Hematology and serum biochemistry of broilers at the initial and growth stages submitted to different levels of digestible sulfur amino acids

Genilson Bezerra de Carvalho[1*]   Poliana Carneiro Martins[1]   Pedro Moraes Rezende[1]
Januária Silva Santos[1]   Evelyn de Oliveira[1]   Thays de Campos Trentin[1]
Danieli Brolo Martins[1]   José Henrique Stringhini[1]   Marcos Barcellos Café[1]

[1]Universidade Federal de Goiás (UFG), Escola de Veterinária e Zootecnia (EVZ), 74690-900 Goiânia, GO, Brasil. E-mail: ge.nilson.bezerra@hotmail.com
*Corresponding author.

ABSTRACT: *The objective of this study was to evaluate the effect of digestible methionine and cystine (Met + Cys) levels on the hematological and serum biochemical parameters of broiler chickens during the initial and growth stages. For this, 1,800 male chicks of the Coob 500 strain were used, with 900 chicks in the initial phase (1 to 21 days old) and 900 chicks in the growth phase (22 to 42 days old), distributed in a completely randomized design of five treatments with six replicates of 30 birds. The treatments consisted of 0.545, 0.616, 0.711, 0.782, and 0.853%; and 0.514, 0.571, 0.647, 0.704, and 0.761% digestible Met + Cys for "1 to 21" and "22 to 42" days of breeding, respectively. Results showed that digestible Met + Cys levels in broiler feed altered some hematological parameters (erythrocyte, hematocrit hemoglobin, total leukocytes, heterophile: lymphocyte) and serum biochemistry (uric acid, PST, total LDL, and TG). The digestible Met + Cys levels in the diet of broilers affected the hematological parameters and serum biochemistry, especially at higher levels. From the inclusion level 0.761 of Met + Cist in the broiler diet, red blood cells, hemoglobin and hematocrit changes begin to appear.*
Key words: *amino acid metabolism, cysteine, methionine, immune system, poultry.*

Hematologia e bioquímica sérica de frangos de corte submetidos a diferentes níveis de aminoácidos sulfurados digestíveis

RESUMO: *Teve-se como objetivos neste estudo avaliar o efeito dos níveis de metionina + cistina (Met+Cis) digestível na dieta sobre os parâmetros hematológicos e bioquímicos séricos de frangos de corte na fase inicial e de crescimento. Para isso, utilizou-se 1.800 pintos, machos, da linhagem Coob 500, sendo 900 pintos para a fase inicial (1 a 21 dias de idade) e mais 900 pintos para a fase de crescimento (22 a 42 dias de idade), distribuídos em delineamento inteiramente casualizado composto de cinco tratamentos, com seis repetições de 30 aves. Os tratamentos consistiram em 0,545; 0,616; 0,711; 0,782 e 0,853% e 0,514; 0,571; 0,647; 0,704 e 0,761% de Met+Cis digestíveis para a fase inicial e de crescimento, respectivamente. Os resultados demonstraram que níveis de Met+Cis digestível na alimentação de frango de corte, acarretam alterações em parâmetros hematológicos como: hemácia, hematócrito, hemoglobina, leucócitos totais, relação heterófilo: linfócito; e bioquímico sérico como: ácido úrico, albumina, colesterol total, proteínas séricas totais (PST), lipoproteína de baixa densidade (LDL) e triglicerídeos (TG). Os níveis digestíveis de Met + Cys na dieta de frangos de corte afetam os parâmetros hematológicos e a bioquímica sérica, principalmente em níveis mais elevados. A partir do nível de inclusão 0.761 de Met + Cist na dieta de frangos de corte, começam a aparecer alterações nos glóbulos vermelhos, hemoglobina e hematócrito.*
Palavras-chave: *aves, cistina, metionina, metabolismo aminoacídico, sistema imune.*

INTRODUCTION

The health conditions of birds are reflected by their blood constituents. Their evaluation is thus essential to determine the effect of the various conditions in which chickens are being bred, since several factors, including metabolism (BORSA, 2006; MINAFRA et al., 2010) affect the results. In broiler nutrition, methionine is the first limiting factor in corn and soybean meal-based diets, as they are deficient in that amino acid (WEN et al., 2014; ZHANG et al., 2015). Therefore, methionine should be supplemented in these diets to provide the methionine + cystine (Met + Cys) content required for optimum performance.

