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Nutritional Requirements in Methionine + Cystine for White-Egg Laying Hens During the First Cycle of Production

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Abstract: A total of 288 leghorn laying hens were used in an experiment to determine methionine+cystine (TSAA) requirements. The addition of six levels of DL-methionine to a basal diet containing 14.4% of crude protein resulted in 0.484, 0.534, 0.584, 0.634, 0.684, and 0.734% TSAA contents. This experiment lasted from 22 to 38 week of age. Increasing TSAA from 0.484 to 0.734% had a quadratic effect on egg production, egg weight, feed intake, feed conversion, and body weight. Based on quadratic regression, TSAA requirements for maximum egg production, egg weight, egg mass, and body weight gain and minimum feed conversion were 0.658, 0.681, 0.664, 0.683, and 0.6665%, respectively. White-Egg laying hens required 0.67% TSAA in diets or 737 mg TSAA per hen daily from 22 to 38 wk of age (with feed consumption of 110 g).

Key words: Methionine, cystine, TSAA, requirement, laying hens

Introduction

Efficiency of protein utilization in a diet depends on the amount, composition and digestibility of the amino acids that contained in the diet. Methione+cystine (TSAA) perform a number of functions in enzyme reactions and protein synthesis. Methionine is the first limiting amino acid in corn soybean meal diets in laying hens (Schutte *et al*., 1978). Cystine can supply no more than 52% of the TSAA in chicks (Baker *et al*., 1996). Based on the ideal protein concept, a protein supplies amino acids in exactly the amounts and proportions required by animals and can be 100% utilized under appropriate circumstances (Emmert and Barker, 1997; Barker, 2003). If one or two of essential amino acids are deficient, the excess amino acids will be wasted as N excretion. To efficiently use excess amino acids, the crystalline amino acids such as methionine and lysine can be added to the diets to balance the amino acids. Novak *et al*. (2004), Liu *et al*. (2005), and Wu *et al*. (2005a) reported that increasing lysine or TSAA had positive effect on performance of laying hens.

It is important to know the TSAA and methionine requirements of laying hens. There are contradicting results in methionine and TSAA requirements of laying hens. NRC (1994) reported that white-egg laying hens with 100 g daily feed consumption required 0.30% methionine and 0.58% TSAA in the diets, or 300 and 580 mg of methionine and TSAA per hen daily, respectively. Similarly, Rostagno (1990) suggests that hens with daily feed consumption of 105 g required 0.311% methionine and 0.567% TSAA or 327 and 595 mg of methionine and TSAA daily, repectively. Ahmad *et al*. (1997) reported that TSAA ranging from 580 to 660 mg per hen daily had no effect on performance of laying hens. However, Schutte *et al*. (1994) reported that the requirement for TSAA was about 740 mg per hen daily, of which about 440 mg was methionine. Cao *et al*. (1992) also reported that the requirement of methionine and TSAA were 424 and 785 mg per hen daily, respectively. In addition, there are differences in TSAA requirements estimated from different production parameters. Novak *et al*. (2004) reported that dietary TSAA level for maximum egg production was 811 mg per hen daily while TSAA for feed efficiency was 699 mg per hen daily.

Technological advances in genetics, managements, animal health and behavior have allowed laying hens to have better feed efficiency, bigger egg size, and longer persistence of the peak production. Thus, it is necessary to conduct research in nutritional requirement determination and in optimization of the use of nutrients so that laying hens can have the maximum genetic potential expression. Therefore, this experiment was designed to determine the nutritional requirements of TSAA for commercial white-egg laying hens.

Materials and Methods

This experiment was conducted in Department of Zootechnia of the Agrarian Science Center of the Federal University of Viçosa, MG, Brazil. 288 Lohmann hens were used. During the initial and growth phases the birds were managed following the recommendations of Lohmann Tradition Management Guide (Anonymous, 2005). After 17 weeks of age, the birds were transferred from the growth house to a production house (60 x 9m^2) with covered ceramic roofing tiles. Two hens were

Table1: Ingredient (%) and nutrient content of basal diet

Ingredient	Amount
Corn	44.72
Soybean meal	17.94
Sorghum-LT	25.00
Limestone	9.55
Dicalcium phosphate	1.71
Iodized salt	0.33
Corn gluten	0.26
Corn starch	0.25
Vitamin premix[1]	0.10
Mineral premix[2]	0.05
L-Lysine -Hcl	0.05
Choline chloride	0.02
Soyben Oil	0.01
BHT [3]	0.01
Calculated analysis	
ME (kcal/kg)	2750
Calcium (%)	4.00
Available Phosphorus, (%)	0.385
Crud protein, (%)	14.40
Methionine, (%)	0.234
Methionine+cystine, (%)	0.484
Lysine, (%)	0.710
Threonine, (%)	0.557
Tryptophan, (%)	0.179

