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## Influence of Sex, Age, and Fasting on Blood Parameters and Body, Bursa, Spleen and Yolk Sac Weights of Broiler Chicks

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### ■ Keywords

Blood parameters, broiler chicks, bursa of  
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### ABSTRACT

The effects of water and feed fasting for 24, 48 and 72 hours post-hatching on blood parameters (mean corpuscular volume, MCV; red blood-cell, RBC; hematocrit, HCT; hemoglobin, HGB; plasma glucose, CGP; plasma total protein, PP, and differential leukocytes count), and on body, liver, spleen, bursa, and yolk sac weights were analyzed. Erythrogram data were obtained with a blood cell counter. Total plasma protein and plasma glucose were determined by using the Bradford method (1976) and a glucose PAP liquiform kit (Labtest, cat. n. 84), respectively. Specific leukocyte counts were carried out on blood smears stained with Rosenfeld solution. According to the obtained data, water and feed post-hatching fasting reduced MCV values, which also were lower in males than that in females. Fasting for 48 hours promoted an increase in PP, while fasting for 72 hours reduced HCT. Chicks submitted to fasting presented lower body weights as compared to fed chicks, but their liver weight did not increase between 48 and 72 hours of age. Fasting decreased spleen weight, but bursa and yolk sac weight were not affected. Data showed that female and male chicks react in a similar way to post-hatching fasting, which affects body weight, liver and spleen weight, and HCT and PP values. Moreover, 72 hours of fasting affected more intensely HCT and MCV values.

### INTRODUCTION

The first post-hatching week is an essential period for broiler development and corresponds to approximately 17% of its growth period before slaughter (Lilburn, 1998). During this period, most of the energy and proteins (amino acids) are utilized for the development and morphological and functional maturation of the organic systems, especially of the mid-gut, and overall body growth of the young chicks (Maiorka, 2002).

Water and food fasting reduces intestinal digestive and absorptive area as a result of slower development of the intestinal villi and of an increase in cell extrusion rate (Yamauchi *et al.*, 1996; Gomide *et al.*, 2004). Body weight also decreases in 5 to 10% because of water loss by respiratory evaporative process (Baião & Cançado, 1998), which can affect bird maximal growth potential.

After placement, the chick intestinal mucosa is exposed to a diversified microflora, to which the body reacts by activating its immune system. Therefore, it is important that, at placement, young chicks do not present changes in the hematological profile that can affect the quality of their immune reaction to new intestinal microbiota or make them susceptible to enteric diseases.

In the present study, the effects of post-hatching water and feed fasting on blood parameters and body, spleen, liver, and yolk sac weights of the male and female chicks were analyzed.



## MATERIALS AND METHODS

Approximately 200 fertile eggs ( $57.5 \pm 2.1$  g) from 29-week-old broiler breeders (Cobb500®) were obtained from a commercial hatchery, and incubated at 37.8°C and 60% humidity in two forced-air rotating incubators (IP120, Premium Ecologica) with automatic control of egg turning (1 turn every 2 hours) and temperature. Immediately after hatching chicks were sorted by sex (using sex-linked feather phenotype), and male and female chicks were divided into 4 groups of 15 birds per sex, with two groups fed water and feed (2,800 kcal/Kg and 22% protein) *ad libitum*, and two groups submitted to water and feed fasting. All groups were reared in commercial cages (Premium Ecologica, 50cm x 90cm), containing two 40W light bulbs (one green and another blue). Twenty four, 48 and 72 hours after the beginning of the experimental treatments, birds were weighed and 6 birds from each treatment were randomly selected (3 birds per replicate) and slaughtered after blood collection.

Data for the erythrogram were followed a 2 (fed *ad libitum* with water and feed, or submitted to water and feed fasting) x 2 (female and male) x 3 (24, 48 and 72 hours of treatment) factorial arrangement in a completely randomized experimental design. Body and organ weight data followed a 2 (fed *ad libitum* with water and feed or submitted to water and feed fasting) x 2 (female and male) x 2 (48 and 72 hours of treatment) factorial arrangement in a completely randomized experimental design.