The four main commercial sources of synthetic methionine used in broiler feed are DL-methionine (DL-Met) as a powder or liquid form as sodium salt (DL-methionine-Na) and methionine hydroxy analogue (MHA) as a powdered salt (MHA-Ca) or in liquid form as a free acid (MHA-FA) (LEITE et al., 2009).

Several studies have been conducted to evaluate the performance and determine the nutritional requirements for methionine in broiler chickens fed with corn and soybean based diets (LEITE et al., 2009; LUMPKINS et al., 2007; GOULART et al., 2011; TAVERNARI et al., 2014; CONDE-AGUILERA et al., 2016). However, the effects of Met + Cys levels in the diet and the sources of methionine available on the chick health market are not well known.

Few papers studying the influence of Met + Cys levels on the blood parameters of chicken have been published since methionine was discovered on 1922. The addition of adequate amounts of methionine to the diet improves the immunity of poultry against different diseases (EDUARDO et al., 2009). YODSERANEE & BUNCHASAK (2012) studied the effects of the dietary supplementation of DL-Met and MHA-FA on the blood profile of broilers in tropical conditions and reported that methionine concentration in the plasma increased with DL-Met supplementation, while concentrations of taurine and plasma uric acid increased significantly with MHA-FA supplementation.

Dietary supplementation with methionine increased blood HDL and decreased serum triglycerides in broilers (KALBANDE et al., 2009; ANDI, 2012). Low protein and amino acid levels in broiler diet have been associated with decreased total serum protein and albumin (EMADI, 2010). FALUYI et al. (2015) studied the effect of different methionine levels on the diet of Ross 308 broilers, and observed in the biochemical analysis of serum proteins that only total protein, among the measured serum metabolites (albumin, globulin and protein), was significantly different. Serum analysis in poultry may be considered a good indicator when checking for changes in their physiological systems (BATINA et al., 2005). Blood analysis provided the opportunity to investigate and identify several metabolites and other constituents in the body. Blood carries nutrients and materials to different parts of the body and plays a vital role in the physiological, nutritional, and pathological state of the animal (AL-MAYAH, 2006; TARAZ & DASTAR, 2008; ETIM et al., 2014).

The analysis of blood components is a readily-available and rapid mean of knowing and interpreting the clinical, nutritional and metabolic health state of animals in food trials, as the ingestion of dietary components has measurable effects on blood composition (ETIM et al., 2014). Therefore, the objective of this study was to evaluate the hematological and serum biochemical parameters in broilers at the initial and growth stages with a diet containing different levels of digestible Met + Cys.

MATERIALS AND METHODS

The experiment was conducted in the School Aviary of the Animal Science Department of the Federal University of Goiás in Goiânia, Goiás, Brazil (latitude 16º40'43"S, longitude 49º15'14"W at an altitude of 749 m a.s.l.) - DZO/EVZ/UFG, from December 2014 to January 2015. A total of 1,800 one-day-old male Cobb 500 broilers were used, with 900 in experiment 1 for the initial phase (1 to 21 days old) and 900 in experiment 2 for the growth phase (22 to 42 days old). The experiments were conducted in a completely randomized design consisting of five treatments (digestible Met + Cys levels), each with six replicates of 30 birds.

Broilers were housed in 30 individual boxes measuring 1.80×1.60 m (2.88 m²), mounted in the central area of the commercial warehouse measuring 12 m × 125 m (width × length) (1,500 m²), under negative pressure with seven exhaust fans, nebulization system, and air intake with an evaporative plate. The boxes had 0.40 m wide masonry side walls and a wire screen of 2.80 m height, height of 3.20 m and an east-west orientation. The housing density in each box was 12 birds/m² (30 birds/box). Each box contained a line with 10 nipple-type drinkers and a tube feeder until the seventh day of age and an adult feeder from eight to 42 days of age. The ratio of feeder and drinkers was 1:30 and 1:3 per individual bird, respectively. The birds were vaccinated in the hatchery against Marek's disease and were vaccinated against Infectious Bursal Disease at 14 days of age via drinking water. The bedding used was rice hulls (first use).

