Utilisation of synthetic amino acids by broiler breeder hens

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Abstract

This study was conducted to examine the response of broiler breeder hens to feeds supplemented with synthetic lysine and methionine when fed once or twice daily during peak production. Replacing intact protein with increasing amounts of free lysine and methionine, up to 2.3 g/kg feed, had no effect on feed intake, bodyweight gain, egg weight or efficiency of lysine utilisation, but reduced the crude protein content in the diet up to 3.3 percentage units and improved the efficiency of protein utilisation by 22.3%. However, for each extra gram of dietary free amino acid content/kg diet, the rate of lay and egg output decreased by 3.0% and 2.5 g per day, respectively, and the efficiency of methionine utilisation decreased by 4.3%. There was no interaction between frequency of feeding and amino acid supplementation. These results suggest that free amino acids are not utilised by broiler breeders.

Keywords: Free amino acids, egg production, broiler breeder hens, methionine utilisation
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Introduction

Crystalline amino acids are used increasingly to meet the lysine, methionine and threonine requirements of poultry. Initially this was on economic grounds but their use is now being encouraged by concerns over N-pollution (Fisher, 2000). When modelling the amino acid requirements of broiler breeder hens, a question that requires closer scrutiny is the efficiency with which synthetic amino acids are used for egg production. In spite of the benefits brought about by the use of synthetic amino acids in poultry feeds, there are still some unresolved issues about their utilisation. Two issues, considered here, are whether egg production would be affected when these ingredients are included at high levels, and whether their use could affect egg production when feed is provided for only a short period each day, such as in feed-restricted broiler breeder hens. Previous experiments conducted with pigs and broilers fed one meal a day have indicated a significantly lower utilisation of free synthetic amino acids than of those bound to protein (Batterham, 1974; Batterham & O'Neill, 1978; Batterham & Murison, 1981; Baker & Izquierdo, 1985) this being overcome when meals were offered at least twice daily (Batterham & Murison, 1981). Because broiler breeders throughout the world are feed-restricted, fed once a day and may eat their meal in as little as 20 - 30 minutes, the possibility arises that supplemental crystalline amino acids may be used less efficiently by these birds under such circumstances.

The objective of this experiment was to evaluate the efficiency of use of DL-Methionine and L-Lysine HCl in broiler breeder hens during the peak production period. In order to balance the arrival of the protein-bound amino acids and free amino acids at the site of absorption by delaying the rate of absorption of free amino acids, the effect of frequency of feeding on the utilisation of these free amino acids was also studied.

Materials and Methods

Two hundred and forty Cobb broiler breeder hens aged 27 weeks were housed in individual cages. The birds had been reared on two growth curves, the first as recommended by the primary breeder (Cobb 500 breeding guide, 2001) designed to achieve 2100 g at 20 weeks, while the other was a fast growth curve to achieve the same weight, but at 15 weeks. One hundred and twenty broiler breeder females from each growth curve were randomly allocated to individual cages arranged in six rows, back to back, each row having two levels of 48 cages. Each cage was supplied with one nipple drinker and drip cup, and one feeder. The house was cross-ventilated using six fans. The lighting program was 16L : 8D (04:00 - 20:00) throughout the experimental period.

Throughout the 10-week experiment, each hen was fed one of five dietary treatments obtained by blending two basal feeds (B0 and B100) appropriately (Table 1). These feeds were formulated to contain no synthetic amino acids (B0) or with the maximum amount of synthetic lysine and methionine whilst maintaining a minimum of 150 g protein/kg in the feed. In formulating these basal feeds, minimum contents of all essential amino acids for broiler breeders laying at their maximum output (Fisher, 1998) were specified, thereby ensuring that no amino acids were more limiting than lysine or methionine in either of the basal feeds. Treatments involved feeding five levels of crude protein (183, 175, 167, 158 and 150 g/kg feed), supplemented with synthetic lysine (0, 0.43, 0.85, 1.28 and 1.70 g/kg) and methionine (0, 0.15, 0.30, 0.45 and 0.60 g/kg) in order to maintain constant lysine and TSAA levels, respectively. Diets were isoenergetic and all birds received 160 g daily. The feeds were given in mash form either once or twice daily. The once-daily feeding was at 07:00 and the twice-daily feeds (80 g per feeding) were given at 07:00 and 13:00.

