measured and calculated (Table 4.13). When data (grower diets 2-10) were analyzed in a factorial manner, no significant differences in all of the parameters measured and calculated were observed in birds fed different grower diets (Table 4.14). Male birds produced significantly more ($P < 0.05$) excreta than female birds but the percentage of N was significantly higher ($P < 0.05$) in the females.

Table 4.12 Factorial comparison: The effect of different levels of threonine and tryptophan on nitrogen (N) excretion and retention by 2-week-old broiler chicks.

Factors	Excreta output [2] (g DM/24h/pen)	N output [2] (g DM/24h/pen)	N in excreta (% DM)	N retention (%)
Threonine [1]				
83% NRC	122	5.33	4.39	63.2
92% NRC	113	5.12	4.54	65.1
101% NRC	124	5.55	4.49	62.6
Tryptophan [1]				
102% NRC	123	5.57	4.53	61.8 [b]
113% NRC	116	5.09	4.39	65.5 [a]
125% NRC	119	5.33	4.50	63.6 [ab]
Male	121	5.22	4.30 [b]	64.4
Female	117	5.44	4.64 [a]	62.9
Overall mean	119	5.33	4.47	63.6
Pooled SEM [3]	2.1	0.09	0.04	0.6
Source of variation [4]				
Threonine	*NS*	*NS*	*NS*	*NS*
Tryptophan	*NS*	*NS*	*NS*	***
Sex	*NS*	*NS*	***	*NS*
Interactions	*NS*	*NS*	*NS*	*NS*

[1] % of the NRC (1994) recommendations
[2] Data represent mean of four pens (two pens of each sex) with eight birds per pen.
[3] Pooled standard error of the mean
[4] NS = not significant, $P > 0.05$ or *** = significant, $P < 0.05$;
[ab] Treatment means with different superscripts within a column for threonine or tryptophan are significantly different at $P < 0.05$.

Table 4.13 The effect of different dietary treatments on nitrogen (N) excretion and retention by 5-week-old broilers.

Diet	Threonine [1]	Tryptophan [1]	Excreta output (g DM/48h/bird)	N output (g DM/48h/bird)	N in excreta (% DM)	N retention (%)
1	Control	Control	99.8 [a]	5.66 [a]	5.73	67.3 [bc]
2	90% NRC	96% NRC	74.3 [b]	4.17 [b]	5.45	69.4 [a]
3	90% NRC	107% NRC	76.7 [b]	4.03 [b]	5.01	71.6 [ab]
4	90% NRC	118% NRC	76.3 [b]	4.28 [b]	5.66	71.1 [ab]
5	100% NRC	96% NRC	83.3 [ab]	4.75 [b]	5.23	69.5 [a]
6	100% NRC	107% NRC	75.0 [b]	4.00 [b]	5.37	71.5 [ab]
7	100% NRC	118% NRC	79.4 [b]	4.53 [b]	5.44	69.2 [a]
8	110% NRC	96% NRC	77.9 [b]	4.08 [b]	5.26	70.8 [ab]
9	110% NRC	107% NRC	75.9 [b]	4.29 [b]	5.64	70.2 [a]
10	110% NRC	118% NRC	67.1 [b]	4.01 [b]	5.66	69.5 [a]
		Male	82.8 [a]	4.52 [a]	5.22 [b]	70.5 [a]
		Female	73.8 [b]	4.21 [b]	5.67 [a]	69.6 [b]
		Overall mean	78.6	4.36	5.45	70.1
		Pooled SEM [2]	1.5	0.09	0.06	0.2

[1] % of the NRC (1994) recommendations

[2] Pooled standard error of the mean; data represent mean of eight birds (four males and four females)

[ab] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 4.14 Factorial comparison: The effect of different levels of threonine and tryptophan on nitrogen (N) excretion and retention by 5-week-old broilers.

Factors	Excreta output (g DM/48h/bird)	N in excreta (% DM)	N output (g DM/48h/bird)	N retention (%)
Threonine [1]				
90% NRC	75.8	5.40	4.16	70.7
100% NRC	79.3	5.35	4.43	70.1
110% NRC	73.7	5.52	4.12	70.2
Tryptophan [1]				
96% NRC	78.5	5.31	4.33	69.9
107% NRC	75.9	5.36	4.11	71.1
118% NRC	74.4	5.59	4.27	70.0
Male	79.7 [a]	5.21 [b]	4.36	70.7
Female	72.7 [b]	5.63 [a]	4.12	69.9
Overall mean	76.2	5.42	4.24	70.3
Pooled SEM [2]	1.3	0.06	0.07	0.2
Source of variation [3]				
Threonine	*NS*	*NS*	*NS*	*NS*
Tryptophan	*NS*	*NS*	*NS*	*NS*
Sex	***	***	*NS*	*NS*
Interactions	*NS*	*NS*	*NS*	*NS*

[1] % of the NRC (1994) recommendations

[2] Pooled standard error of the mean; data represent mean of eight birds (four males and four females)

[3] NS = not significant, $P > 0.05$ or *** = significant, $P < 0.05$

[ab] Treatment means with different superscripts within a column for threonine or tryptophan are significantly different at $P < 0.05$.

Layer Production Study

The CP contents of layer diets were closer to the calculated values (Table 4.6). Results from AA analysis indicated that the levels of threonine and tryptophan in the experimental layer diets (diet 2-10) were same as planned (Table 4.6). There were no strain differences (Hyline versus H & N) in any of the parameters measured. Therefore, the data were pooled together and treated and analyzed as one strain. No significant differences ($P > 0.05$) were found in egg production, mean egg weight, daily egg mass and feed conversion ratio during the 8 week layer study (Table 4.15). The control birds ate significantly more ($P < 0.05$) feed than the birds fed diet 9 (105% threonine and 100% tryptophan). When the data (layer diets 2-10) were analyzed in a factorial manner, no significant differences were found in all of the parameters measured among layers fed diets contained different levels of threonine and tryptophan (Table 4.16).

Layer Balance Study

The percentage of N in the excreta and the amount of N excreted by layers fed the control layer diet were significantly higher ($P < 0.05$) than that of layers fed the experimental layer diets 2-10 (Table 4.17). Laying hens fed the high protein control diet also excreted significantly more ($P < 0.05$) excreta than laying hens fed diets 4, 5, 6, 7, 9 and 10. The proportion of N retained by layers fed the control diet was significantly lower ($P < 0.05$) than that of layers fed the experimental diets (Table 4.17). There were no significant differences ($P > 0.05$) in any of the parameters measured when the data (layer diets 2-10) were analyzed in a factorial manner (Table 4.18).

Table 4.15 The effect of different dietary treatments on the performance of 42-50 week old laying hens.

Diet	Threonine [1]	Tryptophan [1]	Egg production (%)	Mean egg weight (g)	Egg mass per day (g)	Feed intake (g/bird/day)	Feed conversion ratio
1	Control	Control	83.2	62.5	52.0	117.3 [a]	2.26
2	95% NRC	95% NRC	80.6	62.8	50.7	112.2 [ab]	2.22
3	95% NRC	100% NRC	80.8	62.3	50.4	112.4 [ab]	2.23
4	95% NRC	105% NRC	79.7	62.7	50.1	108.0 [ab]	2.16
5	100% NRC	95% NRC	81.2	62.6	50.8	111.4 [ab]	2.19
6	100% NRC	100% NRC	80.1	62.9	50.3	110.4 [ab]	2.19
7	100% NRC	105% NRC	79.3	63.3	50.2	109.5 [ab]	2.18
8	105% NRC	95% NRC	80.8	62.6	50.6	109.5 [ab]	2.16
9	105% NRC	100% NRC	78.9	63.2	49.9	105.3 [b]	2.11
10	105% NRC	105% NRC	79.1	62.3	49.3	109.2 [ab]	2.22
		Overall mean	80.4	62.7	50.4	110.5	2.19
		Pooled SEM [2]	0.5	0.2	0.3	0.8	0.01

[1] % of the NRC (1994) recommendations

[2] Pooled standard error of the mean; data represent mean of two replicates (44 layers per replicate)

[ab] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 4.16 Factorial comparison: The effect of different levels of threonine and tryptophan on 42-50 week old laying hen performance.

Factors	Egg production (%)	Mean egg weight (g)	Egg mass per day (g)	Feed intake (g/bird/day)	FCR[1]
***Thr*eonine [2]**					
95% NRC	80.4	62.6	50.4	110.9	2.20
100% NRC	80.2	62.9	50.5	110.4	2.19
105% NRC	79.6	62.7	49.9	108.0	2.16
***Tryptophan* [2]**					
95% NRC	80.9	62.7	50.7	111.0	2.19
100% NRC	79.9	62.8	50.2	109.4	2.18
105% NRC	79.4	62.8	49.9	108.9	2.19
Overall mean	80.1	62.8	50.3	109.8	2.18
Pooled SEM [3]	0.5	0.2	0.3	0.7	0.01
***Source of variation* [4]**					
Threonine (Thr)	*NS*	*NS*	*NS*	*NS*	*NS*
Tryptophan (Trp)	*NS*	*NS*	*NS*	*NS*	*NS*
Thr x Trp	*NS*	*NS*	*NS*	*NS*	*NS*

[1] Feed conversion ratio
[2] % of the NRC (1994) recommendations
[3] Pooled standard error of the mean; data represent mean of two replicates (44 layers per replicate)
[4] NS = not significant, at $P > 0.05$ or *** = significant, at $P < 0.05$

Table 4.17 The effect of different dietary treatments on nitrogen (N) excretion and retention by 47-week-old laying hens.

Diet	Threonine [1]	Tryptophan [1]	Excreta output (g DM/48h/bird)	N output (g DM/48h/bird)	N in excreta (% DM)	N Retention (%)
1	Control	Control	62.1 [ac]	3.31 [a]	5.35 [a]	56.4 [b]
2	95% NRC	95% NRC	53.2 [bc]	1.78 [b]	3.36 [b]	64.3 [a]
3	95% NRC	100% NRC	52.4 [bc]	1.86 [b]	3.57 [b]	63.6 [a]
4	95% NRC	105% NRC	49.1 [b]	1.70 [b]	3.48 [b]	66.8 [a]
5	100% NRC	95% NRC	50.6 [b]	1.90 [b]	3.74 [b]	63.3 [a]
6	100% NRC	100% NRC	49.6 [b]	1.74 [b]	3.56 [b]	63.2 [a]
7	100% NRC	105% NRC	51.4 [b]	1.82 [b]	3.53 [b]	63.3 [a]
8	105% NRC	95% NRC	56.6 [bc]	1.87 [b]	3.32 [b]	62.0 [a]
9	105% NRC	100% NRC	46.7 [b]	1.77 [b]	3.82 [b]	62.2 [a]
10	105% NRC	105% NRC	48.2 [b]	1.63 [b]	3.39 [b]	65.4 [a]
		Overall mean	52.0	1.94	3.71	63.1
		Pooled SEM [2]	1.1	0.06	0.07	0.5

[1] % of the NRC (1994) recommendation

[2] Pooled standard error of the mean; data represent mean of eight birds.

[ab] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 4.18 Factorial comparison: The effect of different levels of threonine and tryptophan on nitrogen (N) excretion and retention by 47-week-old laying hens.

Factors	Excreta output (g DM/48h/bird)	N output (g DM/48h/bird)	N in excreta (% DM)	N retention (%)
Threonine [1]				
95% NRC	51.6	1.78	3.47	64.9
100% NRC	50.5	1.82	3.61	63.3
105% NRC	50.5	1.76	3.51	63.2
Tryptophan [1]				
95% NRC	53.5	1.85	3.47	63.2
100% NRC	49.6	1.79	3.65	63.0
105% NRC	49.6	1.72	3.47	65.2
Overall mean	50.9	1.79	3.53	63.8
Pooled SEM [2]	1.1	0.04	0.04	0.5
Source of variation [3]				
Threonine (Thr)	*NS*	*NS*	*NS*	*NS*
Tryptophan (Trp)	*NS*	*NS*	*NS*	*NS*
Thr x Trp	*NS*	*NS*	*NS*	*NS*

[1] % of the NRC (1994) recommendations
[2] Pooled standard error of the mean; data represent mean of eight birds.
[3] NS = not significant, at $P > 0.05$ or *** = significant, at $P < 0.05$.

Discussion

The growth performance of the broilers on the experimental broiler diets used in this study was not as good as that obtained with the commercial control broiler diets, contrary to our previous experience. Commercial broiler diets purchased from a feed manufacturer gave superior results during both stages of the experiment. All starter feeds (infestation of mold was less serious in the commercial feed) became infested and this factor undoubtedly had an influence on the feed intake of the birds on the growth trial. This problem was attributed to the feeds being bagged before being cooled sufficiently after pelleting. Furthermore, the CP contents in the experimental starter diets were much lower than planned (21% versus 18.4%).

Fritz *et al.* (1973) and Sharby *et al.* (1973) reported that broiler chicks fed diets containing corn infested by various fungi showed a depressed weight gain and feed efficiency. In another study, Beasley *et al.* (1980) found that chicks fed corn inoculated with *Penicillium lanosum* developed diarrhea and grew at a slower rate than the controls. The metabolizable energy of the experimental starter diets in the present study might have been lower than planned as Bartov *et al.* (1982) showed that a decreased energy level in diets containing ground moldy grains (not containing mycotoxins) is an important factor for their reduced nutritional value. In addition, the CP content in experimental starter diets was 3% CP lower than planned. The mean body weight gain and mean feed intake of starter chicks were 83% and 87% lower respectively than the NRC (1994) standard for the first 3 weeks of age. The much lower CP contents in the starter diets and perhaps the reduction in feed intake by the molds reduced the growth performance of birds fed the experimental starter diets. In an attempt to meet their energy and protein needs, birds receiving the experimental starter feeds, on average, consumed 3% more feed than birds fed the control starter diet.

During the starter period (0-3 weeks), weight gain improved with the increase of dietary threonine. Moreover, feed/gain ratio improved significantly as dietary threonine reached 92% of the NRC (1994) estimated requirements. This indicated that broiler chicks at 0-3 weeks of age required at least 0.74% (or 4.04% of CP) dietary threonine to maximize feed efficiency. This is in good agreement with the 0.73% of the diet (4.13% of CP) reported earlier (Chapter III). Several other studies also reported similar requirement values for threonine. For instance, Thomas *et al.* (1992) reported that male and female broilers aged 0-3 weeks need 0.77% and 0.71% of dietary threonine (average is 0.74% of the diet). On the other hand, 0.72% of threonine

was required to maximize weight gain for broiler chicks up to 14 days of age (Rangel-Lugo *et al.*, 1994). Studies conducted by Holsheimer *et al.* (1994) indicate that, when low protein corn-soybean meal diets supplemented with AA were fed to male and female broiler chicks until 3 weeks of age, improvements in gain and feed/gain ratio were obtained when dietary threonine content was increased to 0.725% of the diet. Kidd *et al.* (1996) found that threonine levels ranging from 92% (0.736% of the diet) to 112% (0.896% of the diet) of the NRC (1994) recommendations failed to improve weight gain of 1-21 day old broiler chicks, indicating that broiler chicks required not more than 0.736% of dietary threonine. More recently, Yamazaki *et al.* (1997a) reported a requirement value of 0.65% digestible threonine or 0.74% total threonine for 1-3 weeks starting period.

However, Rhone Poulenc (Rhodimet Nutrition Guide, 1993), Austic (1994) and the NRC (1994) recommended a higher requirement value of 0-3 week old broilers for threonine (0.80% of diet), whereas Leeson and Summers (1997) suggested a lower level of 0.70% of the diet. Robbins (1987) clearly showed that when expressed as a percentage of the diet, the estimated threonine requirement increased as dietary CP increased. However, when expressed as a percentage of protein, the estimated threonine requirements remained constant relative to dietary protein content. This was confirmed by Austic and Rangel-Lugo (1989) and Rangel-Lugo *et al.* (1994), who found that the requirement for threonine is dependent on the CP level of the diet, and requirement values of 0.63% to 0.77% were reported by these researchers. When expressed as a % of CP, the estimated requirement value found in the present study for threonine is higher than that of Austic and Rangel-Lugo (1989) and Rangel-Lugo *et al.* (1994), who found that the threonine requirement ranges from 2.72% to 3.70% of CP. However, Holsheimer *et al.* (1994) reported a value of 3.95% of CP, which is close to the estimated value found in the current study. Data from several recent studies, from the previous experiment (Chapter III) and from the present experiment indicate that the NRC (1994) may have overestimated the requirements of 0-3 week old broilers for threonine.

Broiler chicks (0-3 weeks old) fed the 113% tryptophan diet gained significantly more ($P < 0.05$) weight than the one fed the lower level (102% of NRC (1994) recommendation). Increasing the level of tryptophan to 125% of the NRC (1994) recommendation did not further improve the weight gain. It seems that the NRC (1994) has underestimated the requirement for tryptophan. The current study indicated that 0.23% of tryptophan is required in the diet or 1.22% of CP for optimal weight gain. However, this is higher than the value (0.19% of diet) obtained in

the first study (Chapter III). The reasons for the discrepancy are unclear. Using purified diets containing five graded levels of tryptophan, Kim *et al.* (1997) found that 1-3 week old broiler chicks required 0.173% of dietary tryptophan. Rogers and Pesti (1990) reported that the requirement of the broiler chicks aged 7 to 21 days for tryptophan was estimated to be 0.80% of CP for growing chick. This equates 0.184% of the diet with 23% CP. On the other hand, Leeson and Summers (1997) recommendation for tryptophan (0.20% of the diet) agreed with that of the NRC (1994).

Several recent studies supported the results of the present study that 0-3 week old broiler chicks need more than 0.20% of dietary tryptophan. In an experiment conducted by Steinhart and Kirchgessner (1984), the birds fed a diet containing 0.22% tryptophan gave the best results with regard to weight gain, feed intake and feed efficiency. Han *et al.* (1991) carried out studies to determine requirements for digestible histidine and tryptophan in 8 to 22 day old chicks fed a histidine and tryptophan deficient intact protein diet containing 25% CP and 3,200 kcal AME_n/kg. They found that the requirement for digestible tryptophan was 0.20% of the diet (or 0.80% of CP) for maximal weight gain and feed efficiency. This indicates that a total tryptophan level of 0.22% in the diet is necessary for broiler chicks fed a 23% CP corn soybean meal diet. Thomas *et al.* (1992) proposed a dietary tryptophan requirement value of 0.225%, whereas the Rhodimet Nutrition Guide (1993) and Austic (1994) recommended values of 0.23% and 0.24%, respectively. When expressed as a % of CP, the estimated requirement obtained in the present study is higher than most of the reported values and only in agreement with that of Abebe and Morris (1990) and Austic (1994). Data from the previous experiment (Chapter III) suggested a lower requirement value of broiler chicks for tryptophan (0.19% of the diet) and agreed with that recommended by the NRC (1994). However, data from the present experiment and from several recent studies indicate that the NRC (1994) may have underestimated the requirements of 0-3 week old broilers for tryptophan.

Broilers fed a control grower diet ate significantly more and gained significantly more weight than the broilers fed the experimental grower diets. The inferior growth performance of the birds fed the experimental grower diets might be related to the poorer growth performance of these birds during the 0-3 week study period. Although feed intake and weight gain of 0-3 week old broiler chicks fed the experimental starter diets were reduced by mold and low dietary protein content, birds fed the experimental grower diets, on average, consumed 9.3% more feed and gained 23% more weight than the NRC (1994) estimation. Moreover, the overall weight gain

(0-6 week) of birds fed the experimental diets was 10% higher than the NRC (1994) estimation. The result of the present study indicated that body weight gain of 3-6 week old broilers did not increase and feed/gain ratios did not improve as dietary threonine and tryptophan increased beyond 90% and 96% of the NRC (1994) recommendations, respectively. This is equal to 0.67% of the diet or 3.4% of CP for threonine and 0.17% of the diet or 0.89% of CP for tryptophan.

Only a limited number of studies have been conducted to evaluate the requirement of 3-6 week old broilers for threonine. Recently, Thomas *et al.* (1992) reported that the performance of male broilers aged 3-6 weeks receiving 0.61% threonine in the diet was similar to those fed higher threonine levels. Leeson and Summers (1997) recommended a value of 0.60% of the diet, whereas Yamazaki *et al.* (1997a) suggested a value of 0.54% digestible threonine for 3-6 week old broiler chicks. A higher value of 0.70% of the diet has been reported by the Rhodimet Nutrition Guide (1993) and Webel *et al.* (1996). On the other hand, Kidd and Kerr (1997) found that weight gain and feed efficiency of broilers (30-42 day of age) did not further improve as the dietary threonine level increased beyond 0.65% of the diet. Penz *et al.* (1997) recommended 0.68% and 0.70% of dietary threonine for maximizing weight gain and feed efficiency, respectively. Interestingly, when the requirement value was expressed as a % of CP, the estimated requirement for threonine (3.4% of CP) obtained in the present study was lower than that of Kidd (1996) but close to the Penz *et al.* (1997) estimation. Data from several recent studies and from the present experiment indicate that the NRC (1994) may have overestimated the requirements of 3-6 week old broilers for threonine.

For tryptophan, comparison with other studies is difficult because not much data on tryptophan requirement are available for broilers beyond 3 weeks of age. The earliest reported by Hunchar and Thomas (1976) found that 4-7 week old broilers required 0.176% and 0.167% of dietary tryptophan for maximum growth and optimum feed efficiency, respectively. Using the ideal protein concept, Baker's (1997) estimation for tryptophan requirement was 0.15% of digestible tryptophan for 21 to 42 day old male broilers. The tryptophan requirement value suggested by Leeson and Summers (1997) (0.17% of diet or 0.85% of CP) is similar to the finding obtained in the present study. The Rhodimet Nutrition Guide (1993), however, recommended a higher level of tryptophan (0.20% of diet) for 3-6 week old broilers. A value as low as 0.131% was reported by Hurwitz *et al.* (1978). However, data from Hurwitz *et al.* (1978) were derived from a computer model that has not been vigorously examined. Data from several

recent studies and from the present experiment indicate that the NRC (1994) recommended tryptophan requirement (0.18% of diet) for 3-6 week old broilers is valid.

For the layer study, performance of the layers (egg production, mean egg weight, daily egg mass and feed conversion ratio) during the production study were not affected by different dietary treatment. These results suggested that the threonine and tryptophan levels in layer diets should not be targeted at more than 448 mg of threonine/hen/day and 152 mg of tryptophan/hen/day for 42-50 week old laying hens. Limited data were available concerning the threonine and tryptophan requirements of laying hens. Using diets consisting mainly of corn, wheat and milo, Yamazaki *et al.* (1997b) reported that the requirement of laying hens at 32-42 weeks of age for digestible threonine was 329 mg/hen/day. Ishibashi *et al.* (1998) recommendations for threonine were 455 and 462 mg/hen/day for egg mass and feed efficiency, respectively. This is in good agreement with the NRC (1994) estimate for the threonine requirement of laying hens (470 mg/hen/day). On the other hand, Coon (1998), Rhodimet Nutrition Guide (1993), Huyghebaert and Butler (1991) and Leeson and Summers (1997) recommended values of 495, 517, 522 and 627 mg/hen/day, respectively. These recent data indicate that laying hens should have a daily threonine intake at the NRC (1994) recommended level or higher.

Results from this experiment showed that laying hens consuming 152 mg of tryptophan per hen daily could maintain a high level of egg production. However, a further increase in tryptophan intake failed to increase the level of egg production. This is in close agreement with the recommendations of Leeson and Summers (1997) and the NRC (1994). Leeson and Summers (1997) recommendation for tryptophan is 154 mg/hen/day, whereas the NRC (1994) recommendation is 160 mg/hen/day. A value as low as 122 mg/hen/day was reported by Coon (1998), whereas 165 mg/hen/day was recommended by the Rhodimet Nutrition Guide (1993). In another study, Jensen *et al.* (1990) conducted four experiments and found that the requirement of laying hens for tryptophan ranges from 95 mg to 168 mg/hen/day. Data from these recent studies and from the present experiment indicate that the NRC (1994) recommended tryptophan requirement for laying hens is valid.

One of the most important factors affecting the utilization of dietary protein is the balance of AA in the feed. The closer the AA composition of the diet matches the requirement for maintenance and growth, the less protein the animal needs and wastes. It is well known that reduced protein diets improve protein utilization by minimizing the excesses of AA and result in

the reduction of N excretion (Waldroup *et al.*, 1976; Fancher and Jensen, 1989; Blair *et al.*, 1999). The results of the present study confirm our previous findings that reducing dietary CP appears to be a practical way of reducing N excretion (Blair *et al.*, 1999). Based on the findings of the balance study, N output over the broiler starter period (0-21 days) would be about 112 g/(8 birds) with the reduced protein starter diets used in this study and would be 175 g/(8 birds) with the commercial starter diets used in this study. This represents a 36% reduction in the N output in the excreta. No trend was observed with different threonine and tryptophan levels in the first N balance study. Nitrogen output over the grower period (22-42 days) would be about 45 g/bird with the reduced protein grower diets used in this study and would be 59 g/bird with the commercial grower diets used in this study. This represents a 25% reduction in N output in the excreta. Increasing the level of dietary tryptophan resulted in a decrease in excreta output, and an increase in the N content of excreta. No trend was observed with dietary threonine level. When compared to the control broiler diets, the amount of N retained was increased by 16% and 4.5% in the starter and grower periods, respectively, indicating better N utilization.

