

## Effect of Dietary Energy and Sulphur Amino Acids Level on Egg Production Traits in the Tropics

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**Abstract:** A 3 × 2 factorial experiment was conducted with commercial hens to determine the effect of dietary energy (2600, 2700 and 2800 kcal of ME kg<sup>-1</sup>) and total sulphur amino acids (TSAA, 0.65 and 0.71%) concentrations on egg traits of laying hens from 28-37 weeks of age. Birds (n = 240) were randomly assigned into 6 treatments. The energy × TSAA interaction was significant (p<0.05) on feed, methionine, TSAA and energy intakes (p<0.05). At 0.71% TSAA, feed (104.6, 102.1 and 95.9 g), methionine (0.42, 0.41 and 0.38 g) and TSAA (0.74, 0.73 and 0.68 g) intakes decreased as the dietary energy level of the diet increased, but were similar among levels of dietary energy for the hens fed 0.65% TSAA. The energy intake increased as the level of dietary energy of the hens fed 0.65% TSAA increased (261.1, 276.4 and 284.3 kcal) and it was similar among levels of dietary energy for hens fed 0.71% TSAA. Eggs from hens fed 0.71% TSAA were heavier than eggs from hens fed 0.65% TSAA (p<0.05) (58.3 and 59.2 g, respectively). The level of energy had a quadratic effect on egg weight (58.5, 59.3 and 58.4 g). The results indicate that hens firstly fitted their feed intake based on TSAA content in the diet and secondly by the content of energy in the diet. Both dietary energy and TSAA intakes influenced the egg weight. Hens fitted their feed intake to 0.37 g of methionine and 0.66 g of TSAA per day.

**Key words:** Dietary energy, sulphur amino acids, egg production, efforts, TSAA

### INTRODUCTION

It has been reported that high levels of dietary energy for laying hens reduces the feed intake (Peguri and Coon, 1991). Grobas *et al.* (1999) mentioned that hens fed 2680 kcal of ME kg<sup>-1</sup> in the diet, ate 4% less food than hens fed 2810 kcal. Wu *et al.* (2005) informed that when the energy of the diet increased from 2719-2956 kcal of ME kg<sup>-1</sup> the hens fitted their feed intake from 107.6-101.1 g/hen/day to keep a similar energy intake. These adjustments in feed intake affect the protein and amino acids intakes.

The efficient use of dietary protein depends on the diet composition, especially on the content of methionine, which is the main limiting amino acid in birds (Novack *et al.*, 2004). The National Research Council (1984) recommends a minimum of 0.60 g of total sulphur amino acids (methionine and cystine) in diets for hens. Schutte *et al.* (1994) estimated the requirement of Total Sulphur Amino Acids (TSAA) in 0.74 g/hen/day. Novack *et al.* (2004) reported that the TSAA levels to obtain higher egg production were 0.86 and 0.72 g of

TSAA/hen/day for phases 1 and 2, respectively. Recently, Fuente-Martinez *et al.* (2005) found that the optimal level of digestible sulphur amino acids in diets for hens was 0.62% (equivalent to 0.74% TSAA), in diets with 2900 kcal of EM. Considering that dietary energy level, the intake of digestible sulphur amino acids per day was 0.53 g (equivalent to 0.64 g of TSAA).

Because the content of both amino acids and energy of the diet affects substantially the feed intake and in consequence the efficient use of the diet, an appropriate handling of the energy and amino acids concentration is needed in order to increase the productivity of laying hens.

The present experiment was conducted to determine the effect of dietary energy and TSAA concentrations on egg traits of laying hens from 28-37 weeks of age.

