

Sulfur amino acids requirements in broiler breeders weighed during the pre-initial growth phase

ARTÍCULO DE INVESTIGACIÓN

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ABSTRACT: Determine the nutritional requirements in Met+Cys for broiler breeder hens during the pre-initial growth phase. Birds were subjected to a basic diet deficient in Met+Cys with the minimum nutritional levels in essential amino acids. It contained 21,5% CP, 3050 kcal ME/kg and 0.688% Met+Cys supplemented with six levels of DL-methionine (0,00; 0,06; 0,12; 0,18; 0,24 and 0,30 %) resulting in diets with 0,688, 0,748, 0,808, 0,868, 0,928 and 0,988% of total Met+Cys, in randomized design blocks with twelve repetitions and eight birds per experimental unit. Body weight (BW), feed conversion (FC) and the effect of the treatments used in the initial phase on the reproductive performance in the period from 24 to 40 weeks of age were evaluated. The requirements in Met+Cys were calculated by polynomial regression and Plateau Linear Response models. Supplementing with DL-methionine was significantly influenced by BW, FC and the chemical composition of the shell at 5 weeks of age. The levels of Met+Cys used in the initial phase influenced only egg production in the period from 24 to 40 weeks and hatching of eggs at 40 weeks, with no effect either during the start of production, egg weight and hatching to 32 weeks nor in the body weight of one day old chicks. The recommendation of total Met+Cys in diets with 3050 kcal of ME/kg can be suggested in an amount of 0,75%, and this corresponds to 0,66% of digestible Met+Cys in the diet.

Key words: broiler chick, cystine, eclosion, methionine, nutrition

Requerimientos nutricionales de aminoácidos azufrados en reproductoras pesadas durante la fase preinicial de crecimiento

RESUMEN: determinar los requerimientos en Metionina+Cistina para aves reproductoras durante la fase pre-inicial de desarrollo. Las aves fueron sometidas a una dieta básica deficiente en Met+Cis con los niveles nutricionales básicos de aminoácidos; conteniendo 21,5% de CP, 3050 g/kal ME/g y 0,688% de Metionina+Cistina complementada con 6 niveles de DL-Metionina (0,00; 0,06; 0,12; 0,18; 0,24 y 0,30%); resultando dietas con 0,688, 0,748, 0,808, 0,868, 0,928 y 0,988 % del total de Met+Cisen, bloques aleatorios diseñados con doce repeticiones y ocho aves/unidad experimental. Fueron evaluados peso corporal (PC), conversión alimenticia (CA) y el efecto de los tratamientos usados en la fase inicial del comportamiento reproductivo durante el período de 24 a 40 semanas de edad. Los requerimientos en Met+Cis se calcularon a través de la regresión polinomial y los modelos de respuesta lineal de Plateau. El complemento con DL-metionina fue influenciado significativamente por PC, CA y la composición química del cascarón a las 5 semanas de edad. Los niveles de Met+Cis utilizados en la etapa inicial, únicamente influyeron en la producción de huevos en el período comprendido entre las 24 a las 40 semanas y la eclosión de los huevos a las 40 semanas, sin tener efecto durante el inicio de la producción, peso y eclosión de los huevos hacia la semana 32 ni en el peso corporal de los polluelos de un día de nacidos. La recomendación total de Met+Cis en dietas con 3050 kcal de ME/kg puede ser sugerida en una cantidad de 0,75%, lo cual corresponde al 0,66 % de la Met+Cis digestible en la dieta.

Palabras clave: pollo de engorde, cistina, eclosión, metionina, nutrición

Introduction

The continuous genetic progress obtained in broiler breeder hens, as a result of the great investments in the animal improvement, has significantly allowed a higher hatch egg production, with better feed efficiency and bigger persistence of egg production. So, it has demanded more intense scientific advances in nutrition, management, behavior and health security with the intention to obtain a high quality and economically viable final product. However, the high pressure in the selection of the birds for fast growing led to feed intake increase, made it necessary to control rate. Thus, many feed restriction programs were used looking for synchronization of sexual maturity and weight of the birds without affecting the uniformity of the flock so that it could have an excellent performance during the productive phase. Nevertheless, the accurate definition of the nutritional requirements of the birds is particularly essential for the success of the adopted feeding program.