Table 1 Composition (g/kg) of the two basal feeds (B0 – no synthetic amino acids ; B100 – with synthetic amino acids) and amino acid requirements (g/kg feed). Amino acid contents are given as digestible

	B0	B100	Amino acid requirements[2]
Ingredient			
Maize	491	509	
Wheat bran	119	198	
Soyabean full fat	178	129	
Sunflower 37	129	70.7	
L-lysine HCL[1]		1.7	
DL-methionine		0.6	
Vit + min premix	1.5	1.5	
Limestone	71.1	72.2	
Salt	3.1	3.2	
Monocalcium phosphate	7.9	6.6	
Oil - sunflower		7.9	
Nutrient requirement[2]			
AME (MJ/kg)	11.3	11.4	
Crude protein	183.0	150.0	
Lysine	6.9	7.0	7.0
Methionine	2.8	2.9	2.9
Methionine + Cystine	5.4	5.3	5.0
Threonine	5.5	5.1	4.4
Arginine	10.5	9.7	6.2
Tryptophan	1.7	1.5	1.5
Isoleucine	6.4	5.5	4.8
Phenylalanine + tyrosine	11.9	10.0	8.2
Valine	7.6	6.5	5.5

[1] Activity of L-lysine HCl = 78.4 g lys/100 g; [2] Fisher (1998), for a bird consuming 160 g feed/d

Bodyweight was recorded at the beginning of the trial, after six weeks and finally at the end of the trial (after 10 weeks). Weekly food intake was calculated by subtracting the amount remaining at the end of each week from the amount fed. Egg weight was recorded on three days of each week and egg production for the remaining four days.

The means for all treatments were calculated, using the general analysis of variance in Genstat (1997), for the final four weeks of the experiment, on the assumption that by this time the responses of birds would have stabilised on each treatment. A linear regression analysis was performed, to determine the effect of dietary synthetic amino acid content on rate of lay, egg output, egg weight, bodyweight change, feed intake and efficiency of utilisation of protein, lysine and methionine. The efficiency of utilisation of protein was calculated for each individual bird in the experiment as follows: efficiency = protein content in eggs/protein available for egg production. The protein content of egg was calculated on the assumption that the proportions of yolk, albumen and shell in an egg were 27.3, 63.6, and 9.1 g/100 g weight respectively at 35 weeks of age (University of KwaZulu-Natal, unpublished) and, from Fisher (1994), that the N content of these components was 27, 17 and 5.3 g/kg, respectively. The protein available for egg production was calculated as protein intake – protein required for maintenance. The maintenance protein requirement is related to feather-free body protein weight, as suggested by Emmans & Fisher (1986). The expression used was MPr = Mp BPm^0.73 u where MPr = maintenance protein requirement, kg/day, BPm = feather-free body protein weight at maturity, kg, u = BP/BPm and BP = body protein weight, kg. In this case u = 1, as the birds were mature. The constant, Mp, has been estimated as 8 g/unit day (Emmans & Fisher, 1986). For the calculation of body protein weight, the bodyweight was assumed to contain 180 g protein/kg (Wilson *et al*., 1995; Fisher, 1998). The efficiency of utilisation of lysine and methionine was calculated for each bird in the experiment in the same way as was the efficiency of utilisation of protein. The values for the lysine and methionine contents of whole egg (Table 2) were based on the amino acid composition of egg components (Lunven *et al*., 1973). The lysine and methionine required for maintenance was given by the same maintenance scaling rule used for protein: Mlys or Mmet = Mp BPm^0.73 u. The amino acid composition of ideal protein for maintenance was assumed to be the same as that of body protein. So, the lysine and methionine content of the body were estimated as 75 and 25 g/kg of body protein (Emmans, 1989; Fisher, 1998).

