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Effect of different metabolizable energy and soybean oil levels in the diet of laying hens on the egg chemical composition and lipid profile

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ABSTRACT - This study was carried out to evaluate the effects of increasing metabolizable energy and soybean oil levels on the egg chemical composition, total lipids and cholesterol contents and fatty acids profile in the egg yolk. Three hundred and sixty 29 week-old Bovans Goldline semi-heavy commercial layers were used during three periods of 28 days. A completely randomized design were used in a 3 × 3 double factorial arrangement with three soybean oil levels (1, 2 and 3%) and three metabolizable energy levels in the diet (2,600, 2,750 and 2,900 kcal/kg), totalizing nine diets with five replicates of eight birds. No interaction soybean oil × metabolizable energy levels was observed. The metabolizable energy levels did not affect any of the determined characteristics. The moisture, ash and protein contents in the egg yolk and albumen and the lipid and cholesterol contents in the yolk were not affected by the soybean oil levels in the diet, while linoleic acid (C18:3) and linolenic acid (C18:2) levels responded linearly. The use of 2,600 kcal ME/kg and 3% of soybean oil in the diet of laying hens with consumption of 120 g/bird/day is justifiable, since this feeding strategy improves the fatty acids profile of the egg.

Key Words: cholesterol, egg composition, fatty acids

Efeito de diferentes níveis de energia metabolizável e óleo de soja na dieta de poedeiras sobre a composição química e o perfil lipídico do ovo

RESUMO - Este estudo foi conduzido com o objetivo de avaliar os efeitos de níveis crescentes de energia metabolizável e de óleo de soja sobre a composição bromatológica do ovo, o teor de lipídios totais e de colesterol e o perfil de ácidos graxos na gema. Foram utilizadas 360 poedeiras comerciais da linhagem Bovans Goldline, com 29 semanas de idade, durante três períodos de 28 dias. O delineamento utilizado foi o inteiramente casualizado, em arranjo fatorial duplo 3 × 3, com três níveis de óleo de soja (1, 2 e 3%) e três de energia metabolizável na ração (2.600, 2.750 e 2.900 kcal/kg), totalizando nove dietas com cinco repetições de oito aves. Não foi observada interação níveis de óleo de soja × energia metabolizável. Os níveis de energia metabolizável não influenciaram nenhum dos parâmetros avaliados. Os teores de umidade, cinzas e proteína na gema e na clara e os teores de lipídio e colesterol na gema não foram afetados pelos níveis de óleo de soja da dieta, enquanto os teores dos ácidos linoléico (C18:3) e linolênico (C18:2) responderam de forma linear. Justifica-se a utilização de 2,600 kcal EM/kg e de 3% de óleo de soja em dietas para poedeiras com consumo de 120 g/ave/dia, uma vez que essa estratégia de alimentação melhora o perfil de ácidos graxos do ovo.

Palavras-chave: ácidos graxos, colesterol, composição do ovo

Introduction

Researches have been developed in recent years aimed at improving productivity through the use of nutrients that can keep the quality of products and even enrich them with components beneficial to human health. The quality of fats ingested is defined by the relationship between saturated and unsaturated fatty acids. The higher this ratio is, the greater the amount of unsaturated fatty acids will be and the more their consumption will be advisable. Mono and polyunsaturated fats do not increase the cholesterol level in the blood and are linked to lower risks of cardiovascular diseases (Van Elswik, 1997). Therefore, the use of vegetable oils (rich in unsaturated

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fatty acids) is recommended to replace saturated fats in the diet.

Egg is a food of high nutritional value, estimated at 96% its biological value. It is very rich in essential amino acids; egg yolk is rich in lipids (about 33% of its chemical composition), which is its main nutritional component, providing important energy source in the human diet, high digestibility and low price. Its cholesterol content and contradictory information make its consumption by the population to be reduced (Fennema, 1996). These lipids are used as source of energy for the embryo, being the reason of unsuccessful attempts in reducing its cholesterol level (Van Elswik, 1997).