Further reductions in the CP content of layer diets to about 12% in the experimental layer diets resulted in 46% lower excretion of N per day than layers fed the commercial layer diet, but the lower dietary CP gave a slightly reduced egg production. Layers fed the experimental diets also excreted on average 18% less excreta than layers fed the commercial diet. The excreta excreted by layers fed the experimental diets also contained 34% less N than that of layers fed the control diet. Nitrogen retained as a percentage of intake was 13% higher with reduced-CP layer diets than with the control layer diet. There was a trend for N retention to decrease with increased dietary threonine, and the N output to decrease with increased tryptophan. The results of the N balance studies are in agreement with the conclusions of FEFANA (1992), Moran *et al.* (1992), Summers (1993), Deschepper and De Groote (1995) and Ibrahim (1997) that by reducing the CP of a diet, N excretion could also be significantly reduced and this provides an option for poultry producers to minimize N pollution from the farm.

Conclusions

Findings in the present studies indicate that when formulating diets for 0-3 week old broilers, dietary levels of threonine and tryptophan should be targeted at 0.74% of the diet (4.04% of CP) and 0.23% of the diet (1.22% of CP), respectively. For 3-6 week old broilers,

dietary levels of threonine and tryptophan should be targeted at 0.67% of the diet (3.20% of CP) and 0.17% of the diet (0.89% of CP), respectively. Laying hens aged 42-50 week old, as indicated by the present study should be targeted at a daily intake of 448 mg threonine/hen and 152 mg tryptophan/hen. The results of this trial confirm our previous findings that reducing dietary CP improves the utilization of protein and appears to be a practical way of reducing N excretion. The results of the balance studies clearly showed that crystalline AA supplementation of the reduced-protein diets improved the AA balance in the diet, hence improving the protein utilization efficiency and resulting in reduced N content in the excreta. Results from this and other studies also indicated that the NRC (1994) has overestimated the requirements of threonine in 0-3 week and 3-6 week old broilers and underestimated tryptophan requirements of 0-3 week old broiler chicks. On the other hand, the NRC (1994) recommendations of threonine and tryptophan for layers and tryptophan for 3-6 week old broilers are supported by a number of recently reported values. These results are valuable to poultry nutritionists for feed formulation and have economic implications with regard to the supplementation of reduced protein diets with feed-grade threonine and tryptophan. Clearly, more research is necessary to determine the requirement data for threonine and tryptophan in the future, particularly for laying hens and broilers during the growing period beyond 21 days of age.

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CHAPTER V

Evaluation and Enhancement of Palm Kernel Cake as a Poultry Feedstuff

Summary

Several *in vitro* and *in vivo* experiments were conducted to investigate the nutritive value of palm kernel cake (PKC) for poultry and the effect of enzyme supplementation on its nutrient quality. Palm kernel cake samples were collected from various palm kernel crushers in Malaysia. All samples were subjected to proximate analysis and amino acid (AA) analysis. The physical structure of palm kernel and PKC were studied under a scanning electron microscope. Apparent metabolizable energy (AME) and nitrogen corrected true metabolizable energy (TME_n) values of PKC were determined using Sibbald's (1986) precision-fed procedure. True AA digestibility (TAAD) of PKC was determined using cecectomized cockerels. Several enzymes supplied by Alltech Inc. were used for the enzyme studies. The effects of pretreatment of PKC with the most effective enzyme were also investigated. Intact and cecectomized cockerels were employed in the study of nutrient digestibility. On average, PKC was found to contain about 17% crude protein, 15% crude fiber, 8% ether extract, 5% ash, 0.40% calcium and 0.77% phosphorus on a dry matter basis. Screw-pressed PKC contained higher amounts of residual oil, gross energy and acid detergent lignin than solvent-extracted PKC. Scanning electron micrographs of the palm kernel revealed the rectangular honeycomb-like cell walls. The content and the digestibility of most AA in the screw-pressed PKC were significantly lower ($P < 0.05$) and this probably was attributed to the formation of Maillard products during the high temperature oil extraction process. When compared to the NRC (1994) AA requirements for poultry, PKC was limiting in lysine, sulfur AA, threonine, isoleucine, valine and histidine. Apparent metabolizable energy and TME_n of PKC were 1,492 kcal/kg and 1,800 kcal/kg, respectively. Alltech PKCase (mannanase, α-galactosidase and protease) was the most effective enzyme for PKC saccharification. PKCase supplementation significantly increased ($P < 0.05$) the release of reducing sugars in PKC and soybean meal by 26.8% - 67.4% and 20% - 30%, respectively. Even though the crude fiber content of pretreated PKC was reduced, pretreatment of PKC with PKCase was not justified in terms of its effects on animal production. PKCase supplementation significantly increased ($P < 0.05$) the AME and TME_n of PKC in both intact and cecectomized cockerels, however,

cecectomy significantly reduced ($P < 0.05$) the AME and TME_n of PKC. It was concluded that PKCase has great potential in increasing the performance of birds fed PKC-based diets.

Key words: palm kernel cake, amino acids, AME, TME_n, enzymes, PKCase

Introduction

Based on the United Nations (1998) estimates, the world population will reach 8.9 billion in 2050. Meanwhile, agricultural production is declining or stabilizing in many areas. This indicates that competition for food resources between animals and humans will increase steadily in the near future. In fact, Duke (1996) stated that the use of conventional feedstuffs such as corn and soybean meal might be impractical 20 years from now. The continued increase in population and loss of farm land (for roads, buildings, etc.) may force farmers to save better quality foods for humans and poultry producers would have to feed lower quality diets to poultry. This scenario is very likely to happen especially in the developing countries, where most of the human population is found.

Most developing countries produce an abundant amount of non-conventional feed ingredients and by-products such as rice bran, peanut meal, rapeseed meal and palm kernel cake (PKC). In 1996, the production of palm oil was second largest in the world following soybean oil (PORLA, 1997). Palm kernel cake is a by-product of the oil palm industry and about 1.4 million tonnes were produced in Malaysia alone in 1996 (PORLA, 1997). Not much research has been done on PKC regarding its use as poultry feed. From a number of available reports (Nwokolo *et al.*, 1977; Yeong, 1985; Onwudike, 1986; Siew, 1989), the nutrient composition of PKC seems to be quite variable, especially its oil, protein and energy contents. There are also great discrepancies in the reported digestibility of amino acids (AA) in PKC (Nwokolo *et al.*, 1976; Onwudike, 1986; Yeong, 1983). The variation in AA digestibility values might be due to different varieties of oil palm and methods of processing palm kernel oil. Processing of PKC involves much heat and pressure to break the kernel and extract as much oil as possible. Thus, there are possibilities for the formation of Maillard compounds between the reducing sugars and AA in PKC, particularly lysine (Mauron, 1981; Hurrell and Finot, 1985).

Knowledge of the AA composition of feed ingredients is useful but usually inadequate, because the amounts of AA that are digestible and available to the animals are often much lower

than the quantity contained in the ingredient. This is especially true for less digestible non-conventional feed ingredients such as PKC. As a result, it is necessary to know both the concentration and digestibility of AA in feedstuffs in order to formulate diets that will meet the animals' AA requirements most efficiently. However, the main criticism of digestibility or balance studies concerns the effects of the hindgut microflora on AA excretion. Consequently, several researchers have proposed the use of cecectomized cockerels for determining AA digestibility of feedstuffs (Parsons, 1985, 1986; Johns *et al.*, 1986; Green *et al.*, 1987). The problems of low availability of energy and AA should be resolved by obtaining accurate values for metabolizable energy and AA digestibility and then using these values in dietary formulation to compensate for any deficiencies in PKC.

Although the beneficial aspects of exogenous enzyme addition to cereal-based poultry diets are well documented (Bedford and Classen, 1992; Campbell and Bedford, 1992; Cheeson, 1993; Grimes *et al.*, 1997), the supplementation of exogenous enzymes to improve the digestion of non-conventional feedstuffs such as PKC is not well established. Recently, several studies have indicated that enzymes with mannanase activity could partly break down the non-starch polysaccharide (mannans) of PKC, hence improving its nutritive quality (Dusterhoft *et al.*, 1993a,b,c; Daud *et al.*, 1997). Therefore, the objectives of this study were to determine the physical and chemical characteristics of Malaysian PKC as a poultry feedstuff and to determine whether its nutritive value could be improved with enzyme supplementation.

Materials & Methods

Collection and Analyses of Palm Kernel Cake Samples

Samples of PKC were collected from palm kernel crushers in various states in Malaysia, namely Johor (six samples), Penang (one sample), Sabah (two samples) and Selangor (four samples). Nine samples were obtained from screw-press plants, while four were obtained from a solvent extraction plant (from the same plant over 4 months). Proximate analyses for crude protein (CP), crude fiber, ether extract, ash and calcium were carried out following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 1984). Phosphorus was measured using a spectrophotometric method (Estrin and Brammell, 1968). Gross energy was measured with an adiabatic oxygen bomb calorimeter (Parr, USA). Neutral

detergent fibre (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to Robertson and Van Soest (1981). All of the samples were analyzed in triplicate.

Palm kernel cake samples were sent to Rhone Poulenc's laboratory in Commentry, France, for AA analysis according to the procedure outlined by Green *et al.* (1987). Amino acid analyses were conducted in duplicate with a Beckman Multicrom B 4255 AA analyzer, after 24 h hydrolysis with 6N hydrochloric acid. Before the analysis of the sulphur-containing AA, a performic acid oxidation treatment was employed to prevent destruction of methionine, cysteine and cystine during acid hydrolysis. For tryptophan, alkaline hydrolysis with sodium hydroxide was followed by high-pressure liquid chromatography with external calibration. Feed and excreta samples that were obtained from the digestibility trials were analyzed in triplicate for crude protein, ADF, NDF and ADL. Glycine was omitted in all calculations of AA digestibility due to the breakdown of uric acid to glycine during acid hydrolysis of excreta (Soares *et al.,* 1971).

Scanning Electron Microscopy of Palm Kernel and Palm Kernel Cake

Fresh palm kernels were cut into 0.5 cm x 0.5 cm cubes. They contained a high amount of oil, making it difficult to study the palm kernel structure under a scanning electron microscope. As a result, samples were immersed in petroleum ether for either 10 s or 10 min to extract the oil. Later, the samples were fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer for 24 h and post-fixed in buffered 1% osmium tetroxide at 4 °C for 2 h. They were then dehydrated in an acetone series (10 min, in 35%, 50%, 75%, 95% and 15 min in 100% with three changes) and critical point dried (BAL-TEC CPD 030, Critical Point Dryer, Switzerland). They were then mounted on 12 mm x 5 mm aluminum stubs (Agar Scientific, UK) and coated with 20 nm gold (Polaron Equipment Ltd. SEM Coating Unit E5100, England). Solvent-extracted PKC was also prepared in the above manner for viewing under an electron microscope, except it was not treated with petroleum ether. Samples were examined with a JEOL 6400 Scanning Microscope (Japan) operated at 15 kV.

Metabolizable Energy and Amino Acid Digestibility of Palm Kernel Cake for Poultry

Three PKC samples obtained from three of the largest palm kernel crushers in Malaysia were selected for the study (Huplee, Lee and Premium). Both Huplee and Lee were obtained from plants using the screw-press technique for oil extraction, whereas Premium was obtained from a plant using the solvent-extract technique. Both the nitrogen corrected true metabolizable energy (TME_n) and the true amino acid digestibility (TAAD) were determined according to Sibbald's procedure (Sibbald, 1986). Briefly, all birds used in the experiment were fasted for 48 h to remove all digesta in the gastro-intestinal tract. Six cockerels were assigned to each treatment. Thirty grams of test feedstuff was given to each bird via crop intubation. A similar number of cockerels of each type were fasted throughout the experimental period to measure endogenous excretion. A plastic tray was placed under each cage and excreta were collected quantitatively for 48 h. Birds had free access to clean water.

Thirty-week-old intact Lohmann Brown cockerels were used for the TME_n study. The excreta samples were dried at 60 °C, re-weighed (dry weight) and ground to pass through a 1 mm mesh screen. Gross energy was measured with an adiabatic oxygen bomb calorimeter. Total N content in the dry excreta was analyzed according to the AOAC (1984) procedure. A correction was made for nitrogen retention, which was either positive or negative. It was done to bring the TME_n data to a basis of nitrogen equilibrium. A correction factor of 8.73 kcal/g of nitrogen was used (Titus *et al.*, 1959).

However, 30-week-old cecectomized Lohmann Brown cockerels were used for the TAAD study to minimize the effect of microbial fermentation on AA digestibility. Cecectomy was performed on cockerels when they were16-week-old according to the procedure of Parsons (1985). All cockerels were given 14 weeks to recover from surgery prior to being used in the experiments. Examination of the cockerels at the end of the experiment indicated that little or no cecal growth had occurred in the cecectomized cockerels. Excreta from two cockerels were pooled and freeze-dried and reweighed. All dried excreta and PKC samples were ground to pass through a 0.5-mm screen mesh and analyzed in duplicate for AA as described above.

Selection of Suitable Enzymes for Palm Kernel Cake Saccharification

Cellulase, pentosanase, β-glucanase, protease and PKCase were obtained from Alltech Inc., (Kentucky, USA). PKCase is an enzyme mixture that contains 107,000 U/g of alpha-galactosidase activity, 2,300 HUT/g of protease activity (HUT = hemoglobin unit on the tyrosine base) and 12,081 U/g of mannanase activity. Manannase activity was determined by the procedure outlined by Araujo and Ward (1990), using locust bean galactomannan as the substrate. The mannanase assay mixture contained 0.5 ml of 1% (w/v) galactomannan, prepared in 0.1 M sodium acetate buffer, pH 5.8, 0.4 ml of 0.1 M sodium acetate buffer and 0.1 ml of enzyme solution (0.05 g PKCase in 250 ml deionized water). The reaction mixture was incubated at 50 °C for 30 min. Reducing sugars produced were determined as mannose reducing equivalents by using the dinitrosalicylic acid (DNS) method of Miller *et al.* (1960). One unit (U) of mannanase activity is defined as the amount producing 1 mol of product per min. The mannanase assay was also carried out in quadruplet at various temperatures to determine the optimum temperature for PKCase. In order to determine the effect of pH on PKCase's mannanase activity, assays were carried out in quadruplet at various pHs (glycine HCL buffer for pH 2.02; sodium acetate buffer for pH 3.6 to 5.8; phosphate buffer for pH 6.0 to 8.0) under the optimum temperature.

To study the effect of different enzymes (cellulase, pentosanase, β-glucanase, protease and PKCase) on solvent-extracted PKC, an enzyme was added to 10 g of PKC at 2kg/t in 50 ml of 0.1 M phosphate buffer solution (pH 6.0). The mixture was incubated in a shaking water bath at 40 °C for 3 h. After incubation, the solution was centrifuged at 1500 revolutions per min for 5 min, then 200 µl of the supernatant solution was mixed with 1.80 ml of phosphate buffer and 3.0 ml of DNS solution in a screw-cap test tube. Test tubes were put into boiling water for exactly 5 min and then cooled for 5 min in cold water. Absorbance was read against the blank at 540 nm using a Hitachi U-2000 spectrophotometer (Kyoto, Japan). Standard mannose solutions with different concentrations were also prepared and a standard curve was prepared for every test. There were three replications for each enzyme. Combinations of various enzymes were also tested to investigate the possible interactions among enzymes.

Since PKCase was found to be the most effective enzyme for the saccharification of PKC, a second experiment with PKCase was then carried out simulating the gastro-intestinal

tract conditions (pH 5.5 in crop; pH 3.0 in proventriculus; pH 6.5 in small intestine) in chickens according to Tervila-Wilo *et al.* (1996). The procedures were similar to those discussed above, except that solvent-extracted PKC was incubated with or without PKCase and exposed to different digestion stages (acidic condition with pepsin (Merck, 7179) and alkaline condition with pancreatin (Merck, 7133)). The treatments were as follows: Control = PKC incubated in phosphate buffer solution (40°C, pH 6.0, 3 h); PKCase control = PKC incubated with PKCase in phosphate buffer solution (40°C, pH 6.0, 3 h); Simulated control = PKC incubated under gastro-intestinal tract conditions (40°C, 3 h); Simulated PKCase = PKC incubated with PKCase under gastro-intestinal tract conditions (40°C, 3 h). There were three replications for each treatment.

Another experiment was carried out to determine the effect of PKCase on different PKC samples (screw-pressed samples: Lee and Huplee; and solvent-extracted samples: Pre2, Pre3 and Pre4) and other feed ingredients namely corn and soybean meal. PKCase was added at either 1 kg/t or 2 kg/t. Five grams of samples were mixed with enzyme in 50 ml of phosphate buffer solution and incubated at 40°C for 3 h. The solution was then measured for reducing sugars released (Miller *et al.*, 1960). There were five replications for each sample.

Solvent-extracted PKC (Premium) was also incubated with PKCase to study the effects of enzyme pretreatment on its nutritive quality. Five hundred grams of PKC were mixed with PKCase (at 1 kg/t) in 2 L of distilled water in duplicate. The mixture was left at room temperature (30 °C) for 24 h. The wet samples were then dried in a 60°C force-draft oven until they reached a constant weight; they were labeled as PretPKC and analyzed for proximate constituents as above.

Effect of PKCase on the Digestibility of Nutrients in Palm Kernel Cake

Both cecectomized (24 birds) and intact Lohmann Brown cockerels (18 birds) used in the TME_n and TAAD study above were also used in this study to determine the effect of PKCase on the digestibility of energy, ADF and NDF in solvent-extracted PKC (Premium and PretPKC). Eighteen cecectomized cockerels were assigned to Premium, Premium + 1kg/t PKCase or PretPKC (six cockerels per sample). Another 12 cockerels (six intact and six cecectomized) were fasted for endogenous excreta collection. Twelve intact cockerels were assigned to either Premium or Premium + 1kg/t PKCase (six cockerels per sample). The digestibility of PretPKC for intact cockerels was not determined because not enough samples were available.

Calculations

TME_n (kcal/kg), and TAAD (%) were calculated as followed (Sibbald, 1979, 1986):

TME_n / kg of feed = $[(F_i \times GE_f) - (E \times GE_e) - (NR \times K) + (FE_m + UE_e)] / F_i$

Where,

F_i = feed intake, g

GE_f = gross energy of feed, kcal/kg

E = excreta output, g

GE_e = gross energy of excreta, kcal/kg

$FE_m + UE_e$ = metabolic fecal and endogenous urinary energy (fasted birds), kcal/kg

NR = nitrogen retention, i.e. nitrogen input minus nitrogen output, g

K = is a constant (8.73 kcal/g of retained nitrogen)

The AME assay was not conducted in this experiment. However, it could be estimated by the theoretical equation outlined by McNab (1990):

AME (kcal/kg) = TME – (EEL / Food intake)

where,

EEL is the endogenous energy loss (energy excreted by fasted birds, kcal/kg)

TAAD (%) = 100 x {[(AA in feed – AA in excreta) + AA excreted by fasted bird] / AA in feed}

Statistical Analyses

This was a completely randomized design experiment. Data were subjected to ANOVA (SAS Institute, 1996) and treatment means separated using Tukey's multiple range tests (Snedecor and Cochran, 1980).

Results

Collection and Analyses of Palm Kernel Cake Samples

The results of the chemical analysis are presented in Table 5.1. No significant differences in dry matter, CP, crude fiber, ash, calcium, phosphorus, NDF, ADF and nitrogen free extract contents were detected between the PKC from plants because of the different methods of extraction except for ether extract, gross energy and ADL. Palm kernel cake obtained from solvent extraction plants contained significantly lower ($P < 0.05$) amounts of oil residue (3.29% versus 8.93%), gross energy (4,504 kcal/kg versus 4,718 kcal/kg), and ADL (7.94% versus 10.26%) when compared to PKC from screw-press plants. Most AA except alanine, histidine and tryptophan were significantly lower ($P < 0.05$) in the screw-pressed than in the solvent-extracted PKC (Table 5.2). However, when expressed as a percentage of CP, most AA except alanine, histidine, methionine, total sulfur AA and tryptophan were significantly lower ($P < 0.05$) in the screw-pressed than in the solvent-extracted PKC.

Table 5.1 Nutrient composition of Malaysian palm kernel cake[1,2] (DM basis)

Nutrients	Mean ± S.D.	Range
Dry matter, %	93.76 ± 2.14	91.38 - 96.31
Crude protein, %	16.58 ± 0.49	15.73 - 17.19
Crude fiber, %	14.61 ± 1.23	12.47 - 16.09
Ether extract[2], %	8.03 ± 2.12	1.43 - 11.39
Gross energy[2], kcal/kg	4,688 ± 122	4,422 – 4,843
Ash, %	4.91 ± 0.36	4.26 - 5.56
Calcium, %	0.41 ± 0.13	0.25 - 0.62
Phosphorus, %	0.77 ± 0.10	0.64 - 0.92
Neutral detergent fiber, %	70.07 ± 2.07	67.95 - 74.25
Acid detergent fiber, %	43.08 ± 1.96	40.33 - 45.65
Acid detergent lignin[2], %	9.51 ± 1.35	6.38 - 11.57
Hemicellulose [3], %	26.98 ± 2.39	22.57 - 30.46
Nitrogen free extract, %	49.32 ± 2.77	45.12 - 54.37

[1] Data represent mean of three replications of 13 palm kernel cakes samples (nine screw-pressed palm kernel cakes and four solvent-extracted palm kernel cakes); ± standard deviation.
[2] Comparison between solvent-extracted and screw-pressed palm kernel cakes was not significant for most nutrients except ether extract, gross energy and acid detergent lignin.
[3] Neutral detergent fiber - acid detergent fiber.

Table 5.2 Nitrogen, crude protein and amino acid content of palm kernel cake (DM basis).

	% of dry matter (± S.D.) [1]			% of crude protein (± S.D.)		
	Solvent [2]	Screw-press [2]	Mean	Solvent	Screw-press	Mean
Nitrogen	2.67 ± 0.10	2.58 ± 0.09	2.61 ± 0.10	-	-	-
Crude protein	16.66 ± 0.63	16.15 ± 0.55	16.31 ± 0.60	-	-	-
Amino acids						
Asp + Asn [3]	1.33 ± 0.05 [a]	1.21 ± 0.04 [b]	1.24 ± 0.06	7.97 ± 0.07 [a]	7.48 ± 0.28 [b]	7.63 ± 0.26
Arginine	2.11 ± 0.12 [a]	1.86 ± 0.11 [b]	1.93 ± 0.14	12.63 ± 0.38 [a]	11.51 ± 0.82 [b]	11.86 ± 0.78
Lysine	0.50 ± 0.03 [a]	0.43 ± 0.03 [b]	0.45 ± 0.04	2.99 ± 0.08 [a]	2.63 ± 0.21 [b]	2.74 ± 0.22
Methionine + cystine	0.50 ± 0.02 [a]	0.45 ± 0.02 [b]	0.47 ± 0.02	2.98 ± 0.03	2.79 ± 0.18	2.85 ± 0.15
Methionine	0.32 ± 0.02 [a]	0.30 ± 0.01 [b]	0.30 ± 0.02	1.93 ± 0.06	1.85 ± 0.11	1.87 ± 0.10
Cystine	0.18 ± 0.01 [a]	0.15 ± 0.01 [b]	0.16 ± 0.02	1.06 ± 0.04 [a]	0.94 ± 0.12 [b]	0.98 ± 0.08
Threonine	0.51 ± 0.02 [a]	0.47 ± 0.02 [b]	0.48 ± 0.02	3.08 ± 0.04 [a]	2.88 ± 0.04 [b]	2.94 ± 0.11
Tryptophan	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	1.01 ± 0.02	0.99 ± 0.02	0.99 ± 0.02
Serine	0.72 ± 0.03 [a]	0.65 ± 0.02 [b]	0.67 ± 0.03	4.30 ± 0.04 [a]	4.03 ± 0.17 [b]	4.11 ± 0.16
Glu + Gln [3]	3.03 ± 0.14 [a]	2.81 ± 0.12 [b]	2.88 ± 0.13	18.17 ± 0.29 [a]	17.43 ± 0.62 [b]	17.66 ± 0.54
Proline	0.55 ± 0.02 [a]	0.50 ± 0.02 [b]	0.52 ± 0.02	3.32 ± 0.02 [a]	3.13 ± 0.10 [b]	3.19 ± 0.10
Glycine	0.73 ± 0.03 [a]	0.67 ± 0.02 [b]	0.69 ± 0.03	4.36 ± 0.03 [a]	4.17 ± 0.14 [b]	4.23 ± 0.12
Alanine	0.66 ± 0.04	0.62 ± 0.02	0.63 ± 0.03	3.93 ± 0.15	3.85 ± 0.11	3.88 ± 0.12
Valine	0.86 ± 0.03 [a]	0.79 ± 0.04 [b]	0.81 ± 0.04	5.15 ± 0.06 [a]	4.89 ± 0.19 [b]	4.97 ± 0.16
Isoleucine	0.58 ± 0.03 [a]	0.53 ± 0.02 [b]	0.55 ± 0.03	3.47 ± 0.05 [a]	3.30 ± 0.10 [b]	3.35 ± 0.09
Leucine	1.05 ± 0.05 [a]	0.97 ± 0.04 [b]	0.99 ± 0.04	6.27 ± 0.10 [a]	6.02 ± 0.18 [b]	6.09 ± 0.16
Tyrosine	0.45 ± 0.02 [a]	0.42 ± 0.02 [b]	0.43 ± 0.02	2.72 ± 0.04 [a]	2.58 ± 0.10 [b]	2.62 ± 0.09
Phenyalanine	0.66 ± 0.03 [a]	0.62 ± 0.03 [b]	0.63 ± 0.03	3.97 ± 0.05 [a]	3.84 ± 0.14 [b]	3.88 ± 0.12
Histidine	0.27 ± 0.04	0.24 ± 0.04	0.25 ± 0.04	1.57 ± 0.19	1.50 ± 0.21	1.52 ± 0.20

[1] Data represent mean of four solvent-extracted palm kernel cakes and nine screw-pressed palm kernel cakes; ± standard deviation.
[2] Type of processing, solvent-extract or screw-press technique
[3] Asp = aspartic acid; Asn = asparagine; Glu = glutamic acid; Gln = glutamine
[a,b]Treatment means with different superscripts within a row (within a calculation method) are significantly different at $P < 0.05$.