### MATERIALS AND METHODS

Two hundred and forty, 28 week old hens (Isa-Babcock B-300) were used. The hens were randomly distributed in cages of 40×40 cm, handling a density of

**Table 1: Ingredient and nutrient composition of experimental diets for laying hens**

	0.65			0.71		
	2600	2700	2800	2600	2700	2800
Sulphur amino acids (%)						
Metabolizable energy (Kcal kg <sup>-1</sup> )						
Sorghum	63.20	58.50	56.80	55.90	54.50	52.00
Soybean meal 44% CP	25.10	25.60	25.20	30.50	29.60	30.00
CaCO <sub>3</sub>	9.90	9.90	9.80	9.90	9.90	9.80
Dicalcium phosphate	1.90	1.90	1.90	1.85	1.86	1.85
Soybean oil	0.60	2.80	5.00	0.60	2.80	5.00
Sodium chloride	0.30	0.30	0.03	0.30	0.30	0.30
Mineral premix <sup>1</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Vitamins premix <sup>2</sup>	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.11	0.12	1.30	0.12	0.13	0.14
Choline chloride <sup>3</sup>	0.05	0.05	0.05	0.05	0.05	0.05
Pigment	0.03	0.03	0.03	0.03		0.03
Calculate analysis						
ME (Mcal kg <sup>-1</sup> )	2.60	2.70	2.80	2.60	2.70	2.80
Calcium (%)	4.19	4.20	4.16	4.20	4.20	4.16
Available phosphorous (%)	0.45	0.45	0.45	0.45	0.45	0.45
Calculate analysis of CP and aminoacids (%)						
Methionine	0.36	0.36	0.37	0.39	0.40	0.40
Methionine + Cystine	0.65	0.65	0.65	0.71	0.71	0.71
Lysine	0.86	0.86	0.86	0.95	0.92	0.93
Crude protein	17.00	16.99	16.67	19.00	18.50	18.50
Crude protein:TSAA	26.20	26.10	25.60	26.80	26.10	26.10
Lysine:TSAA	1.32	1.33	1.33	1.33	1.30	1.31

<sup>1</sup>Vitamin Premix: vitamin A, 8000 UI; vitamin D, 2500 UI; vitamin E, 8 UI; vitamin K, 2 mg; vitamin B<sub>12</sub>, 0.002 mg; riboflavin, 5.5 mg; calcium pantothenate 13 mg; niacine, 36 mg; Choline chloride, 500 mg; folic acid, 0.5 mg; thiamine, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg. <sup>2</sup>Mineral Premix: Mn, 65 mg; I, 1 mg; Fe, 55 mg; Cu, 6 mg; Zn, 55 mg; Se, 0.3 mg. <sup>3</sup>700 g kg<sup>-1</sup> of Choline chloride

either 2 or 3 hens by cage. There were 16 replicates by treatment; 8 cages with 2 hens and 8 cages with 3 hens by cage in each treatment were used. Three levels of energy (2600, 2700 and 2800 kcal of ME kg<sup>-1</sup>) and 2 levels of TSAA (0.65 and 0.71%) in a 3×2 factorial experiment were used. The average maximum and minimum temperatures during the experiment were 28.9±2.5 and 20.3±2.8°C, respectively. The experiment lasted 8 weeks. Ingredients and nutrient composition of experimental diets are shown in Table 1.

The diets were elaborated with sorghum, soybean meal and soybean oil. The content of calcium and phosphorus available was similar for all diets: 4.2 and 0.45%, respectively (Table 1). A similar relationship of TSAA: Lysine and crude protein: TSAA was kept for all diets.

The birds had *ad libitum* access to feed and water throughout the experiment. The offered feed was registered daily and the rejected one was recorded weekly to calculate feed intake by cage. Eggs per cage were collected every day and every week one egg randomly selected from each cage was weighed (scale of 0,1 g). Immediately after being weighed eggs were broken carefully and their content (yolk and albumen) smoothly deposited on a plate. The length and width of albumen and yolk were measured with a Vernier and the height was measured with a micrometric screw. The yolk and albumen of each egg were weighed individually.