Table 2 Lysine and methionine content of whole egg (Lunven *et al*., 1973)

	Lysine	Methionine
mg/g N	439	195
mg/g egg	8.209	3.646

Those hens that laid for not more than one week during the final four weeks of the trial were excluded from the analysis because their efficiencies of utilisation of protein and amino acids would be extremely low (Fisher, 1980), leading to discontinuous protein synthesis and hence large differences in protein and amino acid utilisations between birds.

Results

The mean responses in laying performance, food intake, body weight, gain in weight, and efficiencies of utilisation of protein, methionine and lysine, to the five dietary treatments and two feeding frequencies, over the final four weeks of the trial, are presented in Table 3. The interactions between the dietary synthetic amino acid content and the shape of the growth curve were not significant for the responses in performance or in efficiency of utilisation of protein, lysine and methionine, indicating that the response to dietary synthetic amino acid content was the same for birds reared on the control and the fast growth curves. Consequently, these data were pooled to increase the number of replications of each dietary treatment. The resultant regression equations for each dependent variable obtained by linear regression are presented in Table 4.

A number of hens did not lay for more than a week during the final four weeks of the trial, the numbers being 1, 3, 1, 4 and 3 on feed treatments 1 to 5, respectively. A Chi Square test revealed that the treatments imposed did not influence these numbers, the Chi Square value for such observations being 2.867, which was not statistically significant ($P > 0.05$).

The replacement of lysine and methionine in intact protein with the synthetic form, up to 2.3 g/kg feed, had no effect on feed intake, bodyweight gain, egg weight or efficiency of lysine utilisation. The regression analysis of ep on dietary synthetic amino acid content showed that a significant ($P < 0.001$) linear relationship existed between the two: ep increased with increasing concentrations of synthetic amino acid, an extra gram of synthetic amino acid /kg diet resulting in an increase of 2.4% in ep. Moreover, increasing levels of synthetic amino acids reduced nitrogen excretion by 24.4% (Table 5). Supplements of 2.3 g/kg synthetic amino acids had a protein-sparing effect of 3.3 percentage units with a better utilisation of protein and a reduction in N excretion.

Table 3 Mean responses, in performance and efficiency of nutrient utilisation, to dietary treatments over the final four weeks of the trial

	Rate of lay (%)			Egg weight (g)			Egg output (g/d)		
Frequency of feeding (/d)	1	2	Mean	1	2	Mean	1	2	Mean
Synthetic amino acid (g/d)									
0.0	74.9	76.2	75.6	66.0	67.7	66.8	48.8	51.1	50.0
0.6	71.5	76.4	74.0	64.0	65.4	64.7	44.8	49.5	47.2
1.2	74.0	75.6	75.0	64.3	66.9	65.6	47.3	50.2	48.8
1.7	72.0	75.2	73.6	66.5	65.9	66.2	46.2	49.3	47.8
2.3	67.1	71.1	67.1	65.5	64.9	65.2	39.9	45.1	42.5
Mean	72.0	74.9	73.1	65.2	66.2	65.7	45.4	49.0	47.2
s.e.d.			5.2			1.7			3.9

	Body weight (g)			Body weight change (g/d)			Feed intake (g/d)		
Frequency of feeding (/d)	1	2	Mean	1	2	Mean	1	2	Mean
Synthetic amino acid (g/d)									
0.0	3533	3722	3628	-0.3	-3.6	-1.9	159.9	159.8	159.8
0.6	3617	3694	3655	-1.2	-4.6	-2.9	159.9	159.9	159.9
1.2	3504	3607	3555	-2.4	-1.7	-2.0	159.7	159.4	159.5
1.7	3515	3541	3528	-3.3	-2.8	-3.0	160.0	160.0	160.0
2.3	3434	3521	3478	-0.6	-0.4	-0.5	158.9	160.0	159.4
Mean	3521	3617	3569	-1.5	-2.6	-2.1	159.7	159.8	159.7
s.e.d.			100.4			2.0			0.8

