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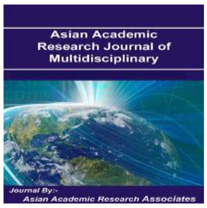

EFFECT OF L-GLUTAMINE AND ZINC SUPPLEMENTATION ON THE BIOCHEMICAL PARAMETERS OF BROILER READER UNDER THERMAL STRESS

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Abstract

The objective of this study was to evaluate the effect of the addition of L-glutamine and zinc on plasma biochemistry. Birds were distributed in a completely randomized design, in factorial scheme 2 (1 and 2% L-glutamine) and 3 (0, 90 and 120 mg of Zn / kg of feed) were used, and a control, with five replications. The animals fed with rations supplemented with 1% of L-glutamine and 120mg of zinc and with 2% of that amino acid without zinc supplementation were superior when compared to the control. In the interaction there was an increasing linear effect for zinc levels in the diets with the addition of 1% of L-glutamine. For globulin, there was a linear decreasing effect for the zinc level in diets containing 2% L-glutamine. The addition of 1% of L-glutamine without zinc supplementation presented higher value than the control treatment for the enzyme aspartate transaminase. The other parameters were not influenced by the tests nutrients. The addition of L-glutamine and zinc in diets of broiler chickens under thermal stress in the period of 1 to 21 days of age increases the plasma concentration of total proteins and does not interfere with liver and renal function.

Keywords : uric acid, creatinine, globulin, ross lineage

1. Introduction

Biochemical profiles of birds are presented in the literature for different diets, age, breeding environment, lineages and analytical methodologies employed. In addition, the values for serum enzymes differ among some authors, due also to the apparatus used for the dosages and the method of obtaining the sérum [1].These parameters can be used as indicators of the physiological state of the animals and may be useful in determining changes caused by heat stress, since high temperatures can induce different changes in the body's homeostasis by causing biochemical and metabolic changes in animals, such as production of free radicals [2].Among nutrients related to positive results so much performance parameters, development of intestinal structures, immune response and biochemical parameters of poultry and pigs, we can mention L-glutamine and zinc, both supplemented in isolation, are related to higher levels of total protein and albumin [3; 4], reduction of aspartate and alanine transaminase, improving the liver detoxification function [5], L-glutamine in particular, by acting as a metabolic regulator contributing to protein synthesis and reducing protein catabolism, if supplemented in the diet, may reduce the muscle damage caused by the action of creatine kinase [6; 7].In the absence of information on the effects of the addition of zinc-associated L-glutamine in diets of broiler chickens raised in environments exposed to high temperatures on biochemical parameters, this work was conducted with the objective of evaluating the effect of this combination on the total protein, albumin, globulin, alanine transaminase, aspartate transaminase, creatine kinase, glucose, uric acid and creatinine at 21 days of age.

II. Materials and Method

The study was carried out in the poultry sector of the Department of Animal Science of the Agricultural Sciences Center of the Federal University of Piauí, Teresina, Piauí, Brazil, in June and July 2015, whose geographical coordinates are: latitude of 5 5 'south, longitude 42 °

48' West, altitude of 74.4 meters and semi-humid tropical climate, with annual precipitation around 1396 mm [8]. The procedures were approved by the Ethics Committee on Animal Experimentation/EAEC/UFPI, under protocol number 087/12.

A total of 630 male broilers from the Ross lineage were used during the period from 1 to 21 days of age with a mean initial weight of 38,17±0,77g. The birds were distributed in a completely randomized design, in a 2x3 + 1 factorial scheme, with two levels of L-glutamine (1 and 2%) associated to three levels of zinc (0.90 and 120mg / kg of feed) in the organic form , a control diet and five replicates. These animals were housed in boxes of 2.7m2, equipped with tubular feeders and pendulum drinkers, located in masonry sheds covered with ceramic tiles and cemented floor.