Scanning Electron Microscopy of Palm Kernel and Palm Kernel Cake

The scanning electron micrographs (Figures 5.1, 5.2) show that palm kernel is made up of oily rectangular honeycomb-like cell walls. Unfortunately, it was difficult to identify and differentiate the different physical structure of PKC in the electron micrograph (Figure 5.3). Fresh palm kernels consist of white oily flesh. However, PKC samples collected in this study were brown in color. In general, PKC samples obtained from solvent extraction plants had a light brown color, while samples obtained from screw-press plants had a medium to dark brown color. Palm kernel cake samples for the proximate analysis were obtained from palm kernel crushers using different oil extraction methods (solvent extraction and screw-press extraction).

Metabolizable Energy and Amino Acid Digestibility of Palm Kernel Cake for Poultry

No significant differences ($P > 0.05$) were found in the AME and TME_n values even though Huplee and Lee contained higher oil residue and gross energy than Premium (Table 5.3). When metabolizable energy values (AME and TME_n) were expressed as percentages of GE, the values were constant for all samples. The digestibility coefficients for six AA were found to be significantly different ($P < 0.05$) among Lee, Huplee and Premium (Table 5.4). The AA digestibility coefficients for arginine, glutamic acid plus glutamine were significantly lower ($P < 0.05$) in Lee. The AA digestibility coefficients for lysine and cystine were significantly higher ($P < 0.05$) in Premium, but tryptophan digestibility was significantly lower ($P < 0.05$) in Premium. There were no significant differences in tryptophan digestibility between Lee and Premium or between Lee and Huplee. On the other hand, the AA digestibility coefficient for histidine was significantly lower ($P < 0.05$) in Huplee. However, no significant difference in histidine digestibility was detected between Lee and Premium. For the overall AA digestibility coefficient, it was significantly higher for Premium when compared to Lee (Table 5.4). But no significantly differences in overall AA digestibility coefficient were found between Premium and Huplee or Lee and Huplee.

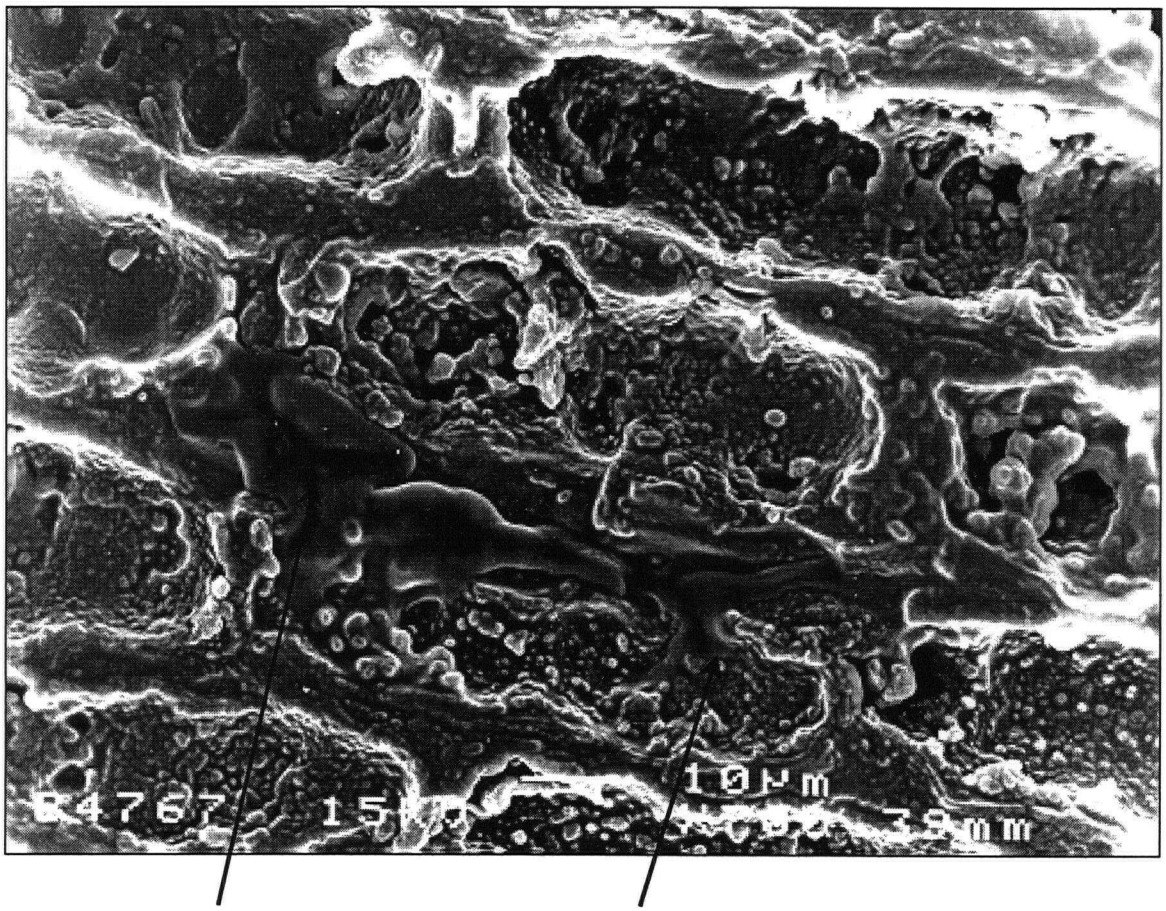

Figure 5.1 Cell wall structures (700 x, 15 kV) of palm kernel (extracted with petroleum ether for 10 s) under a scanning electron microscope showing honeycomb-like palm kernel cell walls covered with oil.

Figure 5.2 Cell wall structures (100 x, 15 kV) of palm kernel (extracted with petroleum ether for 10 min) under a scanning electron microscope showing oil-free cell walls.

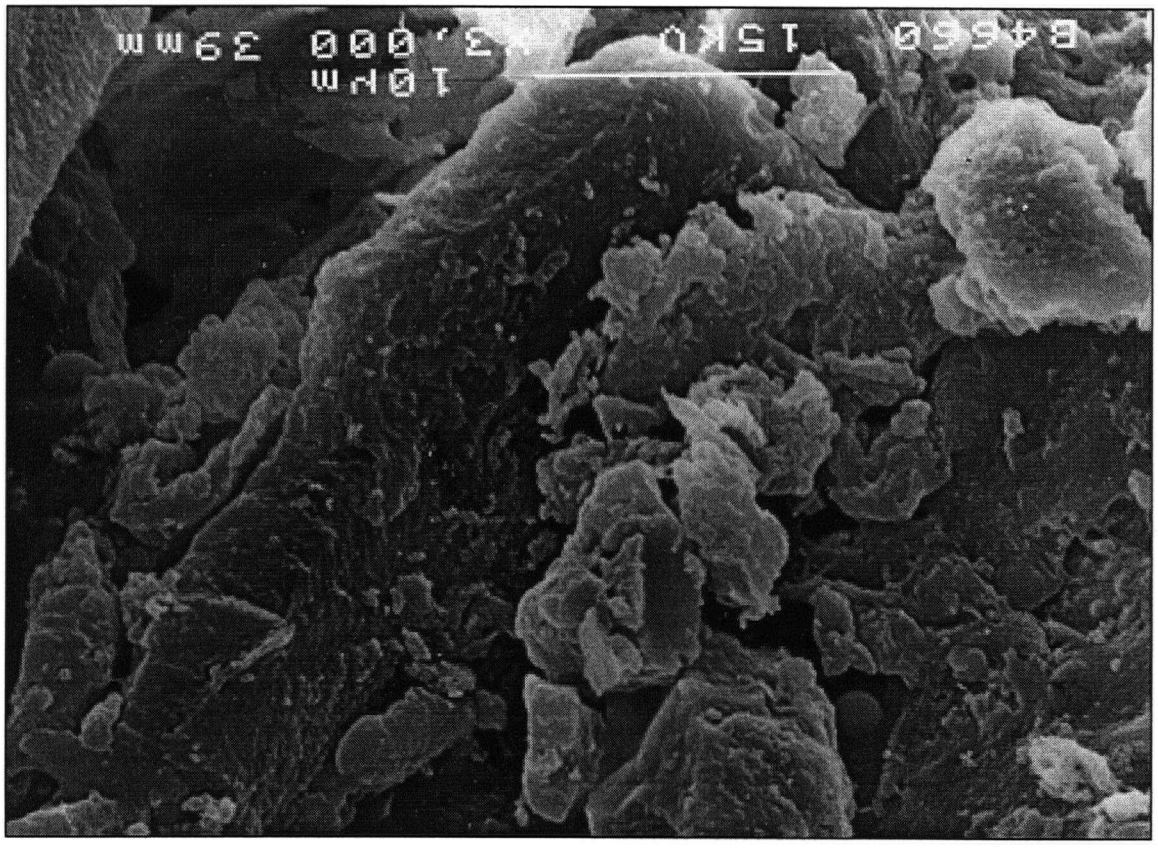

Figure 5.3 Palm kernel cake under a scanning electron microscope (3,000 x, 15 kV).

Table 5.3 The effect of different amounts of oil residue (ether extract) in palm kernel cake (PKC) on its metabolizable energy values (DM basis ± S.D.) [1,2]

PKC	EE % of DM	GE kcal/kg	TME_n kcal/kg	AME kcal/kg	AME (% of GE)	TME_n (% of GE)
Huplee	7.29 ± 0.05 [a]	4,628 ± 6 [a]	1,816 ± 90	1,531 ± 92	33	39
Lee	8.35 ± 0.04 [a]	4,764 ± 19 [a]	1,820 ± 102	1,492 ± 98	31	38
Premium	3.04 ± 0.02 [b]	4,422 ± 18 [b]	1,762 ± 97	1,452 ± 91	33	40

[1] EE = ether extract; GE = gross energy; TME_n = nitrogen corrected true metabolizable energy; AME = apparent metabolizable energy; Data represent mean of six cockerels; ± standard deviation.

[2] Huplee and Lee were screw-pressed PKC; Premium was solvent-extracted PKC.

[a,b] Treatment means with different superscripts within a row are significantly different at $P < 0.05$.

Table 5.4 True amino acid digestibility (%) of samples of palm kernel cake (Mean ± S.D.) [1]

	Lee	Huplee	Premium	Mean
Processing method	Screw-press	Screw-press	Solvent	
Color of samples	Dark brown	Brown	Light brown	-
Amino acids				
Asp + Asn [2]	52.8 ± 3.5	59.5 ± 6.1	61.1 ± 1.4	57.8 ± 5.3
Arginine	80.9 ± 1.2 [b]	83.1 ± 0.6 [a]	82.8 ± 0.7 [a]	82.3 ± 1.3
Lysine	38.8 ± 5.3 [b]	42.0 ± 3.7 [b]	50.4 ± 1.5 [a]	43.8 ± 6.2
Methionine	69.7 ± 1.1	72.5 ± 0.7	73.2 ± 3.2	71.8 ± 2.4
Cystine	33.9 ± 3.3 [b]	32.6 ± 6.2 [b]	49.7 ± 10.0 [a]	38.7 ± 10.2
Threonine	53.5 ± 4.6	59.6 ± 12.6	58.4 ± 1.4	57.2 ± 7.3
Tryptophan	56.6 ± 2.1 [ab]	59.9 ± 3.7 [a]	51.3 ± 5.7 [b]	55.9 ± 5.2
Serine	65.2 ± 4.8	68.1 ± 5.4	69.2 ± 1.7	67.5 ± 4.1
Glu + Gln [2]	63.9 ± 2.5 [b]	74.5 ± 1.2 [a]	74.1 ± 1.1 [a]	70.8 ± 5.4
Proline	56.4 ± 3.9	57.6 ± 4.1	63.5 ± 3.0	59.2 ± 4.6
Alanine	39.2 ± 6.0	40.8 ± 9.0	48.1 ± 6.2	42.7 ± 7.5
Valine	62.4 ± 3.7	68.3 ± 3.2	67.7 ± 2.2	66.1 ± 3.9
Isoleucine	64.6 ± 3.6	68.8 ± 0.3	66.9 ± 3.7	66.8 ± 3.1
Leucine	66.5 ± 2.5	70.1 ± 1.2	69.1 ± 2.9	68.6 ± 2.6
Tyrosine	70.2 ± 3.5	73.5 ± 1.3	71.7 ± 0.3	71.8 ± 2.4
Phenyalanine	71.8 ± 3.2	74.6 ± 1.4	72.6 ± 2.8	73.0 ± 2.6
Histidine	59.0 ± 1.0 [ab]	54.0 ± 6.9 [b]	64.2 ± 2.4 [a]	59.1 ± 5.7
Mean	59.2 ± 2.7 [b]	62.3 ± 2.6 [ab]	64.4 ± 2.4 [a]	62.0 ± 3.3

[1] Data represent mean of three replications of two cecectomized cockerels; ± standard deviation.
[2] Asp = aspartic acid; Asn = asparagine; Glu = glutamic acid; Gln = glutamine.
[a,b] Treatment means with different superscripts within a row are significantly different at $P < 0.05$.

Selection of Suitable Enzymes for Palm Kernel Cake Saccharification

PKCase was found to contain 12,081 U/g of mannanase activity. The optimum temperature and pH for the mannanase activity were 60 °C and 5.0, respectively (Figures 5.4 and 5.5). Alltech enzymes such as cellulase and PKCase significantly increased ($P < 0.05$) the release of reducing sugars from PKC (Table 5.5). PKCase released the highest amount of reducing sugars from PKC, and combining PKCase with other enzymes did not yield a higher level of reducing sugars than PKCase alone. The fact that PKCase activity was maintained under the gastro-intestinal conditions (Table 5.6) indicates that PKCase still yielded significant amounts of reducing sugars under these conditions. PKCase significantly increased ($P < 0.05$) the release of reducing sugars from various PKC samples and soybean meal (Tables 5.7 and 5.8). However, PKCase failed to increase the release of reducing sugars from corn.

Pretreatment of PKC with PKCase at 30 °C did not affect most of the nutrient components of PKC (Table 5.9). However, the crude fiber level was significantly reduced ($P < 0.05$) by PKCase pretreatment. Drying wet pretreated PKC samples is a time consuming and energy-driven process. In addition, some of the pretreated samples became infested with molds during the drying processes.

Effect of PKCase on the Digestibility of Nutrients in Palm Kernel Cake

PKCase supplementation significantly increased ($P < 0.05$) metabolizable energy (AME and TME_n) of PKC in both intact and cecectomized birds (Table 5.10). Cecectomy significantly reduced ($P < 0.05$) the AME and TME_n contents of PKC. However, PKCase supplementation and pretreatment alleviated the negative effect. Cecectomy also significantly reduced true DM retention (Table 5.10). While cecectomy did not have any effect on NDF retention, it improved ADF retention significantly ($P < 0.05$).

Figure 5.4 Relative mannanase activity of Alltech PKCase at different temperatures

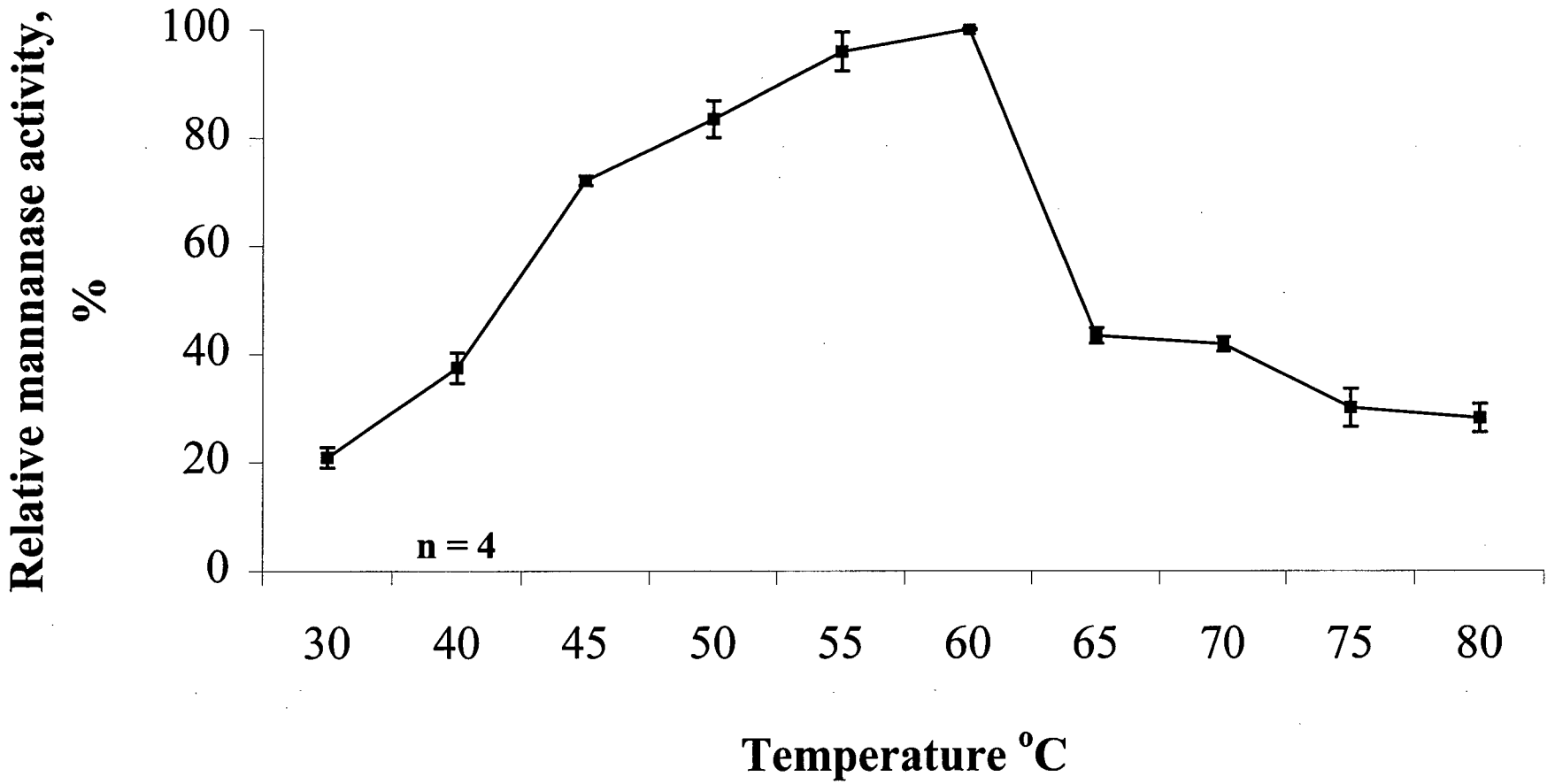

Figure 5.5 Relative mannanase activity of Alltech PKCase at different pH values

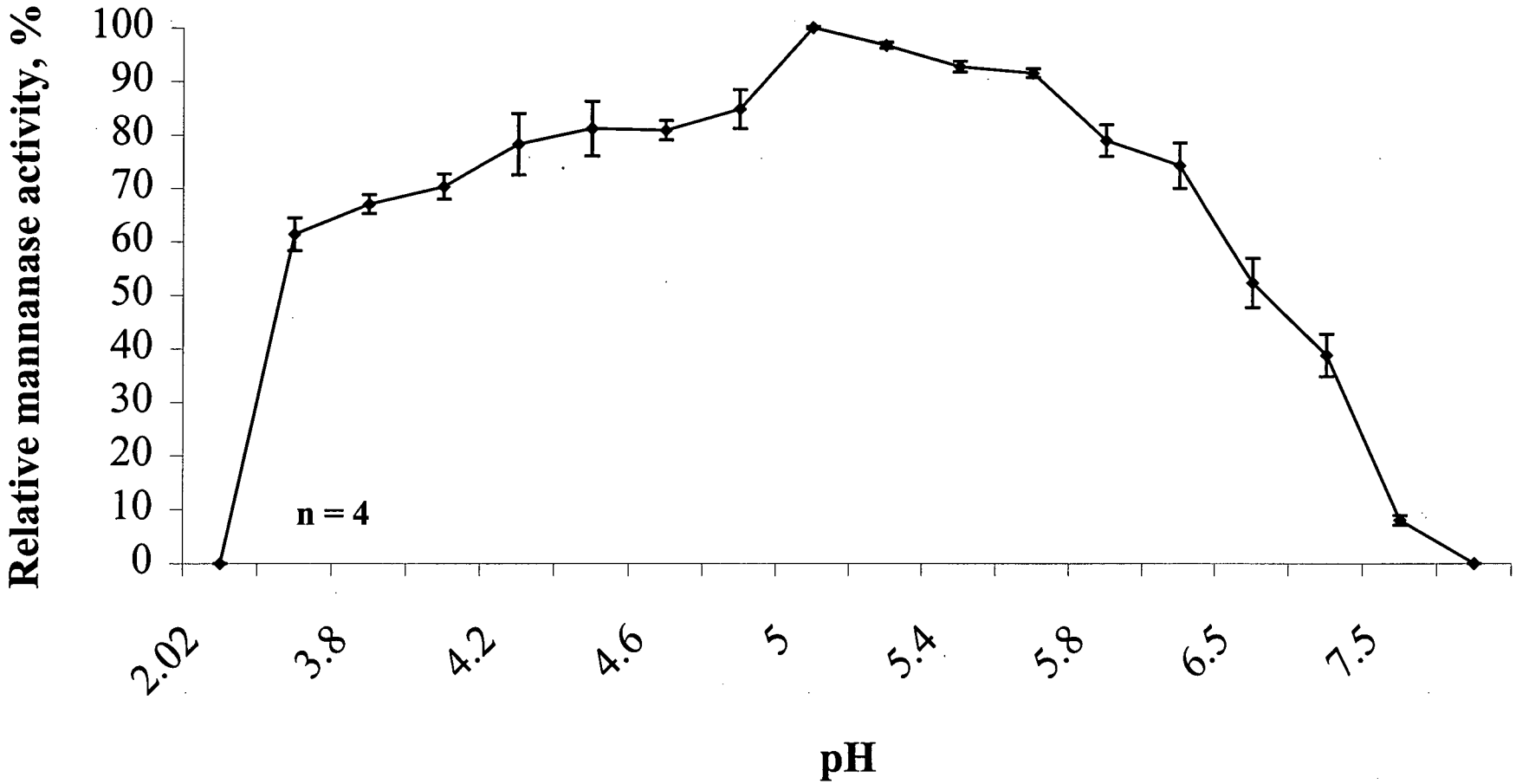

Table 5.5 Release of reducing sugars (expressed as mannose equivalents) from solvent-extracted palm kernel cake (PKC) after incubation with different Alltech enzymes.