[1]Rovimix matrizes (Roche). Composition/kg.: Vit.A- 12.000.000 U.I.; Vit D_3- 3.600.000 U.I.; Vit.E- 3.500 U.I.; Vit.B_1-2.500 mg.; Vit. B_2-8.000 mg.;Vit.B_6-3.000 mg.; Pant. Ac-12.000 mg.; Biotin- 200 mg.; Vit.K-3.000 mg.; Folic ac. 3.500 mg.; Nicot. Ac. -40.000 mg.; Vit.B_{12}-20.000 mcg.; Selenium 130 mg.; Excipients q.s.p.- 1.000 g.
[2]Roligomix Aves (Roche) Composition/kg.: Mn -160 g.; Fe -100 g.; Zn - 100 g.; Cu - 20 g.; Co 2 g.; I - 2 g.; Excipiente q.s.p. - 1.000 g.
[3]Butil-Hidroxi-tolueno (Antioxidant).

housed in conventional type cage (25 x 40 x 45cm^3). From the 18 weeks of age, the birds had received increased stimulation of light until 17 hours of light daily at 27 week of age. Then 17 hours of light daily was maintained until the end of the experimental period. The temperature of air in the interior of the house was recorded at 7:00 AM for whole trial period. The average maximum (daytime) and minimum temperatures (night) were 28.0 and 16.1°C, respectively.

Before experiment, birds were randomly distributed to 6 treatments (6 replicates /treatment and 8 hens/replicate) based on body weight and egg production. From 22 week of age, hens were submitted to the treatments. Hens can drink water freely. This experiment lasted 16 weeks.

The treatments consisted of 6 diets. The addition of six levels of DL-methionine (0, 0.05, 0.10, 0.15, 0.20, 0.25%) to a basal diet containing 14.4% of crude protein resulted in 0.484, 0.534, 0.584, 0.634, 0.684, and 0.734% TSAA contents. DL-methionine was produced by Degussa Corp. (Sao Paulo, Brazil), and contained at least 99% methionine activity. The basal diet (Table 1) was formulated to satisfy nutritional requirements, except methionine and TSAA, according to recommendations of Rostagno (1990) and NRC (1994). The proximal analysis for dietary energy, calcium, phosphorus, and protein contents of the basal diet was conducted at lab of animal nutrition Federal University of Viçosa, MG, Brazil. The amino acid compositions in ingredients including corn, soybean, and sorghum were analyzed by Degussa corp. (Sao Paulo, Brazil) to confirm TSAA and methionine content in basal diets.

Eggs were collected every day. Egg production, egg weight (g), egg mass (g/hen d), feed consumption (g/hen d), feed conversion (g of feed/g of egg) were determined each 4 weeks. Eggs from each replicate were sampled for Haugh units, albumen index, and yolk index each 4 weeks. Yolk index = yolk diameter/ yolk height. Albumen index = Albumen height/average of short and long diameter of albumen.

All data was analyzed in SAS (1996). Once differences among treatments were detected by one-way ANOVA, linear and quadratic effects were tested by contrast statements. The requirements of TSAA had been determined by the polynomial regression models and least square broken line analysis.

Results and Discussion

Increasing TSAA in the diet had a quadratic effect egg production (Table 2). Hens fed with the diet without supplementation of methionine had the lowest egg production (70.2%). Methionine supplementation in the basal diet increased egg production until the level of 0.684% of TSAA. Increasing TSAA level from 0.684% to 0.734% had no improvement of egg production. These results are in agreement with those of Bertram et al. (1995), Bertechini *et al.* (1995), Liu *et al.* (2005), and Wu *et al.* (2005a), who reported that increasing levels of TSAA improved egg production. Low egg production of hens fed the low TSAA levels of in the present study can partially be attributed to amino acid imbalance, which causes the decrease of protein synthesis, inhibits absorption, and increases catabolism of the essential amino acid (Harper, 1956; Elehjem, 1956).

The increase of the TSAA level had a quadratic effect on egg weight and egg mass (Table 2). Increasing TSAA level from 0.484% to 0.684% increased egg weight and egg mass. However, future increase of TSAA from 0.684% to 0.734% had no improvement on egg weight and egg mass. These results are consistent with those of Bertram *et al.* (1995), Liu *et al.* (2005), Wu *et al.* (2005a), who reported that increasing levels of TSAA increased egg weigh or egg mass.

Increasing TSAA level had a quadratic effect on feed intake (Table 2). Hens fed the diet containing 0.484% had lower feed intake than hens fed other diets.