Feed intake was calculated weekly as the average feed intake/bird/day/cage. Feed conversion was calculated as the weekly feed intake per cage, divided by the kilograms of egg produced weekly per cage. The cage-day egg production (%) was calculated as the ratio of the total number of egg per cage divided by the experimental period (8 weeks). Egg mass was calculated as the ratio of the average weight of all eggs and the cage-day-egg production for the whole experimental period. The hens were weighed at initial and final time of the experiment.

Data on weekly measured variables were analysed in a 3×2 factorial experiment in a randomised complete block design with repeated measures using the MIXED procedure of the Statistical Analysis System (SAS, 1991). Dietary energy (2600, 2700 and 2800 kcal kg<sup>-1</sup>) and TSAA (0,065 and 0,71%) were fixed and blocks and cages were random.

Data on egg production (%) and egg mass (during the whole period of study) and average hen-weight per cage, at the end of the study, were analysed in a 2×3 factorial experiment in a complete randomised blocks design, using the general linear model procedure of SAS (1999). In this case, all factors, except the random term, were fixed.

If differences in energy level means were detected by MIXED or GLM procedures, contrast statements were utilized to test for linear or quadratic dietary energy effects. A significance level of p<0.05 was used during analysis.

**RESULTS AND DISCUSSION**

Dietary energy level of the diet affected ( $p < 0.05$ ) egg weight and yolk weight and height (Table 2). The 2700 Kcal  $\text{kg}^{-1}$  level was the best treatment. Energy level had no effect on the other egg components measured ( $p > 0.05$ ). The 0.71% TSAA treatment improved egg weight and albumen weight and width ( $p < 0.05$ ); however, TSAA had no effect ( $p > 0.05$ ) on the other egg component measured (Table 2). Dietary energy level had a quadratic effect on egg weight and albumen length and linear and quadratic on yolk weight and yolk height ( $p < 0.05$ ). The increase in egg weight could be associated with the increase in yolk weight by effect of the dietary energy level and by the increase of the albumen weight by effect of the dietary TSAA content. Shell *et al.* (1987) pointed out that young hens use more lipids for yolk development, because the lipoprotein synthesis in the liver is insufficient in this type of birds. In agreement with the results of this study, Shafer *et al.* (1996) found a significant increase in albumen weight when they fed hens with 0.82 g of TSAA/hen/day in comparison with hens fed 0.62 g. Shafer *et al.* (1998) reported an increase in albumen weight when hens consumed either 0.82 or 0.85 g/hen/day of TSAA in comparison with hens fed 0.72 g. However, other researchers have found no increase in the amount of albumen by the increase of TSAA from 0.64-0.89 g/bird/day (Novak *et al.*, 2004) or from 0.52-0.80 g/bird/day (Scheideler *et al.*, 1998). These discrepancies could be the result of differences in requirements of amino acids between the genetic lines evaluated. Novak *et al.* (2004) and Scheideler *et al.* (1998) worked with Dekalb hens having lower body weights than other leghorn hens, reason why they require less amount of TSAA for maintenance and leave more TSAA to be used for protein synthesis in the egg (Wu *et al.*, 2005). Scheideler *et al.* (1998) mentioned that the Dekalb hens typically have more albumen in their eggs.

High dietary energy levels increased egg weight in agreement with the results of Peguri and Coon (1991). The increase in the egg weight, as the dietary energy level increased, was probably associated with more soybean oil in the diet as the dietary energy level increased (Table 1). It has been reported that the increase of fatty acids in the diet can increase the production of estrogens that stimulate the protein synthesis in the oviduct (Whitehead *et al.*, 1993; Whitehead, 1995). Also, it has been mentioned that a positive association exist between hen weight and egg weight. In this study, the weight of the hens and the eggs increased according the dietary energy level. Summers and Lesson (1983) found that heavy-hens lay significantly heavier eggs than light hens. In this study, the egg weight increased as the energy and TSAA levels increased. The quadratic effect of dietary energy on egg weight could be associated with the decrease of feed and TSAA intakes as the dietary energy level of the diet increased.