Blood samples were collected from wing vein, placed in plastic vials containing EDTA (15µl/ml blood), and put on ice, and submitted to the lab in order to determine hematocrit, red blood cell count, hemoglobin, mean corpuscular volume, white blood cells count, and plasma glucose and protein. Immediately after blood collection, liver, spleen, bursa of Fabricius, and yolk sac were removed and weighed.

Hematocrit (HCT, %), hemoglobin (HGB, g/dL), mean corpuscular volume (MCV, fl), and red blood cell (RBC,  $\times 10^6$ /mL) values were obtained using a blood cell counter (Celm, mod. 550), with one reading per bird. For each reading, 20µL of blood were used.

For plasma protein (g/dL) and glucose (mg/dL) determination, blood samples were centrifuged at 1,500 x g at 4°C for 15 min. Plasma samples were placed into plastic vials and stored at -20°C until analysis. Total plasma protein was determined using the Bradford method (1976), whereas plasma glucose was determined using a glucose PAP liquiform kit (Labtest,

cat. n. 84). Two readings per bird were made for glucose and total protein in 505nm and 595nm, respectively.

Specific leukocyte counts were carried out in blood smears stained with Rosenfeld solution. Monocyte, lymphocyte, heterophil, eosinophil, and basophil counts were determined by counting the number of each leukocyte type in 100 analyzed cells. Data are expressed as estimated percentages of the 100 analyzed cells (González *et al.*, 2003).

Data were submitted to analysis of variance, and significant treatment means were separated by Tukey's test ( $p < 0.05$ ), using the SAS software package (2002).

## RESULTS AND DISCUSSION

In the present study, body weight was significantly ( $p < 0.05$ ) influenced by sex, age, and treatment. Body weight was higher in females than in males, in 72-hour-old chicks than in 48-hour-old chicks, and in chicks that received water and feed than in fasted chicks (Table 1). However, there was a significant ( $p < 0.05$ ) interaction between age and treatment, showing that chicks submitted to fasting did not increase in body weight from 48 to 72 hours of age (Table 3). Liver weight was not affected by sex, but it was significantly ( $p < 0.05$ ) influenced by age and treatment, being higher in 72 h-old than in 48 h-old chicks, as well as being in fed as compared to fasted chicks. In addition, significant interaction ( $p < 0.05$ ) was observed between age and treatment (Table 3): liver weight of fasted chicks did not increase from 48 to 72 hours, and these chicks reached 72 h of age with lower liver weight as compared to fed chicks. Sex and age did not affect ( $p > 0.05$ ) spleen weight, but treatments had a significant ( $p < 0.05$ ) on this parameter (Table 1). Spleen was heavier in fed than in fasted chicks. No significant ( $p > 0.05$ ) effect of sex, age, or treatment was observed on yolk sac weight (Table 1), and there were no significant interactions among sex, age, and treatment for this parameter.

These data show that chicks submitted to post-hatching fasting for 48 and 72 hours did not gain weight, and did not increase liver and spleen weights, as observed in fed chicks after hatching. In addition, fasted chick body weight was 12% and 40% lower, and liver weight 15.4% and 52.3% lower as compared to fed chicks at 48 and 72 hours post-hatching, respectively. The post-hatching period is characterized by rapid body growth and by morphological and



functional differentiation of the organic systems, which utilize a large part of the ingested energy and proteins (Noy & Sklan, 1999). It is well-known that yolk is the largest source of nutrients for chick during their first days of life outside the egg. Therefore, it would be expected that yolk nutrients were utilized more quickly by fasted chicks than by fed chicks, allowing young chicks to maintain their physiological functions. However, similarly to the observations of Baião & Cançado (1998), and Maiorka *et al.* (2003), no difference in yolk sac weight due to fasting was observed in the present study, indicating that feed ingestion after hatching is essential for chick body growth and organ development. Negative effects of the post-hatching food and water deprivation on liver weight were also reported by Donaldson & Christensen (1991) and Maiorka (2002).

**Table 1** - Effects of sex (female or male), age (48 or 72 hours), and treatment (fed with water and feed, water and feed fasted) on body weight, and liver, spleen, bursa, and yolk sac weights in newly-hatched broiler chicks.