The animals received a pre-start diet up to 7 days (Table 1) and a diet for the initial phase (Table 2), from 8 to 21 days of age. The diets were formulated to meet the nutritional requirements recommended by [9]. Zinc was supplemented in the diets to replace the inert material and L-glutamine was introduced into the diet formulation. The birds received feed and water at will.

Table 1. Percentage and calculated composition of the experimental diets for broilers in the pre-initial phase (1 to 7 days of age)

Ingredients	Levels of glutamine (%)/ zinc (mg/kg)						
(%)	0/0	1	1/90	1/120	2	2/90	2/120
Corn	52,000	54,500	54,500	54,500	57,400	57,400	57,400
Soybean	33,430	30,170	30,170	30,170	26,810	26,810	26,810
Vegetable	4,827	4,322	4,322	4,322	3,678	3,678	3,678
Dicalcium	1,770	1,805	1,805	1,805	1,842	1,842	1,842
Calcitic	0,975	0,975	0,975	0,975	0,980	0,980	0,980
NaCl	0,508	0,508	0,508	0,508	0,510	0,510	0,510
L-Lysine-	0,063	0,168	0,168	0,168	0,278	0,278	0,278
L-	0,000	0,000	0,000	0,000	0,011	0,011	0,011
Valine	0,155	0,215	0,215	0,215	0,277	0,277	0,277
Threonine	0,007	0,055	0,055	0,055	0,104	0,104	0,104
Nucleus[a]	6,000	6,000	6,000	6,000	6,000	6,000	6,000
Zinc[b]	0,000	0,000	0,090	0,120	0,000	0,090	0,120
Glutamine[c]	0,000	1,000	1,000	1,000	2,000	2,000	2,000
Kaolin	0,264	0,282	0,192	0,162	0,109	0,019	0,000
TOTAL	100,000	100,000	100,000	100,000	100,000	100,000	100,000

Calculated Composition							
Crude	22,399	22,401	22,401	22,401	22,402	22,402	22,402
ME	2959,99	2959,99	2959,99	2959,99	2960,00	2960,00	2960,00
Lysine dig.	1,324	1,324	1,324	1,324	1,325	1,325	1,325
Methionine	0,669	0,653	0,653	0,653	0,637	0,637	0,637
Threonine	0,861	0,861	0,861	0,861	0,862	0,862	0,862
Tryptophan	0,253	0,234	0,234	0,234	0,226	0,226	0,226
Valine (%)	1,020	1,019	1,019	1,019	1,020	1,020	1,020
Calcium (%)	0,921	0,920	0,920	0,920	0,921	0,921	0,921
Match	0,470	0,470	0,470	0,470	0,471	0,471	0,471
Sodium (%)	0,219	0,219	0,219	0,219	0,219	0,219	0,219
Zinc (mg/kg)	146,869	145,961	235,961	265,961	145,094	235,094	265,094
Glutamine	0,000	1,007	1,007	1,007	2,014	2,014	2,014

[a]Guarantee levels per kg of product: moisture (max.) 120g / kg; crude protein (min.) 340g / kg; ethereal extract 45g; crude fiber 10g; mineral matter 300g; calcium 22g; calcium 28g; phosphorus 5,200mg; methionine 65g; lysine 45gg; threonine 27g; tryptophan 3,780mg; vitamin A 250,000UI; vitamin D3 60,000 IU; vitamin E 833UI; vitamin K3 50mg; vitamin B1 50mg; vitamin B2 133mg; vitamin B6 83mg; vitamin B12 333mg; niacin 100mgg; pantothenic acid 233mg; folic acid 25mg; biotin 0.66mg; biotin 0.66mg; choline 5,900mg; manganese 1,666mg; zinc 1600mg; chelated zinc 400mg; iron 837mg; copper 1,667mg; iodine 21mg; selenium 6mg; BHT 1764 mg; 8.335U phytase; protease 2.500UN; amylase 2.500UN; β-glucanase 2.083UN; xylanase 4.165UN; cellulase 3.750UN; senduramycin + nicarbazine 1.100mg.
[b]Availa®Zn 100.000mg/kg
[c] Metabolizable energy based [10] and crude protein analyzed (119,74).