The laying hen modifies the egg lipid profile in function of the source of fatty acids administered in the diet, being an important food consumed by most of the population. On the other hand, there is a large part of the population that is malnourished, for which the egg could become important component of the diet, given its low cost and excellent nutritional value (Latour et al., 2000).

Thus, eggs with higher polyunsaturated fatty acids content would represent a clear alternative to the consumption of fish. Fish oils, rich in such acids, have high price, are rather of little pleasant ingestion, and in many countries, are scarce and expensive, or do not make part of the population diet (Briz, 1997).

Therefore, this study was carried out to evaluate the effects of different metabolizable energy and soybean oil levels in the diet of semi-heavy laying hens on the egg chemical composition, total lipids and cholesterol contents and fatty acids profile in the egg yolk.

Material and Methods

The experiment was conducted at the Poultry Sector, Department of Animal Sciences - Federal University of Paraiba in the city of Areia, state of Paraiba. The city has the following coordinates: 6°58'554" S and 35°43'047" W and altitude of 618 m above sea level. The maximum, minimum and average temperature values, relative humidity and rainfall were recorded during the trial period, respectively, 27.4, 21.4 and 23.6°C, 86% and 138.4 mm.

Three hundred and sixty 29 week-old Bovans Goldline semi-heavy commercial layers were used during three periods of 28 days. A completely randomized design, in 3 × 3 double factorial arrangement with three soybean oil levels (1, 2 and 3%) and three metabolizable energy levels in the diet (2,600, 2,750 and 2,900 kcal/kg), totalizing nine diets with five replicates of eight birds.

The corn and soybean meal-based diets were formulated following the recommendations of Rostagno et al. (2000), except for the energy levels used.

The hens were housed in conventional laying shed, with gutter-type feeders and nipple-type water fountain. The birds were submitted a period of adaptation to diets assessed for seven days, starting the experiment immediately after. The experiment was divided into 3 periods of 28 days each.

The light program adopted was natural light, water was provided *ad libitum* and the experimental diets were provided in the amount of 120g/bird/day.

At the end of each of the three experimental periods, the eggs were conducted to the Bromatology Laboratory from the Department of Nutrition - Campus I, UFPB, where the chemical determinations were processed. Three eggs per type of diet were mixed to obtain a composite sample (pool).

In the laboratory, protein (Micro Kjedahl method, with factor 6.38 being multiplied by the nitrogen percentage found), mineral matter (oven-dried at 550ºC, AOAC, 2000), total and defatted dry extract (AOAC, 2000) and cholesterol levels (according to Bragagnolo & Rodriguez-Amaya, 1992) were assessed. To determine the moisture content in oven at 105°C for 18 hours, the samples were weighed and pre-dried in forced ventilation oven at 60 ± 5°C for 48 hours, according to the Analytical Standards of the Adolfo Lutz Institute (1985).

To identify the fatty acids profile, aliquots of 0.5 grams of fat were removed for the performance of the methyl esters preparation process, being followed by method described by Hartman & Lago (1973).

The transmethylated samples were analyzed in gas chromatograph model CG Master, with flame ionization detector, capillary column (DB-WAX), polyethylene glycol stationary phase (Carbowax 20M), with 30 m of length by 0.53 mm of internal diameter and 0.25 mm of film thickness. Hydrogen was used as drag gas at a flow rate of 5 mL/minute, nitrogen (30 mL/minute) and hydrogen (30 mL/minute) (detector) and synthetic air (300 mL/minute). The initial oven temperature program was 60°C, initial time of 5 minutes until the 1st ramp with graduation of 7ºC/min. up to 110ºC (remaining for 5 minutes), 2nd ramp with graduation of 4ºC/minutes to reach the final temperature of 19ºC, totaling 37 minutes.

An aliquot of l ì L of the esterified extract was injected into the chromatograph and the fatty acids identification was done by comparing the retention times and the percentages of fatty acids that were obtained through the Peaksimple software (SRI Instruments - USA).