Few researches have been carried through the determination of nutritional requirements in specific nutrients for broiler breeder hens in the period from birth until sexual maturity (Powell & Gehle, 1975; Harms, 1980; Harms & Wilson, 1987; Ambrozini, 1991), being in general recommendations of nutritional requirements obtained from strain management manuals through acquired practical experiences. The lack of information in the literature about the topic motivated this research with the aim to present information concerning to nutritional requirements in Met+Cys for broiler breeder hens in the period from 0 to 7 weeks of age.

Materials and Methods

Location: The present experiment was developed in the dependences of the Genetic Improvement of Birds Section of the Department of Animal Production at Federal University of Viçosa.

Animals: 576 a-day broiler breeder chicks Cobb® 500 were used. They had an initial average weight of 40g and from the 0 the 5 week they were submitted to a basal diet (Table 1) with 21,5% of CP, supplemented with six levels of DL-methionine resulting in diets with 0,688, 0,748, 0,808, 0,868, 0,928 and 0,988% of total sulfur amino acids (Met+Cys). Those were isoenergetic diets (3050 kcal of ME/kg) with the minimum nutritional requirements except the ones of Met+Cys and CP in accordance with the Breeder manual recommendations for Cobb® 500. The supplementation with DL-methionine was done as a substitution of the starch corn. The result was all isoproteic diets in relation to the basal diet.

Table 1. Basal Diet composition.

Ingredients	%
Corn	55,929
Soybeanmeal	36,694
Soya oil	3,301
Limestone	1,118
Dicalcium phosphate	1,959
Mineral premix ^{1/}	0,050
Vitamin premix ^{2/}	0,100
Salt	0,389
BHT	0,010
Starch com	0,350
Choline chloride (60%)	0,100
Total(%)	100,00
-----Calculated composition-----	
Crude protein (%)	21,50
Metabolizable Energy(kcal/kg)	3050
Calcium)	1,000
Available Phosphorus (%)	0,450
Methionine (%)	0,334
Methionine+cystine (%)	0,688
Lysine (%)	1,182
Threonine (%)	0,844
Triptophane (%)	0,291
Arginine (%)	1,454
Fenilalanine (%)	1,092
Valine (%)	1,002
Glicine+serine (%)	2,140
Isoleucine (%)	0,979
Leucine (%)	1,973

1/ - Iron, 80 g, cooper, 10 g, Co, 2 g, Mn, 60 g, Zn, 50 g, I, 1 g e excipients qsp - 500 g.

2/ - vit. A - 15, 000,000 UI, vit. D3 - 1, 500,000 UI, vit. E - 15,000 UI, vit. B1 - 2.0 g, vit. B2 - 4.0 g, vit. B6 - 3.0 g, vit. B12 - 0.015 g, nicotinic acid - 25 g, pantothenic acid - 10 g, vit. K3 - 3.0 g, folic acid - 1.0 g, Zn bacitracin - 10 g, selenium - 250 mg, antioxidant BHT - 10 g and excipients qsp - 1,000.

Procedure: The diet supply was offered in the period from 0 to 5 weeks of age according to the Cobb® Manual recommendations. In the end of the fifth week of age the body weight, feed conversion, and the chemical composition of the carcass of the birds were evaluated. It was used a bird of each experimental unit to determine the chemical composition of the carcass being selected the one with an equivalent average body weight of the whole experimental unit considering a deviation of ± 10 g. The chosen birds were slaughtered, their digestive system and inner organs were removed and the carcass was ground to determine the dry mater levels, ether extract and crude protein, according to traditional methods described by Silva (1990).

From the fifth week on, the remaining birds of each treatment were raised until 24 week of age, according to the management and feeding recommendations made by the Cobb® Manual, time in which a bird of each experimental unit was slaughtered to evaluate the chemical composition of the carcass in the beginning of the period of egg production. In the period from 24 to 40 weeks of age it took place the evaluation of the treatment effects performed in the initial phase about the beginning of egg production (production at least of one egg/pen during three days in a row), percentage of egg production, egg

weight, eclosion of fertile eggs and, initial body weight of the chicks that were born. The standard feeding for this period followed the recommendations of the trademark Manual.

