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Effect of Protein: Lysine Ratio on Energy and Nitrogen Metabolism in Broiler Chickens

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Abstract: The present study was performed to test the assumption that imbalanced dietary amino acid mixtures must lead to increased heat production (HP). The experiment was based on diets formulated to have a wide range of crude protein (CP) concentrations but a fixed concentration of lysine, formulated to be the first-limiting amino acid. 21 d old male broiler chicks ($n = 50$) were divided in to 5 groups of 5 replicates with 2 bird in each. Five dietary treatments with CP levels of 18, 21, 24, 27 and 30% and with a constant lysine level (1.1%) were formulated. Feed intake, water intake and body-weight gain were measured and heat production were recorded by using indirect calorimetry system. There was no significant effect of varying CP concentration on feed intake. However, CP concentration had a significant effect on the water intake ($p < 0.01$). With constant lysine and varying CP, there was a 75% increase in N intake as CP concentration increased. This led to a 150% increase in N excretion, with no significant change in HP. Simulated HP agreed with the empirically determined results in not showing a trend with dietary CP rather correlated with the first limiting amino acid intake.

Key words: Crude protein, lysine, heat production, nitrogen retention, broiler

INTRODUCTION

Dietary protein quality influences growth but also affects the environment because of different rates of nitrogen (N) excretion. A direct relation in reduction of fecal and total N excretion with the reduction of dietary protein has been studied by Meluzzi *et al.* (2001) and Hernandez *et al.* (2011). A reduction in N excretion and increased efficiency of N deposition can be achieved by matching the amino acid (AA) composition of the diet with AA requirements. This would also be expected to have implications for energy metabolism. MacLeod (1997) reported that heat production on an imbalanced, lysine-limited, AA mixture was not higher than on a balanced AA source with the same lysine concentration and there was no indication of stimulation of heat production by excess AAs. Boorman and Ellis (1996) found no adverse effect of protein quality on lysine utilization and detected some indication of increasing net utilization with decreased protein quality. Morris *et al.* (1999) recommended that the requirement for lysine and other essential AA (EAA) should be expressed as a fixed proportion of the dietary crude protein (CP) content because a deficiency or excess of dietary CP could affect the requirement of the broiler for several EAA. However, the experiment of MacLeod (1997) used a rather heterogeneous series of diets and it was felt that a more linear series of diets would lead to more secure conclusions. The effect of diets with increasing concentration of CP at fixed essential amino acid concentrations on performance was also examined. Some studies have indicated that the requirement for the EAA is a function of

the total CP content of the diet if maximum growth rate is required (e.g., Grau, 1948; Almquist, 1952; Morris *et al.*, 1987; Abebe and Morris, 1990). Morris *et al.* (1999) discussed six alternative explanations for the limited response to the critical amino acids and concluded that the most likely explanation was that the limited response stems from a general imbalance of amino acids at high CP intakes. They also reported that an amino acid imbalance leads to the decline in the efficiency of utilization of the limiting amino acid at high CP intake where all amino acids except one are supplied at amounts exceeding the requirements. However, many reports (e.g., D'Mello, 1990; MacLeod, 1997) demonstrated that the utilization of the first limiting amino acid was not affected by amino acid imbalance. Although there have been many hypotheses, no complete explanation has been provided so far. This experiment was, therefore, based on diets formulated to have a wide range of linearly varying protein concentrations but equal concentrations of lysine, the first-limiting amino acid. The hypothesis being tested was that growth rate would be fixed by lysine concentration and that the energy cost of N excretion would therefore vary with excess N intake. The detailed relationships among amino acid balance, N metabolism and energy metabolism were investigated in a computer-controlled chamber calorimetry system. Effects of N metabolism on water intake were of practical importance and were also quantified. The experimental results were compared with simulations using the mechanistically based model of energy metabolism described by MacLeod (1994).

MATERIALS AND METHODS

Experimental birds: The experiment was performed with growing broiler chickens (21 d old) to examine five diets. Male broiler chicks (1 d old) from the same commercial line (Ross) were obtained in batches at 14 d intervals (so that they were of identical age in each period) and reared up to 20 d of age on a common diet in a brooder. At 21 d of age, chicks were randomly allocated (in pairs) to calorimeter chambers, where they remained for 14 d for the experiment. All animal procedures were in accordance with the United Kingdom Home Office regulations.