Alltech enzyme(s)	Mean reducing sugars released (μmol mannose per ml) ± S.D.[1]
Control	5.81 ± 0.17 [c]
Cellulase	8.25 ± 0.22 [b]
Pentosanase	6.02 ± 0.19 [c]
Protease	5.98 ± 0.15 [c]
β-glucanase + cellulase	8.52 ± 0.15 [b]
PKCase[2]	13.34 ± 0.35 [a]
PKCase + cellulase	13.53 ± 0.10 [a]
PKCase + pentosanase	13.32 ± 0.16 [a]
PKCase + protease	13.30 ± 0.18 [a]
PKCase + β-glucanase + cellulase	13.64 ± 0.15 [a]

[1] Each value represents the mean of three replicates; ± standard deviation.
[2] Alltech, Inc. (Kentucky, USA). It contains mannanase, α-galactosidase and protease activity.
[a,b,c] Means with different superscripts differ significantly ($P < 0.05$)

Table 5.6 Effect of incubating solvent-extracted palm kernel cake (PKC) with Alltech PKCase under gastro-intestinal (GI) tract conditions [1].

Treatments [2]	Mean reducing sugars released (μmol mannose per ml) ± S.D.[3]
Control	5.81 ± 0.36 [d]
PKCase control	11.95 ± 0.29 [c]
Simulated control	15.26 ± 0.53 [b]
Simulated PKCase	20.80 ± 0.17 [a]

[1] PKCase is an enzyme mixture designed by Alltech, Inc. (Kentucky, USA) especially for PKC. It contains mannanase, α-galactosidase and protease activity.
[2] Control = PKC incubated in phosphate buffer solution (40°C, pH 6.0, 3 h); PKCase control = PKC incubated with PKCase in phosphate buffer solution (40°C, pH 6.0, 3 h); Simulated control = PKC incubated under GI tract conditions (40°C, 3 h); Simulated PKCase = PKC incubated with PKCase under GI tract conditions (40°C, 3 h).
[3] Each value represents the mean of three replicates; ± standard deviation.
[a,b,c,d] Means with different superscripts differ significantly ($P < 0.05$).

Table 5.7 The effect of incubating several palm kernel cake (PKC) samples with Alltech PKCase [1].

PKC samples	Reducing sugars released (μmol mannose/ml)	Enzyme effect (μmol mannose/ml)	Enzyme effect % increase
Screw-pressed PKC			
Huplee	6.51 ± 0.15 [b]	-	
Huplee + 1kg/t PKCase	9.91 ± 0.78 [a]	3.40 ± 0.78	52%
Lee	5.27 ± 0.03 [b]	-	
Lee + 1kg/t PKCase	8.82 ± 0.28 [a]	3.55 ± 0.28	67%
Solvent-extracted PKC			
Pre2	9.82 ± 0.12 [b]	-	
Pre2 + 1kg/t PKCase	12.45 ± 0.11 [a]	2.63 ± 0.11	27%
Pre3	4.45 ± 0.01 [b]	-	
Pre3 + 1kg/t PKCase	6.61 ± 0.31 [a]	2.18 ± 0.31	49%
Pre4	6.04 ± 0.12 [b]	-	
Pre4 + 1kg/t PKCase	9.32 ± 0.37 [a]	3.28 ± 0.37	54%

[1] PKCase is an enzyme mixture designed by Alltech, Inc. (Kentucky, USA) especially for PKC. It contains mannanase, α-galactosidase and protease activity; Mean of five replicates, ± standard deviation.
[a,b] Means in the same row and same PKC samples with different superscripts differ significantly ($P < 0.05$)

Table 5.8 The effect of incubating corn, soybean meal (SBM) and solvent-extracted palm kernel cake (PKC) with Alltech PKCase [1].

Feed ingredient	Reducing sugars released (μmol mannose/ml)	Enzyme effect (μmol mannose/ml)	Enzyme effect % increase
Corn	9.62 ± 0.27	-	-
Corn + 1kg/t PKCase	9.49 ± 0.14	0.00	0%
Corn + 2kg/t PKCase	9.58 ± 0.19	0.00	0%
SBM	3.05 ± 0.09 [c]	-	
SBM + 1kg/t PKCase	3.66 ± 0.09 [b]	0.61 ± 0.09	20%
SBM + 2kg/t PKCase	3.98 ± 0.07 [a]	0.92 ± 0.07	30%
PKC	4.42 ± 0.08 [c]	-	
PKC + 1kg/t PKCase	6.63 ± 0.25 [b]	2.20 ± 0.25	50%
PKC + 2kg/t PKCase	7.37 ± 0.37 [a]	2.94 ± 0.37	67%

[1] PKCase is an enzyme mixture designed by Alltech, Inc. (Kentucky, USA) especially for PKC. It contains mannanase, α-galactosidase and protease activity; Mean of five replicates, ± standard deviation.
[a,b] Means in the same row and same PKC samples with different superscripts differ significantly ($P < 0.05$)

Table 5.9 Proximate analysis of untreated palm kernel cake (PKC) and PKCase-treated palm kernel cake [1,2].

Sample	Ash	CP	EE	GE (kcal/kg)	CF
Untreated PKC (% DM)	5.84 ± 0.04	17.19 ± 0.46	5.49 ± 0.11	4,591 ± 16	12.6 ± 0.2 [a]
PKCase-treated PKC (% DM)	5.70 ± 0.09	17.21 ± 0.33	5.37 ± 0.06	4,611 ± 18	10.9 ± 0.1 [b]

[1] Solvent-extracted PKC was incubated with or without 1kg/t of PKCase under 30 °C for 24 h. PKCase is an enzyme mixture designed by Alltech, Inc. (Kentucky, USA) especially for PKC. It contains mannanase, α-galactosidase and protease activity; values represent mean of six analyses (± standard deviation)
[2] CP = crude protein; EE = ether extract; GE = gross energy; CF = crude fiber.
[a,b] Means in the same column with different superscripts differ significantly ($P < 0.05$)

Table 5.10 The effect of enzyme (PKCase) supplementation on nitrogen corrected true metabolizable energy (TME_n), true dry matter (DM) retention, neutral detergent fiber (NDF) and acid detergent fiber (ADF) retention [1].

Solvent-extracted Palm kernel cake	AME (kcal/kg)	TME_n (kcal/kg)	True DM retention, %	NDF retention, %	ADF retention, %
Normal birds					
Premium (P)	1,438 [b]	1,929 [b]	37.64 [a]	17.74	4.13 [b]
P + 1kg/t PKCase	1,624 [a]	2,115 [a]	38.17 [a]	20.59	4.03 [b]
Cecectomized birds					
Premium (P)	1,039 [a]	1,703 [c]	26.33 [c]	17.97	5.05 [a]
P + 1kg/t PKCase	1,269 [c]	1,933 [b]	32.27 [b]	19.94	5.29 [a]
PretPKC [2]	1,225 [c]	1,853 [b]	25.33 [c]	18.12	5.46 [a]
Overall mean	1,322	1,908	32.18	19.94	5.29
Pooled SEM [3]	40	28	1.05	0.81	0.21

[1] Data represent mean of six cockerels

[2] Solvent-extracted PKC was incubated with 1kg/t of PKCase under 30 °C for 24 h. PKCase is an enzyme mixture designed by Alltech, Inc. (Kentucky, USA) especially for PKC. It contains mannanase, α-galactosidase and protease activity.

[3] Standard error of the mean

[a,b,c] Means in the same column with different superscripts differ significantly ($P < 0.05$)

DISCUSSION

Palm kernel cake samples used in this study contained a high dry matter content, a moderate amount of protein, a ratio of calcium to phosphorus of about 1:2 and a high level of crude fiber, NDF and ADF. Electron micrographs reveal that palm kernels contained a large amount of oil, hence the efficiency of the oil extraction process will have a major impact on the amounts of oil residue in PKC. The high values of ADF, NDF and ADL indicate that PKC is comprised mainly of cell wall material and this is confirmed by the appearance of the palm kernel under the electron microscope. The cell walls in PKC are mainly made up of complex carbohydrates such as mannans (Dusterhoft and Voragen, 1991; Dusterhoft *et al.,* 1992; Daud and Jarvis, 1992). Poultry do not possess the enzyme (mannanase) required to hydrolyze mannans in PKC. Therefore, the crystalline and insoluble mannans are not likely to be depolymerized under the digestive tract conditions in fowl (Daud and Jarvis, 1992). Thus, the potential of feed enzyme (with mannanase activity) supplementation of PKC-based diets remains promising.

It is very likely that during the extraction of oil from palm kernels that some of the cell wall structures observed in the electron scanning micrographs will be distorted and damaged. This should facilitate the release of some of the nutrients within the cells. However, it is also likely that large amounts of nutrients will remain trapped within the cells and be unavailable to the animals. The high fiber content, the lack of mannanase to break down mannans and the likelihood of entrapment of nutrients in the cells could be the reasons for the poorer performance of birds fed PKC-based diets as reported by Ahmad (1982), Longe (1984) and Osei and Amo (1987).

The gross energy content of PKC is dependent on the amount of oil residue, which is dependent on the efficiency of palm kernel oil extraction. Solvent extraction plants are more efficient in extracting oil and this is reflected in the lower oil content and energy level of its PKC. It was interesting to note that PKC from solvent extraction plants contained significantly lower amounts of ADL. I believe that this is not due to different varieties of oil palm used. Due to the nature of oil extraction conditions in the screw-press plants, palm kernels are exposed to extremely high temperature and pressure, which is likely the reason for the darker color of the screw-pressed PKC. The reactions of proteins with reducing sugars (Maillard or browning reactions) are perhaps the most common cause of nutritional damage to proteins during high

temperature and pressure food processing (Hurrell and Finot, 1985). It is likely that the reactions between proteins and reducing sugars (Maillard reactions) during the oil extraction process led to the formation of heat-damaged products that are more resistant to acid digestion in the ADL procedure, leading to a higher ADL content in screw-pressed PKC. The heat-damaged protein, in turn, might also be more resistant to acid hydrolysis during AA analyses and may have reduced the release of individual AA and their recovery. This might be the reason for the lower quantity of most AA in screw-pressed PKC.

When compared with corn or soybean meal, the ratio of AA in PKC in relation to the NRC (1994) requirement was far from balanced (Tables 5.11, 5.12 and 5.13). The order of limiting AA (in descending order) in PKC is cystine, lysine, threonine, methionine, isoleucine and histidine for starter broiler chicks (0-3 week old). On the other hand, corn is limiting only in lysine, tryptophan and arginine whereas soybean meal is limiting only in the sulfur AA (Table 5.11). For grower broiler chicks (3-6 week old), PKC is limiting in lysine, cystine, threonine, isoleucine, histidine and methionine (in descending order). Corn is limiting in lysine, tryptophan, arginine, threonine and isoleucine whereas soybean meal is limiting only in the sulfur AA (Table 5.12). For the laying hens, PKC is limiting in cystine, lysine, isoleucine, tryptophan, methionine, threonine and valine. Corn, on the other hand, is limiting only in lysine, tryptophan, isoleucine and arginine whereas soybean meal is limiting only in the sulfur AA (Table 5.13). It is important to note that PKC contains a high level of arginine (approximately 200% of NRC (1994) requirement) and a low level of lysine. Special attention should be paid to the adverse arginine : lysine ratio during feed formulation because excess arginine has been shown to depress growth of chicks fed a lysine-deficient diet and this adverse effect was reversed by supplementary lysine (D'Mello and Lewis, 1970). Soybean meal protein has a better AA balance than PKC protein. As a result, it is not very likely that PKC can replace much soybean meal in feed formulation. Palm kernel cake could replace corn to a greater extent if oil could be brought in to offset the low metabolizable energy of PKC. The failure of PKC to substantially replace soybean meal in feed formulation has been demonstrated in the studies of Ahmad (1982), Yeong and Mukherjee (1983) and Onifade and Babatunde (1998). With the commercial availability of crystalline AA such as lysine, methionine, threonine and tryptophan, the quality of PKC could be greatly improved.

Table 5.11 Amino acid composition (% of CP) of palm kernel cake (PKC), corn and soybean meal (SBM, 44%CP) in relation to the NRC (1994) requirements for the growth of 0-3 week old broilers [1] (90% DM).

Amino acids	NRC (1994)	PKC (% of NRC)	Corn (% of NRC)	SBM (% of NRC)
Arginine	5.43	10.67 (197%)	4.57 (84%)	7.29 (134%)
Lysine	4.78	2.47 (52%)	3.13 (65%)	6.23 (130%)
Methionine	2.17	1.68 (78%)	2.17 (100%)	1.44 (66%)
Cystine	1.74	0.88 (51%)	2.17 (125%)	1.53 (88%)
Threonine	3.48	2.65 (76%)	3.49 (100%)	3.99 (115%)
Tryptophan	0.87	0.89 (102%)	0.73 (83%)	1.71 (197%)
Valine	3.91	4.47 (114%)	4.82 (123%)	4.80 (123%)
Isoleucine	3.48	3.02 (87%)	3.49 (100%)	4.54 (130%)
Leucine	5.22	5.48 (105%)	12.07 (231%)	7.86 (151%)
Histidine	1.52	1.37 (90%)	2.77 (182%)	2.71 (179%)
Phenylalanine	3.13	3.49 (112%)	4.57 (146%)	5.01 (160%)

[1]Values for Corn and SBM were obtained from the National Research Council (NRC, 1994)

Table 5.12 Amino acid composition (% of CP) of palm kernel cake (PKC), corn and soybean meal (SBM, 44%CP) in relation to the NRC (1994) requirements for the growth of 3-6 week old broilers [1] (90% DM).

Amino acids	NRC (1994)	PKC (% of NRC)	Corn (% of NRC)	SBM (% of NRC)
Arginine	5.50	10.67 (194%)	4.57 (83%)	7.29 (132%)
Lysine	5.00	2.47 (49%)	3.13 (63%)	6.23 (125%)
Methionine	1.90	1.68 (89%)	2.17 (114%)	1.44 (76%)
Cystine	1.70	0.88 (52%)	2.17 (128%)	1.53 (90%)
Threonine	3.70	2.65 (72%)	3.49 (94%)	3.99 (108%)
Tryptophan	0.90	0.89 (99%)	0.73 (81%)	1.71 (190%)
Valine	4.10	4.47 (109%)	4.82 (117%)	4.80 (117%)
Isoleucine	3.65	3.02 (83%)	3.49 (96%)	4.54 (124%)
Leucine	5.45	5.48 (101%)	12.07 (221%)	7.86 (144%)
Histidine	1.60	1.37 (86%)	2.77 (173%)	2.71 (170%)
Phenylalanine	3.25	3.49 (107%)	4.57 (141%)	5.01 (154%)

[1]Values for Corn and SBM were obtained from the National Research Council (NRC, 1994)

Table 5.13 Amino acid composition (% of CP) of palm kernel cake (PKC), corn and soybean meal (SBM, 44%CP) in relation to the NRC (1994) requirements for laying hens [1] (90% DM).

Amino acids	NRC (1994)	PKC (% of NRC)	Corn (% of NRC)	SBM (% of NRC)
Arginine	4.67	10.67 (229%)	4.57 (98%)	7.29 (156%)
Lysine	4.60	2.47 (54%)	3.13 (68%)	6.23 (136%)
Methionine	2.00	1.68 (84%)	2.17 (108%)	1.44 (72%)
Cystine	1.87	0.88 (47%)	2.17 (116%)	1.53 (82%)
Threonine	3.13	2.65 (85%)	3.49 (111%)	3.99 (127%)
Tryptophan	1.07	0.89 (83%)	0.73 (68%)	1.71 (160%)
Valine	4.67	4.47 (96%)	4.82 (103%)	4.80 (103%)
Isoleucine	4.33	3.02 (70%)	3.49 (81%)	4.54 (105%)
Leucine	5.47	5.48 (100%)	12.07 (221%)	7.86 (144%)
Histidine	1.13	1.37 (121%)	2.77 (245%)	2.71 (240%)
Phenylalanine	3.13	3.49 (112%)	4.57 (146%)	5.01 (160%)

[1]Values for Corn and SBM were obtained from the National Research Council (NRC, 1994). Requirement values were based on 100 g intake per hen per day.

The Maillard reactions may also lead to the formation of inter- and intra-molecular cross-linkages within PKC proteins, which reduces the overall protein digestibility and the bioavailability of all AA. The lysine molecule is destroyed during advanced Maillard reactions, as are other AA such as tryptophan, methionine and cystine, presumably through reacting with active intermediate compounds such as dicarbonyls and aldehydes (Finot *et al.*, 1982; Hurrell *et al.*, 1983). These cross-linkages also reduce the rate of protein digestion by preventing enzyme penetration or by masking the sites of enzyme attack (Hurrell and Finot, 1985). This may explain the significantly lower digestibilities of some AA in screw-pressed PKC (Table 5.4). The relative amounts of individual AA in PKC and their digestibility values found in this study are in close agreement with those of Yeong (1983). However, the mean AA digestibility coefficient found in the present study was lower than those reported by Nwokolo *et al.* (1976) and Onwudike (1986). The average digestibility of AA in PKC in the present study was 61.7% compared to 48%, 64.4%, 84.5% and 83.3% reported by Vilarino *et al.* (1996), Yeong (1983), Nwokolo *et al.* (1976) and Onwudike (1986), respectively. It was also interesting to find that the lysine digestibility in PKC was approximately 90% as reported by Nwokolo *et al.* (1976) and Onwudike (1986), whereas the values of 58.6%, 26% and 43.8% were reported by Yeong (1983), Vilarino *et al.* (1996) and the present study, respectively. The reasons for these

discrepancies are not clear. However, the AA digestibility values determined in the present study were derived from cecectomized cockerels, whereas those reported by others were obtained using intact cockerels. Johns *et al.* (1986) have clearly demonstrated that an excreta digestibility technique gave appreciably higher AA digestibility values when heat-damaged meat and bone meal was fed to intact rather than to cecectomized cockerels. Screw-pressed PKC was used in the studies conducted by Nwokolo *et al.* (1976), Onwudike (1986) and Vilarino *et al.* (1996), whereas solvent-extracted PKC was used by Yeong (1983). Both types of PKC were used in the present study and both were found to have lower AA digestibility than that reported by Nwokolo *et al.* (1976) and Onwudike (1986). In the present study, solvent-extracted PKC was found to contain significantly higher levels of digestible AA than screw-pressed PKC. Probably screw-pressed PKC was exposed to a higher temperature during the oil extraction process. Due to the harsh environment during the processing of palm kernels and the susceptibility of most AA to heat damage, I feel that the AA digestibility values reported by Nwokolo *et al.* (1976) and Onwudike (1986) were somewhat overestimated. The poor AA digestibility in PKC is likely to be attributed to protein entrapment within the cells as well as the high temperatures used during the oil extraction process.

The present study found that only about 33% and 40% of gross energy was available as AME and TME_n respectively to poultry. This is one of the main constraints in using PKC for poultry. Siew (1989) reported that solvent extracted PKC from Malaysia contained about 1,480 kcal/kg AME and 1,760 kcal/kg TME_n, while Rhone Poulenc (Rhodimet Nutrition Guide, 1993) reported an AME value of 1,340 kcal/kg. These values agree with the findings of the present study. However, much higher values for AME have been reported by several researchers, ranging from 1,963 kcal/kg to 3,000 kcal/kg (Nwokolo *et al.*, 1977; Ngoupayou, 1984; Nwokolo, 1986; Onwudike, 1986; Panigrahi and Powell, 1991; Vilarino *et al.*, 1996). Some of the AME values reported were somewhat higher than expected. For instance, Ngoupayou (1984) reported an AME value of 3,000 kcal/kg for PKC with 8% ether extract, whereas an AME value of 2,153 kcal/kg was reported by Nwokolo (1986) for PKC with only 2.09% ether extract. In another study, Nwokolo *et al.* (1977) reported an AME value of 2,796 kcal/kg for PKC containing 43.7% ADF, 21.1% ADL and 4,680 kcal/kg of gross energy. These AME values were even higher than the TME_n values reported in the present study. It is not clear how broilers in the study conducted by Nwokolo *et al.* (1977) could metabolize about 60% of the gross energy in PKC when it contained such a high level of fiber and lignin.

Knowledge of the polysaccharide composition of targeted feed ingredients is one of the most important factors for the successful employment of feed enzymes. Recently, the polysaccharide components of PKC have been characterized by Dusterhoft and Voragen (1991), Dusterhoft *et al.* (1992) and Daud and Jarvis (1992). Palm kernel cake was found to contain mainly linear mannans (polymer of mannose), moderate amounts of cellulose, and small amounts of other polysaccharides. As a result, it is not surprising to find that PKCase (containing mannanase, protease and α-galactosidase) is the most effective enzyme mixture for breaking down the mannans in PKC. Other enzymes such as β-glucanase, pentosanase and protease were not effective in breaking down the cell walls of PKC. Cellulase worked fairly well with PKC, however it was still inferior to PKCase. These findings reflect the polysaccharide composition of PKC. A synergistic reaction between cellulase and PKCase was not observed in this study. This is in agreement with the conclusions of Dusterhoft *et al.* (1993b) and Daud *et al.* (1997) that neither mannan degradation enhances cellulose hydrolysis nor cellulose degradation enhances mannan hydrolysis. The determined optimum pH and temperature for mannanase activity (in PKCase) were in agreement with the study of Mendoza *et al.* (1994), who found that the optimum pH and temperature ranged from 5.0 to 6.0 and 50 °C to 60 °C, respectively, for a crude enzyme excreted by a mannan-utilizing bacterium. PKCase worked very well under the pH conditions of the gastro-intestinal tract (Figure 5.5). However, only 40% of the optimum enzyme activity was attainable under the 40 °C temperature found in fowl. This implies that a further increase in temperature would be necessary for greater improvements in the saccharification of PKC.

With the exception of one sample, up to 50% more reducing sugars were released from various PKC samples with enzyme treatment. This indicates that PKCase acts consistently across various PKC samples. This is an important feature for the prediction of the improvement in animal performance when PKC from various sources is used. As expected, PKCase did not have any effect on the release of reducing sugars from corn because starch was the only major polysaccharide present. On the other hand, the beneficial effect of PKCase on soybean meal was expected. This is because PKCase also contained α-galactosidase activity that could break down the non-starch polysaccharides (mainly polymers of galactose) of soybean meal. Even though not measured, protease activity present in PKCase should also have a beneficial effect on protein and AA digestion in PKC, corn and soybean meal. In addition, using twice the amount of PKCase

further increased the breakdown of cell walls of PKC and soybean meal. However, the amount of reducing sugars released was not proportional to the quantity of PKCase used.

Most grains and seeds contain endogenous enzymes required for the breakdown of storage polysaccharides during germination. Soaking and wetting barley and wheat have been shown to improve the performance of broiler chicks fed diets containing these pretreated ingredients and this has been attributed to the activation of enzymes in the grain (Svihus *et al.*, 1997; Yasar and Forbes, 1997). Enzyme pretreatment of wheat has also been shown to improve broiler performance by reducing the gut's viscosity, and increasing the digestibility of protein, fat, ash, as well as the energy (Svihus *et al.*, 1997). However, pretreatment of PKC with PKCase did not have a major effect on most of the nutrients. This is perhaps due to low enzyme activities at the room temperature of 30 °C. Crude fiber content in the pretreated PKC was significantly ($P < 0.05$) reduced by 1.7%. Even though this reduction was most likely due to the effect of the enzymes, it is also possible that natural fungus originally present on the PKC samples might have helped the digestion of fiber during the 24 h incubation period. The reduction in fiber content was associated with an increase in AME and TME_n values measured with cecectomized cockerels. Data in the present study clearly showed that PKCase is capable of increasing the AME and TME_n values of PKC, the magnitude of increase being even greater with cecectomized cockerels. The AME and TME_n values of PKC were increased by 13% and 9.6% respectively by PKCase in normal intact cockerels. With cecectomized cockerels, it went up by 22% and 13.5%, respectively. Without PKCase supplementation, the AME and TME_n values of PKC were reduced significantly when measured in cecectomized birds, indicating that the ceca might be taking part in the digestion of fiber.

Thornburn and Willcox (1965) investigated the role of ceca in the digestion of crude fiber using intact and cecectomized birds. They found that the overall dry matter digestibility of four feeds (whole wheat, whole oats, whole barley and mixed feed in pellet form) was reduced by cecectomy. This is in agreement with the findings presented in here. However, Thornburn and Willcox (1965) also stated that even though cellulose digestibility was reduced in individual birds after cecectomy, the reduction was not always apparent. This is because the avian rectum had microflora capable of degrading fiber as Duke *et al.* (1984) reported that cecectomized turkeys still degraded 7% of dietary cellulose.

Because of the presence of ceca in intact cockerels, it was expected that ADF and NDF digestibility values for intact cockerels might be higher than that of cecectomized cockerels, if

ceca are involved in the digestion of fiber. However, this was not observed in this study. Perhaps the ceca is not involved in fiber digestion as Mattocks (1971) found that no fiber entered the ceca in domestic goose and concluded that it seems unlikely that ceca contribute to the feed's utilization by cellulose digestion. Researchers from Japan also concluded that ceca do not play a significant part in crude fiber digestion in domesticated birds (Nakahiro and Isshiki, 1975). Nevertheless, ceca have a definite role in the wild state that they can allow birds to conserve water, nitrogen and energy (McNab, 1973).