A male was introduced in each experimental unit at 21 week of age and it was there until the end of the experiment, being fed in separate feeders without access to the feeders of the females.

The eggs' weight, hatchability percentage and the initial quality of the chicks were evaluated at 32 and 40 weeks of age.

Experimental design: The experimental design used was randomized in blocks, four sections of a house where the treatments consisted of six levels of Met+Cys with 12 repetitions and eight birds for experimental unit. The variation analyses were carried out according to the following model:

$$Y_{ijk} = u + B_i + M_j + ijk$$

where,

Y_{ijk} = observed production in experimental unit k of Met+Cys j level in the block i (i=1, 2, 3 and 4);

u= Average

B_i = effect of block i;

M_j = Met+Cys j level effect (j = 0,688, 0,748, 0,808, 0,928 and 0,988),

and,

e_{ijk} = error of each experimental unit.

The statistic analyses of the characteristics were carried through in accordance with the System for Statistic Analysis (SAEG) program, developed by the Federal University of Viçosa (UFV, 2000), and the calculation of the Met+Cys requirements were established by regression models and linear discontinue model Linear Response Plateau (LRP) (Braga, 1983).

Results and Discussions

The performance results during the period from 0 to 5 weeks of age are presented in Table 2. The birds live body weight obtained at 35 days of age was significantly influenced by the Met+Cys levels of the ration ($P < 0,001$). In works with broilers in the initial phase, Mitchell & Robbins (1984), Huyghebaert & Pack (1994), Albino et al. (1995) and Silva et al. (1997) also had observed significant effect in the levels of Met+Cys in this characteristic. Considering the body weight of 620 g indicated as standard for this lineage at 35 days of age (Manual of management Cobb®, 1996), the two first levels of supplementation of Met+Cys (0,688 and 0,748%) had allowed to have birds with average weight relative to the standard of 98 and 103%, respectively. The feed intake was not statistically analyzed because the supply was controlled so that all experimental units received the same amount feed/bird/day.

Table 2. Effect of different levels of Met+Cys in the diet on the performance, carcass composition and sexual maturity in broiler breeders pullets, from 0 to 5 weeks of age.

TSAA (%)	Performance 5 Wk			Carcass composition 5 Wk			Carcass composition 24 Wk			Sexual maturity days	Performance 24-40 Wk	
	FI (g)	BW (g)	FC (g/g)	% in DM			% in DM				EP %/hen/d	FIP g/hen/d
				DM ¹ %	EE	CP	DM ² %	EE	CP			
0,688	1302	607,5	2,29	31,9	34,0	53,9	30,6	33,2	51,9	177,5	51,7	157,1
0,748	1302	640,5	2,17	29,0	30,6	53,9	29,1	31,2	53,8	181,4	55,3	157,5
0,808	1302	664,1	2,09	29,1	26,6	56,5	28,0	29,0	56,5	181,2	53,0	157,9
0,868	1302	676,0	2,05	29,5	27,6	54,8	28,1	29,6	55,3	181,8	48,5	155,8
0,928	1302	683,3	2,02	27,9	25,2	57,9	28,6	29,3	55,6	184,0	49,5	157,0
0,988	1302	686,9	2,01	28,9	27,5	57,14	29,02	29,6	55,2	185,8	46,3	155,5
							ns	ns	ns	ns	**	ns
TSAA effect ³	--	***Q	***Q	***Q	***Q	*L						
CV %	--	1,34	1,37	5,3	16,3	7,2	9,3	23,7	11,3	4,7	10,3	1,6

1/*** ($P \leq 0,001$), ** ($P \leq 0,01$), * ($P \leq 0,05$) and ns ($P > 0,05$) by F test. Quadratic model (Q), Linear model (L). Coefficient of Variation (CV %). ² Dry matter (DM), crude protein (CP) and ether extract (EE). ³ Level of Met+cys (TSAA), Feed intake (FI), Live body weight (BW), Feed conversion (FC), Age to first Egg (AFE), Egg production (EP), Feed Intake Production (FIP).

