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Diet Selection for Protein Quality by Growing Broiler Chickens

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Abstract: Two free-choice feeding experiments were performed to investigate the sensitivity of chickens to its diet on the basis of amino acid balance, especially related to lysine concentration. In first experiment 21 d old broiler chicks (n = 48) were divided in to four groups (in pairs) and offered different proportion of lysine whereas in second experiment two diets with ideal and high protein was offered to broiler chicks (n = 20). Over the whole period of the experiment, birds consumed some of each diet offered, but preference was shown for the moderate or high lysine diet over the low lysine diet. Growth rate reflected total lysine intake. The birds offered the choice between Ideal Protein diet and High Protein diet consumed about 2.5 times as much of the Ideal protein diet as of the imbalanced diet. So, the broilers are instinctively prone to balanced nutrition and high amino acids are on their priority during various strategic choice feeding programs.

Key words: choice-feeding, amino-acid, lysine, broiler

INTRODUCTION

Animals have a tendency to avoid limiting their diet composition to only one food when given a free access to two or more foods of varying nutrient composition (Forbes, 1995). Most of the investigations state that this tendency is a function of diet composition and nutritional status of animal to meet their various dietary requirements (Fortes-Silva *et al.*, 2012; Mehri, 2012).

Birds select an optimum diet between foods of different protein content to provide them with a diet which is close to the optimal for growth. Factors that influence dietary choices by chickens include genotype, age, physiological state and prior experience (e.g., Rose and Kyriazakis, 1991; Shariatmadari and Forbes, 1993; Forbes, 1995; Sterling *et al.*, 2006). When chickens were offered diets either above or below optimal protein content, they consumed predominantly the diet closest to optimum (Shariatmadari and Forbes, 1993). Gous and Swatson (2000) showed that broiler chickens effectively select a combination of diets that meet their requirements and maximize their biological performance, when provided with two or three foodstuffs with just one protein source and offered on a free-choice basis. Shariatmadari and Forbes (1993) reported reduced protein intake in choice situations involving diets with very low and high protein contents. Broilers on choice feeding selected diets with a lower percentage of crude protein (CP) and had lower live body weights at six wk than those fed the complete diet (Yo *et al.*, 1998). Considering the age factor in broilers, Delezie *et al.* (2009) stated that broilers can make a choice between two feeds differing in protein/amino acid contents starting as early as day one and confirmed that very high protein/amino acid concentrations in the starter period are desirable to improve growth rates in fast-growing chickens.

In an experiment with insect, *Drosophila melanogaster* by Toshima and Tanimura (2012), it was suggested that taste preference for amino acids is dependent on internal nutritional state. Amino-acid-deprived flies demonstrated enhanced preference to an amino acid mixture and to several amino acids. Dietary preference of essential amino acid has also been reported in fish. Fortes-Silva *et al.* (2012) observed that tilapia fish avoid the EAA-deficient diet and chose a diet supplemented with EAA, l-tryptophane, l-methionine, l-threonine. There is not much information on diet selection of poultry with few investigations involving specific amino acids (Newman and Sands, 1983; Edmonds and Baker, 1987; Roth *et al.*, 1990; Steinruck *et al.*, 1990a) and essentially none on genetic variation for diet choice. Picard *et al.* (1993) demonstrated that, when effects of raw materials were removed, broilers were able to distinguish between a balanced diet and one deficient in lysine, methionine and tryptophan. Picard *et al.* (1993) also confirmed this by measuring in two genetic lines, discriminatory food intake and growth responses to diets varying in these amino acids. Newman and Sands (1993) investigated the choice by young broiler chicks between a low-lysine diet and one with excess of lysine. Although the birds consumed some of the supplemented food it was not enough to maintain a growth rate as high as those in a control group which were given a single adequate diet. However, these chicks showed definite preference for the adequate lysine diet, which implies sensory recognition of lysine by birds.

In the present study, free-choice feeding was performed to investigate the sensitivity of the broiler chicken to its diet on the basis of protein quality and amino acid balance, especially related to lysine concentration. The hypothesis tested in these experiments was that birds could select between an adequate or balanced diet and a diet with an amino acid deficiency or excess.