On the other hand a study conducted by Savory (1992) discovered that degradation of cellulose by the intestinal microflora does occur normally in (conditioned) fowls. Other researchers (Gasaway, 1976 and Duke *et al.*, 1984) emphasized that the extent to which measurable cellulose digestion in birds takes place depends on preconditioning with high fiber diets. When not used for the metabolizable energy study, cockerels were fed low-fiber broiler diets (therefore, not preconditioned with high fiber diets) in the present study. This might partly explain the non-significant difference in NDF digestion between the cecectomized and intact cockerels. However, it does not explain why the digestion of ADF was higher in cecectomized birds.

Conclusions

The results of the present study showed that PKC contained a moderate quantity of most nutrients. The digestibility of AA and metabolizable energy is influenced by the method of oil extraction. The AA in PKC are not well balanced to meet poultry requirements and several AA are limiting. However, crystalline AA could be used to improve PKC protein quality. Due to the low digestibility of AA in PKC, formulating poultry diets containing PKC on a digestible AA basis should be an improvement over formulating on a total AA basis. There are indications that the low digestibility of AA in PKC is attributed to protein entrapment within cells as well as to products formed due to the high temperatures used during the oil extraction process. Similarly, the high fiber content and the lack of appropriate enzymes for breaking it down could have led to the low AME and TME_n contents in PKC. PKCase (mannanase, α-galactosidase and protease) was found to be very effective in breaking down the polysaccharide component of PKC. PKCase supplementation increased the AME and TME_n of PKC in both intact and cecectomized cockerels. However, cecectomy reduced the AME and TME_n of PKC. Pretreatment of PKC with

enzymes did not seem to be justified due to the high cost of drying after treatment. In addition, there are risks of mold infestation during the drying processes. Given its beneficial effects on PKC, further studies investigating the effects of PKCase on broiler and layer performance are warranted.

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CHAPTER VI

Effects of Dietary Inclusion of Palm Kernel Cake and Enzyme Supplementation on Broiler and Layer Performance

Summary

A total of 400 day-old female broiler chicks and 392 twenty eight weeks old laying hens was used to study the effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme (mixture of mannanase, α-galactosidase and protease) supplementation of PKC on broiler and layer performance. In the 6 week broiler study, a corn-soybean meal control diet and 20% PKC diets with or without enzyme supplementation (1 or 2 kg/t) were used. Palm kernel cake-based diets were included in either the starter phase, or grower phase or in both phases. In the 8 week layer study, two levels of PKC (12.5% and 25%) and three levels of enzymes (0, 1 or 2 kg/t) were arranged in a factorial manner to form six experimental PKC diets. A corn-soybean meal layer diet was used as the control. Enzyme supplementation improved the digestibility of PKC-based diets and the performance of broilers and layers fed PKC-based diets. The broiler study found that 20% PKC could be used during the broiler starter phase or the broiler grower phase but not during both the starter and grower phases unless supplemental enzyme was used. Mortality due to heat stress was significantly higher ($P < 0.05$) in broilers fed PKC-based grower diets without enzyme supplementation. Broilers fed PKC diets with or without enzyme supplementation deposited significantly more ($P < 0.05$) abdominal fat than controls. Dietary inclusion of 12.5% and 25% PKC in layer diets did not adversely affect mean egg production or daily egg mass. However, layers consumed significantly more ($P < 0.05$) PKC-based diets and had significantly poorer ($P < 0.05$) FCR than controls. Enzyme supplementation of PKC-based layer diets decreased feed intake and FCR significantly ($P < 0.05$). Dietary inclusion of PKC or enzyme did not affect eggshell quality, but egg yolk color became significantly paler ($P < 0.05$) when layers were fed the 25% PKC diet. Parametric linear programming was used to investigate the price at which PKC (with or without 1kg/t enzyme) could be used by a computerized feed formulation program and at what level of inclusion. It was found that the use of PKC in poultry diets is dependent on the metabolizable energy of the diet, the price of PKC, and the AME value

of PKC. Because of its low nutrient contents, PKC is more suited for use in the lower energy layer diets than in the higher energy broiler diets.

Key words: Palm kernel cake, enzyme, broiler, layer, feed formulation

Introduction

As the world's human population continues to increase and the acres of farmland continue to decrease, the use of feedstuffs that are not directly available to humans for food is increasingly important. Similar concerns have been raised by Berepubo *et al.* (1995), who emphasized that in most developing countries, especially Nigeria, feed resources for livestock and poultry production have become increasingly expensive because of the increasing competition between humans and domesticated animals for scarce grains and protein feeds. More recently, Sheldon (1998) has also emphasized the importance of developing alternative and cheaper feed ingredients in order to avoid competition with those essential for humans. From this standpoint, it is therefore logical to look for alternative feeds that are not consumable by humans but are acceptable to farm animals.

Palm oil has become a major vegetable oil in the past few decades and many developing countries including Malaysia have devoted a huge amount of farmland for oil palm (*Elaeis guineensis* Jacq.) plantations (PORLA, 1997). One of the most important by-products of palm oil production is palm kernel cake (PKC). Even though large quantities of PKC are available for feed, the use of PKC in the feed industry is mainly limited to the ruminant sector. Palm kernel cake is not widely used in the poultry industry because of its high fiber and low energy contents. Furthermore, information regarding the nutrient composition of PKC is limited and there are great discrepancies in the reported values. Although the subject of using PKC in poultry diets has been studied by several researchers, the recommended levels of inclusion seem to vary from one study to another. Onifade and Babatunde (1998) found that broiler growth performance was depressed in birds fed diets containing as little as 10% PKC when compared to a corn-soybean meal diet. However, studies conducted by Ahmad (1982) and Garcia and Gernat (1998) indicated that dietary inclusion of 10% PKC did not reduce growth of broiler chicks when compared with a corn-soybean meal control diet. Osei and Amo (1987), on the other hand, reported that inclusion of PKC at 12.5% and 15% of the diet reduced growth and feed efficiency.

Even though Longe (1984) reported poorer egg production for layers fed 20% PKC, other authors found that 20% PKC could be used in broiler and layer diets without reducing performance (Yeong, 1980; Hutagalung, 1980; Yeong and Mukherjee, 1983; Ngoupayou, 1984). Still other researchers have reported that levels as high as 40% for layers and 50% for broilers could be used without reducing performance (Onwudike, 1988; Panigrahi and Powell, 1991).

The reasons for these wide variations in reported inclusion levels are not clear. Perhaps, they are caused by the variety of oil palm used in different countries, methods of processing palm kernel oil, age of the birds, and duration and design of the experiments. Recently, several studies have identified the potential of using exogenous enzymes to degrade the non-starch polysaccharides of PKC (Dusterhoft *et al.*, 1993a,b,c; Daud *et al.*, 1997). Previous studies (Chapter V) also found that an enzyme mixture (PKCase) from Alltech, Inc. (Kentucky, USA) has the ability to increase the metabolizable energy of PKC. Therefore, the objectives of the following experiments were to determine the effects of different inclusion levels of PKC and enzyme supplementation on broiler and laying hen performance under hot and humid tropical conditions.

Materials & Methods

A 6 week broiler study and an 8 week layer study were conducted. True dry matter digestibility (TDMD), nitrogen corrected true metabolizable energy (TME_n) and apparent metabolizable energy (AME) of broiler and layer diets were determined in a digestibility study. Palm kernel cake obtained from a solvent extraction plant was used throughout the study. An enzyme mixture (Alltech PKCase) containing mannanase, α-galactosidase and protease activities was used in the broiler and layer studies.

Broiler Growth Study

A total of 400 female day-old broiler chicks (Hubbard) was used in the study. The chicks were vaccinated against Marek's disease at the hatchery and against Newcastle disease at 7 and 21 days of age during the experimental period. Birds were housed in battery brooders (0.9 m

wide x 1.2 m length x 0.5 m height) in an open-sided building at the Poultry Research Unit at the Universiti Putra Malaysia, Malaysia. All chicks were wing-banded at one day of age and were randomly distributed to 40 battery pens (10 birds per pen). Birds received 24 h of light daily and had free access to feed and water. Individual body weights of the birds were measured at day old and then weekly until 6 weeks of age. Feed intake was also measured weekly. Maximum and minimum temperatures within the building were measured daily. Daily mortalities were recorded and the carcasses were sent to the veterinary pathology laboratory at the Universiti Putra Malaysia for post-mortem examination. On the last day of the experiment, five birds from each dietary treatment were sacrificed for the measurement of abdominal fat. Abdominal fat was removed from the gizzard to the cloaca following the procedures outlined by Cahaner *et al.* (1986). In order to investigate whether broilers fed diets with different level of fiber would result in a higher body temperature, eight birds from each dietary treatment group were also randomly selected and measured for rectal temperature in the afternoon (1:00 p.m.) during the last day of the experiment.

Broiler Dietary Treatment

A total of four starter diets (S1, S2, S3, S4) and four grower diets (G1, G2, G3, G4) were used in the study. Diets S1 and G1 were the control diets that did not contain PKC or enzymes, while the rest of the diets contained 20% PKC with or without enzyme supplementation. The compositions of the starter and grower diets are shown in Tables 6.1 and 6.2, respectively. The levels of nutrients in the diets were formulated according to the NRC (1994) recommendation for broilers.It was also the objective of this study to determine whether feeding chicks diets containing PKC at different phases would yield different results. Three different phases were used: Starter phase = PKC was included only in the starter diets; Grower phase = PKC was included only in the grower diets; Both phases = PKC was included in both starter (0-3 weeks) and grower (3-6 weeks) diets. The enzyme (PKCase) was included in the PKC diets at three levels (0, 1, 2 kg/t). Three phases and three levels of the enzyme were arranged in a factorial manner to yield nine dietary treatments (Table 6.3). Control starter and grower diets were used in the control dietary treatment. As a result, there was a total of ten dietary treatments in the study. There were four replications of ten birds per dietary treatment.

Table 6.1 Nutrient composition of broiler starter (0-3 week) diets.

Ingredients (%)	Diet S1 Control starter	Diet S2 PKC starter	Diet S3 PKC starter + 1E[1]	Diet S4 PKC starter + 2E[1]
Corn	53.89	29.28	29.18	29.08
Soybean meal	36.19	34.18	34.18	34.18
Fishmeal	3.00	3.00	3.00	3.00
Palm kernel cake [2] (PKC)	0.00	20.00	20.00	20.00
Palm oil	3.73	10.44	10.44	10.44
Choline chloride (60%)	0.25	0.25	0.25	0.25
Vit-min premix [3]	0.10	0.10	0.10	0.10
Salt (NaCl)	0.20	0.21	0.21	0.21
Antioxidant [4]	0.01	0.02	0.02	0.02
PKCase [5]	-	-	0.10	0.20
DL-methionine	0.18	0.19	0.19	0.19
Limestone	1.30	1.18	1.18	1.18
Dicalcium phosphate	1.15	1.15	1.15	1.15
Total	100.00	100.00	100.00	100.00
Calculated composition (% air-dry unless otherwise indicated)				
Nutrients				
AME, kcal/kg	3,000	3,000	3,000	3,000
Crude protein	21.30	21.37	21.37	21.37
Arginine	1.45	1.73	1.73	1.73
Lysine	1.25	1.25	1.25	1.25
Methionine	0.50	0.50	0.50	0.50
Methionine + cystine	0.90	0.90	0.90	0.90
Tryptophan	0.32	0.32	0.32	0.32
Threonine	0.85	0.86	0.86	0.86
Crude fat	6.31	12.70	12.70	12.70
Crude fiber	3.80	5.76	5.76	5.76
Calcium	1.02	1.03	1.03	1.03
Available phosphorus	0.45	0.45	0.45	0.45
Determined composition (% air-dry basis otherwise indicated)				
Nutrients				
Dry matter	90.02	91.22	90.73	91.00
AME, kcal/kg	3,176	3,094	3,257	3,189
Crude protein	21.95	22.30	22.23	21.87
Crude fat	6.09	11.90	11.38	12.30
Crude fiber	4.01	6.11	6.06	5.94
Ash	6.08	6.13	6.55	6.37
Calcium	1.00	1.10	1.05	1.12
Total phosphorus	0.70	0.75	0.77	0.74

[1] Levels of enzyme. 1E = 1 kg enzyme per tonne of feed; 2E = 2 kg enzyme per tonne of feed.
[2] Solvent-extracted palm kernel cake.
[3] Supplied per kg diet: Fe 100 mg; Mn 110 mg; Cu 20 mg; Zn 100 mg; I 2 mg; Se 0.2 mg; Co 0.6 mg; santoquin 0.6 mg; vitamin A 6,667 IU; vitamin D 1,000 IU; vitamin E 23 IU; vitamin K3 1.33 mg; cobalamin 0.03 mg; thiamin 0.83 mg; riboflavin 2 mg; folic acid 0.33 mg; biotin 0.03 mg; pantothenic acid 3.75 mg; niacin 23.3 mg; pyridoxine 1.33 mg.
[4] FRA® OX Dry, Franklin Products International B. V.
[5] Enzyme mixture, Alltech Inc. USA: it contains mannanase, α-galactosidase and protease activity.

Table 6.2 Nutrient composition of broiler grower (3-6 week) diets.

Ingredients (%)	Diet G1 Control Grower	Diet G2 PKC Grower	Diet G3 PKC Grower + 1E[1]	Diet G4 PKC Grower + 2E [1]
Corn	60.30	35.96	35.85	35.76
Soybean meal	31.86	29.62	29.62	29.62
Fishmeal	3.00	3.00	3.00	3.00
Palm kernel cake [2] (PKC)	0.00	20.00	20.00	20.00
Palm oil	2.44	9.11	9.11	9.11
Choline Chloride (60%)	0.20	0.20	0.20	0.20
Vit-min premix [3]	0.10	0.10	0.10	0.10
Salt (NaCl)	0.10	0.12	0.12	0.12
Antioxidant [4]	0.01	0.02	0.02	0.02
PKCase [5]	-	-	0.10	0.20
DL-methionine	0.033	0.048	0.048	0.048
Limestone	1.30	1.18	1.18	1.18
Dicalcium phosphate	0.65	0.65	0.65	0.65
Total	100.00	100.00	100.00	100.00
Calculated composition (% air-dry unless otherwise indicated)				
Nutrients				
AME, kcal/kg	3,000	3,000	3,000	3,000
Crude protein	20.00	20.00	20.00	20.00
Arginine	1.34	1.61	1.61	1.61
Lysine	1.15	1.14	1.14	1.14
Methionine	0.38	0.38	0.38	0.38
Methionine + cystine	0.72	0.72	0.72	0.72
Tryptophan	0.29	0.29	0.29	0.29
Threonine	0.80	0.80	0.80	0.80
Crude fat	5.22	11.58	11.58	11.58
Crude fiber	3.65	5.59	5.59	5.59
Calcium	0.90	0.91	0.91	0.91
Available phosphorus	0.35	0.35	0.35	0.35
Determined composition (% air-dry unless otherwise indicated)				
Nutrients				
Dry matter	89.87	91.21	91.28	91.24
AME, kcal/kg	3,146	2,958	3,155	3,029
Crude protein	19.74	20.21	19.72	19.99
Crude fat	5.52	10.55	10.32	10.20
Crude fiber	3.14	5.02	5.13	5.09
Ash	5.39	6.15	5.89	5.71
Calcium	0.92	0.88	0.90	0.92
Total phosphorus	0.59	0.64	0.69	0.66

[1] Levels of enzyme. 1E = 1 kg enzyme per tonne of feed; 2E = 2 kg enzyme per tonne of feed.

[2] Solvent-extracted palm kernel cake

[3] Supplied per kg diet: Fe 100 mg; Mn 110 mg; Cu 20 mg; Zn 100 mg; I 2 mg; Se 0.2 mg; Co 0.6 mg; santoquin 0.6 mg; vitamin A 6,667 IU; vitamin D 1,000 IU; vitamin E 23 IU; vitamin K3 1.33 mg; cobalamin 0.03 mg; thiamin 0.83 mg; riboflavin 2 mg; folic acid 0.33 mg; biotin 0.03 mg; pantothenic acid 3.75 mg; niacin 23.3 mg; pyridoxine 1.33 mg.

[4] FRA® OX Dry, Franklin Products International B. V.

[5] Enzyme mixture, Alltech Inc. USA: it contains mannanase, α-galactosidase and protease activity.

Table 6.3 Arrangement of dietary treatments for the broiler study [1]

	0 kg/t enzyme	1 kg/t enzyme (1E)	2 kg/t enzyme (2E)
Both	20% PKC starter (S2) 20% PKC grower (G2) Treatment 2	20% PKC starter + 1E (S3) 20% PKC grower + 1E (G3) Treatment 5	20% PKC starter + 2E (S4) 20% PKC grower + 2E (G4) Treatment 8
Starter	20% PKC starter (S2) Control grower (G1) Treatment 3	20% PKC starter + 1E (S3) Control grower (G1) Treatment 6	20% PKC starter + 2E (S4) Control grower (G1) Treatment 9
Grower	Control starter (S1) 20% PKC grower (G2) Treatment 4	Control starter (S1) 20% PKC grower + 1E (G3) Treatment 7	Control starter (S1) 20% PKC grower + 2E (G4) Treatment 10

[1] Treatment 1 (control group) = control starter and control grower diets

Digestibility of Broiler Diets

True dry matter digestibility (TDMD), apparent metabolizable energy (AME) and nitrogen corrected true metabolizable energy (TME_n) of all starter and grower diets were determined using Sibbald's procedures (1986) as outlined in Chapter V and six cockerels were assigned to each diet.

Layer Production Study

A total of 392 Lohmann Brown laying hens (28 weeks old) was used in this 8 weeks study. All birds were housed in single-bird wire cages (0.3 m wide x 0.4 m length x 0.4 m height); eight layers fed from a single trough, constituted a replicate. There were seven replications for each diet. A total of seven rows in an open-sided laying house was used and the birds received 16 h of light per day. It was considered that rows closer to the open side might experience higher temperatures. As a result, a randomized block design was used for the study in which the seven diets were randomly assigned to each row (or block). Maximum and minimum temperatures were recorded daily in at least two locations within the building. Feed intake per replicate was determined weekly. Eggs were collected once daily (10:30 a.m.) and egg production was recorded. Eggs collected were classified as normal, cracked, deformed-shell, soft-shell or no shell. All eggs produced during a 2-day period (Tuesday and Wednesday) every week were individually weighed. Egg specific gravity was measured once weekly (Wednesday) on all eggs collected on a single day. The saline solutions for egg specific gravity measurements were in increments of 0.005 and ranged from 1.060 to 1.100. Before every measurement, specific

gravity solutions were verified with a hydrometer (ZEAL, England), and water or salt was added as required. During the last day of the experiment, four eggs from each replicate were broken and measured for yolk color with a Roche's Yolk Color Fan.

Layer Dietary Treatments

A total of seven layer diets was used in this study. Diet L1 was set as control diet (without PKC and PKCase) and fed to layers in the control group. Two levels of PKC (12.5 and 25%) and three levels of enzyme (PKCase) were arranged in a factorial manner to yield another six diets (Table 6.4). Diets L2-L7 were fed to layers in the experimental groups. The compositions of the diets are shown in Table 6.5. Most of the nutrients recommended by the NRC (1994) for brown egg layers are derived from study with white egg layers. Therefore, the NRC (1994) recommendations for brown egg layers might not be accurate. As a result, the nutrient levels recommended by the Lohmann Brown breeder company was followed during the formulation of the layer diets.

Table 6.4 Arrangement of dietary treatments for the layer study [1]

	0 kg/t enzyme	1 kg/t enzyme (1E)	2 kg/t enzyme (2E)
12.5% PKC	12.5% PKC Diet L2	12.5% PKC + 1E Diet L3	12.5% PKC + 2E Diet L4
25% PKC	25% PKC Diet L5	25% PKC + 1E Diet L6	25% PKC + 2E Diet L7

[1] Diet L1 = control corn-soybean meal diet without palm kernel cake (PKC) or enzyme supplementation

Digestibility of Layer Diets

True dry matter digestibility, AME and TME_n of all layer diets were determined using Sibbald's procedures (1986) as outlined in Chapter V and six cockerels were assigned to each diet.

Table 6.5 Nutrient composition of layer diets.

Ingredients (%)	Diet L1 Control	Diet L2 12.5% PKC	Diet L3 12.5% PKC + 1E[1]	Diet L4 12.5% PKC + 2E	Diet L5 25% PKC	Diet L6 25% PKC + 1E	Diet L7 25% PKC + 2E
Corn	58.46	41.18	41.08	40.98	25.79	25.69	25.59
Soybean meal	28.24	28.68	28.68	28.68	27.42	27.42	27.42
Fishmeal	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Palm kernel cake [2] (PKC)	0.00	12.50	12.50	12.50	25.00	25.00	25.00
Palm oil	1.15	5.64	5.64	5.64	9.83	9.83	9.83
Limestone	9.10	8.95	8.95	8.95	8.90	8.90	8.90
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50
PKCase[3]	0.00	0.00	0.10	0.20	0.00	0.10	0.20
Antioxidant[4]	0.01	0.02	0.02	0.02	0.02	0.02	0.02
Salt (NaCl)	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Vit-min premix[5]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.12	0.11	0.11	0.11	0.11	0.118	0.118
Choline choride (60%)	.0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calculated composition (% air-dry unless otherwise indicated)							
Nutrients							
AME, kcal/kg	2,691	2,691	2,691	2,691	2,690	2,690	2,690
Crude protein	18.20	18.08	18.08	18.08	18.13	18.13	18.13
Arginine	1.32	1.37	1.37	1.37	1.54	1.54	1.54
Lysine	1.04	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Methionine + cystine	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Tryptophan	0.19	0.26	0.26	0.26	0.27	0.27	0.27
Threonine	0.73	0.71	0.71	0.71	0.71	0.71	0.71
Crude fat	3.68	7.83	7.83	7.83	11.80	11.80	11.80
Crude fiber	3.05	4.93	4.93	4.93	6.45	6.45	6.45
Calcium	3.96	3.96	3.96	3.96	3.98	3.98	3.98
Available phosphorus	0.45	0.45	0.45	0.45	0.45	0.45	0.45

Determined composition (% air-dry unless otherwise indicated)

Nutrients							
Dry matter	89.89	91.58	90.58	91.24	91.58	91.54	91.56
AME, kcal/kg	2,889	2,742	3,020	2,983	2,756	2,973	3,101
Crude protein	18.42	18.33	18.93	18.70	18.53	18.47	18.55
Crude fat	4.01	7.66	7.47	7.60	10.85	10.73	11.51
Crude fiber	2.86	4.81	4.64	4.58	6.11	6.06	6.16
Ash	10.89	12.84	11.40	14.27	13.57	13.76	13.41
Calcium	4.00	3.89	3.91	3.85	4.10	4.02	3.99
Total phosphorus	0.66	0.70	0.70	0.70	0.73	0.73	0.73

[1] Levels of enzyme. 1E = 1kg enzyme per tonne of feed; 2E = 2kg enzyme per tonne of feed.
[2] Solvent-extracted palm kernel cake.
[3] Enzyme mixture, Alltech Inc. USA: It contains mannanase, α-galactosidase and protease activity.
[4] FRA® OX Dry, Franklin Products International B. V.
[5] Supplied per kg diet: Fe 100 mg; Mn 110 mg; Cu 20 mg; Zn 100 mg; I 2 mg; Se 0.2 mg; Co 0.6 mg; santoquin 0.6 mg; vitamin A 6,667 IU; vitamin D 1,000 IU; vitamin E 23 IU; vitamin K3 1.33 mg; cobalamin 0.03 mg; thiamin 0.83 mg; riboflavin 2 mg; folic acid 0.33 mg; biotin 0.03 mg; pantothenic acid 3.75 mg; niacin 23.3 mg; pyridoxine 1.33 mg.

Economical Analyses of Diets

In order to determine the cost of using corn, soybean meal, PKC, PKC + 1kg/t enzyme and palm oil to supply energy and protein, the concentrations of energy and protein per unit of price for these ingredients were calculated. Since the enzyme (PKCase) is not currently available at the commercial market, the price of PKC + 1E was set to be equal to PKC without enzyme. Parametric linear programming was used to investigate the price and the level of inclusion at which PKC (with or without 1kg/t enzyme) would be used by a computerized feed formulation program (The Brill Corporation, Georgia 30092, USA). The current prices (Ringgit Malaysia, RM) of commonly used ingredients were obtained from a local feedmill in Malaysia. The price of PKC was changed from RM0 to RM700 per tonne to investigate the effect of the price of PKC on its inclusion rate. In order to reflect the practical situation, the maximum level of oil allowed in the diet was set at 5%. As determined in the previous study (Chapter V), AME values of 1,438 kcal/kg and 1,624 kcal/kg were assigned to PKC and PKC + 1kg/t enzyme, respectively. Nutrient requirements of broiler starters, broiler growers and layers were set according to the NRC (1994) recommendations. Since PKC has a low AME content, it is also the objective of this study to determine whether more PKC could be used in a low AME poultry diet (containing 200 kcal/kg less than the NRC (1994) recommended AME level) than in a high AME poultry diet (containing the NRC (1994) recommended AME level).