The calculated feed conversion was significantly influenced by the levels of Met+Cys of the diet ($P < 0,001$). Considering that the amount of ration offered was identical for all the experimental units, the response obtained in this characteristic was not only caused by the variations in the weight gain among treatments.

Regarding the calculated data for body weight and feed conversion based on adjusted equations through polynomial regression and Linear Response Plateau (LRP) models, the Met+Cys requirements for broiler breeder hens from 0 to 5 weeks of age are presented in Table 3.

Table 3. Nutritional requirement in Met+Cys for broiler breeders hens from 0 to 5 weeks of age, considering the body weight and the feed conversion, Carcass Dry matter, ether extract and crude protein, adjusted by regression models.

Model	Regression Equation	Inflection point	Requirements Met+Cys (%)	R ²	SSD
<i>Quadratic</i>					
BW (g)	$Y = -291,11 + 2063,22X - 1089,11X^2$	686,03	0,947	0,99**	15,11
FC (g/g)	$Y = 5,61 - 7,58X + 3,99X^2$	2,01	0,950	0,99**	0,0002
DM (%)	$Y = 81,84 - 18,22X + 65,44X^2$	28,45	0,903	0,73***	2,63
EE (% in DM)	$Y = 169,53 - 17,96X + 176,14X^2$	26,04	0,903	0,92**	3,99
EP (%/bird/d)	$Y = -2,30 + 152,52X - 104,91X^2$	53,13	0,723	0,74**	13,65
<i>Linear</i>					
CP (% in DM)	$Y = 45,26 + 12,48X$	---	≥0,988	0,66**	4,97
<i>Linear Response Plateau</i>	The slope Equation	Maximum Point	Requirement Met+Cys (%)	R ²	SSD
BW (g)	$Y = 476,61 + 284,64X$	681,96	0,834	0,99	76,19
FC (g/g)	$Y = 3,47 - 1,73X$	2,03	0,837	0,99	0,0009
DM (%)	$Y = 66,06 - 49,53X$	28,88	0,751	0,92	1,23
EE (% in DM)	$Y = 73,10 - 56,74X$	26,75	0,817	0,98	3,86
EP (%/bird/d)	$Y = 45,05 + 11,09X$	48,11	0,280	0,13	11,22

The requirements in Met+Cys were estimated in 0,947% and 0,950%, after being adjusted using the quadratic regression model, considering the results of body weight and feed conversion, respectively. The requirements in Met+Cys were estimated in 0,834% for body weight and 0,837% for feed conversion, respectively, when adjusted by LRP model.

The chemical composition of the carcass obtained from slaughtered birds with 5 weeks of age was influenced by the Met+Cys levels of the rations (table 2). There was a quadratic effect ($P < 0,001$) of Met+Cys levels regarding dry matter (MS) and ether extract (EE), being the level for crude protein (PB) linearly influenced ($P < 0,01$).

Calculated Met+Cys requirements based on the results from the carcass chemical composition, are presented in Table 3. The Met+Cys requirement based on dry matter and ether extract was estimated in 0,903% for both characteristics when adjusted through the quadratic model ($P < 0,001$). Even though the significant linear effect of Met+Cys levels were observed on these characteristics, the adjustment provided lower coefficients of determination and a higher sum of squares of the deviation (SSD) when it was compared to the adjustment by the quadratic effect. When the adjustment was made by using the LRP model, the Met+Cys requirement was calculated in 0,751 and 0,817% for dry matter and ether extract, respectively.

Keeping in mind that the significant effect of Met+Cys levels above the CP level of the carcass were linear ($P < 0,01$), the estimated value of the nutritional requirement based on this characteristic can be higher or equal to the last value of the level studied, that means 0,988%. This trend was also observed when the adjustment was made by LRP

model, which resulted in a plateau (1,08%) with a higher value than the biggest level studied before, being the effect inside of the studied levels completely linear.