Table 2. Percentage and calculated composition of the experimental diets for broilers in the initial phase (8 to 21 days of age)

Ingredients	Levels of glutamine (%)/ zinc (mg/kg)						
(%)	0/0	1	1/90	1/120	2	2/90	2/120
Corn	58,300	60,500	60,500	60,500	62,600	62,600	62,600
Soybean	34,414	31,400	31,400	31,400	28,246	28,246	28,246
Vegetable	3,259	2,854	2,854	2,854	2,484	2,484	2,484
Dicalcium	1,530	1,560	1,560	1,560	1,600	1,600	1,600
Calcitic	0,907	0,907	0,907	0,907	0,907	0,907	0,907
NaCl	0,482	0,482	0,482	0,482	0,482	0,482	0,482
L-Lysine-	0,000	0,000	0,000	0,000	0,082	0,082	0,082
L-	0,000	0,000	0,000	0,000	0,002	0,002	0,002
Valine	0,029	0,085	0,085	0,085	0,145	0,145	0,145
Threonine	0,038	0,080	0,080	0,080	0,127	0,127	0,127
Premix min	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Zinc[b]	0,000	0,000	0,090	0,120	0,000	0,090	0,120
Glutamine[c]	0,000	1,000	1,000	1,000	2,000	2,000	2,000
Kaolin	0,041	0,132	0,042	0,012	0,325	0,235	0,205
Total	100,000	100,000	100,000	100,000	100,000	100,000	100,000

Calculated Composition							
Crude Protein	21,200	21,198	21,198	21,198	21,201	21,201	21,201
ME (kcal/kg)	3050,0	3049,99	3049,99	3049,99	3050,00	3050,00	3050,00
Lysine dig.	1,211	1,134	1,134	1,134	1,117	1,117	1,117
Methionine	0,608	0,593	0,593	0,593	0,577	0,577	0,577
Threonine dig	0,792	0,790	0,790	0,790	0,790	0,790	0,790
Tryptophan	0,239	0,222	0,222	0,222	0,206	0,206	0,206
Valine (%)	0,937	0,936	0,936	0,936	0,937	0,937	0,937
Calcium (%)	0,841	0,840	0,840	0,840	0,840	0,840	0,840
Match	0,401	0,400	0,400	0,400	0,401	0,401	0,401
Sodium (%)	0,210	0,210	0,210	0,210	0,210	0,210	0,210
Zinc (mg/kg)	88,568	87,703	177,70	207,703	86,758	176,758	206,758
Glutamine	0,000	1,007	1,007	1,007	2,014	2,014	2,014

[a] Guarantee levels per kg of product: methionine 313,60g; lysine 168g; threonine 29.40g; vitamin A 1,200,000IU; vitamin D3 265,000; vitamin E 2,000UI; vitamin K3 260mg; vitamin B1 191mg; vitamin B2 630mg; vitamin B6 290mg; vitamin B12 1,700mg; niacin 4.200mg; pantothenic acid 1,300mg; folic acid 100mg; biotin 7mg; choline 26g; manganese 7,000mg; zinc 6,000mg; iron 5,000mg; copper 900mg; iodine 100mg; selenium 30mg; phytase 50,000 U; 30,000U amylase; β-glucanase 25,000 U; xylanase 50,000 U; 45,000 U cellulase; 30,000 U protease; ethoxyquin 6.666mg; Bacillus licheniformis 2x10E11UFC; Bacillus subtilis 1x10E11UFC; virginiamycin 1,650mg; maduramicin 500mg.
[b]Availa®Zn 100.000mg/kg
[c] Metabolizable energy based on [10] and crude protein analyzed (119,74).

The monitoring of the temperature and relative humidity of the sheds was carried out by means of maximum and minimum thermometers and thermohygrometer of dry and humid bulb kept in the center of the shed. The readings of the thermometers were performed three times a day (8, 13 and 16 hours), except for maximum and minimum, which were read only in the morning. These data were later converted to Globe and Humidity Temperature Index (ITGU), as proposed by [11]. The light program adopted was the continuous (24 hours of natural + artificial light) using 60W incandescent lamps.