Probability		Weights (g)				
		Body	Liver	Spleen	Bursa	Yolk Sac
S		0.0151	0.6759	0.7263	0.3397	0.4548
A		0.0001	0.0001	0.9031	0.6244	0.3698
T		0.0001	0.0001	0.0012	0.8705	0.7592
SxA		0.6154	0.6759	0.4553	0.2470	0.4641
SxT		0.7390	0.2179	0.1325	0.3093	0.2169
AxT		0.0001	0.0001	0.4152	0.0657	0.5896
SxAxT		0.5626	0.5279	0.9566	0.3886	0.2552
Sex (S)	Female	45.4 a	1.4 a	0.030 a	0.07 a	2.21 a
	Male	43.3 b	1.3 a	0.025 a	0.05 a	1.91 a
Age (A)	48	40.3 b	1.2 b	0.030 a	0.063 a	2.22 a
	72	49.5 a	1.6 a	0.024 a	0.060 a	1.96 a
Treatment (T)	Fed	50.9 a	1.7 a	0.032 a	0.070 a	2.11 a
	Fasted	36.6 b	1.0 b	0.020 b	0.061 a	2.06 a

a-b - Means followed by different letters in the same column are significantly different ( $P < 0.05$ ) by Tukey's test.

**Table 2** - Effects of sex (female or male), age (48 or 72 hours), and treatment (fed with water and feed or water and feed fasted) on RBC ( $\times 10^6/\text{mm}^3$ ), MCV ( $\mu\text{m}^3$ ), HCT (%), HGB (g/dl), plasma protein (PP,  $\mu\text{g}/\mu\text{l}$ ), and plasma glucose (PG, mg/dl) values in newly-hatched broiler chicks.

children:

Probability	RBC	HCT	MCV	HGB	PP	PG	
S	0.1589	0.1866	0.0247	0.0667	0.6041	0.0001	
A	0.0352	0.0553	0.2535	0.1732	0.0001	0.0001	
T	0.7918	0.9350	0.0002	0.5329	0.0001	0.0001	
SxA	0.1409	0.0387	0.6785	0.2997	0.2871	0.0009	
SxT	0.9338	0.7771	0.4631	0.8064	0.1013	0.0001	
AxT	0.0488	0.0196	0.0004	0.1653	0.0001	0.0001	
SxAxT	0.9248	0.8679	0.0976	0.9963	0.3283	0.0007	
Sex (S)	Female	24.9 a	2.2 a	113.1 a	11.61 a	2.00 a	208.81 a
	Male	22.9 a	2.0 a	111.2 b	10.25 a	2.01 a	195.32 b
Age (A)	24	23.0 b	2.0 b	111.6 a	10.61 a	1.82 c	208.03 b
	48	22.7 b	2.0 b	112.1 a	10.34 a	2.24 a	219.14 a
	72	27.0 a	2.4 a	113.3 a	12.25 a	1.94 b	198.46 b
Treatment (T)	Fed	23.8 a	2.1 a	113.6 a	10.70 a	1.89 b	222.51 a
	Fasted	24.2 a	2.1 a	111.0 b	11.26 a	2.12 a	196.47 b

a-b - Comparisons between sexes (columns), among ages (columns), and between treatments (columns). Means followed by different letters in the same column are significantly different ( $P < 0.05$ ) by Tukey's test.

**Table 3** - Effects of interactions between age (48 and 72 hours) and treatment (fed with water and feed, water and feed fasted) on body and liver weights, and on RBC ( $\times 10^6/\text{mm}^3$ ), MCV ( $\mu\text{m}^3$ ), HCT (%), plasma protein (PP,  $\mu\text{g}/\mu\text{l}$ ) values in newly-hatched broiler chicks.

	Age	Treatments	
		Fed	Fasted
Body Weight	48	42.7 bA	37.6 aB
	72	59.0 aA	35.2 aB
Liver Weight	48	1.3 bA	1.1 aB
	72	2.1 aA	1.0 aB
RBC	24	1.9 bA	2.1 aA
	48	1.9 bA	2.2 aA
	72	2.6 aA	2.2 aA
HCT	24	2.3 bA	24.6 aA
	48	2.4 bA	24.0 aA
	72	30.1 aA	24.1 aB
MCV	24	111.2 bA	111.9 aA
	48	113.3 bA	110.9 aA
	72	117.0 aA	109.9 aB
PP	24	1.84 aA	1.81 bA
	48	1.89 aB	2.58 aA
	72	1.95 aA	1.93 bA

a-b, A-B - Comparisons among ages (columns) and between treatments (rows). Means followed by different letters in the same column and row are significantly different ( $P < 0.05$ ) by Tukey's test.