The chemical composition of the carcass from slaughtered birds with 24 weeks of age submitted to the different levels of Met+Cys from 0 to 5 weeks of age is presented in [Table 2](#). The levels of Met+Cys used in the initial phase did not influence significantly the chemical composition of the carcass at this period. Two factors could have contributed to make it happen. On one side, the birds were raised according to the constant feeding standards from the manual for the lineage, below the weight and exposed to a higher restriction for those birds above of the standard body weight, willing to reach the curve of growth during the growth phase and to reach the sexual maturity with the biggest possible uniformity. On the other side, the analyzed characteristics had presented values of coefficient of variation (CV %) higher than those gotten in the analyses carried out with data of the phase from 0 to 5 weeks. Therefore, it can be observed that numerically, the two first levels of supplementation presented higher values of ether extract and lower values of crude protein than the other with higher level of Met+Cys; relative differences that have been considered statistically different ($P < 0,05$) in the phase from 0 to 5 weeks of age.

The effect of different Met+Cys levels of the diet in the phase from 0 to 5 weeks of age in the beginning of egg production, feed consumption and percent of egg production of broiler breeder hens in the period from 24 to 40 weeks of age are presented in [Table 2](#). The age for the first egg deposition was not significantly influenced ($P > 0,05$) by the levels of Met+Cys used in the phases from 0 to 5 weeks, being the obtained average age at the beginning of egg production equals to 182 days.

From the characteristics analyzed in the period from 24 to 40 weeks, only the index of egg production was influenced by the levels of Met+Cys, with exception of the hatchability at the 40 week, being the only one characteristic from this phase used to calculate the nutritional requirement ([table 2](#)). The levels of Met+Cys linearly influenced the egg production index in the period from 24 to 40 weeks ($P < 0,001$) presenting a decrease in the index of egg production when the Met+Cys level had increased which is therefore, a minimum requirement estimated 0,688 %. The LRP model did not provide a proper fit on his feature, because the equation with the lowest sum of squares of the deviation (SSD) originated an interception of the corresponding Met+Cys level below the minimum studied interval ([table 3](#)).

The feed intake (food/bird/day) during the production phase was not significantly influenced by the Met+Cys levels used from 0 to 5 weeks of age ($P > 0,5$) although that had been offered in function of the percentage production which differs among treatments. That indicates a better utilization of the diet by the birds that had a higher percentage of egg production.

The egg weight and the results of the incubations obtained in the 32 and 40 weeks are presented in Table 4. The average egg weight acquired in both the 32d and 40th weeks was not significantly influenced ($P > 0,05$) by the levels of Met+Cys. These results agree with Ambrozini (1991), Summers & Leeson (1985) and Lilburn et al. (1987), whose different methods of feeding and alimentary restriction were used and obtained similar findings to this study.

Table 4. Effect of dietary Met+Cys levels from 0 the 5 weeks of age on the egg weight, chick body weight, relation chick body weight/egg weight and hatchability from the 32 to 40 week of age.

Level	Weight egg (g)		Hatchability (%)		Chick weight (g)		Chick weight/egg weight (%)	
	32 wk	40 wk	32 wk	40 wk	32 wk	40 wk	32 wk	40 wk
TSAA (%)								
0,688	61,25	66,06	91,92	94,49	43,18	48,36	70,35	73,31
0,748	61,24	66,01	92,74	94,77	43,41	47,12	70,88	71,45
0,808	61,57	66,10	94,73	94,54	43,10	47,07	70,01	71,21
0,868	60,12	65,78	93,61	93,39	42,64	46,83	70,90	71,32
0,928	60,55	65,58	89,31	87,43	42,424	46,78	70,01	71,31
0,988	60,94	64,81	94,02	95,34	42,76	46,97	70,15	72,21
TSAA Effect ¹	ns	ns	ns	*	ns	ns	Ns	ns
CV(%)	3,06	2,76	5,88	6,04	4,26	4,24	2,09	2,63

1.* ($P \leq 0,05$) and ns ($P > 0,05$) by F test.