	Efficiency of utilisation of protein (g/g)			Efficiency of utilisation of lysine (g/g)			Efficiency of utilisation of methionine (g/g)		
Frequency of feeding (/d)	1	2	Mean	1	2	Mean	1	2	Mean
Synthetic amino acid (g/d)									
0.0	0.245	0.264	0.254	0.527	0.574	0.551	0.591	0.641	0.616
0.6	0.242	0.251	0.246	0.496	0.517	0.507	0.525	0.546	0.535
1.2	0.280	0.281	0.280	0.539	0.545	0.542	0.569	0.574	0.571
1.7	0.294	0.288	0.291	0.535	0.523	0.529	0.561	0.549	0.555
2.3	0.298	0.301	0.299	0.506	0.513	0.510	0.481	0.487	0.484
Mean	0.272	0.277	0.274	0.521	0.534	0.528	0.545	0.559	0.552
s.e.d.			0.039			0.038			0.040

Table 4 Responses in feed intake, average daily gain (ADG), laying performance (rate of lay, egg weight and egg output) and efficiency of utilisation of protein (ep), lysine (elys) and methionine (emet) to synthetic amino acid content (g/kg), as determined by linear regression analysis

	Linear coefficient	s.e.
Feed intake (g/d)	-0.152	0.161
ADG (g/d)	0.453	0.437
Rate of lay (%)	- 2.950**	1.100
Egg weight (g)	- 0.318	0.360
Egg output (g/d)	- 2.507**	0.820
ep	0.024***	0.004
elys	- 0.010	0.008
emet	- 0.043***	0.009

*** P < 0.001 ** P < 0.01 * P < 0.05

Table 5 Effect of reducing dietary crude protein and supplementing with synthetic amino acids on nitrogen excretion

	Diet	
	183 g CP/kg	150 g CP/kg + 1.7 g Lys/kg + 0.6 g Met/kg
N intake (g/d)	29.3	24.0
Efficiency of utilisation of protein (%)	24.7	30.2
N retained (g/d)	7.2	7.3
N excreted (g/d)	22.1	16.7
Reduction in N excretion (%)	-	24.4

However, the relationships between rate of lay, egg output, emet and dietary synthetic amino acid content were linear (P < 0.01, P < 0.01 and P < 0.001, respectively), with rate of lay, egg output and emet decreasing by 3.0%, 2.5 g/d (Figure 1) and 4.3% (Figure 2) with each additional gram of free amino acid content/kg diet.

Feeding twice-daily *vs*. daily resulted in increases in rate of lay and egg output (P < 0.05 and P < 0.01, respectively) over all feeds used, but no differences in egg weight or in the slopes of the three responses when regressed against synthetic amino acid inclusion. Although the efficiencies of utilisation of protein, lysine and methionine were all numerically higher when birds were fed twice-daily these differences were not significant, nor was there an interaction between frequency of feeding and amino acid supplementation for any of these variables.

Discussion

Supplementing animal feeds with synthetic amino acids is of importance not only on nutritional and economic grounds, but also because of environmental considerations (Han & Lee, 2000). This study showed a protein sparing effect of 3.3 percentage units with a better utilisation of protein and a reduction in N excretion when synthetic amino acids were used. A reduction in nitrogen excretion of 24.4% was obtained with an "ideal amino acid balance", which is of considerable consequence in countries where N pollution is a problem. However, it appeared not possible to obtain the same performance with lower protein diets supplemented with synthetic lysine and methionine. For each extra gram of dietary free amino acid content per kg diet, the rate of lay and egg output decreased by 3.0% and 2.5 g per day, respectively. This means, in agreement with Batterham & Murison (1981) with pigs, that synthetic amino acids are not utilised as well as the protein-bound amino acids when birds were fed a restricted amount of food once a day. The elys had no relationship with the dietary synthetic amino acid content, probably because lysine was not the first-limiting amino acid in the feed offered. Because the methionine efficiency ratio declined in a linear (P < 0.01) fashion as supplemental synthetic amino acids increased from 0 to 2.3 g/kg diet, it is likely that this was first-limiting in the feed. It is likely that under restricted feeding conditions, optimal performance cannot be achieved when extensive amino acid supplementation is used to replace essential amino acid from intact protein.