Table 1 - Composition of the experimental diets[1]

Item	Soybean oil levels (%)								
	1			2			3		
	Metabolizable energy levels (kcal/kg)								
	2.600	2.750	2.900	2.600	2.750	2.900	2.600	2.750	2.900
Corn	58.988	64.009	68.543	56.047	61.068	66.091	53.106	58.127	63.148
Soybean meal (46%)	21.400	20.462	17.229	21.943	21.016	20.061	22.486	21.559	20.627
Corn gluten meal (60%)	0.000	0.000	1.748	0.000	0.000	0.000	0.000	0.000	0.000
Soybean oil	1.000	1.000	1.000	2.000	2.000	2.000	3.000	3.000	3.000
Limestone	9.361	9.373	9.395	9.354	9.366	9.377	9.347	9.359	9.371
Dicalcium phosphate	1.287	1.275	1.274	1.294	1.282	1.270	1.302	1.289	1.277
Salt	0.423	0.421	0.423	0.424	0.422	0.420	0.425	0.423	0.421
Mineral mix[2]	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Vitamin mix[3]	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
L-Lysine HCL	0.000	0.006	0.063	0.000	0.000	0.013	0.000	0.000	0.003
DL-Methionine	0.172	0.165	0.160	0.176	0.169	0.162	0.180	0.173	0.166
Choline chloride (60%)	0.070	0.070	0.070	0.070	0.070	0.070	0.070	0.070	0.070
Etoxiquim[4]	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Inert (sand)	7.214	3.135	0.010	8.607	4.522	0.449	9.999	5.915	1.832
Calculated composition									
Metabolizable energy (kcal/kg)	2,600	2,750	2,900	2,600	2,750	2,900	2,600	2,750	2,900
Protein (%)	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000
Calcium (%)	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000
Available phosphorus (%)	0.326	0.326	0.326	0.326	0.326	0.326	0.326	0.326	0.326
Digestible lysine (%)	0.661	0.652	0.640	0.669	0.656	0.652	0.677	0.663	0.652
Digestible methionine (%)	0.387	0.383	0.390	0.390	0.386	0.381	0.393	0.388	0.384
Digestible Met + Cist (%)	0.600	0.599	0.613	0.600	0.599	0.599	0.601	0.600	0.599
Digestible arginine	0.887	0.876	0.823	0.894	0.882	0.871	0.900	0.889	0.878
Digestible threonine (%)	0.498	0.497	0.492	0.498	0.497	0.496	0.499	0.498	0.497
Chloride (%)	0.284	0.285	0.289	0.283	0.284	0.285	0.282	0.284	0.285
Potassium (%)	0.571	0.569	0.526	0.572	0.570	0.567	0.573	0.571	0.569
Sodium (%)	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200

[1] Calculated values according to Rostagno et al. (2000).
[2] Composition/kg: Mn - 160 g; Fe - 100 g; Zn - 100 g; Cu - 20 g; Co - 2 g; I - 2 g; Excipient q.s.p. - 1.000 g. [3] 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 - 5.000 mg; Pantothenic acid - 12.000 mg; Biotin - 200 mg; vit. K - 3.000 mg; Folic acid - 1.500 mg; Nicotinic acid - 40.000 mg; vit. B_{12} - 20.000 mg; Se - 150 mg; Excipient q.s.p. - 1.000 g.
[4] Antioxidant.

Additional tests using a series of standard alkanes ranging from C6 to C19 under identical analytical conditions were performed to verify the linear retention indexes of fatty acids and to verify the positive identity of the interest components by comparing the results of the samples with the retention rates, using the calculation formula for the Linear Retention Index. The fatty acids were quantified by normalization of areas of methyl esters. The fatty acids results were expressed as percentage of area (%).

Data were compiled into spreadsheets and submitted to analysis of variance and regression, using the SAS program (2000), with a significance level of 5%. Data referring to the fatty acids levels were transformed into X+1 logarithms.

Results and Discussion

According to the analysis of variance, there was no soybean oil levels × metabolizable energy levels interaction (Table 2), which shows that one factor did not interfere in another, so their effects were studied separately. No differences were observed in the moisture, ash and protein contents in the egg yolk and albumen or in the lipids and cholesterol levels in the egg yolk in relation to the soybean oil and metabolizable energy levels evaluated.