Experimental design: The experiment was designed as a Latin Square (5 diets x 5 calorimeter chambers) with five 2-wk measurement periods; there were therefore 5 replicate pairs of birds on each diet, giving a total of 50 birds used in the experiment.

Diets and environmental conditions: The lysine concentration of each experimental diet was a constant 1.1% with CP concentration ranging from 18 to 30% (Table 1). The experiment had an acclimatization period of 7 d in which birds were allowed to become accustomed to the experimental diet and calorimeter chambers. The indirect open-circuit calorimetry system has been described by Lundy *et al.* (1978) and improvements in the gas analysis system by MacLeod *et al.* (1985). The five replicate pairs of birds on each diet were allocated to five 2-wk periods and five treatments. Calorimeter chambers were kept at 20°C and on a lighting cycle of 23L: 1D which is commonly used in commercial broiler husbandry. The one hour of dark period started at 00:00 and ended at 01:00 h. The experimental diet and water were given *ad libitum*.

Measurements and sample collections: Feed intake (FI), water intake (WI), body weight gain (BWG) and heat production (HP) were measured daily. HP was measured by means of the indirect calorimetry apparatus. The measurements were made from d 28 to 32. After the first 3 d of measurement, the birds were fasted for 24 h to allow them to reach a basal level of metabolism. Fasting HP and endogenous faecal and urinary energy and N losses were then measured during a second 24 h period of fasting.

Daily droppings collections were made over 3 d during feeding and over the second day of fasting. Collected samples were stored at -20°C in sealed aluminium dishes until they were freeze-dried, weighed and ground for nitrogen and energy analysis. Each diet and droppings sample was measured in duplicate and the mean was calculated. Results were expressed/bird/day.

Simulation: The experimental results were compared with simulations using the mechanistically based model

of energy metabolism described by MacLeod (1994). The simulations were done using the mean bird weight at time of measurement and the mean FI.

Statistical analysis: Statistical analysis done by analysis of variance, fitting the effects of diet, column (chamber) and row (measurement period) in the model:

$$X = \text{Mean} + \text{Diet} + \text{Chamber} + \text{Period} + \text{Residual}$$

where, least-squares regression was also performed using GenStat.

RESULTS

The results are summarized in Table 2 and 3. There was no significant effect of varying CP concentration on FI. However, CP concentration had a significant effect on the WI ($p < 0.01$). The WI increased with increase in CP concentration. It was about 25% greater with birds on the high protein diet than on the moderate protein diet. Dry matter excretion was also significantly affected by CP concentration ($p = 0.001$). No difference was found between the birds in BWG ($p > 0.01$). However, the birds fed on the 18% CP diet showed about 25% higher BWG compared with the birds fed on the 30% CP diet. Feed conversion efficiency (FCE) was not affected by CP concentration. Although the birds fed on the highest protein diet showed slightly lower FCE, there was no significant difference between treatments ($p > 0.05$).

The increase of CP concentration has increased the N intake ($p < 0.001$). The increase was by about 52% between the lowest and highest CP diets (Fig. 1a). Nevertheless, N retention did not change as diet protein content increased ($p > 0.05$). N loss in excreta increased with the increase in CP content ($p < 0.001$). CP content had highly significant effects on efficiency of N utilization ($p < 0.001$). The birds fed on the highest CP diet had the lowest efficiency of N utilization, resulting in a increase in N excretion of about 150% compared with birds fed on moderate CP diet. The efficiency of N utilization was maximal in Diet 1 and significantly decreased as CP concentration increased (Fig. 1b).

Table 4 and Fig. 2 show the results for HP and energy utilization. There was no significant effect of amino acid balance on HP despite the very large change in N excretion. Simulated HP agreed with the empirically determined results in not showing a trend with dietary CP.

True metabolizable energy (ME corrected for endogenous energy losses) intake values are shown in Table 4. Although no significant difference in TME intake was detected between diets, there was a tendency for TME intake to be lower on higher CP diets. TME intakes were higher on the moderate protein, balanced diets (Diet 1 and 2) than high protein, imbalanced diets (Diet 3, 4 and 5).