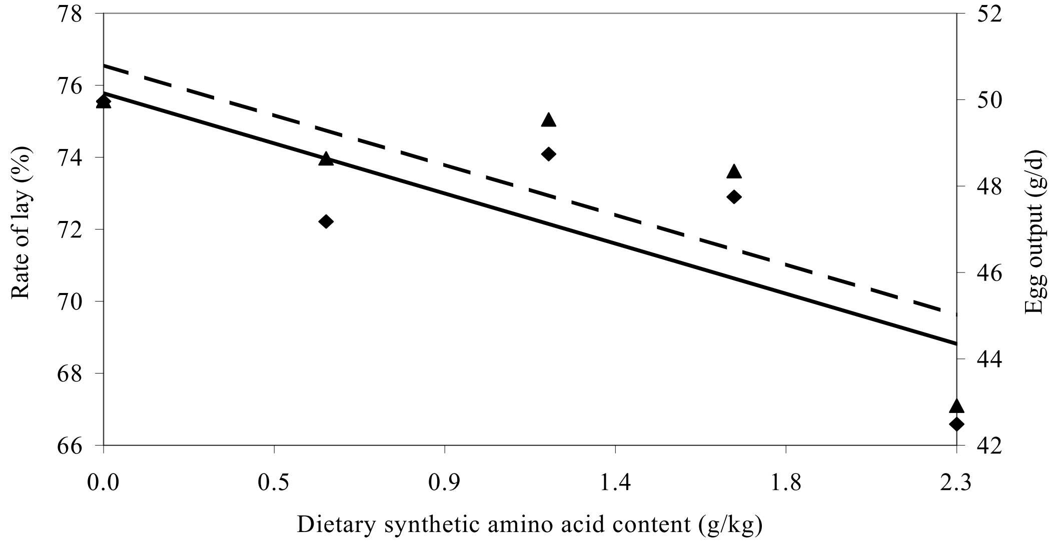

Figure 1 Observed and fitted relationships between rate of lay (%), egg output (g/d) and dietary synthetic amino acid content (g/kg feed). Observed mean rate of lay (▲), egg output (■) and the fitted equations (▬ ▬ rate of lay, ▬ egg output)

