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# The response to dietary threonine in laying-type pullets during growth

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**Abstract** 1. This study aimed to provide information on the response of laying-type pullets to dietary threonine (THR) during three periods of growth prior to the onset of lay. Different batches of Dekalb White pullets were used in three separate trial periods (from 4 to 6, 8 to 11 and 13 to 16 weeks of age) using 8 dietary THR concentrations in each period, using a completely randomised design, and with each treatment being replicated 6 times, using 15 birds per replication in period 1 and 8 birds in periods 2 and 3. In period 1 the THR content (THRc) ranged from 2.3 to 7.6 mg/g, in period 2 from 1.7 to 5.5 mg/g, and in period 3 from 1.4 to 4.7 mg THR/g feed.
2. Body weight gain, food intake and the deposition of protein and lipid in the feather-free body and in the feathers were measured in each period. Linear regressions were fitted to all data falling below the break point defined by the broken stick regression, to estimate the efficiency of utilisation of THR. The maximum protein growth rate was 4.0 ± 0.2, 5.3 ± 0.4 and 3.5 ± 0.5 g/d in periods 1, 2 and 3, respectively.
3. The efficiency of utilisation of dietary THR for THR deposition in each period was the same, at 0.85 ± 0.1 mg/mg. As dietary THRc decreased, the amount of body lipid deposition increased.
4. With this information, it is possible to determine the daily requirement for THR for the potential growth of body and feather protein in growing pullets.

## INTRODUCTION

The conventional method of feeding laying-type pullets during rearing, as recommended by breeding companies such as DEKALB (2012) and LOHMANN (2014), is successful, as evidenced by the exceptional rate of laying seen in hens from such breeding companies, and this is in spite of the considerably different feeding schedules recommended by these two companies, with the first one recommending a change in feed composition after 6, 10, 15 and 17 weeks, whilst the other suggests changes at 3, 8, 16 and 22 weeks. From earlier research in which the effects of the subsequent performance of pullets subjected to qualitative and quantitative changes in feed during rearing were measured, it appears that laying performance is reduced under only the most severe nutritional constraints imposed during rearing (Balnave, 1974; Doran *et al.*, 1983; Chi, 1985; Keshavarz and Jackson, 1992).

The objective in feeding pullets is, according to DEKALB (2012),

> … to attain the greatest number of eggs in the desired weight range at the most efficient cost per dozen or per pound of egg mass. To attain this goal, birds should be fed correctly during both the growing and egg production phases.

This begs the question of how to determine the correct feeding method during growth. Because performance appears to be adequately buffered by minor differences in the nutrient supply during rearing, the "correct" feeding method may be defined as that which supplies the pullets

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with an adequate daily amount of each essential nutrient at the lowest cost. Arguably, the most accurate way of determining this optimal economic feeding schedule for growing pullets is to predict the food intake of these pullets, taking account of genetic, environmental and nutritional factors and interactions (Emmans, 1981).

Of particular importance in predicting food intake is knowing the potential body and feather protein growth rate of pullets throughout the growing period, which provides information on the daily requirement for the amino acids and energy needed to maintain the bird and allow for this potential growth (Emmans, 1981). It is also necessary to know the extent to which the given strain can deposit lipid as a means of overcoming a nutrient deficiency: birds and animals overconsume energy in an attempt to consume sufficient of the limiting nutrient in the feed (Foot, 1972; Emmans, 1981; Sibbald and Rhind, 1997; Whittemore, 1998; Gous *et al.*, 2012), but this overconsumption is limited by the extent to which the genotype can deposit lipid, i.e., lean genotypes are unable to compensate to the same extent as fat genotypes for a marginal nutrient deficiency, and therefore, the amino acid-to-energy ratio in lean genotypes is more critical in such strains. In addition, to calculate a bird's daily nutrient requirements, it is necessary to know whether the efficiency of utilisation of amino acids for protein growth changes during growth. Such information may be used to determine the required daily amounts of the essential amino acids, and energy, to ensure that the pullet will grow at its potential (Martin *et al.*, 1994), as well as the consequences of underfeeding or overfeeding of the pullets at different stages of growth. From such information, it is then possible to determine the most cost-efficient way of meeting these daily requirements for nutrients, i.e., what the balance and concentration of amino acids should be during each feeding phase, the optimum length of each phase and the optimum number of phases.

Unfortunately, information in the literature on the feeding of laying-type pullets is scarce, with little useful evidence of changes that occur in food intake, growth and carcass composition in response to protein supply during different stages of the growing period. But such information is invaluable when attempting to determine the optimum method of feeding pullets using a more formal approach such as that suggested by Martin *et al.* (1994). In none of the pullet growth studies previously conducted in Brazil (Silva *et al.*, 2000*a*, 2000*b*, 2000*c*, 2009*a*, 2009*b*; D'Agostini *et al.*, 2012), or by the authors mentioned above, was the body composition of pullets determined. Food intake and body weight gain were the only measures of interest, other than the subsequent performance of the birds.