When dehydrated, the body tries to maintain its homeostatic balance by ingesting water and by reducing water excretion volume or using interstitial and intracellular fluids (Bruno & Macari, 2002). However, animals submitted to fasting, and that are already dehydrated, utilize tissue substances for metabolic energy production, which can also be used by the animals to maintain homeostasis. In the present study, young chicks were submitted to water and feed fasting. Therefore, it is possible that the utilization of interstitial and intracellular fluids, as well as of metabolic energy, is involved in the absence of weight gain and the lack of increase in liver and spleen weights shown by fasted chicks.



Bursa weight results are shown in Table 1. No significant ( $p>0.05$ ) effect of sex, age, or treatment was observed on this parameter. No significant ( $p>0.05$ ) interaction among sex, age and treatment was detected. The bursa presents considerable development after hatching, particularly up to 8 weeks of age (Verma *et al.*, 1999). Our results seem to indicate that the post-hatching bursa development may begin after 72 h of hatching, which could explain the absence of effects of fasting on this organ during the experimental period. The absence of differences between males and females in bursa weight show that both sexes present a similar pattern of early bursal development.

Tables 2 and 3 show erythrogram results. No significant effect of sex or treatment was observed on RBC and HCT, but there was significant ( $p<0.05$ ) effect of age on both parameters. RBC and HCT values were higher in 72 h-old than in 24 or 48 h-old chicks. There was a significant ( $p<0.05$ ) interaction between age and treatment on RBC and HCT, indicating that chicks submitted to fasting did not increase the values of these parameters between 48 and 72 h of age. These chicks reached 72 h of age with lower HCT values than fed chicks. There were significant ( $p>0.05$ ) effects of sex and treatment on MCV values, but not of age (Tables 2 and 3). Female chicks presented higher MCV values than male chicks, and fed chicks had higher values than fasted chicks. In addition, there was a significant ( $p<0.05$ ) interaction between age and treatment (Tab. 3): fed chicks presented an increase in MCV values between 48 and 72 h of age, which was not observed in fasted chicks, and at 72 h, had higher MCV values as compared to fasted chicks. No significant ( $p>0.05$ ) effects of sex, age, or treatment were observed on HGB values, nor any significant interaction among them (Tables 2 and 3).

According to those data, when chicks were submitted to water and feed fasting for 72 hours, there was no increase in RBC, HCT, or MCV values between 48 and 72 hours of age, as reported in the fed chicks, and in addition presented lower HCT and MCV values at 72 hours of age, but with no simultaneous reduction in HGB values. Lower MCV values were also observed by Maxwell *et al.* (1990) in *Gallus domesticus* submitted to feed restriction starting on the fourth week of life. However, in contrast with our data, these authors reported an increase in RBC and reduction in HGB values in chicks submitted to feed restriction. Therefore, our data suggest that the intensity of the erythrocytic response elicited in an animal depends on

the degree of food deprivation to which this animal is submitted. Considering that the fasted chicks did not receive either water nor feed, our results indicate that the simultaneous occurrence of HCT reduction and HGB increase in these birds was caused by the reduction in the of MCV values as a consequence of water fasting. In addition, they suggest that prolonged fasting induced microcytic anemia in the fasted chicks. Dehydration produces changes in blood parameters, particularly in HCT values, which are abnormally increased when the animal is not able to maintain homeostasis (Swenson, 1996). In the present study, the young chicks that were submitted to fasting maintained MCV and HCT values constant during the entire experimental period (72 hours), indicating that they maintained their homeostatic equilibrium under prolonged water and feed fasting, probably using interstitial and intracellular fluids (Bruno & Macari, 2002). It is well known that, with an isometric increase in size, volume increases faster than surface area (Woods, 1999); the opposite is also true. This seems to explain the occurrence of higher MCV values without an increase in the HGB values in the female chicks; however, this but was not observed in males.