In both experiments, a basal diet without methionine supplementation was prepared, which was then gradually supplemented with DL-Methionine 99% (0.072, 0.168, 0.239, 0.311% and 0.058, 0.134, 0.192, 0.250% in the breeding phases of 1 to 21 and 22 to 42 days, respectively), which replaced the corn starch, to reach the desired digestible Met + Cys levels of 0.545, 0.616, 0.711, 0.782, and 0.853% for the 1 to 21 and 0.514, 0.571, 0.647, 0.704 and 0.761% for the 22 to 42 days of breeding phases. In both phases (1 to

21 and 22 to 42 days), the basal diets were formulated to meet the nutritional requirements of the birds, following the nutritional recommendations of the feed supplier, as proposed by ROSTAGNO et al. (2011) (Table 1). To prevent a possible cumulative effect of the amino acid sources, different birds were used in the initial and in the growth phase, thus preventing residual responses in the following phase.

The broilers received diet and water *ad libitum* throughout the experimental period and were bred following the lighting, temperature, humidity, and management recommendations of the guide management COBB-VANTRESS (2008).

For hematological and biochemical evaluation, 8 mL of blood was collected by cardiac puncture of each bird, six animals per treatment, in a total of 30 birds at 21 and 42 days of age. Of the total blood volume, 0.5 mL was maintained in tubes with ethylenediaminetetraacetic acid anticoagulant (10% EDTA K2) to perform the blood count and 7.5 mL in tubes without anticoagulant for biochemical analysis.

Hemograms were performed manually according to JAIN, 1993. Hematimetric indexes of mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined using indirect calculations, panotic stained blood smears (Laborclin®, Pinhais/PR) were used to visualize the cellular morphology. The heterophile: lymphocyte ratio was calculated using heterophile and lymphocyte results. Hematological analyzes were performed immediately after blood collection.

Table 1 - Percentage nutritional composition of experimental diets used in this study.

Ingredients (%)	Initial phase	Growth phase
	(1 to 21 days)	(22 to 42 days)
Maize (%)	63.12	64.69
Soybean meal 45.5% (%)	28.38	24.56
Meat and bone meal 44% (%)	3.20	2.60
Poultry viscera meal (%)	2.00	2.67
Poultry fat (%)	1.33	3.81
Salt (%)	0.40	0.24
Calcitic limestone 37% (%)	0.62	0.65
DL-Methionine 99% (%)	0.00	0.00
L-lisina 70% (%)	0.40	0.34
L-Threonine 98% (%)	0.10	0.07
Choline 75% (%)	0.06	0.03
Vitamin supplement[2] (%)	0.05	0.05
Mineral supplement[1] (%)	0.05	0.05
Inert (%)	0.29	0.24
Total (%)	100.0	100.0
------Calculated composition------		
Metabolizable energy (kcal/kg)	3109	3300
Crude protein (%)	21.803	21.217
Calcium (%)	0.952	0.952
Available phosphorus (%)	0.451	0.421
Digestible methionine (%)	0.675	0.526
Digestible methionine + cystine (%)	0.545	0.514
Digestible lysine (%)	1.190	1.058
Digestible threonine (%)	0.749	0.667
Sodium (%)	0.201	0.190
Chlorine (%)	1.598	1.399

[1]Mineral supplement per kg of diet (Mineral mix): Mn, 60 g; Fe, 80 g; Zn, 50 g; Cu, 10 g; Co, 2 g; I, 1 g; vehicle q.s.p., 500 g. [2]Vitamin supplement per kg of diet (Protein mix): Vit. A - 15,000,000 lU, Vit. D3 - 1,500,000 lU, Vit. E - 15,000 lU, 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, Zinc bacitracin - 10 g, Selenium - 250 mg, vehicle q.s.p. - 1000 g.

The biochemical profile comprised the measured analytes: albumin (ALB), total serum proteins (TSP), uric acid (UA), high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (CHOL) and triglycerides (TG), as well as aspartate aminotransferase (AST). The levels of these analytes were determined by spectrophotometry with a CM 250 automatic analyzer (Wiener®, Rosário, Argentina), following the manufacturer's recommendations, using commercial analytical kits (Biotécnica®, Varginha, MG).

The data were analyzed using an analysis of variance. The Scott-Knott test with a significance level of 5% was used to compare means. Homogeneity of variances (Bartlett test) and normality of residuals (Shapiro-Wilk test) were considered. R CORE TEAM, (2017) was used for the analyses.