The experiments reported in this paper involved measuring the response of laying-type pullets during rearing to feeds limiting in dietary threonine (THR). THR is considered to be the third limiting amino acid in maize/soya bean diets, being important for maintenance and in the formation of endogenous protein (Fuller, 1991). It is the amino acid in the highest concentration in mucin and in antibodies, and its deficiency may impair the functioning of the immune and digestive system and reduce its availability for muscle protein synthesis (Stoll, 2006). Body and feather proteins contain similar amounts of THR, the amounts ranging from 33.9 to 40.2 mg/g for the feather-free body (Williams *et al.*, 1954; Saunders *et al.*, 1997; Stilborn *et al.*, 2010; Silva, 2012) and from 42.0 to 44.9 mg/g for feathers (Fisher *et al.*, 1981; Stilborn *et al.*, 1997; Silva, 2012), thus eliminating the problem of determining separately the rates of deposition of THR in the body and feathers.

The method used in these experiments to measure the response of laying-type pullets to dietary THR differed in many respects from those used previously. A summit dilution technique (Fisher and Morris, 1970) was used to measure the response of pullets at three different stages of growth to a range of dietary THR concentrations; this technique has a number of advantages over the more commonly used graded supplementation technique (Gous and Morris, 1985). Body composition of birds on the various dietary treatments was measured at the start and end of each of the three periods to enable the separate calculation of body and feather protein gain. By measuring the response at different stages of growth, the efficiency of utilisation of THR for protein growth during these periods could be compared, thereby determining whether the efficiency changed as the birds grew. Digestible and not total THR intakes were used when reporting the results of the response trials, as the digestible THRc of each basal feed used in these trials was measured using caecectomised roosters.

This study was aimed therefore at providing information on the response of pullets to dietary THR that could be used to evaluate different feeding options with a view to determining the optimum economic method of feeding laying-type pullets during rearing.

## MATERIALS AND METHODS

The trials conducted at the Department of Animal Sciences, São Paulo State University, UNESP, Jaboticabal, SP, Brazil, were designed to measure the response of pullets to dietary THR during the

three periods of growth. The first trial was conducted over the period from 4 to 6 weeks of age, the second from 8 to 11 weeks and the third from 13 to 16 weeks. A total number of 1488 Dekalb White pullets was used in these trials. A completely randomised design was used with 8 dietary concentrations of THR (treatments) and with each treatment being replicated 6 times, using 15 birds per replication in period 1 and using 8 birds in periods 2 and 3, respectively.

Prior to the start of each experimental period, the birds received diets formulated according to the recommendations of Rostagno *et al.* (2005) for growing pullets. Management of the pullets and the lighting programme used followed the recommendations of the Dekalb White supplier, namely 22-h light during the first week of age, decreasing gradually until the 10th week, after which only natural light was used until the 18th week (average of 11-h light/day). The average temperature decreased from 31°C at the age of one day to 28°C at 3 weeks of age and was maintained at an average of 25°C thereafter. Different birds were used in each trial. At the beginning of each trial period, the birds were standardised according to body weight and distributed to cages with a floor space of 0.053 $m^2$/bird in period 1 and 0.113 $m^2$/bird in periods 2 and 3.

The experimental feeds were formulated using a dilution technique (Fisher and Morris, 1970). A high-protein summit feed was formulated to contain approximately 1.3 times the digestible THR concentrations suggested by Rostagno *et al.* (2005) for pullets during the three periods, with the minimum concentrations of all other essential amino acids being set at 1.5 times their suggested concentrations. These summit feeds were diluted sequentially with isoenergetic, protein-free feeds (N-free) (Fisher and Morris, 1970), as shown in Table 1, to create a range of feeds increasing in THRc (period 1: 2.5 to 8.2; period 2: 1.8 to 5.9; and period 3: 1.5 to 5.1 mg THR/g feed).

To confirm that THR was limiting in each series, an additional treatment was included in the design in which the feed with the lowest concentration of THR was supplemented with 0.86 g, 0.62 g and 0.53 g of L-THR/kg in periods 1, 2 and 3, respectively.