Plasma protein (PP) values observed in the present study show significant ( $p<0.05$ ) effects of age and treatment on this parameter (Tables 2 and 3). PP values were higher in chicks submitted to fasting than in fed chicks, and in 48 h-old than in 24 and 72 h-old chicks. However, the significant ( $p<0.05$ ) interaction between age and treatment showed that fasted chicks did not present higher PP values at 48 h as compared to 24 and 72 h of age, and that they presented higher PP values than fed chicks at 48 h of age. This result is consistent with previous reports of Warris *et al.* (1992) that chicks submitted to fasting for 48 hours presented higher PP values as compared to chicks that received water and feed. This increase in the PP values may have been caused by an increase in protein breakdown promoted by the body to maintain its physiological functions during fasting using metabolic energy (Katanbaf *et al.*, 1988). On the other hand, chicks submitted to prolonged fasting (72 hours) presented PP values similar to those obtained by fed chicks at the same age (our data). This result is very interesting and suggests that protein depletion could be used by fasted chicks to obtain energy and/or metabolic water.

Plasma glucose (PG) values were significantly ( $p<0.05$ ) influenced by sex, age, and treatment (Table 2). PG values were higher in female than in male chicks, in 48 h-old than in 24 and 72 h-old chicks, and in fed





than in fasted chicks. Moreover, there was a significant ( $p < 0.05$ ) interaction among sex, age, and treatment for this parameter. Fed females presented higher PG values than fed males only at 72 h of age, while fasted females presented higher values than fasted males in all three analyzed ages. The interaction among sex, age, and treatment also showed that fed females presented an increase in PG values between 48 and 72 h of age, which was not observed in fed males. Fasted females and males presented an increase in this parameter values between 24 and 48 h of age followed by a decrease from 48 to 72 h of age. In addition, it was also shown that fed females presented higher PG values than fasted females at 24 and 72 h of age, while fed males presented lower PG values than fasted males at 48 h of age, and higher PG values than fasted males only at 72 h of age.

These results show that post-hatching fasting influenced PG values both in females and in males, but not at the same time. Females presented a reduction in PG values after 24 hours fasting, indicating an early PG depletion, but the values returned to those observed in fed chicks after 48 hours fasting. On the other hand, males presented an increase in the PG values after 48 h fasting. In both cases, the increase in PG values may be related with liver glycogen utilization, considering that glucose is the main energy metabolite of domestic chicken (Engku-Azahan & Forbes, 1989). However, both sexes presented a marked reduction in the PG values after prolonged fasting (72 h) as compared to the values obtained in chicks submitted to fasting for 24 h and those fed for 72 hours. Moreover, female chicks submitted to prolonged fasting did not present the same increase in PG values between 48 to 72 hours as those observed in fed females. This reduction in PG values indicates that liver glycogen depletion occurs after 48 hours of fasting and in both sexes. These data are very important from a physiological and practical point of view as they show that post-hatching fasting must not be longer than 48 hours. The data obtained with females is consistent with the results reported by Warris *et al.* (1992) in fasted chicks, according to which glucose values remain low under fasting. On the other hand, our results are different from those observed in 7 week-old broilers submitted to water and feed deprivation for 24 hours by Knowles *et al.* (1995). These authors verified a reduction both in PG and in PP values, whereas we observed reduction only in PG. Our data support the findings of Gomide *et al.* (2003), Sterzo *et al.* (2003), and Pires *et al.* (2003), who showed that female and

male chicks present different reactions to post-hatching fasting. In addition, they are consistent with Almeida (2002) and Pires *et al.* (2003), who demonstrated that prolonged fasting (72 hours) has a markedly negative effect on chicks, and recommend that this should be avoided.

**Table 4** – Effects of the interactions between sex (female and male), age (24, 48, and 72 hours) and treatment (fed with water and feed, water and feed fasted) on plasma glucose values (PG, mg/dl) in newly-hatched chicks.