Analyses of Samples

Feed and excreta samples were treated and analyzed as in Chapters IV and V.

Statistical Analyses

The broiler study was a factorial experiment in a completely randomized design whereas the layer study was a factorial experiment in a completely randomized block design. All data generated for the experimental diets were subjected to analysis of variance (ANOVA) by using the General Linear Models (GLM) procedure of SAS® software (SAS Institute, 1996). If treatments were found to be significantly different, Tukey's multiple range test (Snedecor and

Cochran, 1980) was used to determine the statistical significance among treatment least-square means.

The results obtained for birds fed the control diet were compared to those from birds fed the experimental diets using a one-way ANOVA by using the General Linear Models (GLM) procedure of SAS® software (SAS Institute, 1996). If treatments were found to be significantly different, the Bonferroni (Dunn) T test (SAS Institute, 1996) was used to determine the statistical significance between treatment least-square means. The Bonferroni (Dunn) T test was used because it will avoid detecting random differences when comparing a large group of treatments (ten dietary treatments in the broiler study and seven dietary treatments in the layer study). Mortality was subjected to Chi-Square analysis.

Results

Broiler Study

During the 0-3 week of the broiler study, inclusion of 20% PKC in the starter diet significantly reduced ($P < 0.05$) the body weights of the 3 week old broiler chicks (Table 6.6). Enzyme supplementation of the PKC-based starter diets improved body weight, and it was significant ($P < 0.05$) when the enzyme level was 2kg/t. There were no significant differences ($P > 0.05$) in 3 week body weight between birds fed control starter diets and PKC-based diets containing 2kg/t of enzyme. Body weight gain (0-3 weeks) was significantly lower ($P < 0.05$) in birds fed PKC-based diets without enzyme supplementation than the other diets. Birds fed control starter diets and PKC-based starter diets containing 2kg/t of enzyme gained significantly more ($P < 0.05$) weight than birds fed PKC-based starter diets containing 0 or 1kg/t of enzyme. Enzyme supplementation of PKC-based diets significantly improved ($P < 0.05$) body weight gain at 3 weeks of age (Table 6.6). Feed intake and feed conversion ratio (FCR) were significantly lower ($P < 0.05$) in birds fed the control diet (S1). Enzyme supplementation of the PKC-based diets (S3 and S4) did not reduce feed consumption but significantly reduced ($P < 0.05$) the FCR of birds fed diet S4 (Table 6.6)

During the 3-6 week of the broiler study, body weight gain was significantly lower ($P < 0.05$) for birds fed PKC-based grower diets without enzyme supplementation than for birds fed the control grower diet (G1) (Table 6.7). Again, enzyme supplementation alleviated the poorer

performance of PKC-fed birds. There were no significant differences in body weight gain (3-6 weeks) between birds fed the control grower diets and enzyme supplemented PKC-based grower diets (Table 6.7). Feed intake was significantly higher ($P < 0.05$) for birds fed enzyme supplemented PKC-based grower diets than for those fed the control grower diets. Feed conversion ratio was significantly lower ($P < 0.05$) for birds fed the control grower diets than for those fed the other grower diets.

Table 6.6 The effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on live body weight (g), weight gain (g), feed intake (g) and feed conversion ratio (FCR) of female broilers at 3 weeks of age.

Starter diet	Body wt. Week 0	Body wt. Week 3	Weight gain Week 0-3	Feed intake (g) Week 0-3	FCR Week 0-3
Control (S1)	44	714 [a]	670 [a]	1090 [b]	1.62 [c]
20% PKC (S2)	44	677 [b]	633 [c]	1143 [a]	1.81 [a]
20% PKC + 1E[1] (S3)	43	691 [b]	648 [b]	1145 [a]	1.77 [ab]
20% PKC + 2E (S4)	43	710 [a]	666 [a]	1156 [a]	1.74 [b]
Overall mean	43	698	654	1134	1.74
Pooled SEM[2]	0.2	2.7	2.7	8.2	0.02

[1] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity; 1E = 1kg/t and 2E = 2kg/t

[2] Pooled standard error of the mean; data represent mean of four replications of 10 birds.

[abc]Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 6.7 The effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on live body weight (g), weight gain (g), feed intake (g) and feed conversion ratio (FCR) of female broilers at 6 weeks of age.

Grower diet	Body wt. Week 6	Weight gain Week 3-6	Feed intake (g) Week 3-6	FCR Week 3-6
Control (G1)	1831 [a]	1132 [a]	2511 [b]	2.22 [b]
20% PKC (G2)	1771 [b]	1084 [b]	2591 [ab]	2.41 [a]
20% PKC + 1E[1] (G3)	1822 [a]	1117 [ab]	2627 [a]	2.35 [a]
20% PKC + 2E (G4)	1831 [a]	1116 [ab]	2629 [a]	2.37 [a]
Overall mean	1814	1112	2590	2.34
Pooled SEM[2]	6.9	5.9	18.1	0.02

[1] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity; 1E = 1kg/t and 2E = 2kg/t

[2] Pooled standard error of the mean; data represent mean of four replications of 10 birds.

[abc]Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

When the whole experimental period was considered (0-6 weeks), no significant differences in 0-6 week weight gain and 0-6 week feed intake were found among birds in dietary treatments 1-10 (Table 6.8). However, birds in dietary treatments 2 and 5 (fed 20% PKC diets throughout with no or 1 kg enzyme per tonne) had significantly poorer ($P < 0.05$) FCR than birds in dietary treatment 1 (control). No significant differences ($P > 0.05$) in FCR were found among birds in dietary treatments 2-10 (Table 6.8).

When the overall data (dietary treatments 2-10) were analyzed as a factorial experiment, overall weight gain (0-6 week) of the birds was not influenced by dietary inclusion of PKC at different phases (both, starter, grower) (Table 6.9). However, enzyme supplementation (1 or 2 kg/t) significantly increased ($P < 0.05$) weight gain of birds fed PKC-based diets. There was no significant effect of the inclusion of PKC during different phases and enzyme supplementation on feed intake. However, the trend with enzyme supplementation was for increased feed intake and decreased FCR with increased amount of enzymes (Table 6.9). Using PKC-based diets during the starter or grower phase significantly reduced ($P < 0.05$) FCR than when PKC-based diets were used in both phases.

For the broiler starter diets, true dry matter retention was significantly higher ($P < 0.05$) with the control starter diet (Table 6.10) than with the other starter diets. When compared to birds fed the PKC-based diet without enzyme supplementation, adding enzyme at 1 or 2 kg/t to PKC-based diets significantly improved ($P < 0.05$) the true dry matter retention of birds. Both AME and TME_n of PKC-based diets were significantly higher ($P < 0.05$) with 1kg/t enzyme supplementation (Table 6.10) than without enzyme supplementation. Increasing the levels of enzyme in the PKC-based starter diets from 1 kg/t to 2 kg/t did not further increase its AME and TME_n values.

For the broiler grower diets, true dry matter retention was significantly higher ($P < 0.05$) with the control grower diet (Table 6.11) than with the other PKC-based diets. Even though the AME and TME_n of the control grower diets (G1), and enzyme supplemented PKC-based grower diets (G3 and G4) were not significantly different, the AME and TME_n of the control grower diet and enzyme supplemented (1kg/t) PKC-based diet were significantly higher ($P < 0.05$) than PKC-based grower diet without enzyme supplementation (Table 6.11).

Table 6.8 Effect of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on female broilers overall performance (weight gain, feed intake and feed conversion ratio (FCR)) during the 6 week experimental period.

Dietary Treatment	Arrangement of diets	Weight gain (g)	Feed intake (g)	FCR
1	Control	1820	3609	1.97 [b]
2	PKC Starter PKC Grower	1727	3771	2.19 [a]
3	PKC Starter Control Grower	1759	3608	2.05 [ab]
4	Control Starter PKC Grower	1728	3622	2.10 [ab]
5	PKC Starter + 1kg/t PKCase[1] PKC Grower + 1kg/t PKCase	1766	3739	2.12 [a]
6	PKC Starter + 1kg/t PKCase Control Grower	1765	3614	2.05 [ab]
7	Control Starter PKC Grower + 1kg/t PKCase	1792	3742	2.09 [ab]
8	PKC Starter + 2kg/t PKCase PKC Grower + 2kg/t PKCase	1819	3787	2.10 [ab]
9	PKC Starter + 2kg/t PKCase Control Grower	1810	3707	2.05 [ab]
10	Control Starter PKC Grower + 2kg/t PKCase	1754	3662	2.09 [ab]
	Overall mean	1774	3688	2.08
	Pooled SEM[2]	6.9	21.3	0.01

[1] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity; 1E = 1kg/t and 2E = 2kg/t

[2] Pooled standard error of the mean; data represent mean of four replications of 10 birds.

[ab] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 6.9 Factorial comparison: Effect of dietary inclusion of solvent-extracted palm kernel cake (PKC) at different phases and enzyme supplementation on female broilers overall performance (weight gain, feed intake and feed conversion ratio (FCR)) during the 6 week experimental period.

Treatment	Weight gain (g)	Feed intake (g)	FCR
Phase [1]			
Both	1770	3766	2.14 [a]
Starter	1778	3643	2.05 [b]
Grower	1758	3675	2.09 [b]
Enzyme [2]			
0 kg/t	1739 [b]	3667	2.11
1 kg/t	1774 [a]	3698	2.09
2 kg/t	1796 [a]	3719	2.08
Overall mean	1769	3695	2.09
Pooled SEM [3]	7.3	22.8	0.01
Factorial effects [4]			
Phase	NS	NS	***
Enzyme	***	NS	NS
Phase x Enzyme	NS	NS	NS

[1] Both = PKC was included in both the starter and grower diets; Starter = PKC was included only in the starter diets; Grower = PKC was included only in the grower diets.

[2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity.

[3] Pooled standard error of the mean; data represent mean of four replications of 10 birds.

[4] NS = not significant ($P > 0.05$) or *** = significant at $P < 0.05$

[ab] Treatment means with different superscripts within a column for phase or enzyme are significantly different at $P < 0.05$.

Table 6.10 The effect of dietary enzyme supplementation on true DM retention and apparent metabolizable energy (AME) and nitrogen corrected true metabolizable energy (TME_n) of broiler starter diets [1].

Starter diets	True DM retention, %	AME, kcal/kg	TME_n, kcal/kg
Control (S1)	74.2 [a]	3,176 [ab]	3,669 [ab]
20% PKC (S2)	62.6 [c]	3,094 [b]	3,580 [b]
20% PKC + 1E[2] (S3)	67.3 [b]	3,257 [a]	3,745 [a]
20% PKC + 2E (S4)	66.4 [b]	3,189 [ab]	3,676 [ab]
Overall mean	67.6	3,179	3,667
Pooled SEM [3]	1.1	20.2	20.3

[1] DM basis

[2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity; 1E = 1kg/t and 2E = 2kg/t

[3] Pooled standard error of the mean; data represent mean of six cockerels.

[abc] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 6.11 The effect of dietary enzyme supplementation on true DM retention and apparent metabolizable energy (AME) and nitrogen corrected true metabolizable energy (TME_n) of broiler grower diets [1].

Grower diets	True DM retention, %	AME, kcal/kg	TME_n, kcal/kg
Control (G1)	73.2 [a]	3,146 [a]	3,639 [a]
20% PKC (G2)	62.2 [c]	2,958 [b]	3,444 [b]
20% PKC + 1E[2] (G3)	66.8 [b]	3,155 [a]	3,641 [a]
20% PKC + 2E (G4)	62.6 [bc]	3,029 [ab]	3,515 [ab]
Overall mean	66.1	3,070	3,558
Pooled SEM [3]	1.1	29.9	30.1

[1] DM basis

[2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity; 1E = 1kg/t and 2E = 2kg/t

[3] Pooled standard error of the mean; data represent mean of six cockerels.

[abc] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Broilers in dietary treatment 1 deposit significantly less ($P < 0.05$) abdominal fat than broilers in dietary treatments 2-10 (Table 6.12). Factorial analysis revealed that neither inclusion of PKC during different phases nor enzyme supplementation significantly ($P > 0.05$) affected abdominal fat deposition (Table 6.13). Rectal temperatures of the 6-week-old broilers fed different grower diets were not significantly different ($P > 0.05$) (Table 6.14). Even though not significantly different, rectal temperature of birds fed enzyme supplementation PKC diets was numerically lower when compared to the birds fed PKC diet without enzyme supplementation. A total of 22 broilers was found dead when the temperature inside the building reached 36°C during days 32, 37 and 40 of the experiment. Veterinarians from the post-mortem laboratory concluded that all of the birds died of heat stress. The data for mortality due to heat stress were subjected to Chi-square analysis. The result of the analysis showed that the birds fed 20% PKC grower diets without enzyme supplementation had significantly higher ($P < 0.05$) mortality due to heat stress than those fed the control diet (Table 6.15). When birds fed the enzyme supplemented PKC-based grower diets were compared with those fed the control diets, no significant differences ($P > 0.05$) in mortality due to heat stress were found.

Layer Study

There were no significant interactions between blocks and diets. Therefore, the data were analyzed as a completely randomized design experiment. When comparing data from layers fed layer diets L1-L7, no significant ($P > 0.05$) dietary effects were observed on mean egg production, hen-day egg production or daily egg mass (Table 6.16). Birds fed enzyme supplemented (2kg/t) 25% PKC-based layer diets (L7) produced significantly heavier ($P < 0.05$) eggs than birds fed diets L1, L3, L4, L5 and L6. On the other hand, birds fed a 25% PKC-based diet (L5) produced significantly smaller ($P < 0.05$) eggs than the rest of the birds, except those fed an enzyme supplemented (2kg/t) 12.5% PKC-based diet (L4). Birds fed the control (L1) and enzyme-supplemented (1kg/t) 12.5% PKC-based (L3) diets consumed significantly less ($P < 0.05$) feed than other birds (Table 6.16). Birds fed the 12.5% PKC diets (L2, L3 and L4) also consumed significantly less ($P < 0.05$) feed than those fed the 25% PKC diets (L5, L6 and L7). The best FCR (feed intake/egg mass) was obtained from birds fed diets L1 and L3, whereas significantly poorer ($P < 0.05$) FCR were found in birds fed diets L2, L4, L5, L6 and L7 (Table 6.16).

Table 6.12 Effect of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on broiler abdominal fat deposition at week 6.

Dietary treatment	Arrangement of diets	Abdominal fat (% body weight)
1	Control	1.02 [b]
2	PKC Starter PKC Grower	1.60 [a]
3	PKC Starter Control Grower	1.52 [a]
4	Control Starter PKC Grower	1.73 [a]
5	PKC Starter + 1kg/t PKCase[1] PKC Grower + 1kg/t PKCase	1.48 [a]
6	PKC Starter + 1kg/t PKCase Control Grower	1.41 [a]
7	Control Starter PKC Grower + 1kg/t PKCase	1.57 [a]
8	PKC Starter + 2kg/t PKCase PKC Grower + 2kg/t PKCase	1.78 [a]
9	PKC Starter + 2kg/t PKCase Control Grower	1.62 [a]
10	Control Starter PKC Grower + 2kg/t PKCase	1.59 [a]
	Overall mean	1.51
	Pooled SEM [2]	0.05

[1] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity.

[2] Pooled standard error of the mean; data represent mean of five broilers.

[ab] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 6.13 Factorial comparison: Effect of dietary inclusion of solvent-extracted palm kernel cake (PKC) at different phases and enzyme supplementation on broiler abdominal fat deposition at week 6.

Treatment	Abdominal fat (% body weight)
Phase [1]	
Both	1.60
Starter	1.49
Grower	1.65
Enzyme [2]	
0 kg/t	1.60
1 kg/t	1.52
2 kg/t	1.62
Overall mean	1.58
Pooled SEM [3]	0.05
Factorial effects [4]	
Phase	NS
Enzyme	NS
Phase x Enzyme	NS

[1] Both = PKC was included in both the starter and grower diets; Starter = PKC was included only in the starter diets; Grower = PKC was included only in the grower diets.
[2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity.
[3] Pooled standard error of the mean; data represent mean of five broilers.
[4] NS = not significant ($P > 0.05$) or *** = significant at $P < 0.05$

Table 6.14. The effect of broiler grower diets on six-week-old broilers' rectal temperature.

Grower diets	Body temperature °C (± S.D.) [1]
Control (G1)	44.22 ± 0.53
20% PKC (G2)	44.49 ± 0.45
20% PKC + 1E [2] (G3)	44.41 ± 0.48
20% PKC + 2E (G4)	44.21 ± 0.59
Overall mean	44.31 ± 0.52

[1] Data represent mean of eight broilers; ± standard deviation. PKC = palm kernel cake.
[2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity; 1E = 1kg/t and 2E = 2kg/t

Table 6.15 Chi-square analysis: Comparing the effect of different broiler grower diets on broiler mortality due to heat stress during 3-6 weeks of age [1].

Diet comparison	Significant level [2]
G1 vs G2	***
G1 vs G3	NS
G1 vs G4	NS
G1 vs G3 + G4	NS
G1 vs G2 + G3 + G4	NS
G2 vs G3	NS
G2 vs G4	NS
G2 vs G3 + G4	NS
G3 vs G4	NS

[1] G1 = grower diet without palm kernel cake (PKC); G2 = grower diet with 20% PKC; G3 = grower diet with 20% PKC + 1kg/t Alltech PKCase; G4 = grower diet with 20% PKC + 2kg/t Alltech PKCase. PKCase is an enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity.
[2] *** = siginficant at $P < 0.05$; NS = not significant or $P > 0.05$.

Table 6.16 The effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on the mean performance of laying hens over 8 weeks [1].

Layer diet	Eggs produced per replicate	Hen-day egg production (%)	Egg weight (g)	Daily egg mass (g)	Feed intake (g/bird/day)	FCR
Control (L1)	410	91.5	55.1 [bc]	50.4	98.7 [c]	1.96 [d]
12.5% PKC (L2)	411	91.7	55.6 [ab]	51.0	105.3 [b]	2.07 [bc]
12.5% PKC + 1E [2] (L3)	412	92.0	54.9 [c]	50.5	101.5 [c]	2.01 [cd]
12.5% PKC + 2E (L4)	417	93.1	54.7 [cd]	50.9	104.7 [b]	2.06 [bc]
25% PKC (L5)	413	92.1	54.3 [d]	50.0	111.0 [a]	2.22 [a]
25% PKC + 1E (L6)	413	92.3	55.1 [bc]	50.8	108.6 [a]	2.14 [ab]
25% PKC + 2E (L7)	415	92.6	55.7 [a]	51.6	108.5 [a]	2.10 [bc]
Overall mean	413	92.2	55.1	50.7	105.5	2.08
Pooled SEM [3]	1.2	0.2	0.1	0.2	0.3	0.01

[1] Layers were 28 weeks old at the start of the experiment, FCR = feed conversion ratio.

[2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity; 1E = 1kg/t and 2E = 2kg/t.

[3] Pooled standard error of the mean; data represent mean of seven replications of eight layers.

[abcd] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Factorial analysis (diets L2-L7) revealed that the levels of PKC or enzymes have no significant effect on mean egg production, hen-day egg production, egg weight or egg mass (Table 6.17). Significantly more ($P < 0.05$) feed was consumed by birds fed the 25% PKC diet than with the 12.5% PKC diet. When compared with layers fed PKC diets without supplemental enzyme, enzyme supplementation at 1kg/t significantly reduced ($P < 0.05$) feed intake of birds fed PKC diets. However a significant difference in feed intake was not observed in layers fed PKC diets supplemented with either 1kg/t or 2kg/t of enzyme (Table 6.17). A significant interaction was found between PKC level and enzyme level for egg weight and FCR, the interactions being shown in Figures 6.1 and 6.2 for egg weight and in Figures 6.3 and 6.4 for FCR. Inconsistent results were found for egg weight (Figures 6.1 and 6.2). Egg weight was significantly reduced with enzyme supplementation when the 12.5% PKC diet was fed. However, the reverse was true for the 25% PKC diet (Figure 6.1). When no enzyme was used, birds fed the 12.5% PKC diet produced significantly heavier ($P < 0.05$) eggs than those fed the 25% PKC diet (Figure 6.2). No significant effect in egg weight was found at the 1kg/t enzyme level but birds fed the 25% PKC diet produced significantly heavier ($P < 0.05$) eggs than those fed 12.5% PKC diets with the 2kg/t enzyme level (Figure 6.2). For FCR (Figures 6.3 and 6.4), no effect of the enzyme was found at 12.5% PKC, although there was a trend for better FCR with enzyme supplementation (Figure 6.3). Enzyme supplementation, on the other hand, significantly improved ($P < 0.05$) the FCR of birds fed 25% PKC diets (Figure 6.3). Birds fed 25% PKC diets had significantly poorer ($P < 0.05$) FCR at 0 and 1 kg/t enzyme levels. However, the differences were alleviated at 2kg/t enzyme level (Figure 6.4).

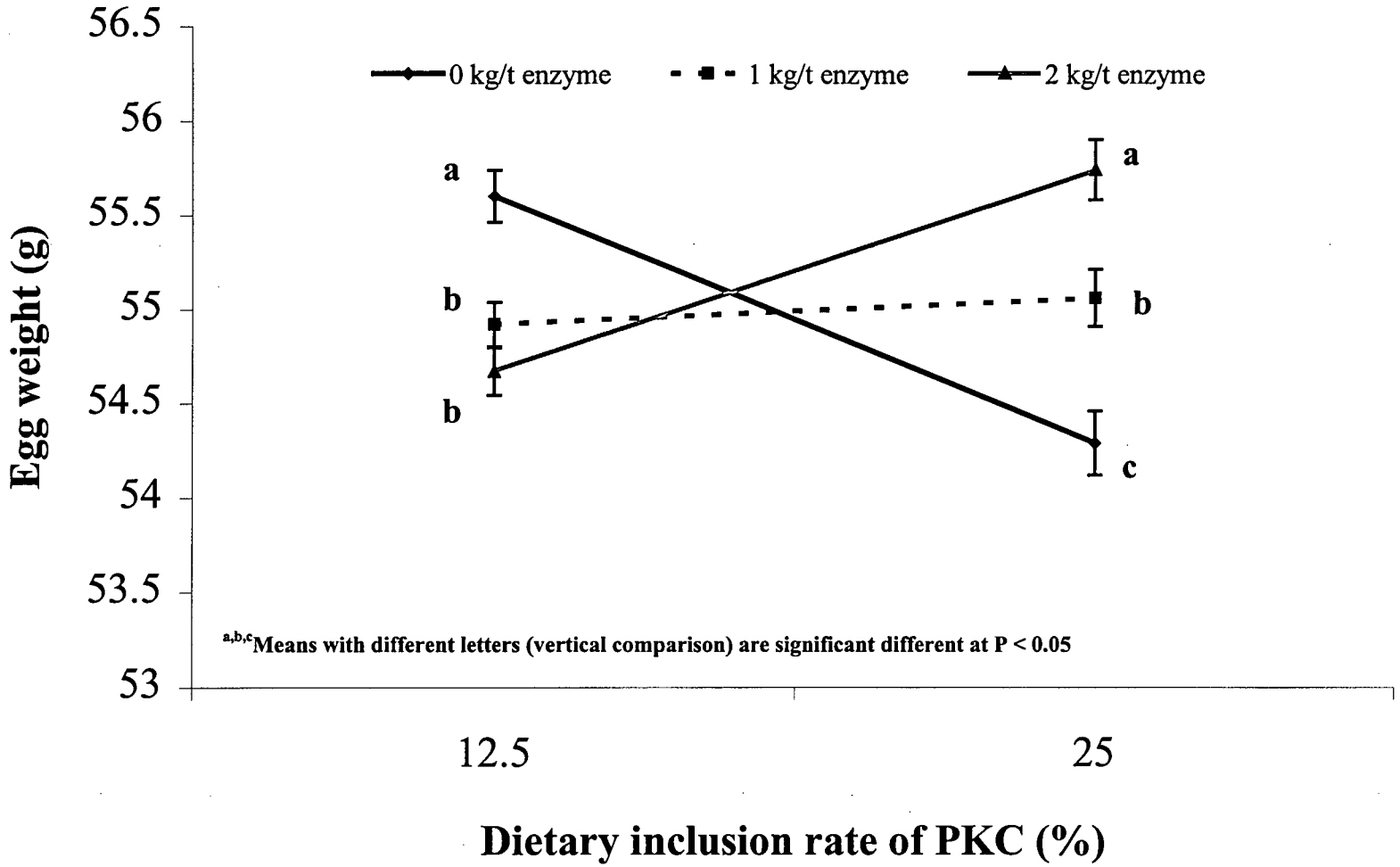
When comparing birds fed diets L1-L7, no significant difference was found in eggshell quality among diets (Table 6.18). Egg yolk color was significantly paler ($P < 0.05$) for eggs produced by birds fed 25% PKC-based diets (L5, L6 and L7). Factorial analysis (comparing diets L2-L7) revealed that the levels of PKC and enzyme supplementation had no significant effect on eggshell quality (Table 6.19). However, egg yolk color was significantly paler ($P < 0.05$) for birds fed 25% PKC diets.