Table 1: Specifications and ingredients of diets in calorimetry experiment

Diet	1	2	3	4	5
TME ¹ , MJ/kg	13.4	13.4	13.4	13.4	13.4
CP ² , (%)	18.0	21.0	24.0	27.0	30.0
Lysine concentration, (%)	1.1	1.1	1.1	1.1	1.1
Lysine: CP ratio	0.061	0.052	0.046	0.041	0.037
CP:TME ratio	0.0056	0.0066	0.0075	0.0084	0.0094
Ingredients, (%)					
Wheat meal	58.60	55.25	51.90	48.55	45.20
Barley	15.00	11.25	7.50	3.75	-
Maize meal	8.10	6.08	4.05	2.03	-
Maize gluten meal (60% CP)	-	5.45	10.90	16.35	21.80
Soya bean meal (48% CP)	-	6.10	12.20	18.30	24.40
Casein	10.10	7.51	5.01	2.50	-
Choline chloride	0.03	0.03	0.03	0.03	0.03
Fat blend	2.80	3.10	3.41	3.71	4.01
Dicalcium phosphate	1.15	1.21	1.26	1.32	1.37
Sodium chloride	0.38	0.37	0.36	0.35	0.34
Limestone flour	1.47	1.44	1.41	1.38	1.35
Vitamin/mineral supplement ³	0.50	0.50	0.50	0.50	0.50
Pellet binder	1.00	1.00	1.00	1.00	1.00
Lysine	0.08	0.06	0.04	0.02	-
Arginine	0.470	0.350	0.235	0.118	-
Methionine	0.270	0.2.03	0.135	0.068	-
Threonine	0.050	0.038	0.025	0.013	-

¹TME: true metabolisable energy, CP: crude protein, Vitamin/mineral supplement contains following nutrients per kg of diet: vitamin A, 15,000IU; vitamin D₃, 1,500IU; vitamin E, 20 mg; vitamin K₃, 0.7 mg; vitamin B₁₂, 0.02 mg; niacin, 22.5 mg; thiamin, 5 mg; folic acid, 0.7 mg; pyridoxine, 1.3 mg; riboflavin, 5 mg; pantothenic acid, 25 mg; choline chloride, 175 mg; Mn, 60 mg; Zn, 45 mg; I, 1.25 mg; Se, 0.4 mg; Cu, 10 mg; Fe, 72 mg; Co, 2.5 mg

Table 2: Effect of crude protein content on feed intake, water intake, weight gain, feed conversion efficiency and dry matter excretion

Diet	1	2	3	4	5	SED	p-value
CP (%)	18	21	24	27	30		
Feed intake (g/bird/d)	132.1	127.3	126.6	123.7	129.1	4.99	NS
Feed intake (g/kgW ^{0.75} /d)	119.8	113.4	112.7	111.6	117.0	3.99	NS
Water intake (ml/bird/d)	170.5 ^b	172.8 ^b	181.8 ^b	195.0 ^{ab}	214.7 ^a	11.65	0.016
Water intake (ml/kgW ^{0.75} /d)	154.7 ^b	153.6 ^b	161.8 ^b	175.7 ^{ab}	194.7 ^a	10.03	0.008
Water: Feed ratio	1.29 ^c	1.35 ^{bc}	1.44 ^b	1.57 ^{ab}	1.66 ^a	0.05	<0.001
Weight gain (g/bird/d)	80.0	74.0	76.9	75.0	67.7	6.62	NS
FCE ²	0.61	0.58	0.61	0.60	0.53	0.04	NS
Excretion, DM ³ (g/bird/d)	30.7 ^b	32.8 ^b	34.9 ^b	39.0 ^a	41.6 ^a	2.11	0.002
Excretion, DM (g/kgW ^{0.75} /d)	27.9 ^b	29.2 ^b	31.1 ^b	35.2 ^a	37.7 ^a	1.84	0.001

¹CP: crude protein, ²FCE: feed conversion efficiency, ³DM: dry matter
^{a,b,c}Means with different superscripts within a row differ significantly

Protein, fat and total energy retentions are presented in Table 4. Total energy retention differ significantly ($p < 0.05$) between varying CP diets. Energy retention as protein was similar between diets. Energy retention as fat between diets were significantly different ($p = 0.001$) and there was a tendency to be lower on higher CP diets.