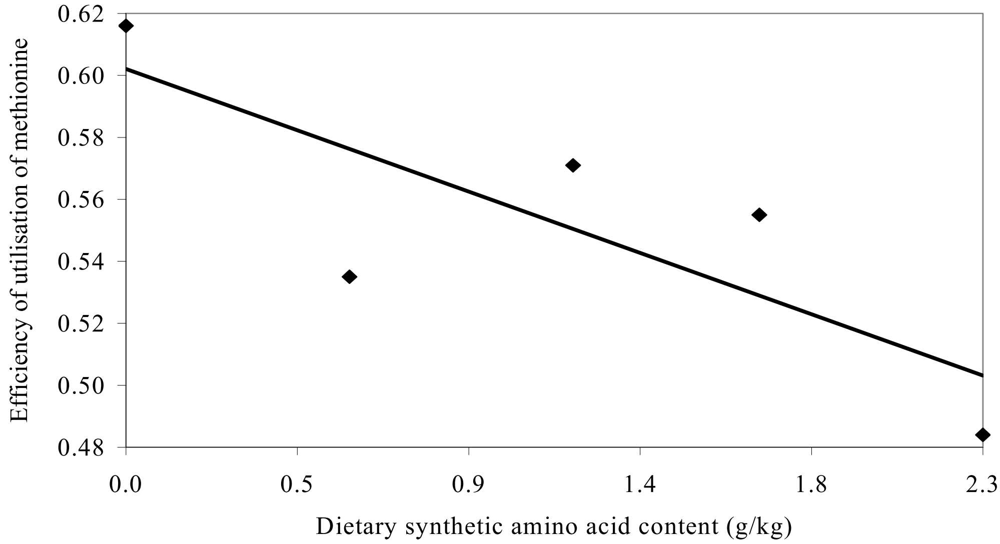

Figure 2 Observed and fitted relationship between efficiency of utilisation of methionine and dietary synthetic amino acid content (g/kg feed)

There have been many reports of a depression in growth or performance when low protein feeds, supplemented with amino acids to the same level as those in intact protein, are fed to poultry (e.g. Ferguson *et al*., 1998; Kerr & Kidd, 1999a; b; Waibel *et al*., 2000). One reason given for this is that one of the amino acids not normally considered in a feed formulation program may inadvertently be first-limiting in such a feed. To ensure that this was not the case here, all essential amino acids were considered when formulating feed B100, making use of the requirements specified by Fisher (1998), thus taking care that the amino acid balance in the two basal feeds was relatively constant. Of the non-test amino acids only tryptophan was not in excess of requirement. However, the small difference in tryptophan content between the two basal feeds suggests that it is unlikely that the large differences in response measured here could have been a response to tryptophan or indeed to any of the other non-test amino acids, especially as the same ingredients were used to supply these amino acids in both basal feeds.

It is more likely that the observed response was due to the more rapid absorption of the synthetic amino acids once digested compared with the amino acids from intact protein. This phenomenon, which has been measured in growing pigs (Yen *et al*., 2004), would result in an unbalanced amino acid mixture being available for incorporation into protein once the intact proteins had been digested and absorbed. It is for this reason that frequent feeding of low-protein feeds has been successful with growing pigs (Cook *et al*., 1983).

With frequent meals, one would assume an equilibrium would be established between gut, blood and tissues with regard to amino acid utilisation for protein synthesis (Baker & Izquierdo, 1985). However, our results with twice-daily feeding showed that there was no interaction between frequency of feeding and amino acid supplementation. This finding is not in general agreement with Batterham's more recent work with pigs (1974, 1978 and 1981) where he showed that growing pigs meal-fed once daily utilised crystalline lysine no better than 50 to 67% of that achieved by pigs meal-fed six times daily. Although differences in efficiency of utilisation of protein and the test amino acids were not significant when comparing once- and twice-daily feeding, they were numerically greater where intact protein was fed than at the other end of the scale. This is contrary to the theory that more frequent feeding would result in greater efficiency of utilisation of free amino acids. But given that the synthetic amino acids were not utilised at all by the hens (see below) one could expect less variation in performance between birds fed lower amounts of the limiting amino acid, as performance would be equally constrained in all birds. It is possible that feeding more frequently than twice-daily may overcome the poorer efficiency of utilisation of synthetic amino acids, but this was not tested in the present experiment.

In practical applications of the Reading Model (Fisher *et al.*, 1973) and in subsequent publications pertaining to this model (Pilbrow & Morris, 1974; Morris & Blackburn, 1982; McDonald & Morris, 1985) the assumption has been, for the sake of simplicity, that all amino acids are utilised by laying hens with an efficiency of between 0.80 and 0.85. However, this applies to laying hens fed *ad libitum*. The efficiency of utilising methionine from bound protein in this trial was 0.62 (Table 3) but only 0.484 with maximum inclusion of synthetic methionine. With 0.21 of the methionine in feed 5 (B100) being in the synthetic form and the remainder (0.79) being bound, the utilisation of free methionine in this feed was zero (calculated as [0.484-(0.79*0.62)]/0.21), effectively reducing the methionine content to only (0.79*2.9) = 2.3 g/kg, which would adequately explain the decrease in performance on this feed. This has important consequences both in designing feeds for broiler breeders and when modelling their responses to amino acids during the laying period. It would appear that free amino acids should not be used in feeds for broiler breeders.