Dietary energy and TSAA content of the diet had no effect on egg production and egg mass ( $p > 0.05$ ). Nevertheless, the increase in the dietary energy level did not improve the percentage egg production, which agrees with results from Sell *et al.* (1987) and Keshavarz and Nakajima (1995). However, the final body weight of the hens increased linearly ( $p < 0.05$ ) as the dietary energy level of the diet increased (Table 3). Hen weight was not affected by the dietary TSAA level, which agrees with the results of Sohail *et al.* (2002) and Novak *et al.* (2006). Probably, the TSAA intake obtained in this experiment (0.64 g/bird/day) was about to cover the requirements for the growth of the birds. According to Novak *et al.* (2004), the growth of the hens was maximized for the consumption of 0.69 g/bird/day of TSAA.

Dietary energy x TSAA interaction ( $p < 0.05$ ) was observed on feed, energy, methionine and TSAA intakes (Table 4). At 0.71% TSSA: high levels of energy decreased feed (104.6, 102.1 and 95.9 g  $\text{day}^{-1}$ ), methionine

**Table 2: The effects of dietary treatments on weight of egg components**

Factor		Weight (g)		Height (mm)		Width (mm)		Length (mm)	
		Egg	Albumin	Yolk	Albumin	Yolk	Albumin	Yolk	Albumin
Energy	2600	58.5	36.2	13.3	12.0	35.9	55.8	14.9	69.5
(Kcal $\text{kg}^{-1}$ )	2700	59.3	36.4	13.7	12.1	36.4	56.4	15.0	70.1
	2800	58.4	36.1	13.6	12.1	36.3	55.9	14.8	69.2
TSAA (%)	0.65	58.3	35.7	13.6	12.0	36.2	55.6	14.9	69.5
	0.71	59.2	36.7	13.5	12.1	36.2	56.4	14.9	69.8
SEM		0.29	0.31	0.11	0.14	0.15	0.31	0.11	0.39
Main effects and interactions									
Block		0.6254	0.8127	0.5167	0.3740	0.3912	0.7233	0.3955	0.9364
Energy		0.0266	0.4944	0.0044	0.7992	0.0022	0.1470	0.3641	0.0712
TSAA		0.0036	0.0001	0.1400	0.5285	0.6777	0.0009	0.7999	0.3736
Energy x TSAA		0.0872	0.1634	0.4068	0.6088	0.8596	0.4895	0.1509	0.1516
Contrast									
Energy linear		0.6524	0.7129	0.0130	0.5181	0.0207	0.9123	0.5999	0.5014
Energy quadratic		0.0081	0.2623	0.0299	0.8631	0.0088	0.0505	0.1871	0.0288

**Table 3: The effects of dietary treatments on rate of egg production, egg mass and weight of the hens**

Factor		Egg production (%)	Egg mass	Final weight of the hens (kg)
Energy	2600	95.3	56.0	1.55
(Kcal kg <sup>-1</sup> )	2700	95.2	55.7	1.61
	2800	95.5	56.2	1.62
TSAA (%)	0.65	95.9	56.2	1.60
	0.71	94.7	55.7	1.60
SEM		1.27	0.83	0.027
Main effects and interactions				
Block		0.0002	0.0072	0.6859
Energy		0.9703	0.8182	0.0212
TSAA		0.2319	0.4316	0.6752
Energy x TSAA		0.4121	0.4580	0.6024
Contrast				
Energy lineal		0.9104	0.7571	0.0098
Energy quadratic		0.8280	0.5817	0.2990