At 21 days of age, in the morning, 3 ml of blood was collected from the jugular vein of 5 birds per treatment, one bird from each replicate, being randomly selected. Serum obtained after coagulation and centrifugation of the blood was stored at -20 ° C for further analysis of plasma protein, albumin, globulin, glucose, creatinine, uric acid, alanine transaminase, aspartate transaminase and creatine kinase plasma concentrations. Biochemical determinations were performed by means of commercial enzymatic kits on Bio-200F semi-automatic biochemical analyzer, Bioplus®, following the protocols described by the manufacturer.

The data on environmental variables were submitted to mean and standard deviation calculations. The other parameters were subjected to analysis of variance, and when significant, L-glutamine levels were compared by Duncan's test and zinc levels by regression analysis. In the comparison of each treatment with the control diet, the Dunnett test was applied, according to PROC GLM procedures of the SAS software (2002). With the exception of the histomorphometry of the cloacal pouch, which violated the principles of normality and homoscedasticity and, for this reason, the test used was Kruskal-Wallis. A = 0.05 was used.

III. Results and Discussion

Considering the values of the environmental variables obtained during the experimental trial (Table 3), the animals were raised under heat stress conditions, and during the first and second week, they were exposed to temperatures above and below the thermal comfort range.

Table 3. Environmental conditions observed during the experimental period

Age (week)	Humidity (%)	Temperatures (°C)			ITGU[1]
		Maximum	Minimum	Average	
1º	67,61±12,84	32,27±1,06	22,68±0,95	27,60±0,49	80,13±2,48
2º	63,71±15,35	32,69±0,48	22,44±1,06	27,56±0,54	80,11±2,55
3º	69,82±18,63	32,20±1,05	23,60±0,83	27,90±0,64	79,18±4,89

[1]ITGU- Globe and Humidity Temperature Index

The environment in which the research was carried out shows that the birds were raised under conditions of heat stress mainly during the third week of life, during the first and second week temperatures were below and above the thermal comfort range, since according to [12] comfortable temperatures for birds are 31.3; 26.3 - 23.2 and 22.5-23.2 ° C, respectively in the first, second and third week of life, and the temperature index of the globe and humidity in the first week ranges from 77 to 81 and in the second and the third week of life the interval is 74.5 to 77 [13].

Regarding the studied nutrients, interaction between L-glutamine and supplemental zinc was verified for total proteins (P <0.05). A linear effect was observed for increasing levels of zinc in the diets with the addition of 1% of L-glutamine, represented by the equation: $\hat{Y} = 0.087x + 2.5673$ ($P < 0.05$; $R^2 = 0.85$). With the unfolding of the interaction, it was verified that the level of 2% of L-glutamine without the addition of supplementary zinc presented higher concentration of total proteins when compared to the level of 1% of that amino acid and to the control group. Likewise, treatment with the addition of 1% of L-glutamine associated with 120mg of zinc presented a higher value for this variable when compared to the control (Table 4).

Table 4. Blood biochemistry of broiler chickens fed different levels of L-glutamine and zinc in the phase 1 to 21 days of age