A digestibility trial was conducted to determine the digestible amino acid contents of the summit feeds used in each period, using the method described by Sakomura and Rostagno (2007). Caecectomised roosters (6 per treatment) were given 40 g of feed by intubation after 48-h fasting. Excreta collection continued for 48 h after feeding. In order to quantify the metabolic and endogenous losses of amino acids in the body, 6 roosters were given only glucose in water during the 48-h period. Amino acid contents were quantified in samples of feeds and excreta that were hydrolysed with 6 N HCl during 24 h. The amino acids released in the hydrolysate were separated by high-performance liquid chromatography (HPLC) reverse phase and detected by UV at 254 nm (AOAC, 2000).

Body weight gain, feed intake, digestible THR intake, feed conversion efficiency (FCE) and the absolute (g) and relative weights (% of body weight) of feathers were calculated from the measurements of body weight and feed intake made during each trial period. The rates of deposition of protein and lipid in the feather-free body and in the feathers were determined by the comparative slaughter technique using representative samples of birds at the beginning and end of each trial period. The number of birds sampled at the beginning of each trial was 18, whilst 96 birds were sampled at the end of each trial (two birds from each of the 6 replications of 8 treatments). These sampled birds were weighed before they were killed by asphyxiation in $CO_2$ without loss of blood, then again after plucking. The feather-free bodies of the two birds from each replication were minced together, a sample was taken, and this was used to determine the contents of water first by freezing the samples at −20°C and then vacuum-drying at −50 °C and −80 kPa (VLP20, Thermo Fisher) for 72 h. Protein content was determined using the Kjeldahl method (Kjeltec$^{TM}$ 8400, Foss), lipids by Soxhlet extraction (XT15, Ankom) and ash using a furnace by the procedures outlined by AOAC (1990).

The THRc of protein in the feather-free body and in the feathers of the birds sampled at the beginning and end of each trial period was measured using HPLC from which the amount of THR deposited during each period was calculated.

In order to determine the means and standard errors of the variables measured or calculated for each treatment, an analysis of variance (SAS 9.1 software, 2009) was conducted on food intake (g/d), body weight gain (g/d), FCE (g food/kg gain), body protein deposition (BPd, g/d), feather protein deposition (FPd, g/d), total protein (body plus feathers) deposition (PDt, g/d), body lipid content (BLc, g) and THR deposition (THRd, mg/d).

A broken stick regression described by Robbins *et al.* (1979) was fitted, using PROC NLIN in SAS 9.1 software (2009), to determine the maximum rate of deposition of protein in each period: $Y = R_{max} + u\ (r–x) + €$, where $Y$ is the response variable (BPd or THRd); $x$ is the

**Table 1.** *Composition (g/kg) of the basal feeds used in each trial together with their analysed nutrient contents*

| | Diets | | | | | |
|---|---|---|---|---|---|---|
| | Period 1 | | Period 2 | | Period 3 | |
| Ingredient | Summit | N-free | Summit | N-free | Summit | N-free |
| Maize | 555 | ——— | 622 | ——— | 669 | ——— |
| Soya bean meal | 391 | ——— | 223 | ——— | 157 | ——— |
| Starch | ——— | 543 | ——— | 509 | ——— | 540 |
| Rice husk | ——— | 120 | ——— | 148 | ——— | 150 |
| Sugar (sucrose) | ——— | 120 | ——— | 150 | ——— | 120 |
| Sand | ——— | 118 | ——— | 100 | ——— | 100 |
| Wheat bran | ——— | ——— | 87.0 | ——— | 111 | ——— |
| Soya bean oil | 13.3 | 50.0 | 30.0 | 50.0 | 30.0 | 50.0 |
| Dicalcium phosphate | 17.4 | 23.6 | 15.0 | 21.2 | 10.6 | 16.8 |
| Limestone | 10.5 | 6.60 | 9.90 | 4.70 | 12.2 | 6.60 |
| Sodium chloride | 4.60 | 4.20 | 3.50 | 3.60 | 3.30 | 3.40 |
| Potassium chloride | ——— | 11.5 | ——— | 11.3 | ——— | 10.9 |
| DL-methionine (98 %) | 3.50 | ——— | 2.60 | ——— | 2.00 | ——— |
| L-lysine (78.5 %) | 2.60 | ——— | 2.40 | ——— | 2.30 | ——— |
| L-tryptophan (93.5 %) | ——— | ——— | 0.40 | ——— | 0.50 | ——— |
| L-valine (98 %) | ——— | ——— | 0.80 | ——— | 0.50 | ——— |
| L-isoleucine (98 %) | ——— | ——— | 1.00 | ——— | 0.80 | ——— |
| Vitamin/mineral premix[1] | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Choline chloride | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 |
| Antioxidant | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Nutrients | | | | | | |
| Metabolisable energy[4] (MJ/kg) | 12.1 | 12.1 | 12.1 | 12.1 | 12.1 | 12.1 |
| Crude protein[2] | 223 | 6.70 | 180 | 6.70 | 153 | 6.70 |
| Lysine[2,3] | 14.0 | ——— | 9.93 | ——— | 8.32 | ——— |
| Methionine + cystine[2,3] | 10.3 | ——— | 8.05 | ——— | 6.95 | ——— |
| Methionine[2,3] | 6.70 | ——— | 5.12 | ——— | 4.27 | ——— |
| Tryptophan[2,3] | 2.83 | ——— | 2.57 | ——— | 2.25 | ——— |
| Threonine[2,3] | 8.15 | ——— | 5.94 | ——— | 5.10 | ——— |
| Arginine[2,3] | 15.9 | ——— | 11.2 | ——— | 9.38 | ——— |
| Valine[2,3] | 10.5 | ——— | 8.53 | ——— | 7.20 | ——— |
| Isoleucine[2,3] | 9.89 | ——— | 7.90 | ——— | 6.55 | ——— |
| Leucine[2,3] | 19.4 | ——— | 15.2 | ——— | 13.7 | ——— |
| Phenylalanine + tyrosine[2,3] | 11.8 | ——— | 8.59 | ——— | 7.37 | ——— |
| Calcium | 9.40 | 9.40 | 8.30 | 8.30 | 8.00 | 8.00 |
| Sodium | 1.80 | 1.80 | 1.60 | 1.60 | 1.50 | 1.50 |
| Available phosphorus | 4.40 | 4.40 | 3.90 | 3.90 | 3.10 | 3.10 |
| Potassium | 8.70 | 8.70 | 6.60 | 6.60 | 5.70 | 5.70 |
| Crude fibre | 30.7 | 47.5 | 30.6 | 58.7 | 30.0 | 59.4 |

