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CHARACTERIZATION OF THE METABOLIC RESPONSE OF LOHMANN BROWN LAYERS TO FEEDING DIETS WITH DIFFERENT PROTEIN LEVELS

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Abstract

40-week-old Lohman Brown layers were used in this study. Three diets with different levels of crude protein allowed the consumption of 15.38, 13.58 and 11.78 g/d/cap., respectively. The experimental feeding lasted four weeks. Laboratory determinations were performed at the end of the experimental period. Triglycerides, total cholesterol, GOT, GPT and γ-GT transaminases, HDL-cholesterol, protein, albumin, γ-globulins and uric acid were determined in the blood serum. Nitrogen intake, nitrogen excretion in manure, nitrogen retention in the body and in the egg were also determined. Thus, following four weeks of experimental feeding, serum transaminases and HDL-cholesterol remained within normal limits. Triclycerides and uric acid presented an increase of their serum values. As a consequence of the decrease in daily nitrogen intake, nitrogen excretion and nitrogen retention as total in the body decreased while nitrogen excretion as total in the egg remained unmodified.

Key words*: Lohmann Brown layers, diet protein level, metabolic response.*

INTRODUCTION

Housing and feeding are among the multitude of factors that influence the productivity of laying hens in general, the Lohmann Brown tetra-line laying hybrid in particular (Koreleski & Świątkiewicz, 2009; Wang et al., 2014). As early as 1972, Hurwitz and Bornstein started research on the protein requirements of laying birds establishing the basic benchmarks for calculating the protein requirements of laying hens. Later, Wang et al. (2014) searched the effects of protein source and nutrient density in the diets of broiler breeders on their subsequent growth, blood constituents, and carcass compositions. In Romania, Marmandiu et al. (2020) published a recent study on the influence of rearing system on laying in Lohmann Brown. Usturoi et al. (2022) studied the adaptability of the same Lohmannn Brown hybrid in different production systems. The two most important components of a diet in poultry are energy and protein. Nutritional requirements for crude protein and related amino acids are provided by NRC 1994.

Dietary protein is important in its level and origin. These two properties of dietary protein exert energetic influence but also influence digestibility, absorption, its use in intermediate metabolism, and excretion. The sources of protein used in the diet of birds are relatively numerous: from plant-based proteins such as soybean meal, cotton seed meal, alfalfa meal and sunflower meal, to animal-based protein such as fish-meal and poultry products. Numerous studies try to establish the optimal levels of protein in the diet, depending on the age and physiological state of the birds (Macelline et al., 2021). The metabolic influences of these protein sources are different. This is because some proteins are absorbed faster, others slower, some more and some less. The most preferred source of protein used in poultry diet formulation is the soybean. It contains up to 49% crude protein, depending on the removed hulls and the process of oil

extraction. The ultra-specialized commercial Lohmann Brown hybrid for egg production still presents many unknown particularities regarding nutritional physiology, especially protein requirements and intermediate protein metabolism. The present study aims to determine the effect of different dietary protein levels/sources on nitrogen balance in 40-week-old Lohmann Brown hens.

MATERIALS AND METHODS

Animals and experimental design

A total number of 120 40-week-old Lohmann brown hens devised in three equal groups were monitored in this experiment. The birds were raised in an industrial system benefiting of a program of feed restricted to 110 g/day, free access to water and a light program of 14:30 hours per day, from 5:30 to 20:00. The animals in the three groups were fed diets differing in the level of protein in the feed. The structure and chemical composition of the diets used in the experiment are presented in Table 1. Two diets were formulated: the first providing 15.38 g of protein at an average daily consumption of 110 g forage/capita and the second providing an amount of 11.78 g of protein at a consumption of 100 g of forage/capita. The combination of the two diets resulted in a third, the intermediate diet, which provided 13.58 g protein/capita.

The duration of the experimental monitoring period was from 40 to 44 weeks of age of the hens. The entire mass of faeces (mixture of faeces and urine) was collected daily, weighed and stored a -20°C until drying. Excreta samples were dried in a forced-air drying oven at 115°C for 72 h and finally ground for chemical analyses. Daily forage intake was restricted for 110 g/capita/day during the experimental period.