Parameters	Control	L-Glutamine	Zinc (mg/kg)			Average	CV (%)	Valeu P	
			0	90	120			L	Q
PT (g/dL)	2,660	1	2,630b	3,100a	3,800a*	3,176	20,3	0,013	0,747
		2	3,810a*	2,960a	2,840a	3,203		0,390	0,334
Média			3,220	3,030	3,320			0,390	0,334
Glob (g/dL)	1,066	1	0,9740b	1,056a	1,792a	1,274	44,4	0,284	0,450
		2	1,932a	1,494a	1,024a	1,483		0,031	0,961
Média			1,453	1,275	1,408				
Alb (g/dL)	1,594	1	1,656	2,044	2,008	1,902	25,5	0,733	0,349
		2	1,878	1,466	1,816	1,720			
Média			1,767	1,755	1,912				
Alb/glob	1,597	1	1,486	1,918	1,287	1,543	57,8	0,780	0,451
		2	1,054	1,235	1,468	1,237			
			1,246	1,539	1,367				
ALT (UI/L)	26,140	1	28,240	20,900	26,140	25,093	38,5	0,356	0,125
		2	18,800	18,820	28,750	21,650			
Média			23,520	19,860	27,300				
AST (UI/L)	274,480	1	492,800*	315,520	344,660	384,200a	43,1	0,229	0,405
		2	216,840	235,720	220,000	224,190b			
Média			354,820	275,520	282,33				
Ck (UI/L)	5368,600	1	7742,750	5209,800	6550,200	6412,000	51,8	0,934	0,687
		2	3460,500	5187,000	4876,600	4583,000			
Média			5602,000	5198,000	5713,000				
Gli (mg/dL)	158,700	1	165,320	168,040	166,800	166,720	6,8	0,362	0,352
		2	171,840	172,780	160,880	168,500			
Média			168,580	170,410	163,840				
AU (mg/dL)	7,880	1	8,020	9,240	9,300	8,821	20,5	0,302	0,863
		2	9,020	8,440	9,520	8,993			
Média			8,520	8,840	9,422				
Cr (mg/dL)	0,280	1	0,240	0,260	0,280	0,260	18,7	0,192	0,798
		2	0,260	0,280	0,280	0,273			
Média			0,250	0,270	0,280				

Means followed by asterisks differ from the control treatment by Dunnett's test (P <0.05). Means followed by the same lowercase letter in the column for the same variable do not differ by Duncan's test (P> 0.05).

L, Q: probability of linear and quadratic order regarding the inclusion of zinc in the diet.
PT- total protein; Globoglobulin; Albumin albumin; ALT-alanine transaminase; AST-aspartate transaminase; CK-creatine kinase, Glyc glucose; Uric acid; Cr creatinine.

Although animals fed diets supplemented with 1% L-glutamine combined with 120 mg zinc / kg feed and 2% L-glutamine without supplemental zinc supplementation deferred from the control treatment, it is found that total plasma protein concentrations in all treatments are within normal values in birds, 2.5 to 4.5 g / dL [14]. These findings suggest a good state of health of the animals, since the dosage of total plasma proteins allows the indication of alterations of hepatic failure, intestinal and renal disorders, bleeding or feeding deficiency [15].

The linear increase of total proteins in the blood of broilers, as a function of zinc levels, in animals fed diets with the addition of 1% of L-glutamine can be justified by the fact that this mineral is a structural component of a large number of enzymes , which participate in the synthesis of proteins and nucleic acids [16].

Regarding the concentration of globulin, it can be verified that the test treatments did not differ from the control, however, there was interaction between the levels of L-glutamine and supplementary zinc, which presented a linear decreasing effect for the zinc level in the diets containing 2% of L-glutamine, according to the equation: $\hat{Y} = -0.0069x + 1.9694$ ($P < 0.05$; $R^2 = 0.91$). In the unfolding of the interaction, it was verified that the level of 2% of L-glutamine without the addition of supplementary zinc provided the highest concentration of plasma globulin ($P < 0.05$).

The addition of L-glutamine and supplementation zinc in the diets had no effect on the levels of globulin, albumin and albumin / globulin ratio, in addition, it was observed that in all treatments, including control, the values presented are below (3.1 g / dL) and (2.5 g / dL), and above (0.8) the normal limits referenced [14], respectively. These results indicate a low immune response, since the low albumin / globulin ratio and the high concentration of globulin mean better immune response and disease resistance [17], even if the total protein concentration is found within the normal reference parameters [18].

These findings may be related to the high temperatures observed in this experiment, in which neither zinc nor L-glutamine were able to alter this response, because under heat stress conditions, digestion and nutrient absorption are impaired in function of the reduction in feed intake by the bird, as a physiological adjustment for maintenance of homeothermia, consequently compromising protein synthesis [19].