[1]Provided per kg diet: retinol 2.7 mg, menadione 1.8 mg, α-tocopherol 12.4 g, cholecalciferol 92 μg, thiamine 2.4 mg, riboflavin 5.95 mg, pyridoxine 2.5 mg, vitamin B12 12 μg, nicotinic acid 38 mg, pantothenic acid 12 mg, folic acid 0.95 mg, biotin 60 mg, selenium 300 mg, manganese 200 mg, iron 100 mg, zinc 160 mg, copper 16 mg, iodine 1.5 mg, antioxidant 250 mg. [2]Values analysed by HPLC. [3]Digestible amino acid composition determined by trials with caecectomised roosters. [4]Predicted value calculated according to Rostagno *et al.* (2005).

independent variable (THRi) associated with $Y$; $R_{max}$ is the maximum expected value for deposition (BPd or THRd); $u$ is the slope of the function; $r$ is the break point on the horizontal axis, representing the point where, for an intake $x$, the response is maximal; and $\epsilon$ is aleatory error based on assumptions of normality. Linear regressions were then fitted to all the data falling below the break point defined by the broken stick regression in each period, in order to estimate the coefficient of response to THR intake, this being the inverse of the efficiency of utilisation of THR for protein growth. These slopes were compared using simple linear regression with groups in GENSTAT (2009), and a $P$-value greater than 0.05 indicated significant differences between treatments.

## RESULTS

Mean responses to dietary THR are given in Table 2 for each of the three trial periods. The addition of synthetic THR to the feed with the lowest concentration of THR resulted in higher FI, BWG, FCE, BPd and FPd and lower BLc in all the three trial periods than the feed with the lowest THRc, confirming that THR was the first limiting amino acid in the feeds used in these trials.

On the 3 or 4 feeds with the highest THRcfood intake was relatively stable (Table 2), but as the THRc declined further, food intake decreased, falling to 0.93 of the maximum in period 1 and to 0.83 and 0.77 in periods 2 and 3, respectively. The effect on body weight gain

**Table 2.** *Mean responses in feed intake (FI), body weight gain (BWG), feed conversion efficiency (FCE), feather-free body protein deposition (BPd), feather protein deposition (FPd) and body lipid content (BLc) of pullets to dietary threonine (THRc) in periods 1 (4–6 weeks), 2 (8–11 weeks) and 3 (13–16 weeks)*