**Table 4: The influence of dietary treatments on feed intake and feed conversion**

		Intake/day				Conversion factor		
		Feed (g)	Energy Kcal	Methionine (g)	Methionine + Cystine (g)	Feed (kg kg <sup>-1</sup> )	Energy (Kcal kg <sup>-1</sup> )	TSAA (mg g <sup>-1</sup> )
Energy	2600	102.5	266.6	0.39	0.68	1.85	4.9	12.8
(Kcal kg <sup>-1</sup> )	2700	100.3	276.1	0.39	0.68	1.82	5.2	13.0
	2800	98.7	276.4	0.37	0.66	1.78	5.1	12.3
TSAA (%)	0.65	101.4	273.9	0.37	0.64	1.84	5.0	12.0
	0.71	100.9	272.1	0.40	0.70	1.80	5.1	13.4
SEM		1.22	3.32	0.01	0.01	0.03	165.93	0.24
Main effects and interaction								
Block		0.0002	0.0002	0.0003	0.0002	0.2258	0.2410	0.2301
Energy		0.0518	0.0651	0.0422	0.0414	0.4441	0.4531	0.1966
TSAA		0.6929	0.6322	0.0001	0.0001	0.1983	0.1953	0.0001
Energy x TSAA		0.0204	0.0201	0.0188	0.0180	0.7680	0.7682	0.7297
Contrast Energy lineal		0.0290	0.0403	0.0216	0.0226	0.2645	0.2727	0.2616
Energy quadratic		0.2752	0.2574	0.2976	0.2718	0.1573	0.1500	0.1570

(0.42, 0.41 and 0.38 g day<sup>-1</sup>) and TSSA (0.74, 0.73 and 0.68 g day<sup>-1</sup>) intakes, but energy intakes were similar (272.1, 275.8 and 268.5 kcal for 2600, 2700 and 2800 kcal of ME kg<sup>-1</sup>, respectively); whereas, at 0.65% TSAA: feed (100.4, 102.4 and 101.5 g day<sup>-1</sup>), methionine (0.36, 0.37 and 0.37 g day<sup>-1</sup>) and TSAA (0.65, 0.67 and 0.66 g day<sup>-1</sup>) intakes were similar among levels of energy, even energy intake increased (261.1, 276.4 and 284.3 kcal). There was not interaction for the remaining traits. The decreased feed intake with higher dietary energy levels agrees with previous findings (Peguri and Coon, 1991; Grobas *et al.*, 1999; Wu *et al.*, 2005). The increase in the energy levels of the diet adjusted the energy intake to 276 kcal of ME/bird/day in the diets with 2700 and 2800 kcal of EM kg<sup>-1</sup>. The decrease of TSAA intake was directly associated with the reduction in feed intake as the energy level increased and with the reduction in the concentration of TSAA content of the diet.

The dietary energy x TSAA interaction suggested that hens fitted their methionine and TSAA intakes to 0.37 and 0.66 g, respectively, independently of the dietary energy level. These results support the hypothesis that feed intake is modified by the energy level when the amino acids content of the diet is optimal. Viceversa, hens fit their intake in function of the dietary amino acids level

when the requirements of amino acids are not fulfilled, independently of energy level (Veldkamp *et al.*, 2005).

The level of TSAA have no effect on feed conversion (p>0.05); however hens offered the 0.65% TSAA diet had a better conversion than those fed the 0.71% TSAA (Table 4). Like reported by Novak *et al.* (2004) more TSAA were consumed to produce a gram of egg according concentration of TSAA in the diet increased.

The energy concentration of the diet had no effect on feed and energy conversions (p>0.05). These results agree with Wu *et al.* (2005), who reported that independently of the dietary energy level, the amount of energy required to produce one gram of egg is the same. In the current work, approximately 5 kcal of ME were required to produce one gram of egg, which is lower than the 5.8 kcal of ME g<sup>-1</sup> notified by Wu *et al.* (2005). The difference is probably due to the lower levels of energy used in this experiment (2600-2800 kcal of ME).

## CONCLUSION

The results reported here indicate that hens firstly fitted their feed intake based on the TSAA content and secondly on the energy content of the diet. Variations in the energy and TSAA intakes could influence egg weight.

Hens in this experiment fitted their feed intake at 276 kcal of ME day<sup>-1</sup>, 0.37 g day<sup>-1</sup> of methionine and 0.66 g of TSAA/day.

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