RESULTS AND DISCUSSION

Red blood cell, hematocrit, MCV, MCHC, and TPP values in the blood of the broilers were similar among treatments (Table 2). However, hemoglobin levels were significantly reduced and presented similar results to the basal group for the H: L ratio. presented similar results to the basal group for the H: L ratio ($P<0.05$) by the treatments.

Methionine supplementation during the initial stages of chicken growth significantly improved the hematocrit, red blood cell counts and related parameters (AL-MAYAH, 2006; TARAZ & DASTAR, 2008). However, in 21-day-old chickens, results obtained in this study indicated that digestible Met + Cys dietary supplementation in the levels 0.616, 0.782 and 0.853 decrease ($P<0.05$) the value of hemoglobin, despite the hematocrit and erythrocyte mass values remain constant. At 42 days of age, additional levels of 0.761 Met + Cys increased ($P<0.05$) the concentrations of red blood cells, hemoglobin and hematocrit. No difference was observed between the treatments for MCV, MCHC, and TPP (Table 2). Thus, birds submitted to the different treatments at these stages of development have different oxygen transfer capacity, since hemoglobin plays an important role in the transport of organism gases, which may have significant impacts on the metabolism of the bird (JAIN, 1993).

The H:L ratio seems to be a more accurate indicator, since it is less variable than the number of heterophiles or lymphocytes alone (CAMPBELL, 2015), or even total leukocytes. In adittion, digestible Met + Cys in the diet of 21-day-old chickens reduced ($P>0.05$) heterophil:lymphocyte ratio among treatments 0.616, 0.782 e 0.853 (Table 3).

Table 2 - Effects of different digestible Met + Cys levels on the erythrogram and TPP of broilers at 21 and 42 days of age.

Met + Cys %	Red blood cells (x10^{6})	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fL)	MCHC (%)	TPP (g/dL)
---		21 days			--	
Basal	2.045	7.017 [a]	31.333	154.082	22.370	2.833
0.616	1.967	6.143[b]	30.571	158.676	20.193	2.533
0.711	2.417	6.660[a]	32.000	132.228	20.798	3.000
0.782	2.068	6.233[b]	30.500	149.283	20.415	2.800
0.853	2.136	5.983[b]	31.000	140.797	19.393	2.880
CV%	12.42	9.59	6.96	16.07	8.72	9.79
P	0.191	0.044	0.770	0.359	0.092	0.101
---		42 days			--	
Basal	2.107 [a]	7.425[a]	30.167 [a]	143.580	24.648	4.000
0.571	2.202 [a]	7.980[a]	31.728[a]	143.573	24.653	4.200
0.647	2.158 [a]	7.528[a]	31.000[a]	143.226	24.590	4.333
0.704	2.140 [a]	7.402[a]	30.600[a]	142.690	24.700	3.880
0.761	2.498 [b]	8.810[b]	35.667 [b]	143.540	24.616	4.300
CV%	8.99	9.91	9.29	0.35	0.41	10.86
P	0.012	0.015	0.017	0.085	0.615	0.404

Values followed by different letters in the same column indicate statistically significant differences by the Scott-Knott test. CV% = coefficient of variation. Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC) and Total Plasma Proteins (TPP).

These groups also showed a significant decrease in hemoglobin value. These results differ from those presented by the group 0.711, which was similar to the basal group in erythrogram and leukogram. Feeding birds with protein-deficient diets (i.e. protein malnutrition) reduced blood cell production, leading to bone marrow hypoplasia and induced structural alterations, which interfere with innate and adaptive immunity (BORELLI et al., 2009; FOCK et al., 2010; CUNHA et al., 2013). Methionine deficiency can also cause pathological and ultrastructural alterations of the thymus, reduced the T-cell population, serum levels of interleukin-2 and the proliferative function of T cells, and increase the percentage of apoptotic cells (WU et al., 2012).

For 42-day-old birds, all the Met + Cys levels presented similar results to the basal group for the H: L ratio (Table 3). The H:L ratio has been an important indicator of immune system as well as stress associated with lesions, reproductive cycles and seasonal changes (CAMPBELL, 2015).