The hatchability of fertile eggs incubated in the 32 week was not influenced by the levels of Met+Cys ($P > 0,05$). However, in the 40 week the hatchability of fertile eggs from birds that had received diets with 0,928% of Met+Cys in the initial phase was significantly lower when compared with the others levels ($P < 0,05$).

The body weight of the chick at birth and the relation between chick body weight/egg weight resulting at 32 and 40 weeks of age were not meaningfully influenced by the treatments.

The chemical composition of the carcass results are in accordance with the results of Pfaff (1977), cited by Cabel et al. (1988), who observed the association among diets without or with small DL-methionine supplementation and the increased activity of lipogenic (new lipids production) enzymes and the rate of liver lipogenesis, what can partially explain the decrease in the ether extract content in the carcass as well as the increase of Met+Cys levels of the diet.

Generally, in the determination of nutritional requirements for birds, the calculated values to improve feed conversion are always higher in relation to those that are necessary to maximize the weight gain, independently from the model used for the adjustment (Almquist, 1952; Silva et al., 1984; Schutte & Pack, 1995; Albino et al., 1995). This seems to agree with the research made by Silva et al. (1997), who obtained Met+Cys calculated levels about the feed intake.

Ambrozini (1991) observed that the sexual maturity age was at 172 days, which corresponded to the appearance of the first egg after testing different levels of energy and Met+Cys in the growing phase. Varying values were found by Classen (1979) in the period from 172 to 188 days, while Stringhini (1990) observed that the average age in the beginning of the egg production were 181,5 days.

Powell et al. (1976), Wilson et al. (1983) did not verify statistical differences either ($P>0,05$) in the hatchability of fertile eggs from broiler breeders hens, submitted to different systems and methods of feeding in the growth and production phases which matches up with the results gotten in the 32 week, without the existence of an apparent biological justification for the isolated effect observed in the 40 week.

The calculated values of the requirements in Met+Cys were always lower when they were adjusted by the LRP model compared to the results obtained with the quadratic model for all characteristics evaluated. These results are similar to the ones found by Couto et al. (1990), Lima (1995), Silva et al. (1997) in different works determining nutritional requirements.

The calculated requirements obtained from the corporal weight basis, feed conversion, fat, and protein in the carcass differ from those obtained from the basis of the egg production. Differently from the experimentation with broilers whose goal is to maximize the weight gain with the best possible carcass composition, in the experimentation with broiler breeder hens a balanced product is chased presenting an adequate body weight that makes possible to maximize the production of chicks, more number of a-day-chicks.

Although, the assessments achieved from the basis of the body weight, feed conversion and content of lipids and protein in the carcass were statistically significant with values of determined coefficients that demonstrate a good statistical adjustment which were not adequate because these did not support the main objective of the broiler breeder hens production to maximize the egg and chicks number.

Probably, better results can be found in the phase of production during the period from 0 to 5 weeks with birds that reached proper weight and corporal composition, meaning that those are not necessary birds with higher body weight, higher protein deposition and lower fat deposition in the carcass, considering the results of body weight at 5 weeks of age and egg production obtained in this experiment and the standard body weight recommended by Manual Cobb® 500.

It's important to consider that the birds must present a body weight as close as possible to the ideal, or standard, for a given age. In this study this was achieved with the second level of supplementation (103% relatively to standard 100) corresponding to the highest

egg production index in the period from 24 to 40 weeks, not so statistically different from the first and the third levels of supplementation.

Conclusion

In conclusion the recommendation in total Met+Cys in diets with 3050 kcal of EM/kg can be suggested for broiler breeder hens from 0 to 5 weeks of age equals to 0,75%, what corresponded to 0,66% of digestible Met+Cys or 0,216% of digestible Met+Cys/Mcal of ME.

This recommendation is lower than those mentioned by Leeson (1996) and the Manual of Management Cobb® (1996) which suggests levels of digestible Met+Cys of 0,237% and 0,265% Mcal of ME in the diet, respectively. However, it is very close to the recommendation made by Rostagno et al. (2000) that suggest a minimum level of digestible Met+Cys of 0,203% Mcal of ME.

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