DISCUSSION

The results indicated that there was no effect of CP concentration ranging from 18 to 30% of diets on FI. It seems that control of energy intake or first limiting amino acid took priority over the control of CP intake. MacLeod (1990) reported that there was no effect of CP concentration on feed intake when diets were fed ranging from 13.0 to 26.0%. These results are also in

agreement with Shariatmadari and Forbes (1993) who reported that there was no significant difference in feed intake and growth rate between diets ranging from 17.2 to 28.0% CP. Similarly, Plumstead *et al.* (2007) found no effect of dietary CP concentration on feed intake of broilers when a fixed proportion of dietary lysine (dLys) relative to CP and key indispensable AA was maintained in graded increments of CP from 21.9 to 26.9%. As per Kamran *et al.* (2008) feeding low-CP diets with constant ME:CP ratio had adversely affected the growth performance of broilers. Morris *et al.* (1987) examined the effect of diets varying in CP content (14.0 to 28.0% of diet) on male broiler chicks. They reported that growth rate and efficiency of feed utilization to 21 d of age responded to increasing dietary protein contents up to about 23.0% CP. According to Abebe and Morris (1990),

Table 3: Effect of crude protein content on nitrogen intake, retention and loss and efficiency of nitrogen utilization

Diet	1	2	3	4	5		
CP ¹ (%)	18	21	24	27	30	SED	p-value
N ² intake (g/bird/d)	4.10 ^c	4.18 ^c	5.29 ^b	5.90 ^a	6.18 ^a	0.21	<0.001
N intake (g/kgW ^{0.75} /d)	3.70 ^c	3.72 ^c	4.72 ^b	5.32 ^a	5.61 ^a	0.18	<0.001
N retention (g/bird/d)	2.68	2.43	2.60	2.61	2.60	0.15	NS
N retention (g/kgW ^{0.75} /d)	2.44	2.16	2.32	2.36	2.35	0.12	NS
N loss (g/bird/d)	1.41 ^c	1.75 ^c	2.68 ^b	3.29 ^{ab}	3.59 ^a	0.17	<0.001
N loss (g/kgW ^{0.75} /d)	1.28 ^d	1.56 ^d	2.40 ^c	2.97 ^b	3.26 ^a	0.15	<0.001
Efficiency of N retention	0.66 ^a	0.58 ^b	0.49 ^c	0.44 ^{cd}	0.42 ^d	0.022	<0.001

¹CP: crude protein, ²N: Nitrogen

^{a,b,c,d}Means with different superscripts within a row differ significantly

Table 4: Effect of crude protein content on heat production and energy utilization

Diet	1	2	3	4	5		
CP ¹ (%)	18	21	24	27	30	SED	p-value
TME ² intake (kJ/bird/d)	1870	1918	1824	1844	1763	64.5	NS
TME intake (kJ/kgW ^{0.75} /d)	1696	1708	1622	1663	1598	48.3	NS
Heat production (kJ/bird/d)	1037	1021	1038	988	999	33.4	NS
Heat production (kJ/kgW ^{0.75} /d)	939.7	908.9	928.3	890.7	902.3	26.24	NS
Simulated HP (kJ/bird/d)*	977	977	970	978	979	-	-
E ³ retention (kJ/bird/d)	725	800	680	754	654	45.7	0.056
E retention (kJ/kgW ^{0.75} /d)	657 ^{ab}	712 ^a	604 ^b	680 ^a	593 ^b	37.2	0.039
CP retention (g/kgW ^{0.75} /d)	16.8	15.8	16.3	16.3	16.2	0.92	NS
E retention as protein (kJ/bird/d)	398	360	386	387	384	21.76	NS
E ret as protein (kJ/kgW ^{0.75} /d)	361	320	343	349	348	17.13	NS
E retention as fat (kJ/bird/d)	328 ^b	440 ^a	294 ^{bc}	367 ^b	270 ^c	30.84	0.001
E retention as fat (kJ/kgW ^{0.75} /d)	297 ^b	393 ^a	261 ^{bc}	331 ^{ab}	245 ^c	26.68	0.001