The average cholesterol content (12.22 mg/g egg yolk) observed in this study was similar to that observed by Bragagnolo et al. (2003), who assessed chicken and quail eggs collected from various supermarkets in the state of Sao Paulo and obtained an average content of 12.3 mg of cholesterol per gram of yolk from brown-shell eggs and average weight of 67 g. The absence of significant differences in the cholesterol content is related to the physiological mechanism of egg production, in which cholesterol is added to the yolk to meet the survival requirements of the embryo, being the reason of unsuccessful attempts in reducing its cholesterol levels. Laying hens transfer blood cholesterol for the yolk development and this is the main cholesterol excretion mechanism, followed by excretion through bile.

Table 2 - Content of moisture, ash and protein in albumen and yolk, lipids in yolk and cholesterol in yolk and egg of laying hens according to the dietary soybean oil and metabolizable energy levels

Variable	Metabolizable energy level (kcal/kg)				Soybean oil level (%)				Mean	CV %
	2,600	2,750	2,900	Effect	1	2	3	Effect		
Albumen										
Moisture (%)	49.03	49.05	49.35	ns	48.91	48.98	49.54	ns	49.14 ± 0.91	1.85
Ash (%)	1.66	1.70	1.62	ns	1.65	1.66	1.67	ns	1.66 ± 0.11	6.83
Protein (%)	15.75	15.65	15.37	ns	15.68	15.74	15.36	ns	15.59 ± 0.74	4.75
Yolk										
Moisture (%)	87.22	87.54	87.59	ns	87.42	87.63	87.30	ns	87.45 ± 0.54	0.61
Ash (%)	0.50	0.51	0.48	ns	0.49	0.50	0.50	ns	0.50 ± 0.16	3.69
Protein (%)	10.79	10.41	10.39	ns	11.15	10.20	10.23	ns	10.53 ± 1.08	10.26
Lipids (%)	32.57	33.60	32.93	ns	32.97	33.23	32.89	ns	33.03 ± 1.20	3.62
Cholesterol[1]										
Yolk[1] (mg/g)	11.60	12.53	12.52	ns	12.50	12.21	11.94	ns	12.22 ± 1.23	10.05
Egg[2] (mg/g)	2.90	3.13	3.13	ns	3.13	3.05	2.99	ns	3.05 ± 0.31	10.05
Egg[2] (mg)	197.28	213.04	212.82	ns	212.51	207.59	203.03	ns	207.71 ± 20.88	10.05

[1]Average yolk weight: 17 g; [2]Average egg weight: 68 g; CV - coefficient of variation; ns - not significant.

The results obtained in this study corroborate those from Milinsk (2003), who reported that many studies trying to reduce the cholesterol level in the egg yolk have been unsuccessfully conducted. Although the cholesterol level is really high, the nutritional quality of fat should be assessed not only in relation to its cholesterol level, but also in relation to the saturated fat, monounsaturated fatty acids and polyunsaturated fatty acids contents. High monounsaturated and polyunsaturated fatty acids levels associated with low saturated fat content may reduce the negative effect of the high cholesterol, and reduce the incidence of cardiovascular diseases.

The metabolizable energy levels had no effect on any of the fatty acids identified in the egg yolk (Table 3). The soybean oil levels, however, had linear effect on the linoleic acid (C18:3) and linolenic acid (C18:2) levels. As the soybean oil level increased in the diet, an increase in the linoleic and linolenic acids levels was observed in the egg yolk, which were higher (21.81 and 1.40% respectively) in the highest soybean oil level.

The saturated and monounsaturated fatty acids levels were not influenced by the energy and soybean oil levels used. The mean content of saturated fatty acids obtained in this study (36.21%) was in agreement with those obtained by Simopoulos (2000) and Li-Chan et al. (1995), who reported that the saturated fatty acids content normally ranges from 30 to 38% of the total fatty acids in the egg yolk, depending on the type of fat added in the diet of laying hens, and that the concentrations of these fatty acids in egg, especially palmitic and stearic acids, are very stable and influenced by the polyunsaturated fatty acids content in diet.