MATERIALS AND METHODS

Experiment 1: Forty-eight male broiler chicks were used. The birds were reared in brooders for 20 days after hatching on a common starter diet. At 21 d of age, they were randomly allocated (in pairs) into cages fitted with two feeding troughs and a water container. Three diets with different lysine concentrations were formulated (Table 1 and 2). The lysine concentrations were 0.6 (low), 1.0 (moderate; NRC (1994) recommendation for 3-6 wk old broiler) and 1.4% (high), respectively. Other amino acids were maintained at concentrations 1.5 times the published recommendations, to ensure that no other amino acid became limiting. The diets were made by adding the supplements on the basal diet to minimize the palatability for certain feed ingredient. As supplemental lysine is removed, stepwise, it was replaced with 2X its weight of glutamic acid to keep diets isonitrogenous (Table 2). Birds were divided into 4 treatment groups. Group A was offered the choice between the low lysine diet and the moderate lysine diet. Group B was offered the low and high lysine diets. Group C was given the moderate and high lysine diets. Group D had only the moderate lysine diet, to act as the control group but the food was given in two separate troughs. All diets contained 30% of crude protein and 3200 kcal/kg of true metabolizable energy. Feed and water were provided *ad libitum* to the birds. Diets and fresh water were replenished daily. Two days were allowed for the birds to become accustomed to the experimental set-up. Food and water intake and body weight were measured daily thereafter, for 10 d. The environmental temperature was 22°C and the birds were kept on a 23L:1D lighting cycle. Statistical significance of the choice was tested by one-sample t-test under the null hypothesis that the birds ate equal amounts of the two diets.

Experiment 2: Twenty male boiler chicks were reared to 14 d in brooders on a standard starter diet. At 14 d they were randomly allocated to individual cages fitted with two troughs, one for each of the two diets. Formulations of the two diets with ideal and high protein are presented are in Table 3. Diets and water were available *ad libitum*. Food and fresh water were replenished daily. Food intake and body weight were measured daily thereafter, for 14 d. The environmental temperature was 22°C and the birds were kept on a 23L: 1D lighting cycle. The data were analyzed by one sample t-test and analysis of variance.

All animal procedures were in accordance with United Kingdom Home Office regulations.

RESULTS AND DISCUSSION

Experiment 1: Over the whole period of the experiment, birds consumed some of each diet offered, but preference was shown for the moderate or high lysine diet over the low lysine diet. During the first 5 d period, group A showed a preference for the moderate lysine diet (Table 4). Group B also showed that the bird consumed more of the high lysine diet than the low lysine one but the tendency was not large enough to reach significance. In the second half of the period (d 6-10), the difference in selection reached on highest ($p<0.001$) levels in both group A and B. Both groups consumed about 2.5 times as much of the moderate and high lysine diets as of low lysine diet. When data was pooled for d 1-10, besides having greatest difference statistically birds didn't consume with same intensity as during d 6-10. In groups C and D, there was no significant difference in selection over the whole period of measurement ($p>0.05$).

Group A and B, which had a low lysine diet included in their choice showed lower total food and water intake and growth rate than the groups including high lysine diet (Table 5). Growth rate reflected total lysine intake (Table 6, Fig. 1). Although the birds consumed some of moderate lysine diet in group A, it was not enough to produce a growth rate as high as those in control group D.

In this experiment, groups A and B showed a very similar degree of preference for the higher of the two lysine concentrations which they were offered. The selection became more significant with time. In group B, this led to a lysine intake equal to that of the control group (D). These results agree with Newman and Sands (1993) who reported significant preference for an adequate lysine diet over a low lysine diet. In a study by Kirchgessner *et al.* (1999), they observed that monogastrics that were fed a choice of a lysine-deficient and a lysine-sufficient diet did not select randomly from both diets but selected a significantly higher proportion of the lysine sufficient diet than of the lysine-deficient one. Ettle and Roth (2009) stated that monogastrics have the ability to distinguish between diets differing in Lys content and prefer a better balanced diet over a Lys deficient one. Similarly, lysine deficient rats selected lysine containing solution over other amino acid containing solution (Mori *et al.*, 1991; Tabuchi *et al.*, 1991). According to Murphy and King (1989), birds showed a preference even for a protein-free diet over a high-protein diet with a severe imbalance of amino acids. It is assumed that the birds learn that one food contains too little of an amino acid for its requirements and another doesn't. Hrupka *et al.* (1999) demonstrated that small changes in dietary lysine concentration are a meaningful stimulus for dietary selection when animals are adapted to lysine-deficient diets. Forbes (1995) stated that it is incumbent to give a color cue if the