¹CP: crude protein, ²TME: true metabolizable energy, ³E: energy

^{a,b,c}Means with different superscripts within a row differ significantly

*Simulation results cannot be given a standard error since the model is not stochastic

the concentration of the first limiting AA had the most important effect on growth and feed intake. The results from the present experiment confirm that growth rates were fixed by the concentration of the first limiting AA. Although the FI of birds on different diets did not differ, those on the lowest protein diet (18% CP) tended to have a slightly higher FI than other groups. It has been suggested that with a diet containing slightly less protein than optimal, FI might increase modestly over that for an optimal diet (Boorman, 1979) as the birds try to attain a protein intake commensurate with their needs.

Gleeves *et al.* (1968) reported that feed intake was reduced when the CP:ME ratio was decreased and vice-versa. They attributed the greater feed intake when the ratio increased to the increase in egg production, where egg production was associated with protein level. Protein concentration, therefore, acted on FI in an indirect way. They also suggested that energy in the diet should be adequate, otherwise protein would be used as a source of energy but, when energy is beyond the requirement level, it will serve as the main regulator of FI. Fancher and Jensen (1989) fed male broilers diets varying CP levels below 20% and reported that the minimum CP level needed to optimize body weight ranged from 16.0 to 17.5% whereas feed efficiency required that the CP be greater than 18.9%. When a fixed ratio of dLys to CP was applied and indispensable and

dispensable AA were not limiting, BWG and adjusted FCR of birds responded positively to incremental dLys up to at least 1.32% (27.2% CP) and was independent of the dietary ME over a range from 3,000 to 3,200 kcal/kg (Plumstead *et al.*, 2007).

Although differences were not significant, there was a tendency for growth to be lower on higher CP diets in the present experiment. Sklan and Plavnik (2002) reported that increasing protein content from 18.2 to 24.2% resulted in a linear decrease in feed intake, while weight gain and feed efficiency changed quadratically with a smaller positive effect at the highest CP intakes. They suggested that broiler performance at the lower protein intakes was limited by either non-EAA or EAA intake whereas at high protein intakes the decreased efficiency of amino acid utilization after growth requirements are fulfilled resulted in poorer performance.

Water intake was significantly affected by the CP content of the diet. Water: feed ratio also increased from 1.29 up to 1.66 as CP content increased from 18 to 30%. Marks and Pesti (1984) reported that increasing CP content increased the water intake and water: feed ratios. They found that birds receiving a 26% protein diet consumed more water than birds receiving the 22 and 17% diets (124, 108 and 86 g/d from d 16-18, respectively) and concluded that water: feed ratios were directly proportional to dietary protein content. Whereas,

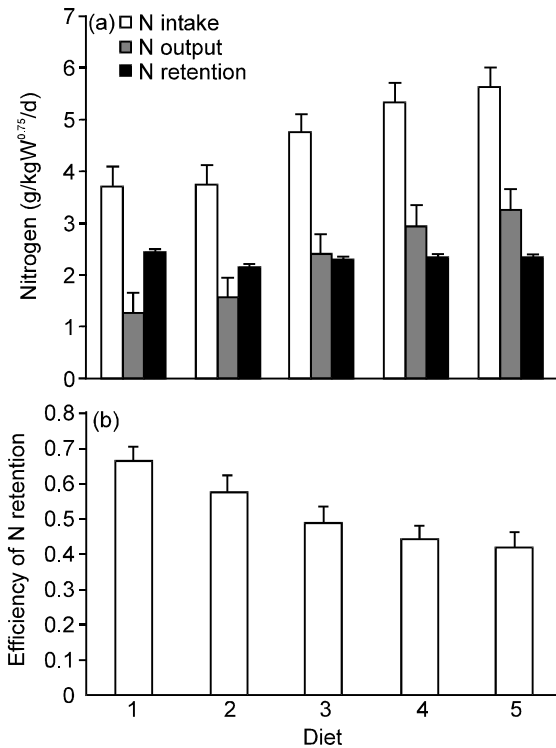


Fig. 1: Effect of crude protein (CP) content on nitrogen (N) utilization. (a) Effect of CP content on nitrogen intake, loss and retention and (b) Effect of CP content on efficiency of nitrogen retention*CP content of diets: 1, 18%; 2, 21%; 3, 24%; 4, 27%; 5, 30%. Standard error bars were calculated from the pooled estimate of the results