For the aspartate transaminase, there was no interaction between the nutrient tests, however, it was observed that the addition of 1% of L-glutamine without zinc supplementation presented higher value than the control treatment (P <0.05). And, regardless of the zinc level, it was verified that the inclusion of 1% of L-glutamine gives a higher concentration of that enzyme when compared to the level of 2% (P <0.05). (Table 4)

Plasma levels of aspartate and alanine transaminase are reported as a sensitive indicator of hepatic injury, whose normal levels for broiler chickens are 202-325 and 14 to 34 IU / L [20], respectively, thus increasing the concentration of these enzymes above the proposed ranges may reflect hepatocyte insults [21].

However, since they are not specific enzymes of hepatocellular disorder, they should be measured along with a muscle-specific enzyme, such as creatine kinase , so that it is possible to differentiate hepatic damage from muscle injury [22].

The other biochemical parameters, albumin, albumin / globulin, alanine transaminase, creatine kinase, glucose, uric acid and creatinine, presented no difference when compared to control (P> 0.05), and were also not influenced by the association of L -glutamine and zinc in the diet (P> 0.05).

Considering the above information, it is noted that the aspartate aminotransferase values obtained for the animals from the treatments with addition of 1% L-glutamine without the addition of supplemental zinc and treatment with 1% L-glutamine independent of zinc levels above normal levels, are not related to hepatic injury, but to some type of muscular disorder, since all treatments, including control, presented creatine kinase values above that proposed by [23], whose range is 2485 ± 1.122U / L.

Thus there are two possible causes that explain elevated levels of creatine kinase, the first is that zinc levels up to 0.1 mM may cause increased creatine kinase activity [24], so it is assumed that the zinc values present in the control ration and in the treatments used may have contributed to the increase in the concentration of this enzyme or are related to the muscular activity due to containment for blood collection and not only by muscle injuries caused by zinc supplementation [25]. and in both hypotheses, L-glutamine supplementation was not able to reduce its activity, even though it had the capacity to act as a metabolic regulator, increasing protein synthesis and reducing protein catabolism if supplemented in the diet [6] and consequently reduce muscle damage caused by the action of creatine kinase.

It was found that the glucose concentration of birds in all treatments, including control, presented values below the recommended range for chickens, which according to [26] is 200 to 500mg / dL. Thus, although L-glutamine supplementation provides increased insulin secretion and consequently reduced plasma glucose concentration by increasing the concentration of the hormone Glucagon Like Peptide-1 [27; 28], these effects can not be attributed to the supplementation of this amino acid, due to the similarity between the test treatments and the control.

Unlike to zinc, which also acts on the synthesis of insulin in β-pancreatic cells, leading to the reduction of blood glucose [29; 30], since all treatments, including the control, presented levels of zinc in the ration, above that recommended by [31] 76.15 and 68.72 mg of inorganic zinc and 33.85 and 30.54 mg of organic zinc / kg of feed and for the phase 1 to 7 and 8 to 21 days of age, respectively, and 100 mg of zinc / kg of feed for all breeding phases according to the Ross lineage manual [32].

The levels of the test ingredients did not change the concentration of uric acid and all treatments presented values considered normal for the species, from 2 to 15mg / dL [33], denoting that such ingredients did not interfere in hepatic conjugation nor in renal excretion of this metabolic.

Os resultados obtidos para creatinina estão abaixo da faixa indicada para frangos, que é de 0,49 a 0,55 mg / dl [23], inclusive no grupo controle, dessa forma considerando que os valores de creatinina são um excelente indicador de função, e que os níveis sanguíneos muito elevados indicam uma deficiência na função renal [34], pode deduzir-se que os níveis de L-glutamina e zinco utilizados neste estudo não interferiram com a função renal.

IV Conclusions

The addition of L-glutamine and zinc in broilers diets in the period from 1 to 21 days of age increases the plasma concentration of total proteins and does not interfere with liver and renal function.

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