| THRc g/kg | FI g/d | BWG g/d | FCE g/kg | BPd[1] g/d | FPd[1] g/d | BLc[2] g/kg |
|---|---|---|---|---|---|---|
| Period 1 (4–6weeks) | | | | | | |
| 2.5 | 23.6 | 6.21 | 262 | 0.87 | 0.62 | 87.1 |
| 3.2[3] | 23.9 | 6.81 | 285 | 1.26 | 0.77 | 78.9 |
| 3.3 | 25.2 | 7.85 | 312 | 1.27 | 0.88 | 61.7 |
| 4.1 | 25.4 | 9.49 | 373 | 1.60 | 1.17 | 52.2 |
| 4.9 | 25.5 | 10.5 | 413 | 1.71 | 1.25 | 28.4 |
| 5.7 | 25.5 | 11.5 | 450 | 2.03 | 1.49 | 21.2 |
| 7.3 | 25.5 | 12.8 | 495 | 2.39 | 1.71 | 19.3 |
| 8.2 | 25.4 | 12.9 | 508 | 2.21 | 1.72 | 17.6 |
| RMS[4] (34 df)[5] | 0.047 | 0.092 | 0.004 | 0.070 | 0.017 | 3.257 |
| Period 2 (8–11weeks) | | | | | | |
| 1.8 | 35.2 | 1.61 | 50.3 | 0.40 | 0.14 | 69.3 |
| 2.3[3] | 37.3 | 3.31 | 82.6 | 0.69 | 0.29 | 66.8 |
| 2.4 | 39.1 | 3.60 | 82.6 | 0.81 | 0.54 | 62.9 |
| 3.0 | 41.6 | 8.45 | 189 | 1.75 | 1.12 | 51.8 |
| 3.6 | 41.8 | 8.10 | 187 | 1.87 | 1.24 | 48.4 |
| 4.2 | 42.3 | 9.30 | 207 | 2.28 | 1.54 | 40.2 |
| 5.4 | 43.6 | 13.4 | 288 | 2.80 | 2.27 | 36.0 |
| 5.9 | 42.3 | 12.9 | 280 | 3.03 | 1.97 | 36.2 |
| RMS (31 df) | 8.57 | 4.40 | 7.64 | 0.94 | 0.15 | 12.97 |
| Period 3 (13–16weeks) | | | | | | |
| 1.5 | 36.9 | 0.95 | 23.4 | 0.24 | 0.07 | 74.4 |
| 1.9[3] | 37.1 | 1.79 | 58.5 | 0.55 | 0.16 | 71.0 |
| 2.0 | 40.2 | 3.47 | 84.0 | 1.08 | 0.53 | 65.4 |
| 2.5 | 44.4 | 7.30 | 150 | 1.46 | 0.64 | 58.4 |
| 3.1 | 47.4 | 7.78 | 152 | 1.53 | 0.82 | 54.1 |
| 3.6 | 47.0 | 9.35 | 188 | 1.78 | 1.38 | 50.0 |
| 4.6 | 48.2 | 10.8 | 206 | 2.12 | 1.55 | 45.2 |
| 5.1 | 48.0 | 9.98 | 202 | 2.25 | 1.32 | 45.7 |
| RMS (33 df) | 14.08 | 3.310 | 26.02 | 1.182 | 0.264 | 23.57 |

[1]Crude protein deposited during the experimental period estimated by the difference between initial reference slaughter and final slaughter; [2]Lipid content (g/kg) in feather-free body of birds sampled at the end of each period; [3]Additional concentration to determine whether threonine was limiting (concentration one plus 0.86 g, 0.62 g or 0.53 g of L-THR/kg in each period); [4]RMS: residual mean square; [5]df: degrees of freedom.

(Table 2) was considerably more severe, with gains on feeds with the lowest THRcs falling to 0.48, 0.12 and 0.01 of the highest gains achieved. Food conversion efficiency reflected these differences in food intake and performance (Table 2), the values on feeds with the lowest THRc being 0.52, 0.18 and 0.12 of the highest FCEs recorded for periods 1, 2 and 3, respectively.

In all the three trial periods, the rate of protein deposition increased with increasing intake of THR up to a maximum, this being reached on 165 ± 8.2 mg THR/d in period 1, 230 ± 25.8 mg/d in period 2 and 168 ± 41.5 mg/d in period 3 as measured by the broken stick equations fitted in each trial. The maximum rate of protein growth (body plus feathers) achieved in each period was 4.00 ± 0.11, 5.28 ± 0.40 and 3.51 ± 0.49 g/d; thus, the amount of THR required/g protein gain was 41.3, 43.6 and 47.9 mg/g in each period, respectively. Whereas the linear slope of the response to THR in each period was 0.023 ± 0.002, 0.028 ± 0.006 and 0.018 ± 0.005 g protein/mg THR intake, respectively, there was no difference ($P < 0.05$) between the slopes of these lines (0.024 ± 0.003 g/mg) when these were compared using simple linear regression with groups. Thus, the amount of THR required per g of protein deposition as measured here was (1/0.024) = 41.7 mg/g. These responses are illustrated in Figure 1 in which protein deposition (body plus feathers, g/d) for each of the three periods has been regressed against THR intake. Note the common slope in each period of growth.