Table 1: Formulation of basal diet in experiment 1

Ingredient	%
Wheat meal	46.98
Barley meal	8.68
Maize gluten meal (60% CP[3])	34.85
Maize oil	1.0
Choline chloride	0.03
Fish meal	0.96
Dicalcium phosphate	2.91
Sodium chloride	0.38
Limestone flour	1.5
Arginine	0.8
Methionine	0.14
Threonine	0.19
Tryptophan	0.08
Vitamin/mineral supplement[1]	0.5
Pellet binder	1.0
Calculated values	
TME[2], (kcal/kg)	3200
CP[3], (%)	30

[1]Vitamin/mineral supplement contain following nutrients per kg of diet: vitamin A, 1,175,000 IU; vitamin D3, 225,000 IU; vitamin E 1,900 IU; vitamin K, 891 mg; vitamin B1, 50 mg; vitamin B2, 2,250 mg; vitamin B6, 750 mg; vitamin B12, 600 mg; Ca-pantothenate, 2,500 mg; niacin, 15,400 mg; biotin, 110 mg; folic acid, 30 mg; Co, 50 mg; Cu, 1,750 mg; Mn, 36,000 mg; Zn, 24,000 mg; I, 600 mg; Se, 25 mg
[2]TME, True Metabolizable Energy
[3]CP, Crude Protein

Table 2: Composition of lysine supplements in experiment 1

	------------- Supplements (% of diet) -----------------		
Diet	Lysine	Glutamic acid	Maize starch
Low	0	1.583	0
Moderate	0.5	0.792	0.291
High	1.0	0	0.583

Table 3: Formulation of experimental diets in experiment 2

	Ideal protein	High protein
Ingredient	------------------- %	--------------------
Wheat meal	66.5	50.28
Soya bean meal (48% CP)	16.05	21.2
Maize gluten meal (60% CP)	4.03	15.0
Fish meal	2.9	5.762
Soya oil	5.08	3.94
Sodium chloride	0.342	0.28
Dicalcium phosphate	1.4	0.874
Choline chloride	0.03	0.03
Limestone flour	0.857	0.734
Titanium dioxide	0.4	0.4
Arginine	0.185	0
Lysine	0.346	0
Methionine	0.252	0
Threonine	0.128	0
Vitamin/mineral supplement[1]	0.5	0.5
Pellet binder	1	1
Calculated values		
TME[2], kcal/kg	3200	3200
CP[3], (%)	20.5	29.4
Lysine concentration, (%)	1.1	1.16

[1]Vitamin/mineral supplement contain following nutrients per kg of diet: vitamin A, 1,175,000 IU; vitamin D3, 225,000 IU; vitamin E 1,900 IU; vitamin K, 891 mg; vitamin B1, 50 mg; vitamin B2, 2,250 mg; vitamin B6, 750 mg; vitamin B12, 600 mg; Ca-pantothenate, 2,500 mg; niacin, 15,400 mg; biotin, 110 mg; folic acid, 30 mg; Co, 50 mg; Cu, 1,750 mg; Mn, 36,000 mg; Zn, 24,000 mg; I, 600 mg; Se, 25 mg
[2]TME, True Metabolizable Energy
[3]CP, Crude Protein