Analysis

Feed and excreta samples were analysed for dry matter (method 930.15; AOAC, 2005), total nitrogen and non-proteic nitrogen (after

Table 1. Ingredients and composition (%) of the diet to provide 13 g of protein, composition of the diet to provide 17 g of protein at 110 g and the composition of the intermediary diet to provide 15 g of protein at 110 g of daily feed intake each diet

Items	Control (diet before experimental feeding)	Diet 1: for 17 g of crude protein/day in 110 g of forage/day	Diet 2: Intermediary diet:15 g of crude protein/day in 110 g of forage/day	Diet 3: for 13 g of crude protein/day in 11 g of forage/day
Ingredients				
Corn meal	72.05	68.66	72.71	76.77
Soybean meal (in which, 48% crude protein)	11.80	13.43	11.87	10.32
Corn gluten meal	2.56	5.50	2.75	—
Soybean oil	2.00	2.00	2.00	2.00
Choline chloride	0.10	0.10	0.10	0.10
Limestone	8.00	8.00	8.00	8.00
Hen eggshell	0.42	0.40	0.43	0.45
Dicalcium phosphate	0.72	0.70	0.73	0.76
Salt	0.38	0.36	0.38	0.40
Premix of vitamins and minerals[1]	0.50	0.50	0.50	0.50
Nutrient contents (%, as calculated values)				
ME	2,925.1	2,867	2,928.5	2,990
Calcium	3.38	3.40	3.40	3.40
Nonphytate phosphorus	0.21	0.22	0.22	0.22
Protein	**13.2**	**15.38**	**13.58**	**11.78**
Nitrogen	2.13	2.44	2.15	1.86
Crude fiber	2.98	2.92	2.89	2.86
Crude fat	3.43	3.64	3.54	3.44
Ash	8.78	8.89	8.80	8.63

[1]Premix vitamino-mineral composition: vitamin A - 30,000 IU; vitamin D_3 - 4,500 mg; vitamin E -102 IU; vitamin K - 8 mg; vitamin B_1 - 8 mg; vitamin B_2 - 35 mg; vitamin B_6 - 22 mg; vitamin B_{12} - 0.10 mg; biotin - 0.20 mg; pantothenic acid - 86 mg; niacin, 70 mg; Fe - 150 mg; Zn- 30 mg and Se - 0.50 mg.

deproteinization by trichloroacetic acid) by Kjeldhal (AOAC, 199). Uric acid in excreta was determined by the enzymatic method of Marquardt (1983). Basically, the method consisted in the determination of the absorbance of a perchloric acid extract of the excreta measured at 285 nm versus an uric acid standard solution. Nitrogen balance including intake, excretion, and retention was calculated based on the method study proposed by Barzegar et al. (2019). Blood samples were taken from the axillary vein at the beginning and end of the experimental feeding period. Serum was obtained from the blood samples after coagulation, decantation and centrifugation at 1500 rpm. The serum samples thus obtained were used to determine serum biochemistry: total cholesterol, HDL-cholesterol, total triglycerides, proteins, total, albumin, globulins, GOT, GPT, γ-GT, inorganic nitrogen, and uric acid. Serum biochemistry determinations were performed according to the methods described by Manta et al. (1976).

Statistics

The differences between experimentally fed groups were statistically processed using a Microsoft Excel 2019 software. The differences between the experimental groups were analysed by Tukey test. The correlation of the dietary protein level evolution and the analysed items was tested by Pearson correlation coefficient. The differences between the values at the end of the experimental feeding period were also statistically compared with those at the beginning of the experimental feeding period. The differences between the groups were considered significant when the probability of the null hypothesis was below 5% ($P < 0.05$).

RESULTS AND DISCUSSIONS

Regarding the metabolic response of the Lohmann Brown adult layers to different levels of dietary protein, the general aspect of the determined parameters in the blood serum is that of their maintaining within the physiological limits. First of all, the maintenance of serum transaminases within physiological limits is noted ($P > 0.05$ for each experimental group), which reveals the maintenance of the normal functional state of the liver cell. These results are in agreement with the results reported by Heo et al. (2023) on pullets and Leghorn laying hens. A special situation is revealed by uric acid in the serum. The blood serum levels of uric acid show a decrease which is proportional with the level of dietary proteins. Uric acid is the main form of protein catabolism in birds. Its decrease with the levels of protein in diets points to a diminution of protein catabolism, with the possible decrease of the use of proteins for energy production.