The analysed THRcs of body and feather protein were 37 and 45; 37 and 43; and 39 and 43 mg/g, for periods 1, 2 and 3, respectively, the mean THRcs of body and feather protein thus being 37.7 and 43.8 mg/g, respectively. These values were used to calculate the rates of THR deposition in body plus feathers by pullets on each of the dietary treatments in each period. The maximum rates of THR deposition in body and feathers, namely 166, 230 and 168 mg THR/d, were reached on 164 ± 4.08, 200 ± 15.7

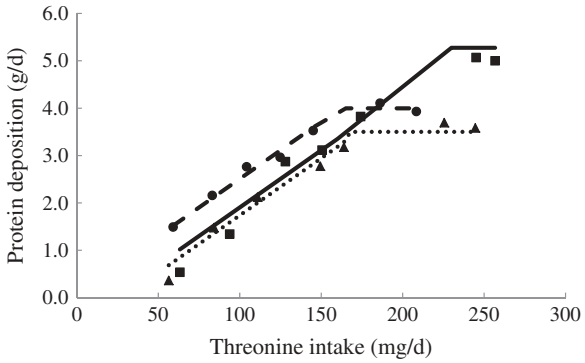


**Figure 1.** *The response in protein (body plus feathers) deposition (g/d) to threonine intake (mg/d) for pullets in periods 1 (4–6 weeks, ●), 2 (8–11 weeks, ■) and 3 (13–16 weeks, ▲).*

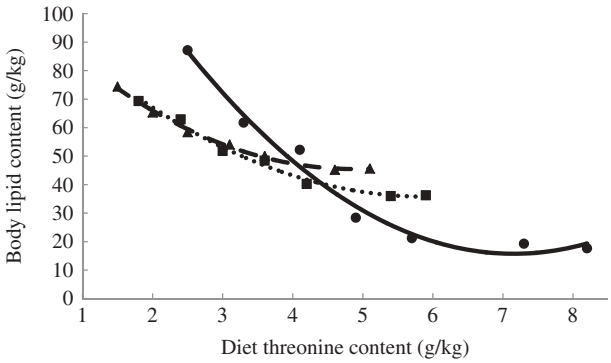


**Figure 2.** *Body lipid content at the end of each period as a function of dietary threonine content, in periods 1 (4–6 weeks,●), 2 (8–11 weeks, ■) and 3 (13–16 weeks, ▲).*

and 108 ± 11.7 mg THR/d. When the linear portion of the response in each period was interrogated, the rate of increase in THR deposition differed in each period (0.934 ± 0.09; 0.966 ± 0.23 and 0.752 ± 0.19 mg/mg), but when the data were combined using simple linear regression with groups, the slopes were found not to differ and the common slope for all periods was 0.855 ± 0.13 mg/mg. Thus, 1.17 mg dietary THR is required per mg of body and feather protein deposited.

As dietary THRc decreased, the amount of body lipid deposition increased curvilinearly (Table 2 and Figure 2), so that by the end of each trial period those pullets on the lowest THRcs were fatter than pullets reared on the higher THRcs.

To determine to what extent the proportions of feather and body protein varied during growth and according to the amount of dietary THR in the feed, feather protein content was expressed as a proportion of body protein at the end of each trial period, and the results are presented in Table 3. These proportions were regressed against dietary THR intake, with trial period as a factor, using simple linear regression with groups. The slopes of these regressions were the same (1.04 ± 0.067 g/100 g body protein per g THR intake) for the first two periods, but differed significantly in the third period (0.38 ± 0.052).

## DISCUSSION

The objective of the research reported here was to measure the response of growing pullets during three periods of growth to dietary THR with a view to determining the efficiency of utilisation of THR for body and feather protein growth and to determine whether this efficiency would change as they became older and approached sexual maturity. The mature body protein content of this strain of laying hen was calculated to be 0.26 kg (Silva, 2012); thus in the first trial period, the degree of maturity of the pullets ($u$, body protein at time $t$/mature body protein) was 0.116; in the second trial, $u$ was 0.282; and in the third period, when growth rate is slowing down, $u = 0.497$. When pullets reach sexual maturity,