nutrient in question is only required in trace amounts and especially if it is colorless. Lysine is colorless and has little taste. In this experiment, however, the birds showed clear preference for high lysine diets although color cue was not used. It appears that birds learned the difference between the two foods primarily by their positions in the cage since the position of two troughs was not changed as the case of Newman and Sands (1993). Steinruck *et al.* (1990b) examined broilers given a choice between a complete diet and methionine-deficient diet (half the recommendation) chose predominantly the former, especially after they had been made methionine-deficient by prior feeding on the low methionine diet. The same authors (1990a) investigated the effect on diet selection and growth of position of complete and methionine-deficient foods in the cage. Deficient birds were either given the two foods in the same position everyday for 36 d or the positions of the foods were reversed every 3, 6 or 9 days. There was a 0.68 choice of the complete diet where the positions were unchanged, but as the changes became more rapid so selection was less appropriate, being 0.65, 0.62 and 0.58 for the 9, 6 and 3 d change over birds, respectively. Uzu *et al.* (1993) also examined the effect of location of feeders by choice feeding between a balanced diet and a methionine-deficient diet in laying hens and reported that when location was reversed, approximately 1 wk was required for hens to again exhibit preference for the balanced diet. Forbes and Shariatmadari (1994) pointed out that some authors have misguidedly reversed the positions of two diets at frequent intervals in the hopes of avoiding positional bias and have seen poor diet selection as a result.

In groups C and D, there was no significant difference between selections over the whole period of measurement. In the case of group C, it appeared that the birds' sensitivity was not high enough to distinguish between moderate lysine and high lysine. This may have been caused by the high protein content of the diets, since the dietary protein concentration can affect the requirements for individual essential amino acids (Sterling *et al.*, 2006; Mehri, 2012; Ospina-Rojas *et al.*, 2012). Even among the published recommendations of lysine for broiler, there are some differences between reports (e.g., Dozier *et al.*, 2010; Ospina-Rojas *et al.*, 2012). Therefore, the high lysine diet (14 g/kg) in this experiment might not be excessive for birds in this circumstance.

Total food and water intake and growth rate was lower in Groups A and B, which had a low lysine diet included in

Table 4: Diet selection between low, moderate and high lysine diets of birds

Group	A		B		C		D	
Lysine content	Low	Mod[1]	Low[2]	High[3]	Mod	High	Mod	Mod
Proportions of intake d1-d5	0.436	0.564	0.465	0.535	0.485	0.515	0.495	0.505
Proportions of intake d6-d10	0.300	0.700	0.281	0.719	0.521	0.479	0.493	0.507
Proportions of intake d1-d10	0.357	0.643	0.362	0.638	0.506	0.494	0.494	0.506
Differences from equality in proportions of intake d1-d5	0.064 (p = 0.058)		0.036 (p = 0.150)		0.015 (p = 0.245)		0.006 (p = 0.807)	
Differences from equality in proportions of intake d6-d10	0.201 (p = 0.002)		0.219 (p<0.001)		-0.021 (p = 0.238)		0.007 (p = 0.785)	
Differences from equality in proportions of intake d1-d10	0.143 (p = 0.002)		0.138 (p = 0.001)		-0.006 (p = 0.064)		0.006 (p = 0.767)	

[1]Moderate; 1.0% lysine (NRC recommendation), [2]Low; 0. 6% lysine, [3]High; 1.4% lysine

*Statistical significance of the choice was tested by one-sample t-test under the null hypothesis that the birds ate equal amounts of the two diets

Table 5: Total food intake and water intake of birds (d1-d10)

Group	A	B	C	D	SED	p
Total food intake (g/b/d)	143.0	139.8	159.9	157.5	6.45	0.003
Water intake (ml/b/d)	246.3	259.0	294.0	252.2	13.49	0.003

Table 6: Daily lysine intake of birds (d1-d10)

	A		B		C		D	
Group	L	M[1]	L[2]	H[3]	M	H	M	M
Lysine intake (g/b/d)	0.261	0.802	0.273	1.114	0.688	0.945	0.663	0.689
Total lysine intake	1.063		1.387		1.633		1.352	
SED	0.079							
p	<0.001							

[1]Moderate; 1.0% lysine (NRC recommendation), [2] Low; 0.6% lysine, [3]High; 1.4% lysine

their choice when compared to those of the groups including high lysine diet. Growth rate reflected total lysine intake. Although the birds consumed some of high lysine diet in group A, growth rate was not as high as those in other groups. Newman and Sands (1983) showed similar results. They offered newly hatched layer chicks a choice between a low-lysine diet and excess-lysine diet. Although the birds consumed some of the supplemented diet it was not enough to maintain a growth rate as high as those in a control group which were given a single adequate food.