Table 6.17 Factorial comparison: The effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on the mean performance of laying hens over 8 weeks [1].

Factor	Egg produced per replicate	Hen-day egg production (%)	Egg weight (g)	Daily egg mass (g)	Feed intake (g/bird/day)	FCR
PKC						
12.5% PKC	413	92.2	55.1	50.8	103.8 [b]	2.05 [b]
25.0% PKC	414	92.3	55.0	50.8	109.3 [a]	2.15 [a]
Enzyme [2]						
0 kg/t	412	91.9	54.9	50.5	108.1 [a]	2.14 [a]
1 kg/t	413	92.1	55.0	50.6	105.0 [b]	2.07 [b]
2 kg/t	416	92.8	55.2	51.2	106.6 [ab]	2.08 [b]
Overall mean	413	92.3	55.0	50.8	106.6	2.10
Pooled SEM [3]	1.2	0.3	0.2	0.2	0.7	0.01
Factorial effects [4]						
PKC	NS	NS	NS	NS	***	***
Enzyme	NS	NS	NS	NS	***	***
PKC X Enzyme	NS	NS	***	NS	NS	***

[1] Layers were 28 weeks old at the start of the experiment, FCR = feed conversion ratio.
[2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity.
[3] Pooled standard error of the mean; data represent mean of seven replications of eight layers.
[4] NS = not significant ($P > 0.05$) or *** = significant at $P < 0.05$
[abcde] Treatment means with different superscripts within a column for PKC or enzyme are significantly different at $P < 0.05$.

Figure 6.1 The effects of enzyme levels at different inclusion rates of palm kernel cake (PKC) on egg weight



[a,b,c]Means with different letters (vertical comparison) are significant different at P < 0.05

Figure 6.2 The effects of dietary inclusion rates of palm kernel cake (PKC) at different enzyme levels on egg weight

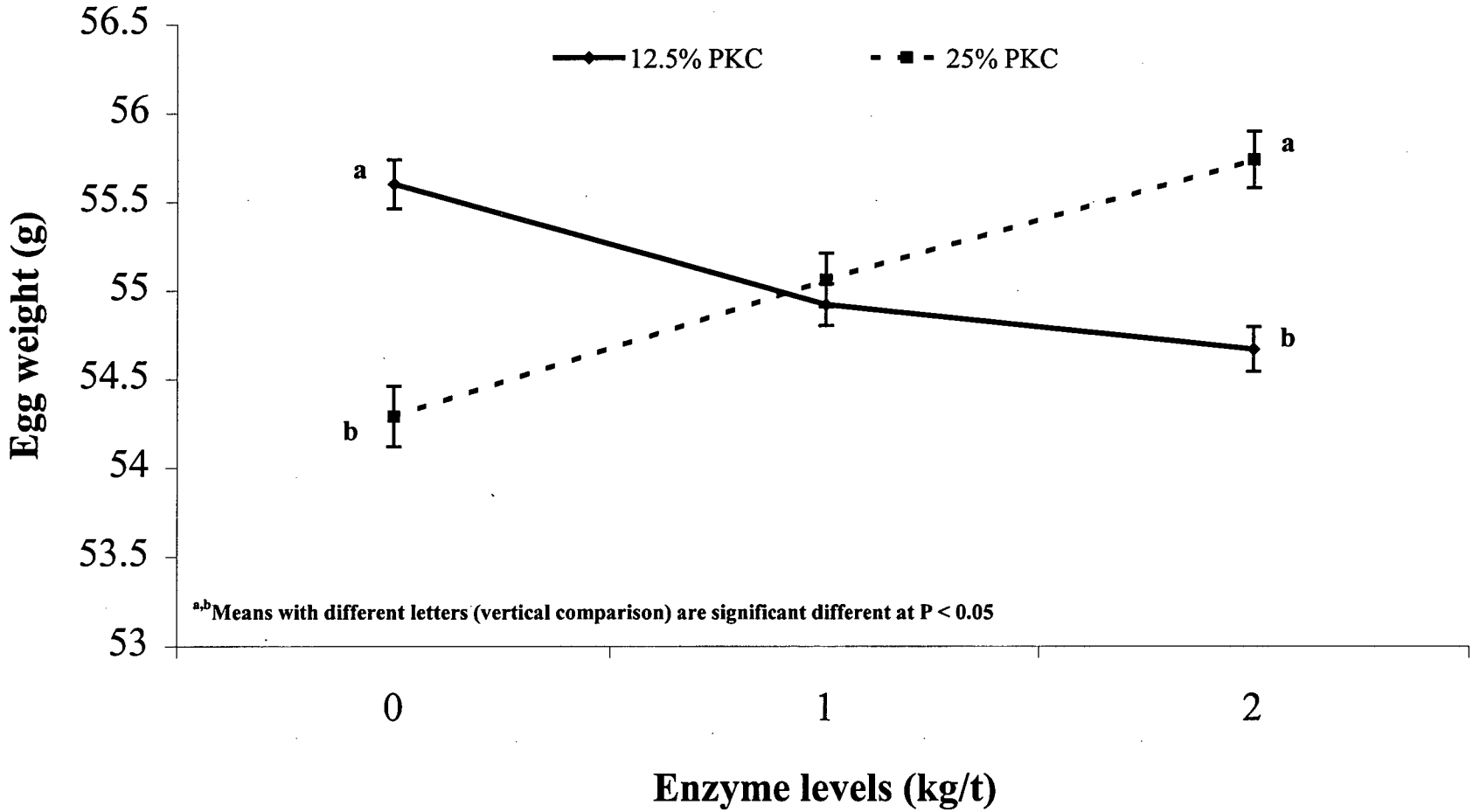


Figure 6.3 The effects of enzyme levels at different inclusion rates of palm kernel cake (PKC) on layer's feed conversion ratio

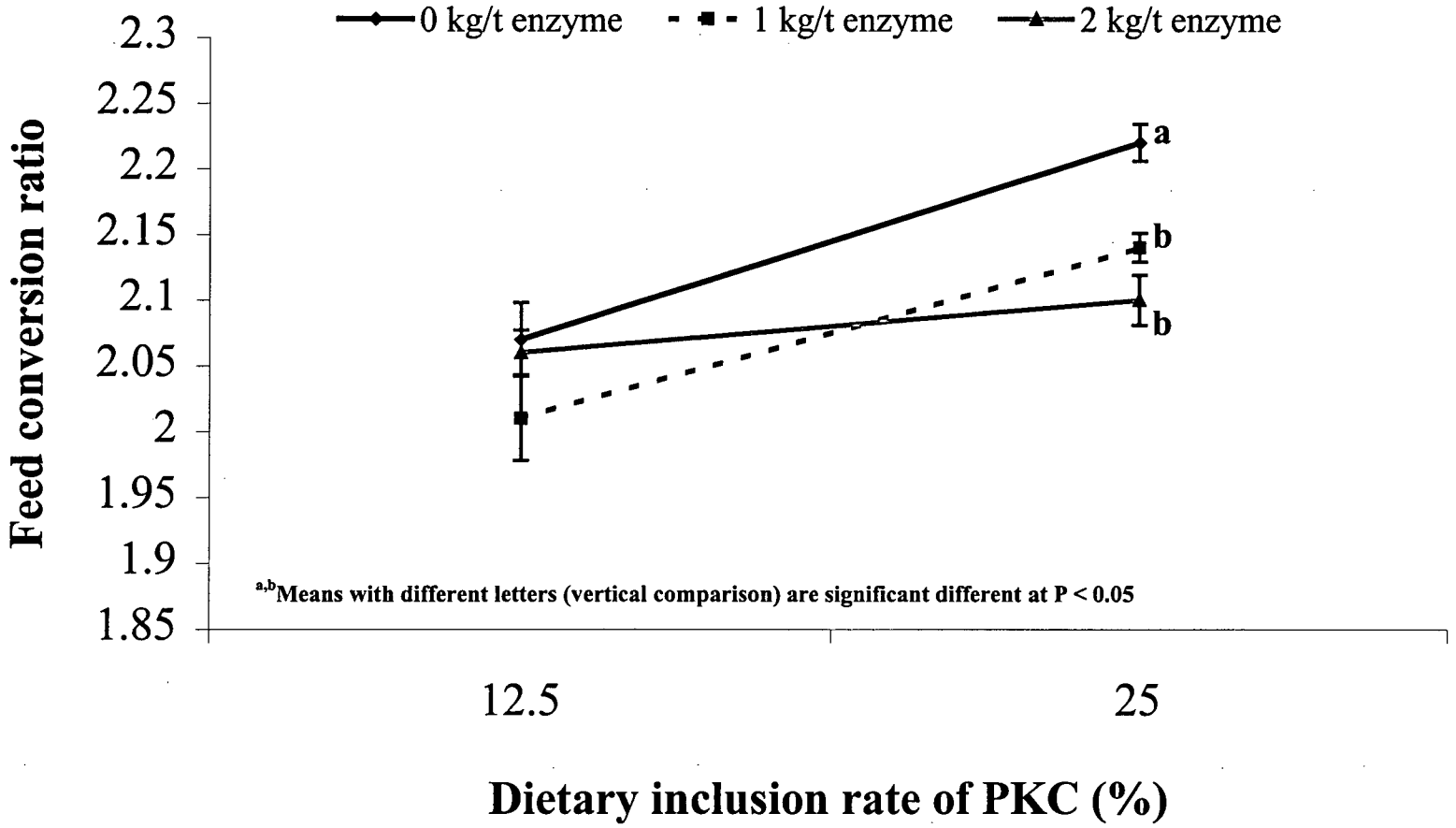


Figure 6.4 The effects of dietary inclusion rates of palm kernel cake (PKC) at different enzyme levels on layer's feed conversion ratio

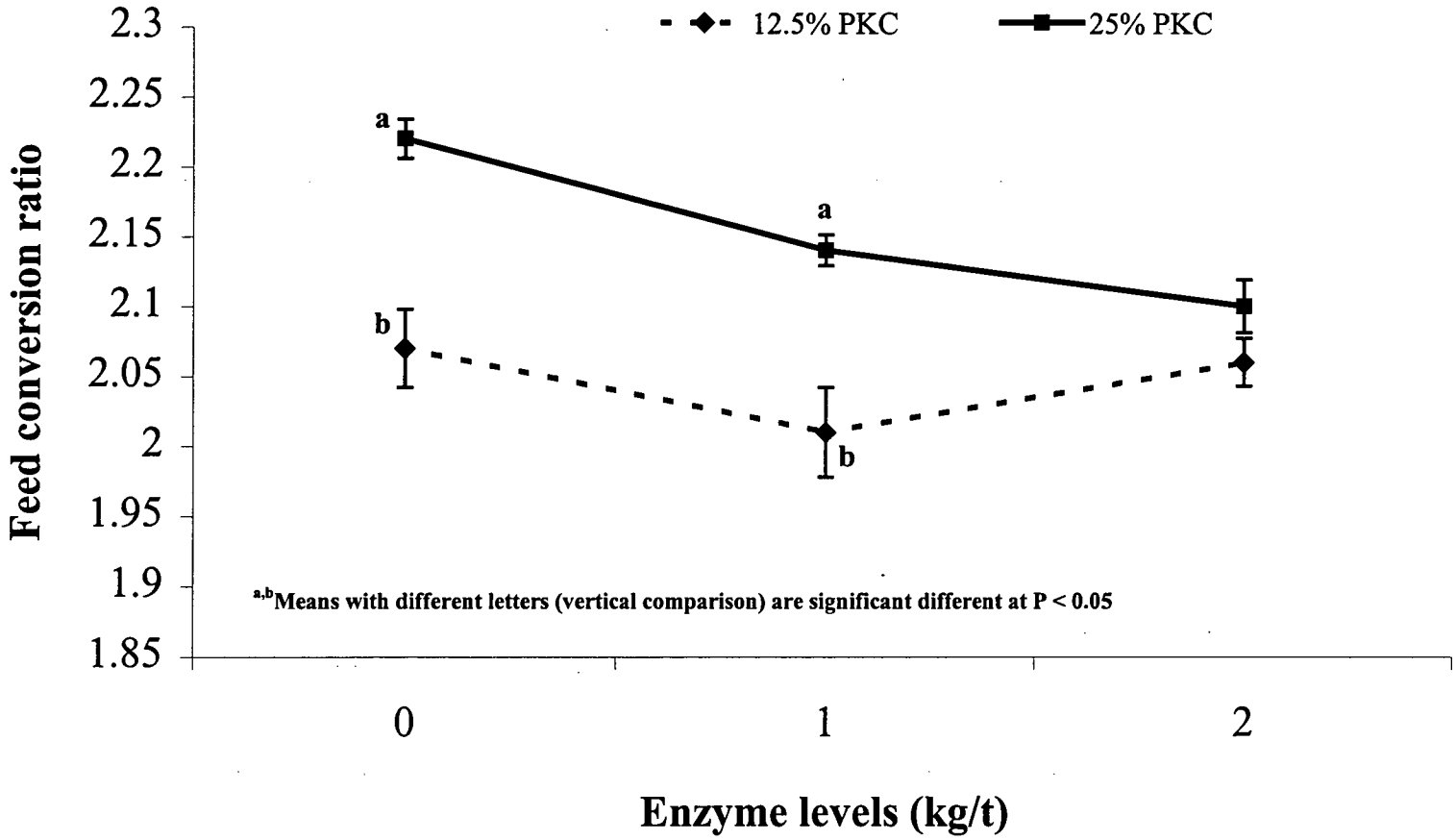


Table 6.18 The effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on the mean egg quality over 8 weeks from 28 weeks of age.

Layer diets	Egg shell specific gravity	Egg yolk color [1]
Control (L1)	1.091	3.9 [a]
12.5% PKC (L2)	1.091	3.9 [a]
12.5% PKC + 1E [2] (L3)	1.092	3.7 [a]
12.5% PKC + 2E (L4)	1.091	3.8 [a]
25% PKC (L5)	1.091	2.3 [b]
25% PKC + 1E (L6)	1.092	2.4 [b]
25% PKC + 2E (L7)	1.092	2.4 [b]
Overall mean	1.091	3.2
Pooled SEM [3]	0.0001	0.1

[1] Measured with Roche yolk color fan; [2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity; 1E = 1kg/t and 2E = 2kg/t
[3] Pooled standard error of the mean; data represent mean of seven replications of eight layers
[ab]Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 6.19 Factorial Comparison: The effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on the mean egg quality over 8 weeks from 28 weeks of age.

Factor	Egg shell specific gravity	Egg yolk color [1]
PKC		
12.5%	1.092	3.8 [a]
25.0%	1.091	2.3 [b]
Enzyme [2]		
0 kg/t	1.091	3.1
1 kg/t	1.092	3.0
2 kg/t	1.091	3.1
Overall mean	1.091	3.1
Pooled SEM [3]	0.0001	0.1
Factorial effects [4]		
PKC	NS	***
Enzyme	NS	NS
PKC X Enzyme	NS	NS

[1] Measured with Roche yolk color fan; [2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity.
[3] Pooled standard error of the mean; data represent mean of seven replications of eight layers
[4] NS = not significant ($P > 0.05$) or *** = significant at $P < 0.05$; [ab]Treatment means with different superscripts within a column for PKC or enzyme are significantly different at $P < 0.05$.

There were no significant differences in true dry matter retention between birds fed the control layer diet (L1) and enzyme-supplemented PKC diets (L3, L4, L6 or L7) (Table 6.20). Birds fed a 25% PKC-based layer diet (L5) had a significantly lower ($P < 0.05$) true dry matter retention than those fed the control layer diet (L1) or enzyme-supplemented PKC diets (L3, L4, L6 or L7). The control diet (L1), 12.5% PKC diet (L2) and 25% PKC diet (L5) had lower AME and TME_n values when compared to 25% PKC diet supplemented with 2 kg/t enzyme. Enzyme-supplemented PKC diets (L3, L4, L6 and L7) had significantly higher AME and TME_n values than PKC diets with no enzyme supplementation (L2 and L5) (Table 6.20). Factorial analysis (diets L2-L7) found that birds fed diets containing 25% PKC retained significantly less ($P < 0.05$) dry matter than those fed the 12.5% PKC diets (Table 6.21). Enzyme supplementation significantly increased ($P < 0.05$) true dry matter retention in birds fed PKC diets. The levels of PKC did not significantly influence AME and TME_n of the diets, but adding the enzyme significantly increased ($P < 0.05$) the AME and TME_n values of PKC diets.

Table 6.20 Apparent and nitrogen corrected true metabolizable energy[1] (AME and TME_n) and true dry matter retention of layer diets.

Layer diet	True dry matter retention (%)	AME kcal/kg	TME_n kcal/kg
Control (L1)	71.4 [a]	2,889 [bc]	3,382 [bc]
12.5% PKC (L2)	62.9 [bc]	2,742 [c]	3,226 [c]
12.5% PKC + 1E [2] (L3)	69.6 [a]	3,020 [ab]	3,509 [ab]
12.5% PKC + 2E (L4)	68.1 [ab]	2,983 [ab]	3,469 [ab]
25% PKC (L5)	56.7 [c]	2,756 [c]	3,240 [c]
25% PKC + 1E (L6)	65.2 [ab]	2,973 [ab]	3,458 [ab]
25% PKC + 2E (L7)	66.2 [ab]	3,101 [a]	3,586 [a]
Overall mean	65.7	2,923	3,410
Pooled SEM [3]	0.9	24	24

[1] Dry matter basis

[2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity; 1E = 1kg/t and 2E = 2kg/t

[3] Pooled standard error of the mean; data represent mean of six cockerels.

[abc] Treatment means with different superscripts within a column are significantly different at $P < 0.05$.

Table 6.21 Factorial comparison: Apparent and nitrogen corrected true metabolizable energy[1] (AME and TME_n) and true dry matter retention of layer diets.

Factor	True dry matter retention (%)	AME kcal/kg	TME_n kcal/kg
Palm kernel cake			
12.5%	66.9 [a]	2,915	3,401
25.0%	62.7 [b]	2,943	3,428
Enzyme			
0 kg/t	59.8 [b]	2,749 [b]	3,233 [b]
1 kg/t	67.4 [a]	2,996 [a]	3,483 [a]
2 kg/t	67.2 [a]	3,042 [a]	3,527 [a]
Overall mean	64.8	2,929	3,415
Pooled SEM [3]	0.8	26	26
Factorial effects [4]			
Palm kernel cake (PKC)	***	NS	NS
Enzyme	***	***	***
PKC X Enzyme	NS	NS	NS

[1] Dry matter basis

[2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity.

[3] Pooled standard error of the mean; data represent mean of six cockerels.

[4] NS = not significant ($P > 0.05$) or *** = significant at $P < 0.05$

[ab]Treatment means with different superscripts within a column for palm kernel cake or enzyme are significantly different at $P < 0.05$.

Economical Analyses of Diets

The relative costs of energy and protein for corn, soybean meal, PKC, PKC + 1kg/t enzyme and palm oil are presented in Table 6.22. Based on the prevailing prices in Malaysia, corn supplies the cheapest source of energy, while soybean meals supply the cheapest source of protein. Even though PKC contains lower AME than soybean meal, it is much cheaper than soybean meal. As a result, PKC provides a cheaper source of energy than soybean meal. On the other hand, PKC contains a moderate amount of protein and costs less per tonne than corn, making it a cheaper protein source than corn. Parametric linear programming revealed that the computerized feed formulation program was not willing to use any level of PKC or PKC + 1kg/t enzyme in the high AME (3,200 kcal/kg) broiler starter and high AME (3,200 kcal/kg) broiler grower diets even when the prices of both PKC and PKC + 1kg/t enzyme were set at zero (Table

6.23). For low AME (3,000 kcal/kg) diets the feed formulation program still rejected PKC in the starter diet. However, the computerized feed formulation program was willing to include 5.0 to 5.7% of PKC + 1kg/t enzyme into the low AME starter diet when the price was less than RM31/tonne. The feed formulation program included 6% PKC into the low AME grower diet when the price of PKC was less than RM700/tonne. On the other hand, a higher level (6.4% - 12.4%) of PKC + 1kg/t enzyme was used in the low AME grower diet (Table 6.23).

For layer diets, the computerized feed formulation program used 21% and 8% PKC in the low (2,700 kcal/kg) and high (2,900 kcal/kg) AME layer diets respectively, when the price of PKC was less than RM700/tonne (Table 6.23). The level of inclusion of PKC + 1kg/t enzyme in the low AME layer diet ranged from 23% to 29%. Lower levels (10% to 16%) of PKC + 1kg/t enzyme were used in the high AME layer diet (Table 6.23).

Table 6.22 The relative costs of apparent metabolizable energy (AME) and protein (CP) in corn, soybean meal (SBM), solvent-extracted palm kernel cake (PKC), PKC + 1kg/t PKCase (PKC + 1E) and palm oil [1,2].

Ingredients	Cost per tonne (RM)	AME [3] (kcal/kg)	CP [3] (%)	Cost per Mcal (RM)	Cost per kg CP (RM)
Corn	786	3,350	8.5	0.23	9.25
SBM	1,071	2,220	44	0.48	2.43
PKC	520	1,452	15	0.36	3.47
PKC + 1E	520	1,624	15	0.32	3.47
Palm oil	2500	8,430	0	0.30	-

[1] RM = Ringgit Malaysia

[2] PKCase is an enzyme mixture obtained from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity. This enzyme is not currently available at the commercial market, therefore the cost of PKC + 1E was assigned to be equal to PKC. The above analysis shows that the enzyme would have to be priced at no more than RM61.6/kg for it to be economic for commercial use.

[3] The AME and CP values for corn and SBM were obtained from the NRC (1994) for poultry, whereas the values for PKC and PKC + 1E were determined in the previous study (Chapter V). The AME value for palm oil was obtained from Yeong and Mukherjee (1983).

Table 6.23 Evaluation of the effect of the price of palm kernel cake (PKC) and PKC + 1kg/t PKCase (PKC + 1E) on its inclusion rate in low and high apparent metabolizable energy (AME) broiler (starter and grower) and layer diets by parametric linear programming [1,2].

	Price of PKC (RM/tonne)	PKC % inclusion	Price of PKC + 1E (RM/tonne)	PKC + 1E % inclusion
Starter (23% CP)				
AME (kcal/kg)				
3,000	0	0	0 – 22	5.7
3,000	-	-	23 – 31	5.0
3,000	-	-	> 31	0
3,200	0	0	0	0
Grower (20% CP)				
AME (kcal/kg)				
3,000	0 - 700	6	0 - 22	12.4
3,000	-	-	23 - 31	11.7
3,000	-	-	32 - 700	6.4
3,200	0	0	0	0
Layer (15% CP)				
AME (kcal/kg)				
2,700	0 - 700	21	0 - 24	29
2,700	-	-	25 - 31	28
2,700	-	-	32 - 700	23
2,900	0 - 700	8	0 - 66	16
2,900	-	-	67 - 74	15
2,900	-	-	75 - 700	10

[1] RM = Ringgit Malaysia. For broiler diets, low AME = 3,000 kcal/kg and high AME = 3,200 kcal/kg. For layer diets, low AME = 2,700 kcal/kg and high AME = 2,800 kcal/kg. Solvent-extracted PKC was used. [2] An enzyme mixture from Alltech Inc., Kentucky, USA. It contains mannanase, α-galactosidase and protease activity.

Discussion

Up to now, no published study has been conducted to investigate the effects of using PKC during different phases of the broiler production cycle or on the effects of enzyme supplementation of PKC-based diets on poultry performance. The broiler study clearly showed that weight gains for 0-3 weeks and 3-6 weeks were significantly reduced when 20% PKC was incorporated into the starter and grower diets, respectively, even though the diets were

formulated to contain similar levels of nutrients. The birds fed 20% PKC starter or grower diets also consumed more feed and had a poorer FCR than those fed the control diet. The reasons for the poorer growth performance included a reduction in dry matter retention and in the metabolizable energy content of PKC based diets. True dry matter retention by birds fed the starter and grower diets containing 20% PKC was 15% lower than in birds fed the control diet. Lower PKC amino acid digestibility as indicated in Chapter V could also have retarded growth.