There was no significant difference (P>0.05) between treatments of the same experiment regarding the number of thrombocytes at 21 and 42 days of age (Table 4). Thrombocytes may be related to innate immunity due to their phagocytosis capacity, as well as their participation in blood coagulation (SCHMIDT et al., 2007). They may also participate in the removal of foreign material from the blood (CAMPBELL, 2015).

Clinical signs of bird disease are non-specific and both hematological and biochemical analyzes have been reported as important sources of information for the immune system state of these animals. There were an increased value (P<0.05) in the levels of UA, ALB, TSP, CHOL, LDL and TG in the blood of the birds at the 21-days-old chicken for diferent treatments when compared to birds fed with the basal diet (Table 5).

HERNANDEZ et al. (2012) reported that plasma UA concentration increased with higher protein levels in the diet of broilers in the pre-initial phase. Therefore, the serum content of this substrate has been widely used as an indicator of amino acid use in broilers (CORZO et al., 2009; DONSBOUGH et al., 2010). Results of the present study showed that UA levels increase as Met + Cys intake increases. This demonstrates that diets with higher Met + Cys levels can be absorbed and metabolized by the birds, thus increasing the transformation of nitrogen into uric acid.

Birds fed with the basal diet had lower levels of ALB in the blood compared to the other treatments. The CHOL, LDL and TG levels in birds fed with the basal diet were similar (P>0.05) to those in birds fed with the second supplementation level,

Table 3 - Effects of digestible methionine + cystine levels in the diet on the leucogram (/μL) of 21- and 42-days old broilers.

Met + Cys %	Total leukocytes (x10³)	H/L	Lymphocytes	Heterophiles
--21 days--				
Basal	13500.000	1.037 [a]	6168.333	4788.400
0.616	9714.286	0.562 [b]	5778.571	3214.286
0.711	13000.000	0.780 [a]	6944.000	5316.000
0.782	11666.667	0.462 [b]	7136.667	3690.000
0.853	9800.000	0.545 [b]	7765.000	2798.000
CV%	31.28	39.78	45.24	44.78
P	0.315	0.009	0.786	0.138
--42 days--				
Basal	23000.00 [a]	1.192	9188.250	10418.167
0.571	13900.00 [b]	1.552	5426.833	7778.333
0.647	14600.00 [b]	1.315	6976.400	8520.333
0.704	14600.00 [b]	1.836	4358.000	8286.000
0.761	11200.00 [b]	1.143	5654.167	4757.500
CV%	28.74	48.46	40.05	38.89
P	0.002	0.437	0.072	0.059

Values followed by different letters in the same column indicate statistically significant   differences by the Scott-Knott test. CV% = coefficient of variation. Heterophile: Lymphocyte ratio (H/L).

Table 4 - Effects of digestible Met + Cys levels in the diet on platelet numbers (/μL) of broilers at 21 and 42 days of age.

Variable	Met + Cys levels (%)						
	21 days						
Thrombocytes	Basal	0.616	0.711	0.782	0.853	CV%	P
	30500	31857	45000	33000	36500	32.88	0.269
	42 days						
	Basal	0.571	0.647	0.704	0.761	CV%	P
	39000	36000	37000	43000	42000	22.07	0.505

CV% = coefficient of variation. No significant differences were found.

and higher in birds fed with the diets with the three highest Met + Cys levels, which were all similar to each other. A reduction in the CHOL, LDL and TG concentrations in the blood of birds was recorded in the basal diet and at the second methionine supplementation level. These results concurred with a study by BOUYEH (2012) who reported an effect on TG, CHOL, LDL and HDL concentrations in Ross 308 male broilers at 21 days of age submitted to different levels of methionine and lysine.

With the results of this study, it can be suggested that diets with deficient levels of Met + Cys reduce the concentrations of CHOL and LDL in the blood. This also suggested that broiler diets with deficient levels of digestible Met + Cys may compromise the metabolism of non-esterified fatty acids, such as LDL (the lower the LDL values, the better) and TG. This is probably because sulfur amino acids inhibit the hydroxymethylglutaryl-CoA reductase enzyme, which is responsible for cholesterol

Table 5 - Serum levels of UA, ALB, AST, TSP, CHOL, HDL, LDL and TG in broilers at 21 and 42 days of age fed with diets with different levels of digestible methionine + cystine.