When modelling the utilisation of synthetic amino acids in broiler breeder feeds, three situations need to be considered: 1) if none are fed, the first-limiting amino acid in the feed may be utilised at a maximum rate of 0.60–0.80; 2) if all amino acids are (theoretically) supplied in synthetic form, the efficiency will also be maximal because all free amino acids will be available for protein synthesis simultaneously, 3) if a portion of the amino acid requirement is supplied in the synthetic form, the efficiency will decrease in relation to the ratio of synthetic to protein-bound amino acids. This decrease may be calculated by assuming that the free amino acid is not utilised: thus in this study the efficiency of methionine utilisation decreased by 5 g/kg for each 1g of free methionine added per kg feed.

When laying in closed cycles, broiler breeders should be as efficient as laying hens in converting dietary amino acids to egg output (Fisher, 1980): an efficiency of between 0.75 and 0.85 has been suggested by McDonald & Morris (1985) and by Emmans & Fisher (1986). Broiler breeders fed intact protein here utilised lysine and methionine for egg production with mean efficiencies of 0.55 and 0.62, respectively, these being lower than the equivalent values estimated for laying hens, but similar to those reported by Bowmaker & Gous (1991) (0.47 for lysine and 0.50 for methionine) and Goddard (1997) (between 0.58 and 0.68 for lysine) in their studies with broiler breeders. It is likely that lysine was not first-limiting in the present trial, given its lower efficiency compared with that for methionine. The range in efficiencies of utilisation of lysine fell between 0.11 and 0.73 and between 0.12 and 0.82 for methionine, with only three birds having an efficiency of utilisation of methionine greater than 0.75. This low efficiency could be accounted for in some birds as being the result of making use of an average protein content in the carcass of 180 g/kg when calculating maintenance requirement, or of hens being inefficient egg producers. A subsequent analysis was performed in which the carcass protein contents were assumed to be either 160 or 200 g/kg. This wide range of protein contents had little effect on the resultant efficiencies, being 0.50 and 0.55 for lysine, and 0.53 and 0.57 for methionine, for the two body protein contents respectively. Not all the birds were laying at a rate greater than 0.5. Their rate of lay varied between 20 and 100%. A subsequent analysis was performed in which data from birds laying at not more than 50% were excluded from the analysis. The resultant efficiency of utilisation of lysine for egg production was 0.54 and of methionine 0.56; values that are no different from the efficiencies calculated using data from those birds that laid not more than one week during the final four weeks of the trial. Two possibilities exist that might explain the lower efficiency of utilisation of the limiting amino acids by broiler breeder hens, although neither is very convincing: one possibility is that these birds have higher maintenance requirements per kg of body protein than laying hens, and the other is that amino acids may be wasted in the processes of yolk formation and subsequent reabsorption or through internal laying, both of which occur to a greater extent in broiler breeders than in laying hens. The question of lower efficiency in converting dietary amino acid in egg output in broiler breeders compared with laying hens needs further investigation.

Conclusions

Whereas crystalline amino acids may enable nutritionists to comply better with constraints in linear programming (least cost formulation) when formulating feeds for broiler breeders, by lowering the cost of the feed and contributing to a reduced nitrogen excretion, the results of the trial reported here suggest that these synthetic amino acids should not be used in such feeds. It is evident that these free amino acids are not utilised by broiler breeders, resulting in a reduced rate of lay compared with birds fed intact protein. It is likely that these amino acids are rapidly absorbed and metabolised before the intact protein has been digested and absorbed, resulting in an unbalanced mixture being available for incorporation into egg protein.

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