Syafwan *et al.* (2012) found 8% higher water intake in broilers fed diet with 21.5% CP. Patrick and Ferrise (1961) stated that greater amounts of water are required for the metabolism of protein than for carbohydrates or fat. A partial explanation for this relationship may be that more water is needed to increase the elimination of N as uric acid. Since the bird is incapable of breaking down uric acid into urea, an appreciable amount of water consumed is, therefore, tied up in the metabolism of the excess of protein ingested.

It is generally recognized that reducing the dietary protein concentration is the most efficient way to lower N excretion. According to Summers (1993), when dietary CP was lowered 17 to 13% for 24-wk-old layers, faecal N excretion was reduced as much as 34% without affecting egg production. Hernandez *et al.* (2012) observed that the reduction in dietary protein content linearly decreased water consumption and when the protein in broiler's diet reduced by 1.5 or 3%, it reduced N excretion to the environment by 9.5 and 17% in males and by 11.8 and 14.6% in females, respectively. Similarly, in another experiment on pigs by Hernandez *et al.* (2011) it was concluded that lowering dietary CP

levels even by small amounts and using CP levels close to those used in commercial diets decreased urinary and total N excretion. Faecal N content significantly and linearly decreased with reduction in dietary protein content (from 17 to 13%) and was about 50% of the intake in brown laying hens (Meluzzi *et al.*, 2001). Several researchers (Moran *et al.*, 1992; Moran, 1994) have shown that reducing the dietary protein content to 10 and 15% while maintaining the required essential amino acid levels within each age period for broilers, will reduce litter N content approximately 24% without impairing weight gain. In this experiment, increasing dietary CP level by 67% and N intake by 1.91 g/d resulted in a 155% (1.98 g/d) increase in N excretion (Fig. 3). Ferguson *et al.* (1998) reported that each 1% reduction in the protein level of the broiler diet resulted in a 7% reduction in N output. Blair *et al.* (1999) showed that reduction in dietary CP content caused a 10-27% reduction in the total amount of N excreted during the 6-wk broiler rearing period and with layers, there was a 30-35% reduction in daily N output. They also reported that reduction in dietary CP from about 21 to 18% resulted in a reduction of more than 20% in daily N output. This is similar to the result in present experiment which showed 20% reduction in N output as CP level decreased from 21 to 18%. The efficiency of N utilization decreased as CP content increased. Overall, reduction of dietary CP level had a beneficial effect in terms of reducing N excretion.

Theoretically birds fed protein in excess of their requirements must catabolize the surplus amino acids, leading to increased excretion of N and this process yields heat. Both the synthesis and degradation of protein and amino acids are energy demanding processes and these costs are necessarily included in model predictions of energy balance. Also, when protein synthesis becomes limited by the first limiting amino acid, amino acids present in excess of the resulting requirement enter the pool of substrates available as energy sources. It has been implied that this would result in a greater heat increment than would occur with a balanced amino acid mixture (MacLeod, 1997). In birds, the excretion of excess amino acid N which is not used for protein synthesis takes place mainly as uric acid. Uric acid synthesis is approximately twice as energy demanding as urea synthesis and so birds inherit an energy cost for excreting an insoluble nitrogenous product. However, uric acid has four of N atoms and urea has two N. Therefore, the total energy cost is about the same per each of N atom excreted. According to MacLeod (1997), the trans-species N correction of 5.63 kJ/g of protein retained (35.2 kJ/g N) agrees closely with the 36.5 kJ/g N conventionally used in poultry.