**Table 3.** *Dietary threonine content (THRc), feather protein content (FPc), body protein content (BPc) and the ratio of feather to body protein (FPc/BPc) at the end of each trial period*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Period 1 (4–6 weeks) | | | | | | | | |
| THRc (g/kg) | 2.5 | 3.2 | 3.3 | 4.1 | 4.9 | 5.7 | 7.3 | 8.2 |
| FPc (g) | 12.5 | 14.8 | 16.1 | 20.1 | 21.1 | 24.6 | 27.7 | 27.9 |
| BPc (g) | 26.4 | 33.3 | 32.1 | 36.6 | 36.7 | 42.7 | 47.8 | 45.2 |
| FPc/BPc[1] | 0.47 | 0.44 | 0.50 | 0.55 | 0.58 | 0.58 | 0.58 | 0.62 |
| Period 2 (8–11 weeks) | | | | | | | | |
| THRc (g/kg) | 1.8 | 2.3 | 2.4 | 3.0 | 3.6 | 4.2 | 5.4 | 5.9 |
| FPc (g) | 30.7 | 33.9 | 35.9 | 44.3 | 44.8 | 49.1 | 55.5 | 56.2 |
| BPc (g) | 70.9 | 77.1 | 74.0 | 88.6 | 86.7 | 92.3 | 89.8 | 106 |
| FPc/BPc | 0.43 | 0.44 | 0.49 | 0.50 | 0.52 | 0.53 | 0.62 | 0.53 |
| Period 3 (13–16 weeks) | | | | | | | | |
| THRc (g/kg) | 1.5 | 2.0 | 2.0 | 2.5 | 3.1 | 3.6 | 4.6 | 5.1 |
| FPc (g) | 54.6 | 56.8 | 59.1 | 66.4 | 69.0 | 76.8 | 79.2 | 75.9 |
| BPc (g) | 112 | 115 | 124 | 133 | 134 | 137 | 142 | 144 |
| FPc/BPc | 0.49 | 0.49 | 0.48 | 0.50 | 0.52 | 0.56 | 0.56 | 0.53 |

[1]FPc/BPc – (feather protein content/body protein content).

which is considerably before they reach somatic maturity, growth of body protein is at a minimum until egg production ceases (Fisher and Gous, 2009), so there is little value in measuring growth responses at a later degree of maturity than was used here. The three trial periods therefore adequately covered the important periods of growth of a laying-type pullet.

Feather protein grows at a faster rate than body protein (Emmans, 1987), so it would be expected that the relative growth rates of body and feather protein would differ in each of these periods, but because the THRc of body protein is so similar to that of feather protein, these relative differences would not have influenced the response as much as might some other amino acids whose concentrations in body and feather protein differ markedly, as is the case with lysine and cysteine (Emmans, 1989).

The values found in the literature for THRc in the body and the feathers range from 33.9 to 40.2 mg/g for the feather-free body (Williams *et al.*, 1954; Saunders *et al.*, 1997; Stilborn *et al.*, 2010; Silva, 2012) and from 42.0 to 44.9 mg/g for feathers (Fisher *et al.*, 1981; Stilborn *et al.*, 1997; Silva, 2012). The THRcs of body and feather protein determined in these trials (37.7 and 43.8 mg/g, respectively) are similar to these published values and could therefore confidently be used to calculate the deposition of THR in the body and feathers.

Of importance in measuring the efficiency of utilisation of THR for protein deposition is to ensure that the linear portion of the response curve is identified and used for this purpose, which then obviates the need to subtract the amount of amino acid required for maintenance from the total intake. We used the broken stick model to identify the point at which no further response to THR intake was obtained and then used all points below this intake to measure the regression coefficient, which was then compared using simple linear regression with groups, the groups in this case being the three periods during which measurements of response were made. Also of importance was the confirmation that all feeds used in the three periods were the first limiting in THR, as indicated by the higher food intake, growth and FCE when synthetic THR was added to the feed with the lowest THRc in each of the response periods. This is one of the major advantages to the use of the summit dilution technique compared to the graded supplementation technique as in the latter technique it cannot be guaranteed that the amino acid under test remains the first limiting in all the feeds in the dilution series, nor is it likely that this would be the case (Gous and Morris, 1985). The efficiencies of utilisation of THR in the three periods under test could be regarded as being accurately estimated using the method applied here.

The degree of maturity of the pullets did not alter the regression coefficient reflecting the response in rate of protein deposition to increasing THR intake, the value being 0.025 ± 0.004 g body and feather protein/mg THR intake when data from all three periods were combined. The efficiency of utilisation of THR for protein deposition therefore remained at 0.417 over all the periods in spite of differences in the rates of growth of body and feather protein. Although this efficiency is relatively low, the efficiency with which dietary THR was converted to THR in the body and feathers was considerably higher, being 0.855 ± 0.13 mg of dietary THR/mg of body and feather protein gain. This is the same efficiency as was reported by Gous and Morris (1985) for the efficiency with which lysine is used for protein growth in 0-to 3-week-old broilers, similar to that reported by Edwards *et al.* (1997) (0.82) for the conversion of THR into whole body protein and higher than the 0.73 reported by Burnham *et al.* (1992) for isoleucine in young broilers.