Table 2. Some biochemical parameters as they are influenced by different protein levels in diet in Lohmann Brown layers following four weeks of experimental feeding

Item	Values in the week before experimental feeding (control)	Values, 4 weeks of experimental feeding			Significance as P, by Tukey test
		Diet 1	Diet 2 (intermediary)	Diet 3	
Total cholesterol, mg/dL	111±11	112±22	106±21	110± 32	0.033
Triglycerides, mg/dL	117.7±19	113.8±15	117.0*±21	214.3*±13	0.017
GOT, U/L	162±32	164±16	157±14	167±23	0.701
GPT, U/L	1.70±0.3	2.00±0.23	1.71±0.23	1.86±0.04	0.093
γ-GT, U/L	31.4±0.2	30.2±1.9	30.9±0.5	30.4±1.0	0.200
HDL cholesterol, mg/dL	78.7±2.2	83.6±3.6	77.8±10.2	72.7±2.4	0.054
Total protein, g/dL	4.04±0.5	4.10±1.0	4.06±0.8	3.97±0.9	0.111
Albumin, g/dL	2.42±0.33	2.49±0.32	2.41±0.28	2.40±0.23	0.212
Globulins, g/dL	1.66±0.32	1.61±0.09	1.60±0.05	1.52±0.32	0.432
Uric acid, mg/dL	3.14±0.43	3.26$^{\#}$±0.76	3.11±0.55	2.70$^{\#}$±0.54	0.003

Complete details for the protein content of each diet are given in Table 1

Values are given as mean ± standard error of mean

Each value represents the mean of a minimum seven samples ($n \leq 7$)

Values in the same line with the same superscript are statistically different ($P < 0.05$)

Abbreviations: GOT for glutamic oxaloacetic transaminase; GPT for glutamic pyruvic transaminase; γ–GT for gamma-glutamyl transaminase; HDL for high-density lipoprotein.

Pearson correlation coefficient was 0.30 for the evolution of uric acid level, which reveals a direct relationship of the serum uric acid and the diet protein levels. The maintaining of the HDL cholesterol levels in the blood serum reveals, together with the transaminases GOT and GPT, the maintaining of the proteo-synthetic capacity of the liver in these birds. These results are again in agreement with those reported by Heo et al. (2023) on growing pullets and on He Line brown layers. In their study on the effects of different dietary protein levels (from 18 to 12% crude protein) on performance, nitrogen excretion, and odour emission of growing pullets and laying hens, Heo et al. (2023) found that lowering of crude protein levels in the diets of the broilers did not modify significantly the main serum compounds. The idea of maintaining blood serum parameters within physiological limits under feeding conditions with different levels of dietary protein (15 and 16.5% crude protein) is also supported by Xin et al. (2022). These authors described a significant increase of triglyceride values, which is in agreement with our results on Lochmann Brown, meaning a particular shift of the energy metabolism.

Table 3 shows the results regarding the nitrogen balance of the three groups of Lohmann Brown hens fed with different levels of protein in the feed: 17, 15.5 and 13%. From the data presented in Table 3, it was found that the modification of the protein levels in the food led to the modification of the nitrogen intake as well as the daily nitrogen excretion, despite the hens consumed the same amount of forage and the three diets were iso-energetic. Daily nitrogen intake decreased by a percentage of 21% in hens fed with diet 2 and by 44.4% in hens fed with diet 3. Nitrogen excretion decreased by 35.5% in hens fed by diet 2 and 41.2% in hens fed by diet 3. Nitrogen retention as total in the body decreased by 1.8% in group fed on diet 2 and by 66% in group fed on diet 3 versus hen fed on diet 1. All the values were significantly different ($P < 0.05$) according to Tukey test. Surprisingly, the different levels of crude protein tested over a period of four weeks in adult Lohmann hens, at the top of laying, did not affect the nitrogen content of the whole egg, revealing the preferential distribution of the nitrogen by the organism in this direction, which is again in agreement with the data