Experiment 2: Table 7 shows the results of the experiment 2. During the first 7 d period, birds consumed more of the Ideal Protein diet than the High Protein diet (p = 0.16). During the second half period, birds showed greater preference for the Ideal Protein diet (p<0.001). Birds consumed about 2.5 times as much of the Ideal Protein diet as of the imbalanced diet over the whole experimental period of 14 d.

The second experiment also confirmed the bird's ability to select an ideal protein diet to high-protein imbalanced diet. When given simultaneous choice of two diets, birds consumed 70% of the ideal-protein diet and 30% of the high-protein diet. In an experiment by Delezie *et al.* (2009), when choice feeding was started, growth rates were increased (+4.2%) at high protein diet, whereas all other choice feeding treatments showed similar or significantly lower final body weights. Emmans (1991) stated that when the formulation of two diets is such that no mixture of them is of adequate composition then the animal will choose from them in such a way that the inadequacy or excess is minimized. Thus, birds offered two diets avoid the higher protein diet and thus avoid the excess protein intake. In the experiment of Shariatmadari and Forbes (1993), birds offered choices between different protein diets and chose their intake almost entirely from the one closer to adequacy and rejected the extreme one. The choice feeding could be one of the methods to provide the birds with a feed that is near to fulfill their daily growth requirements (Gous, 1998).

Table 7: Diet selection between ideal protein diet and high protein diet of birds

	Protein quality choice	
	Ideal protein	High protein
Proportions of intake d0-d7	0.655	0.345
Proportions of intake d7-d14	0.718	0.282
Proportions of intake d0-d14	0.694	0.306
Differences from equality in proportions of intake d0-d7	0.155 (p = 0.16)	
Differences from equality in proportions of intake d7-d14	0.218 (p<0.001)	
Differences from equality in proportions of intake d0-d14	0.194 (p<0.001)	

*Statistical significance of the choice was tested by one-sample t-test under the null hypothesis that the birds ate equal amounts of the two diets

Diets which are imbalanced in the amino acids absorbed from the digestive tract lead to metabolic disturbances and reduced food intake (Sterling *et al.*, 2006). The reduction in food intake is directly proportional to the degree of amino acid deficiency or imbalance (Boorman, 1979). This is thought to be due to

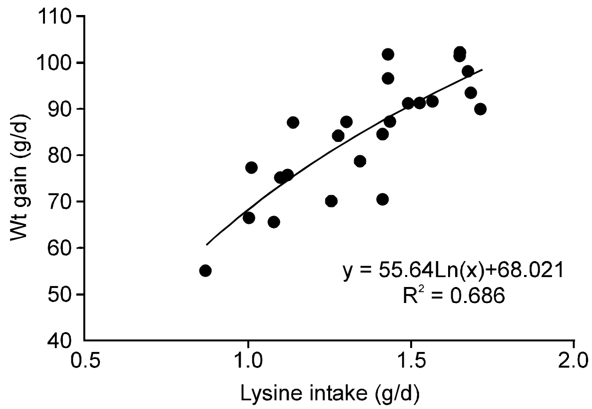

Fig. 1: Relationship between body-weight gain and lysine intake, including all diet choices in experiment 1

the metabolic cost of deaminating the excess of those amino acids which cannot be utilized because of the deficiency of others. Appetites for individual amino acids can therefore be envisaged if the animal learns that one food contains too little of an amino acid for its requirements and another too much. Further, to determine the optimum requirements of amino acids at different growth stages, the response of body weight to a range of intake of lysine particularly must be estimated (Gous, 1998).

As shown in past research on free-choice feeding, it seems that the broilers have a nutritional instinct for balanced amino acid in their diet. Since lysine and methionine are crucial amino acids in poultry nutrition, there are few reports on diet selection related to the other amino acids. However, an ability to select the appropriate diet in relation to other essential amino acids is also likely to exist. This information can be of considerable practical importance in poultry production if it is used to develop alternative strategies for better economic returns. It might be possible to determine most suitable way of economical feeding of broilers.

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