Edwards *et al.* (1997) reported that for each 1 g increase in protein accretion in broiler chicks THR accretion was increased by 44.1 mg, suggesting that THR concentration in whole body protein gain is constant. A similar result was found in these trials, but the mean THRc of body and feather protein gain, taking account of systematic changes over the growing period, was in this case 39.4 mg/g protein. This is surprising given the proportional changes in body and feather protein with THR intake and with age of pullet, but is probably the result of the contents of THR being so similar in the two proteins being formed.

The increase in body lipid content as dietary THRc was reduced has been reported previously for broilers (Gous and Morris, 1985; Burnham *et al.*, 1992) where the summit dilution technique was also applied. The highest lipid content recorded in these trials (87 g/kg, Table 2) is considerably lower than that reported for broilers in the above papers (190 g/kg, with the lowest lipid content being 100 g/kg). The range of lipid contents deposited by pullets was greater in the first period than in the other two periods which may reflect the greater range of dietary THRcs used in the first trial period. The theory of food intake of Emmans (1981, 1987) predicts that birds will get fat when offered a feed with limiting protein or an amino acid because they will overconsume energy in an attempt to consume sufficient of the limiting nutrient in the feed in an endeavour to grow at their potential. This theory has been confirmed many times (Foot, 1972; Emmans, 1981; Gous *et al.*, 1990, 2012; Sibbald

and Rhind, 1997; Whittemore, 1998). The extent to which energy can be overconsumed depends on the amount of lipid that can be stored in the body: if the genotype is such that a large amount of lipid can be stored, then the bird will be capable of consuming relatively more of an imbalanced feed than one whose ability to fatten is limited genetically. The range of food intakes measured in these trials was relatively small, and in no instances did the food intake on marginally deficient feeds increase above that of the pullets on the highest concentration of THR, whereas in the experiments by Burnham *et al.* (1992) food intake increased from 41.8 g/bird d on the feed with the highest isoleucine content to 45.7 g/bird d on a marginally deficient feed and the intake finally decreased to 37.0 g/d on the lowest isoleucine feed. Similarly, in a trial reported by Clark *et al.* (1982) in which the response of broilers to a well-balanced amino acid mixture was measured, food intakes increased from 33.9 g/d on the highest protein concentration to 37.2 g/d on a marginally deficient feed, dropping to 28.0 g/d on the lowest protein concentration used in the trial. Clearly, the pullets in the present trials were not capable of overconsuming energy in an attempt to consume sufficient dietary THR, with the consequence that body weight gains decreased to a greater extent than would have been the case had the birds been capable of consuming excess lipid. Two important conclusions can be drawn from these observations: commercial laying-type pullets will not achieve their potential body and feather protein gain if the dietary protein content is marginally reduced, unlike some broiler strains, and the advantage of measuring protein gain and using this to calculate efficiencies of utilisation rather than working with body weight gains is obvious.

Body and feather protein grow at different rates (Emmans, 1987; Hancock *et al.*, 1995; Gous *et al.*, 1996), but there is less evidence of the relative growth rates of these two proteins when the bird is subjected to feeds differing in protein content. The data in Table 3 are thus of interest, as they demonstrate that the ratio of feather-to-body protein does not remain constant at an age (or degree of maturity) when different dietary protein concentrations are given. Irrespective of the age of the pullets, the ratio of body protein-to-feather protein increased at a rate of 0.0248 ± 0.0036 as the dietary THRc (mg/g) increased. Deficiencies in dietary protein are therefore likely to reduce the rate of feather protein growth relative to body protein growth.

The responses in body and feather protein growth measured in these trials are of value in determining the optimum concentrations of THR to be included in feeds for growing pullets. The objective was not to define the optimum intake of THR in each trial, but to make use of the determined efficiencies of utilisation of THR for protein growth to define the amount of dietary THR that would be required by growing pullets on each day of the growing period, taking account of their daily potential rates of growth of body and feather protein. As the efficiency of utilisation remained the same in all the three trial periods, it is not necessary to use different efficiencies to describe their mean daily THR requirements. By defining the rates of growth of body and feather protein, and calculating the amount of THR required each day to meet these requirements, taking account of the efficiency of utilisation of THR for protein growth and including an amount to meet the requirement for THR to maintain body and feather protein, it is possible to determine the daily requirement for THR to enable a pullet to grow at its potential. These daily requirements need to be converted to dietary concentrations, and some means of predicting food intake is essential if this process is to be successful. A feeding programme can then be devised that will minimise the periods of underfeeding and overfeeding of THR whilst minimising the number of feeds to be used in the feeding programme and the cost of providing the feed required.

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