Table 2: The effect of TSAA on performance of laying hens from 22 to 38 wk of age

Met+Cys (%)	Egg production (egg/hen d)	Egg weight (g/hen d)	Egg mass (g/hen d)	Feed intake (g)	Feed conversion (g feed/g egg)	Body weight gain (g)
0.484	70.2	53.9	37.8	91.9	2.43	19.0
0.534	86.6	57.4	49.7	104.3	2.10	118.5
0.584	90.9	59.7	54.3	108.0	1.99	185.6
0.634	90.3	59.7	54.0	106.8	1.98	179.7
0.684	93.6	60.5	56.6	108.1	1.91	215.2
0.734	90.8	60.5	54.7	107.3	1.96	207.2
CV%	4.54	2.33	4.55	6.01	3.77	3.77
Quadratic effect		**	**	**	**	**

****P<0.01

Table 3: The effect of TSAA on egg quality of laying hens from 22 to 38 wk of age

Met+Cys (%)	Haugh units	Albumen index	Yolk index
0.484	97.07	0.137	0.471
0.534	97.00	0.135	0.454
0.584	99.81	0.144	0.465
0.634	96.89	0.132	0.461
0.684	96.63	0.131	0.462
0.734	94.57	0.125	0.456
CV%	1.72	4.77	1.33
Linear effect	**	NS	NS

**P<0.01

Table 4: Quadratic equation summary of TSAA requirements (%) of white laying hens from 22 to 38 wk of age

Parameters	Quadratic equation	R^2	Requirement of TSAA (%)
Egg production (%)	$Y=214.9+938.0X-712.1X^2$	0.92	0.658
Egg weight (g)	$Y=-16.3+226.1X-165.9X^2$	0.97	0.681
Egg mass (g/hen d)	$Y=-183.6+723.9X-545.1X^2$	0.95	0.664
Feed Conversion (g feed/g egg)	$Y=8.6-20.1X+15.1X^2$	0.95	0.665
Body weight gain (g)	$Y=-1997.3+6478.9X-4744.2X^2$	0.96	0.683

Increasing TSAA from 0.484% to 0.684% increased feed intake. Therefore, Methionine and TSAA level might be able to regulate feed consumption. Harper *et al.* (1970) and Austic (1986) reported TSAA could modify the plasmatic amino acidic profile in animals to activate appetite.

There was a quadratic response of feed conversion to TSAA level (g feed/egg g) with increasing TSAA level (Table 2). Feed conversion was improved as TSAA level increased from 0.484 to 0.684%. Increasing TSAA level from 0.684 to 0.734% had no effect on feed conversion. These observations were in accordance with the results of Bertram *et al.* (1995), Novak *et al.* (2004), Liu *et al.* (2005), Wu *et al.* (2005a). The explanation for the improved feed efficiency with increasing TSAA level might be attributed to more balanced amino acids.

There is a quadratic effect on body weight gain to TSAA level (Table 2). Hen fed 0.484% TSAA had the lowest body weight gain, while hens fed 0.684% had the highest body weight gain. When TSAA increased from 0.484 to 0.734, Haugh unit linearly decreased from 97.07 to 94.57 (Table 3). Increasing TSAA level had no effect on albumen index and yolk index. The Haugh unit is a measure of the internal quality of an egg. This is done by determining the height of the albumen in relation to the egg's weight. The reduction of Haugh unit with increasing TSAA level might be due to increased egg weight. Similarly, Wu *et al.* (2005b) reported Haugh unit might be decreased by increased egg weight.

Quadratic model was used to predict TSAA requirements by taking maximum or minimum asymptote. TSAA requirement determined by quadratic model for maximum egg production, egg weight, egg mass were 0.658, 0.681, and 0.664%, respectively (Table 4). The requirement of TSAA for minimum feed conversion was 0.665%. Laying hens required 0.683% TSAA in the diets for maximum body weight gain. The mean of TSAA requirements for maximum egg production, egg weight, egg mass, and body weight gain, and minimum feed conversion was 0.67%.

Because average feed intake from 30 to 38 wk of age was close to or equal to 110 g/hen d, feed consumption of 110 g/hen d was used to calculate TSAA requirements per hen daily. Based on quadratic model, laying hens required 0.67% TSAA in diets or 737 mg TSAA per hen daily from 22 to 38 wk of age (with feed consumption of

110g). These values are very close to those of Schutte *et al.* (1994), who reported that the requirement for TSAA was about 740 mg per hen daily. NRC (1994) reported that White-egg layers with 100 g daily feed consumption required 0.58% TSAA in diets, or 580 mg of TSAA per hen daily. TSAA values estimated in this experiment are higher than NRC values probably because of improved performance of laying hens in this experiment.

In conclusion, increasing TSAA in the diets deficient in TSAA significantly improved performance of laying hens. White-Egg laying hens required 0.67% TSAA in diets or 737 mg TSAA per hen daily from 22 to 38 wk of age (with feed consumption of 110 g).

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