Emmans (1994) reported the method to predict the effective energy' of diets. He suggested that heat

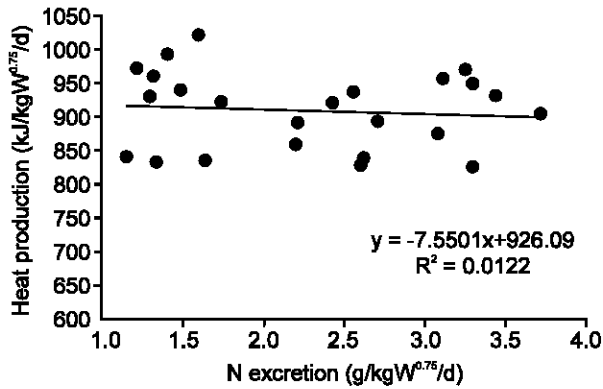


Fig. 2: Relationship between heat production (HP) and nitrogen (N) excretion. Standard errors are shown in square brackets. $HP = -7.55[14.5]N_{Out} + 926.1[35.2]$, ($p = 0.608$)

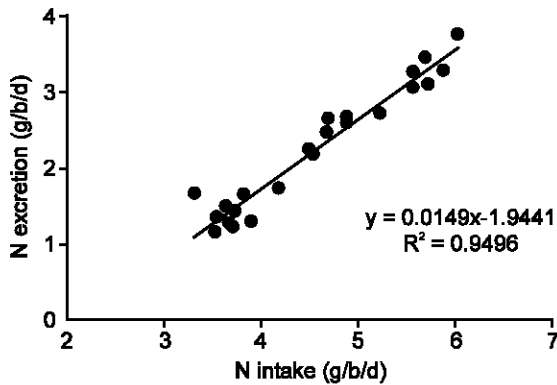


Fig. 3: Linear regression of nitrogen excretion (Y) on nitrogen intake (X) of birds

increment of feeding is linearly related to five measurable traits; faecal organic matter, urinary nitrogen, N retention, lipid retention derived from dietary lipid and fat retention derived from non-lipid feed ingredients. The estimates for these process of the heat increment are 3.81, 29.20, 36.48, 16.40 and 4.39 kJ/g product, respectively. According to his calculation, 29.2 kJ of energy cost is needed for the excretion of g of UN.

Digestibility of protein varies with ingredients of the diet. But typical digestibility of N in the diet is approximately 75% which means about 25% of N intake will be lost in the faeces. Therefore, the amount of urinary N can be estimated from the total N loss in the faeces. In the present experiment, therefore, the heat increment of UN excretion of each treatment can be calculated as 9.93, 18.4, 35.6, 47.9 and 54.3 kJ/bird/d, respectively. HP of each group ranged between 890 and 940 kJ/kgW^{0.75}/d and was not significantly different. The estimated values of energy cost of N excretion are about 1 to 6% of total HP which is relatively small. The largest part of heat increment of feeding is protein retention which costs

36.48 kJ/g. N retention of five treatments was constant in the present experiment. Energy cost of protein retention, therefore, seems to have not affected the total HP.

Although there was fluctuation in fat retention, there was a tendency for it to decrease as protein content increased. The decrease in energy retained as fat, as CP level increases in the diets, was reported by Adams *et al.* (1962) and MacLeod (1990, 1992). The reduction in protein concentration means that a greater proportion of energy will be available from the diet to fuel the different processes which require energy. This, in turn, allows the bird to store a higher proportion of its energy intake as body fat. It was reported that excess dietary protein also forced the birds to use energy to excrete excess N as uric acid (Bartov, 1979). Therefore, less energy would be available for lipogenesis (Buttery and Boorman, 1976).

Conclusion: The experiment was performed to test the hypothesis that growth would be determined by lysine concentration and that the energy cost of nitrogen excretion would vary with excess nitrogen intake. The results showed that varying CP content had no effect on feed intake and growth. This suggests that growth rates were fixed by the concentration of the first limiting amino acid or dietary energy content. Since the ME content of the diet and feed intake were within normal limits, energy intake is unlikely to have been limiting. However, N loss in excreta significantly increased with increasing CP content. The efficiency of nitrogen utilization significantly decreased as CP content increased. This confirmed the beneficial effect of reduced CP diet in terms of reducing N excretion. The estimated energy cost of N excretion was relatively small and there was no indication of a stimulation of HP by excess amino acids. These results suggest that HP was correlated with the first limiting amino acid intake rather than total CP intake. Further experiments should be planned to investigate this relationship between HP and the first limiting amino acid.

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