The polyunsaturated fatty acids levels and polyunsaturated:saturated and linoleic:linolenic ratios differed with the soybean oil levels evaluated. The highest polyunsaturated and polyunsaturated: saturated levels and the lowest linoleic:linolenic ratios were observed for the highest soybean oil level. The improvement in these ratios in the egg yolk increases the nutritional qualities of eggs for human nutrition.

Filard et al. (2005) evaluated the saturated fatty acids, monounsaturated, polyunsaturated, polyunsaturated: saturated and linoleic:linolenic levels on diets with the addition of 2.5% (on average) of different fat sources, including soybean oil, 16 % of crude protein and 2,950 kcal ME/ kg in the diet of second laying cycle ISA Brown hens. The palmitic (23.54%), palmitoleic (1.99%), stearic (8.62%), oleic (38.74%), linoleic (19.31%) and linolenic acid (0.62%) levels reported by Filard et al. (2005), which are in brackets, are lower than those obtained in this research.

The highest effect on the lipids composition of the yolk are related to changes in the fatty acids composition in diet, allowing a partial modification of the yolk lipid profile through qualitative selection of fat in diet.

According to works cited by Warnants et al. (2001), linoleic acid is incorporated into the egg quite easily, presenting incorporation potential higher than that of the linolenic acid. In this work, however, the increase in the linolenic acid content (ω-3) was higher, proportionately, to that occurred in the linoleic acid content (ω-6).

In the 60s and 70s, Sell et al. (1968) and Sim et al. (1973) demonstrated that the inclusion of soybean oil in the diet of laying hens had the power to change the fatty acids composition of the egg yolk. Similar results were obtained by Shafey et al. (1992), who reported that supplementation of diet with soybean oil increased the unsaturated fatty

Table 3 - Profile of saturated fatty acids in the egg yolk of laying hens according to the dietary soybean oil and metabolizable energy levels

Variable (g/day)	Metabolizable energy level				Soybean oil level (%)						
	2,600	2,750	2,900	Effect	1	2	3	Effect	R^2	Mean	CV %
Saturated											
Palmitic	27.79	26.16	27.67	ns	27.98	27.09	26.54	ns	-	27.21 ± 2.11	7.76
Stearic	8.92	8.73	9.37	ns	8.87	9.01	9.14	ns	-	9.01 ± 1.29	14.31
Total	36.72	34.88	37.04	ns	36.85	36.11	35.69	ns	-	36.21 ± 1.76	4.86
Monounsaturated											
Palmitoleic	2.69	2.52	2.36	ns	2.64	2.65	2.28	ns	-	2.52 ± 0.53	21.09
Oleic	39.29	40.28	39.71	ns	40.63	39.83	38.83	ns	-	39.76 ± 2.75	6.96
Total	41.98	42.80	42.08	ns	43.27	42.47	41.11	ns	-	42.28 ± 3.00	7.10
Poliunsaturated											
Linoleic	20.20	21.27	19.73	ns	18.93	20.46	21.81	L**	0.99	20.40 ± 2.58	12.71
Linolenic	1.10	1.05	1.16	ns	0.94	0.96	1.40	L**	0.78	1.10 ± 0.30	27.22
Total	21.30	22.32	20.89	ns	19.88	21.42	23.20	L*	0.99	21.50 ± 2.73	12.73
Linoleic:linolenic	18.97	21.88	18.28	ns	21.30	21.45	16.39	L*	0.73	19.71 ± 4.11	20.83
Poliunsatured:satured	0.58	0.64	0.57	ns	0.54	0.59	0.65	L*	0.99	0.60 ± 0.09	14.38

CV - coefficient of variation; ns - not significant; L - linear (* P<0.05; **P<0.01).

acids (oleic and linoleic acids) and saturated (palmitic and stearic acids) ratio in the egg yolk.

Conclusions

The combination of 2,600 kcal ME/kg and 3% of soybean oil in the diet of laying hens with feed intake about 120 g/bird/day could be used, since it promoted enhancements in the lipid profile, resulting in egg with better nutritional quality.

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