The results of the present studies also demonstrate that adding 20% PKC into both broiler starter and broiler grower diets (0-6 week) results in a poorer FCR when compared to birds fed control corn-soybean meal diets (0-6 week). The lower digestibility of PKC amino acid and energy and the possible entrapment of nutrients within the cells by indigestible cell walls (Chapter V) could have led to the poorer FCR of birds fed the 20% PKC-based diet from 0-6 weeks of age. However, when 20% PKC was included in the broiler diet during the starter phase, and a corn-soybean meal diet was fed during the grower phase (Case I), or in the broiler diet during the grower phase while a corn-soybean meal diet was fed during the starter phase (Case II), the performance of the birds at 6 weeks was not jeopardized. Even though broilers gain less weight during 0-3 week of age when PKC based diet was fed, they were able to compensate the lower weight gain by growing faster when corn-soybean meal diet was fed during 3-6 weeks (Case I, compensatory growth). On the other hand (Case II), birds were better able to utilize PKC during the grower phase because of their better developed digestive tract. This indicates that 20% PKC can be used during the starter or grower phase but not in both the starter and grower phases.

This is in agreement with the findings of several studies that inclusion of high level of PKC in broiler diets for 5 weeks or longer led to poorer bird performance (Ahmad, 1982; Garcia and Gernat, 1998 and Onifade and Babatunde, 1998). Ahmad (1982) found that 10% PKC could be included in a broiler diet from 15 to 56 days of age without reducing growth performance, but as PKC level reached 20%, daily weight gain was significantly reduced and feed/gain ratio was increased. This agrees with the finding in the present study that feed/gain ratio was significantly increased when broilers were fed a 20% PKC-based diet instead of a corn-soybean meal diet from 0-6 week of age. Ahmad (1982) also stated that the high fiber content of PKC reduced its nutrient digestibility, especially for protein, and concluded that there was no economic advantage in using PKC over corn and soybean meal. In another study, Garcia and Gernat (1998) found that birds fed corn-soybean meal or 10% PKC diets from 0 to 6 weeks of age had significantly higher

body weights, higher carcass weights and better FCR than birds fed diets containing 20% or 30% PKC. Other authors (Onifade and Babatunde, 1998) concluded that adding 10%, 15% or 20% PKC (Nigeria, screw-pressed) into broiler diets from 0 to 35 days of age reduced weight gain when compared to broilers fed the corn-soybean meal control diet. However, the authors failed to adjust the metabolizable energy of the diets to that of the control diet and this might have reduced the birds' performance.

These results are contradictory to the findings of Yeong (1980), Hutagalung (1980), Yeong and Mukherjee (1983) and Ngoupayou (1984) stating that broiler growth performance was not jeopardized when the diet contained 20% PKC. Yeong and Mukherjee (1983) found that by adding 9% palm oil to a 20% PKC diet (which brought the metabolizable energy level to that of the control diet), growth performance and feed efficiency of the birds fed PKC diets was not different from that of birds fed the control diet during the 8 week study. In the present study, palm oil was also added to 20% PKC diets (which brought the metabolizable energy level to that of the control corn-soybean meal diet). Even though weight gain of the broilers fed different diets was not significantly different, broilers fed the 20% PKC diets were not as efficient (in utilizing feed) as broilers fed the control diets. It is likely that birds increased their feed intake to compensate the poor nutrient availability in PKC based diets.

During a 4 week feeding trial, Ngoupayou (1984) found that PKC was quite palatable to broiler chicks, and this was confirmed in the present study as feed intake was increased in birds fed PKC-containing diets. But this is in disagreement with Swick and Tan (1997) who reported that PKC is unpalatable to poultry. Given the high fiber content and low digestibility of protein, amino acids and energy (Chapter V) in PKC, it is surprising that some studies used more than 20% PKC in broiler diets without adverse effects on growth performance. Nwokolo *et al.* (1977) concluded that broilers could be fed diets containing up to 30% PKC without any apparent adverse effects on performance. However, this was based only on an 8-day experiment. In another study, Onwudike (1986) found that starter birds were able to utilize 28% screw-pressed PKC without any significant effect on performance (1-7 week of age). It was interesting to note that the daily weight gain of the birds fed the control diet for the 6 week study period was only 21.2 g, which is equivalent to 890.4 g/42days. This is too low when compared to commercial standards. Perhaps this happened because the control diet was based on corn and groundnut cake instead of corn and soybean meal. Even though the dry matter retention of the diet was 73% in

the control group compared to 51% in the 50% PKC group, Panigrahi and Powell (1991) concluded that Malaysian PKC could be incorporated at up to 50% in broiler diets from 0 to 7 weeks of age without depressing growth and feed intake. However, the authors concluded that such diets were uneconomic because of the high inclusion of oil, and they were too oily to be considered practical. The reasons for the discrepancies in dietary inclusion rate of PKC reported in this and other studies are not clear. Perhaps, they are caused by the variety of oil palm used in different countries, methods of processing palm kernel oil, age of the birds, and duration and design of the experiments.

The potential of the enzyme (PKCase) for PKC saccharification was demonstrated in the previous study (Chapter V). In the current study, enzyme supplementation significantly increased true dry matter retention, AME and TME_n in the starter and grower PKC-based diets, leading to a better performance by the birds. The improvement in performance of the broilers fed the enzyme-supplemented PKC-based diets was likely due to the breaking down of the non-starch polysaccharide (mannans) of PKC by mannanase, hence releasing more energy (reducing sugars) and other nutrients which could have been trapped inside the cell. A trend for increased weight gain was observed with increased levels of the enzyme in the PKC-based broiler diet. However, if 20% PKC was added into both the broiler starter and grower phases (0-6 weeks), 2kg/t of enzyme is required for better FCR.

Due to the low AME value of PKC, the addition of PKC into poultry diets inevitably results in the need for greater inclusion of oil to offset the replacement of high-energy corn. High levels of oil in the 20% PKC diets unfortunately also led to a higher abdominal fat deposition. This could be a major drawback for the use of PKC diets in the broiler industry. The effect of diet constituents and environmental temperatures on the animal performance has been the subject of several investigations (Schoenherr *et al.*, 1989 and Black *et al.*, 1993). The interaction between diet and environmental temperature could be attributed to the lower heat increment of dietary fat compared to that of dietary protein and carbohydrates (Black *et al.*, 1993). In the present study, broilers started dying when the temperature inside the building reached 36°C. Mortality due to heat stress was significantly higher in broilers fed the 20% PKC diets without enzyme supplementation (contain 5% crude fiber) than in broilers fed the control corn-soybean meal diets (contain 3% crude fiber). Perhaps, increasing heat production from crude fiber digestion and fermentation led to higher heat load during hot environmental temperature, thus

causing the significantly higher mortality due to heat stress in broilers fed PKC grower diets without enzyme supplementation. No significant differences in mortality due to heat stress were detected between broiler fed the control grower and enzyme-supplemented PKC diets. The reduction in mortality due to heat stress by enzyme supplementation perhaps was accomplished via the reduction in dietary crude fiber content as the previous study (Chapter V) found that crude fiber content was significantly reduced in enzyme-treated PKC.

Not many published studies on PKC have dealt with laying hens. The present study indicated that laying hens were capable of maintaining production performance when 12.5% or 25% PKC was included in their diets. However, the layers consumed significantly more PKC containing diets in order to offset the poorer digestibility of PKC diets. This led to significantly poorer FCR in PKC-fed groups. A direct relationship between feed intake and the level of PKC was found. It has been long established that birds attempt, as a priority, to consume a certain quantity of feed necessary to meet their energy requirements (Larbier and Leclercq, 1994). In the present study, both the AME and TME_n contents of the PKC-containing layer diets (L2-L7) were not lower than those of the control corn-soybean meal diet (L1). Therefore, it is not likely that layers consumed significantly more PKC diets to meet their energy needs.

Other factors might have overridden the bird's tendency to eat to a given energy level. For instance, Parsons *et al.* (1984) and Edmonds *et al.* (1985) found that broilers increased rather than decreased their voluntary feed intake when dietary protein level in a corn-soybean meal diet was reduced from 24% to 16% by replacing soybean meal with corn. Thus, the birds actually consumed more of a low protein-high energy than a high protein-low energy diet. Therefore, the birds appeared to be trying to eat to meet their protein-amino acid needs rather than their energy needs. In the present study, the increase in feed intake of layers fed PKC-containing diets was probably due to the lower amino acid and dry matter digestibility of the PKC-based diets, especially at high levels of PKC. As a result, it is possible that layers consumed more of the PKC-based diets to meet their amino acid needs.

Contrary to the findings of the present study, Longe (1984) found that 24-week-old laying hens fed 20% PKC diets produced fewer eggs than laying hens fed a control corn-soybean meal diet. As the calculated daily energy consumption between layers fed the control and 20% PKC diets were not different, it is not clear why layers fed the PKC diet produced fewer eggs (Longe, 1984). Feed intake of layers fed PKC-based diets was significantly higher, in agreement

with the finding of the present study. However, in this case, the layers consumed more of the 20% PKC diet probably to meet their energy needs because the author (Longe, 1984) failed to adjust the AME of the PKC-containing diet to that of the control diet,. Interestingly, another study from the same country (Nigeria) found that laying hens could tolerate up to 40% PKC in their diets without adverse effects on feed intake or egg production (Onwudike, 1988). However, layers fed either the control or 40% PKC diets only attained a hen-day production of 62% and 36.5 g of daily egg mass for 31-week-old laying hens (Onwudike, 1988). It is not clear whether this is a common and acceptable performance found in Nigeria, but it is certainly unacceptable in many parts of the world including Malaysia. More recently, another study has also reported equivalent performance of laying hens fed either a control or a 40% PKC diet (Panigrahi and Waite, 1998). It was noted that this experiment was carried out in an environment-controlled room in England, it is not sure whether layers fed the 40% PKC would perform equally in hot and humid tropical countries such as Nigeria or Malaysia. Even though the diets in the current study did not contain such high levels of PKC (25% versus 40% PKC used by Onwudike (1988)), excellent performance (92.2% egg production and 50.7g egg mass) was achieved with layers fed 25% PKC layer diets.

The beneficial effects of enzyme supplementation on true dry matter retention (improved by 12.5%), AME (improved by 9.8%) and TME_n (improved by 8.4%) were also observed in the present layer study. Enzyme supplementation significantly reduced feed consumption and FCR in the PKC-fed groups. A positive trend was also found, indicating higher egg production, egg weight and egg mass with enzyme supplementation. The layer study indicates that it is possible to use a 12.5% PKC diet with 1kg/t enzyme supplementation with layers since it results in a FCR equal to that obtained with the control diet. Dietary inclusion of PKC or enzyme supplementation did not influence eggshell quality. The mean egg specific gravity of 1.091 indicates excellent shell quality. Practically, this means that only about 0.7% of the eggs can be expected to crack if the eggs are run through a commercial egg grading and sizing plant (Holder and Bradford, 1979). Egg yolk color, however, was significantly paler as the dietary level of PKC reached 25%, in agreement with the findings of Panigrahi and Waite (1998). Therefore, in countries where consumers prefer a darker color yolk, pigmenting agents would have to be added to layer diets containing 25% PKC.

Cereals provide most of the energy whereas protein concentrates provide most of the protein (amino acids) in a practical poultry diet. However, the type of cereals and protein concentrates used in poultry feeds depend entirely on their availability and the concentration of energy and protein they supply per unit of price. Even though, the price of PKC + 1kg/t enzyme (PKC + 1E) was set to be equal to the price of PKC without enzyme, PKC + 1E contained 172 kcal/kg more AME than PKC without enzyme and the extra energy is worth at least RM0.04 per Mcal (Table 6.22). Therefore the enzyme would have to be priced at no more than RM61.6/kg for it to be economic for commercial use. Parametric linear programming reveals what should be the price of PKC and PKC + 1E for it to be included into a poultry diet at a certain level. For instance, the price of PKC has to be less than RM700 per tonne for a 6% inclusion rate in a 3,000 kcal/kg broiler grower diet. On the other hand, the price of PKC and enzyme should fall between RM32 and RM700 per tonne for a 23% inclusion rate in a 2,700 kcal/kg layer diet. Parametric linear programming revealed that there are three major factors that affect the inclusion rates of PKC into poultry diets. First of all, the metabolizable energy value assigned to PKC. For instance, in the current study, the computerized feed formulation program chose to use more than PKC in the broiler and layer diets. This is because PKC + 1E contains higher AME and it is less expensive than PKC to supply energy. It should be noted that if the 5% constraint (maximum) on palm oil was removed, the feed formulation program would choose to use more palm oil, since it provided a cheaper source of energy than PKC or PKC + 1E.

The second factor is the price of PKC. Due to its low AME value, the cost per unit of energy is very important for its use in commercial feed formulation. When the price of PKC is low, the cost per kilocalorie is also low, and the lower the cost the more PKC can be used. For example, when the price of PKC per tonne is below RM31, 28% - 29% of PKC + 1E is used in the low AME layer diet. The dietary inclusion rate is reduced to 23% when the price of PKC + 1E per tonne exceeds RM31. The dietary level of AME is another factor that affects the inclusion rates of PKC into poultry diets. For instance, more PKC is used in the low AME (2,700 kcal/kg) layer diets than in the high AME (2,900 kcal/kg) layer diets. The feed formulation program does not include much PKC or PKC + 1E in the high nutrient density broiler starter and grower diets, indicating that PKC is probably more suitable for use in lower energy diets such as layer diets than in broiler diets.

The beneficial effect of enzyme (with mannanase activity) on PKC saccharification has been demonstrated by Dusterhoft *et al.* (1993a,b,c), Daud *et al.* (1997) and the previous study (Chapter V). However, the effect of adding enzyme to PKC-based diets on poultry performance has not been studied before. The present study indicates that the release of more energy and nutrients from PKC with enzyme supplementation translates into better performance of both broilers and layers in a hot tropical environment.

Conclusions

The findings in the present study indicate that using 20% PKC in both the starter and grower phases is not recommended, unless supplemental enzyme is used. The lower nutrient digestibility of PKC diets resulted in reduced growth performance when it was included in both the starter and grower phases. There are also higher risks of mortality due to heat stress in hot tropical conditions when PKC-based grower diets are fed, because of a higher heat increment. Broilers fed PKC diets tend to deposit more abdominal fat than broilers fed control diets. Laying hens were able to tolerate 12.5% and 25% PKC in their diets without adversely affecting egg production and egg mass. However, laying hens fed 12.5% and 25% PKC diets consumed significantly more feed and had a poorer FCR than laying hens fed control diets. Enzyme supplementation of PKC-based diets improved growth performance and feed efficiency of broilers and layers by increasing the DM digestibility and AME value. The diminishing yolk color associated with a high inclusion rate of PKC in layer diets could be a major problem for egg producers in Asian countries where customers prefer a darker yolk color. Parametric linear programming found that PKC was not likely to be used in commercial broiler diets under the current price conditions in Malaysia. However, higher levels of PKC could be incorporated into commercial layer diets. Enzyme supplementation of PKC-based remains promising and future studies should be conducted to evaluate whether a higher level of enzyme-supplemented PKC could be used in poultry diets. Several recent studies (Oyofo *et al.*, 1989; Hinton *et al.*, 1990; Allen *et al.*, 1997) have showed that mannose (from the digestion of PKC) greatly reduced the colonization of salmonella in the gastro-intestinal tract. Further study in this area is also warrant.

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CHAPTER VII

General Conclusions

As the world human population continues to increase, it is crucial to reduce the competition between animal and human for food. Data presented in this thesis have proved that by improving utilization of poultry feedstuffs with supplemental amino acids and enzymes, more feed could be conserved. Hence, more food will be available for human consumption.

Protein is a costly item in poultry diets, so maximizing the efficiency of protein and amino acid (AA) utilization is economically important. In addition, the high energy cost of uric acid synthesis and excretion implies several nutritional advantages of balancing the AA composition of absorbed protein close to the requirement. Unfortunately, valid AA requirement values for broilers and laying hens are not available for most of the essential AA, especially for threonine and tryptophan. As a result, when formulating poultry diets, the nutritionist must be concerned about the dietary levels of threonine and tryptophan. Under such circumstances, the nutritionist could use the AA profile (0-3 week old broiler chicks) determined in this study. For instance, the recommended ratio of threonine and tryptophan to lysine is 65% and 16%, respectively. If the nutritionist is formulating a diet with 1.00% digestible lysine, the level of digestible threonine and tryptophan in the diet should be targeted at 0.65% and 0.16%, respectively.

On the other hand, the nutritionist could also use the results from the reduced protein study as a reference. That is, the nutritionist could set the minimum levels of dietary threonine and tryptophan for 0-3 week old broilers at 0.74% of the diet (or 4.04% of CP) and 0.23% of the diet (or 1.22% of CP), respectively. Reducing dietary threonine to 0.74% did not depress growth performance or nitrogen retention in the present study and increasing the level of tryptophan in the diet to 0.23% proved to be beneficial in terms of growth and nitrogen retention in the present study for 0-3 week old broiler chicks. Results from the present and other recent studies indicate that the NRC (1994) has overestimated the requirements for threonine (Thomas *et al.*, 1992; Holsheimer *et al.*, 1994; Rangel-Lugo *et al.*, 1994; Kidd *et al.*, 1996; Leeson and Summers, 1997; Yamazaki *et al.*, 1997) and underestimated the requirement for tryptophan (Abebe and

Morris, 1990; Han *et al.*, 1991; Thomas *et al.*, 1992; Rhodimet Nutrition Guide, 1993; Austic, 1994) for 0-3 week old broiler chicks.

As indicated by the NRC (1994), the AA requirement values of broilers beyond 3 weeks of age and laying hens are based on limited published data. Since broilers consume the most feed during the second phase of the production cycle, it is important for the nutritionist to know exactly what the AA requirements are for 3-6 week old broilers. Based on this study, the level of dietary threonine and tryptophan for 3-6 week old broilers should be targeted at 0.67% of the diet (3.4% of CP) and 0.17% of the diet (0.89% of CP), respectively. Nutritionists should also be aware that most of the recently available data (Thomas *et al.*, 1992; Rhodimet Nutrition Guide, 1993; Webel *et al.*, 1996; Baker, 1997; Kidd and Kerr, 1997; Penz *et al.*, 1997; Yamazaki *et al.*, 1997) point strongly to the fact that the NRC (1994) has overestimated the threonine requirement for 3-6 week broilers. However, the NRC (1994) recommendation for tryptophan is well received (Rhodimet Nutrition Guide, 1993; Baker, 1997; Leeson and Summers, 1997). Laying hens (42-50 weeks of age), as indicated by this study, should be targeted at a daily intake of 448 mg threonine/hen and 152 mg tryptophan/hen. The NRC (1994) recommendations for threonine and tryptophan for laying hens are supported by this and other recent studies (Coon (1998) and Ishibashi *et al.* (1998) for threonine, and Rhodimet Nutrition Guide (1993) and Leeson and Summers (1997) for tryptophan).

The results of the balance studies clearly show that crystalline AA supplementation of the reduced-protein diets improved the AA balance in the diet, hence improving the protein utilization efficiency and reducing nitrogen content in the excreta. Lowering the CP content of the experimental diets resulted in a 36%, 25% and 46% reduction in nitrogen output in the excreta of starting and growing broilers and layers, respectively. In order to improve the utilization of protein, valid data on the requirements for essential AA are crucial. Furthermore, by improving the efficiency of protein utilization, less nitrogen will be excreted into the environment. The latter is becoming an important issue in countries with a high concentration of animals and a limited land base for manure disposal. Future studies should concentrate on determining the requirements of poultry for essential AA other than lysine and methionine, especially for older birds. In addition, further research is needed to more accurately determine the amounts of digestible AA in feedstuffs and the digestible AA requirements of poultry. This is because the use of digestible AA values in the formulation of poultry diets is a means of

improving the utilization of protein sources that are known to be less digestible than soybean meal (Fernandez *et al.*, 1995; Rostagno *et al.*, 1995; Wang and Parsons, 1998).

As the world population continues to increase, improving the utilization of feed ingredients that are not in direct competition with human food is equally important. Before incorporating palm kernel cake (PKC) into poultry diets, nutritionists should be aware that there are large discrepancies in the reported nutritive quality for PKC and that the reasons for these discrepancies are not clear. Therefore, the results presented in this thesis may reflect only the quality of PKC obtained from Malaysia, and some of these findings might not be applied to PKC obtained from another countries. On average, Malaysian PKC was found to contain moderate amounts of most nutrients (16.6% CP; 14.6% CF; 4.9% ash; 0.41% calcium and 0.77% total phosphorus). In general, PKC samples obtained from screw-press extraction plants contained a higher amount of residual oil (8.93% versus 3.29%), gross energy (4,718 kcal/kg versus 4,504 kcal/kg) and acid detergent lignin (10.26% versus 7.94%) than PKC samples from solvent-extraction plants. In addition, the concentration of AA, digestibility of AA and metabolizable energy are dependent on the method of oil extraction. Therefore, because of its higher nutrient digestibility and higher digestible AA contents, poultry nutritionists should try to use PKC obtained from solvent extraction plants.

The low digestibility of PKC AA (62%) indicates that formulating PKC diets using digestible AA values should improve the utilization of protein and should give a better growth performance over PKC diets formulated using total AA. Nonetheless, future research in this area is necessary. Apparent metabolizable energy (AME) and nitrogen corrected true metabolizable energy (TME_n) values for PKC were found to be low compared with that of corn. Therefore, the main constraint in using large quantities of PKC in poultry diets is its low energy content. Therefore, when PKC is included in poultry diets, nutritionists should try to bring the energy content of the diet to the normal standard. Also, future research to further improve the metabolizable energy content of PKC is crucial. In addition, nutritionists should pay special attention to the AA balance in the diet, because PKC contains a high level of arginine and it is also limiting in several AA including lysine and methionine.

Up to now, no published study has been conducted to investigate the effects of using PKC during different phases of the broiler production cycle or on the effects of enzyme supplementation of PKC-based diets on poultry performance. An enzyme mixture (mannanase, α-galactosidase and protease) was found to be very effective in breaking down the non-starch

polysaccharides of PKC. Therefore, nutritionists should consider the use of supplemental enzymes in PKC-based diets if it is cost-effective. Results from the broiler trial demonstrated that 20% PKC could be used in the starter phase or grower phase. However, dietary inclusion of 20% PKC in both the starter and grower phases is not recommended unless supplemental enzyme is used. Even though research conducted in other countries was conducted using higher than 20% PKC without reducing broiler performance, the results of the present study do not recommend the use of more than 20% Malaysia PKC in broiler diets because of its low AME level and low AA digestibility.

When PKC-based grower diets are fed to broilers under elevated environmental temperatures (36 °C), broiler producers should pay attention to the temperature and ventilation inside the building, because these birds are more likely to die from heat stress than those fed corn-soybean meal diets. The exact mechanism regarding how PKC-based diets could have induced higher mortality due to heat stress under elevated environmental temperatures is not clear and further research is necessary to investigate the possible reasons.

Not many published studies on PKC have dealt with laying hens. In the present study, laying hens were able to tolerate 12.5% and 25% PKC without reducing egg production or egg mass. However, layers fed either the 12.5% or 25% PKC diets consumed significantly more feed and had poorer FCR than layers fed the corn-soybean meal control diet. Poultry producers should be aware that even though PKC did not affect eggshell quality, egg yolk color became significantly paler when 25% PKC-based diets were fed to the layers. Therefore, using a high level of PKC (25%) is not recommended in countries where consumers prefer a darker yolk color unless a pigmenting agent is used. In addition, because of the lower energy and AA requirements of laying hens compared with broilers, PKC is more suitable for layer diets. Enzyme supplementation of PKC-based poultry diets looks promising, and further research in this area is warranted. High fiber content, poor protein quality and also low metabolizable energy in PKC indicate that it could be a valuable feed ingredient for ruminants. However, with the commercial availability of synthetic amino acids and enzymes it is now possible to improve the nutritive quality of PKC, thus making it suitable for use as a poultry feed ingredient as indicated by the data presented in this thesis.

In addition, it is not clear whether the mannose released by enzyme treatment will be absorbed by the animal, and further research should be conducted to investigate whether the efficiency of absorption of mannose in the digestive tract is equivalent to that of glucose.

Recently, a few studies have demonstrated that mannose from either a purified source or from the digestion of PKC could bind salmonella (Izat *et al.*, 1990; Allen *et al.*, 1997). It would be interesting to know whether dietary inclusion of PKC could reduce the incidence of bacterial infections in poultry. The use of by-products such as PKC in poultry feeds that are not competing as human food sources will conserve feed resources and should be encouraged.

The findings presented in this thesis are valuable to the poultry industry in both developed and developing countries. The new information on the estimated requirements of poultry for threonine and tryptophan are useful for nutritionists who want to supplement feed-grade threonine and tryptophan in poultry diets. In addition, reduced protein diets are an option for poultry producers who want to reduce the amount of nitrogen in the excreta and minimize nitrogen pollution. The new information on PKC is valuable to nutritionists who want to incorporate PKC into poultry diets. In conclusion, improving utilization of conventional and non-conventional poultry feedstuffs with supplemental AA and enzymes will help ensure long-term profitability and sustainability of the poultry industry in a very competitive environment.

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