Table 3. Nitrogen metabolism in Lohmann Brown layers following four weeks of experimental feeding with different protein level diets

Item	Values in the week before experimental feeding (control)	Values 4 weeks thereafter			Significance as P, by Tukey test
		Diet 1	Diet 2	Diet 3	
Daily feed intake (g/d)	110	110	100	110	ND
Daily manure (g/d, as fresh manure: faeces + urine)[a]	101±11	112.3±8	100±22	92.2±13.4	1.011
Daily nitrogen intake (g/cap./d)	3.65±0.13	3.48$^{\beta}$±0.5	2.78$^{\beta}$±0.21	1.97±0.32	0.000
Nitrogen excretion (g/cap./d)	1.20±0.0	1.89±0.18	1.22±0.05	1.11±0.11	0.505
Nitrogen retention as total in the body (g/cap./d)[b]	1.51±0.09	1.59$^{\$}$±0.22	1.56$^{\$}$±0.32	0.86±0.19	0.005
Nitrogen retention as integral egg (g/d)[c]	0.90±0.03	1.21±0.05	0.89±15	1.09±0.10	0.221
Nitrogen retention in the body (g/cap./d)[d]	0.65±0.11	0.70‡ ±0.21	0.66‡±0.5	0.31±0.04	0.001

Complete details for the protein content of each diet are given in Table 1

[a] Values in table are given as mean ± standard error of values from each week of experimental feeding

[b] Calculated as difference between nitrogen intake and nitrogen excretion

[c] Calculated according to Heo et al. (2023), from Miranda et al. (2015), based on the following equation: egg mass (g/hen/d) Ł N concentration in eggs (%), assumed N concentration in egg as 1.94%

[d] Calculated as difference between nitrogen retention as total and nitrogen retention in the egg.

Daily excreta resulted form daily collection of the faeces.

Values in the same line with the same superscript are statistically different ($P < 0.05$)

Nitrogen balance as intake and retentions was calculated according to Barzegar et al. (2019)

ND - not determined

reported by Heo et al. (2023), in He Line layers. Similar results were reported Alfonso-Avila et al. (2022) for broiler chickens, and Soares et al. (2019) for growing pullets.

According to our findings, excreta ash concentrations were decreased as the dietary crude protein concentrations decreased. According to Adeola et al. (2016), low crude protein-induced decrease of excreta ash can be attributed to a greater contribution of basal endogenous losses of minerals in low ash diet compared with that in high ash diet. Nitrogen in excreta comes from several sources: unabsorbed nitrogen from ingestion, nitrogen resulting from endogenous catabolism, eliminated via the kidneys, as well as microbial nitrogen (Soomro et al., 2018). The decrease of the excreta nitrogen level can be explained by the lower content of diet proteins, respectively, amino acids. Our results regarding nitrogen excretion in hens fed diets with different protein levels are also in agreement with those published earlier by Latshaw and Zhao (2011) who reported lower levels of nitrogen in the excreta of hens fed 17 g protein/ day (5.68% nitrogen of dry matter of manure) compared to hens fed on 13 g of protein per day (3.98% nitrogen of dry matter of manure). However, these authors did not specify nor the age, neither the breed of the chickens. Similarly, according to Heo et al. (2023), excreta output, nitrogen intake, and nitrogen excretion were linearly decreased (−0.12 Pearson correlation coefficient) as the crude protein levels decreased in the diets of the experimental layers, which was to be expected. It is reported that variations in lower limits of crude protein (16.5-18%) do not significantly change uric acid nitrogen in manure (Murakami et al., 2011).

CONCLUSIONS

The application of diets with different levels of crude protein in the feed in 40-week-old Lohmann Brown hens allowed the characterization of metabolic response of the hybrid. Thus, following four weeks of experimental feeding, serum transaminases and HDL-cholesterol remained within normal limits. The parameters that showed increased serum values were triglycerides and uric acid. As a consequence of the decrease in daily nitrogen intake, nitrogen excretion and nitrogen retention as total in the body decreased while nitrogen excretion as total in the egg remained unmodified.

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