Met+ Cys %	UA (mg/dL)	ALB (g/dL)	AST (UI/L)	TSP (g/dL)	CHOL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)
21 days								
Basal	2.585 [b]	1.103 [b]	210.950	2.047 [c]	104.667 [b]	89.217	9.040 [b]	25.000 [b]
0.616	2.737 [b]	1.360 [a]	215.517	2.678 [b]	117.000 [b]	100.717	11.083 [b]	26.000 [b]
0.711	4.187 [a]	1.423 [a]	232.717	2.755 [b]	127.000 [a]	104.650	13.020 [a]	36.667 [a]
0.782	4.857 [a]	1.352 [a]	249.740	3.297 [a]	136.333 [a]	113.950	15.350 [a]	33.400 [a]
0.853	4.697 [a]	1.370 [a]	231.367	3.010 [a]	126.167 [a]	106.950	10.400 [b]	34.500 [a]
CV%	42.61	7.91	12.61	12.63	13.16	12.77	24.68	11.03
P	0.056	<0.001	0.267	<0.001	0.026	0.043	0.016	<0.001
42 days								
Basal	2.642	1.390	524.867	3.238 [c]	108.667	91.283	10.450	34.667
0.571	2.282	1.390	509.450	3.377 [c]	123.000	97.983	16.950	40.333
0.647	2.866	1.458	713.933	4.508 [a]	121.167	88.317	22.183	48.000
0.704	3.324	1.366	720.440	3.792 [b]	115.400	89.580	16.780	45.200
0.761	3.347	1.447	540.540	4.296 [a]	130.500	100.700	21.800	37.000
CV%	31.13	7.73	31.51	6.34	11.86	14.06	42.50	20.00
P	0.280	0.611	0.172	<0.001	0.130	0.425	0.077	0.094

Values followed by different letters in the same column indicate statistically significant differences by the Scott-Knott test. CV% = coefficient of variation. Uric Acid (UA), Albumin (ALB), Aspartate aminotransferase (AST), Total Serum Proteins (TSP), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Triglycerides (TG).

synthesis, thus reducing plasma cholesterol levels in the body. Methionine plays an important role in lipid metabolism in birds (KALBANDE et al., 2009). The significant increase in CHOL, LDL, and TG observed in this study for the highest Met + Cys levels may be an acceptable explanation considering the above-mentioned evidence.

No significative difference was observed in the growth phase for UA, ALB, AST, CHOL, LDL, HDL, and TG levels with increased digestible Met + Cys concentrations in the diets (Table 5). Similar results were reported by RODRIGUEIRO et al. (2000), who did not report a significant effect of Met + Cys levels on UA concentrations in the serum of broilers in the growth phase. HERNANDEZ et al. (2012) reported that plasma UA concentrations were not affected by increased levels of protein in the diet of broilers in the initial, growth and final phases. Results reported in this study for ALB disagree with the results of NIKOOFARD et al. (2016), who showed that ALB levels increased significantly with the increase of crystalline methionine levels in the diet of male Ross 308 broilers at 42 days of age.

TSP values differed (P<0.05) among the different digestible Met + Cys levels. There was a stepwise increase of TSP levels at 21 days of age, similar to what was observed for the higher digestible Met + Cys levels for 42-day-old broilers. This finding disagrees with a study by AL-MAYAH (2006), which did not find a significant difference in TSP and ALB values in 28-day-old broilers receiving different levels of DL-methionine. ZHANG & GUO (2008) demonstrated that DL-2-hydroxy-4-methylthio butanoic acid (LMA) in liquid form, used as a source of methionine in the diet, did not influence serum TSP levels in 21-day old Arbor Acres male broilers.

AST activity was not influenced (P>0.05) by the treatments in any of the phases evaluated. These values are within the values considered normal for 21-day old birds, which ranges from 202 to 325 IU/L[1]. In general, AST values of 350 IU/L indicated moderate elevations of the enzyme and from of 800 IU/L show marked elevations, indicating severe hepatocellular damage if accompanied by biliverdinuria or biliverdinemia (CAPITELLI, 2013).

CONCLUSION

The data of the present study showed that the digestible Met + Cys levels in the diet of broilers affect the hematological parameters and serum biochemistry, especially at higher levels. From the inclusion level 0.761 of Met + Cist in the broiler diet,

red blood cells, hemoglobin and hematocrit changes begin to appear.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

DECLARATION OF CONFLICT OF INTERESTS

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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