Age	Treatment			
	Female		Male	
	Fed	Fasted	Fed	Fasted
24	212.62 bAx	202.78 bAx	263.24 aAx	179.54 bBy
48	210.86 aAy	139.66 cBy	222.50 aAx	215.53 aBx
72	214.24 aAy	217.44 bAx	247.38 aAx	177.65 cBx

a-c, A-B, x-y - comparisons among ages (columns), between treatments within of each sex and age (rows), and between sexes within the same treatment and age (rows), respectively. Means followed by different letters in the same column are significantly different ( $P < 0.05$ ) by Tukey's test.

Data referring to differential leukocyte counts are presented in Table 5. There was significant ( $p < 0.05$ ) effect of treatment on all leukocyte types. However, monocyte and lymphocyte counts were higher in fed than in fasted chicks, while eosinophil, basophil, heterophil counts and H/L ratios were lower. In both treatments, lymphocytes and heterophils were the most frequent leukocyte types in the blood (Table 5). Fed chicks presented higher ( $p < 0.05$ ) lymphocyte than heterophil counts, whereas fasted chicks had higher ( $p < 0.05$ ) heterophil than lymphocyte counts. There was no significant ( $p > 0.05$ ) effect of sex on differential leukocyte counts and on heterophil/lymphocyte (H/L) ratios. Female and male chicks presented the same lymphocyte and heterophil counts, which were higher ( $p < 0.05$ ) than eosinophil, basophil and monocyte counts, the last two being similar. Age significantly ( $p < 0.05$ ) influenced only basophil and eosinophil counts, which increased between 48 and 72 h of age. In the three analyzed ages, lymphocyte and heterophil counts were similar and higher ( $p < 0.05$ ) than the counts of the other leukocyte types. Monocytes and basophils were found in lower percentages in the blood.

In terms of the effects of fasting on leukocytes, the data above demonstrate the presence of basophilia, heterophilia, and eosinophilia, and an increase in the H/L ratios in fasted chicks. A similar leukocytic response was also found by Maxwell *et al.* (1991, 1992) in adult broilers and turkeys submitted to food restriction. Concurrently to changes in the leukocyte parameters

**Table 5** – Effects of sex (female or male), age (24, 48, or 72 hours) and treatment (fed with water and feed, or water and feed fasted) on differential leukocyte counts and H/L ratios in newly-hatched broiler chicks.

	Monocytes	Basophils	Eosinophils	Heterophils	Lymphocytes	H/L
<b>Sex</b>						
Male	3.74 a	1.56 a	21.36 a	34.28 a	39.06 a	0.97 a
Female	3.31 a	2.30 a	17.02 a	35.56 a	41.81 a	1.00 a
P	0.5616	0.1525	0.0607	0.7675	0.4055	0.8478
<b>Age</b>						
24	3.98 a	1.47 b	16.35 b	32.53 a	45.67 a	0.71 a
48	2.91 a	1.31 b	22.66 a	36.50 a	36.62 a	1.12 a
72	3.90 a	3.32 a	19.81 a	35.48 a	37.49 a	1.02 a
P	0.4819	0.0011	0.0431	0.3602	0.0750	0.4276
<b>Treatment</b>						
Fed	5.48 a	1.50 b	15.36 b	29.46 b	48.20 a	0.66 b
Fasted	5.48 a	2.39 a	24.65 a	41.80 a	30.10 b	1.47 a
P	0.0000	0.0209	0.0000	0.0000	0.0000	0.0000

A-B - Comparisons between sexes, among ages and between treatments (columns). Means followed by different letters in the same column are significantly different ( $P < 0.05$ ) by Tukey's test.

described above, our study also found a reduction in monocyte, lymphocyte, and thrombocyte counts in the fasted chicks. In adult broilers and turkeys submitted to feed restriction, Maxwell *et al.* (1991) and Maxwell *et al.* (1992) reported only a reduction in thrombocyte and lymphocyte counts, respectively. These differences and similarities between our results and those of other authors show that bird immune reactions change with the intensity and the type of stress to which they are exposed, and that reduction in the monocyte count occurs under severe stressful conditions (in this case, prolonged water and feed fasting). Our data support the results obtained by Gross & Siegel (1986), who also reported an increase in H/L ratios in chicks submitted to food fasting. However, our results are different from those reported by Hocking *et al.* (1993) and De Jong *et al.* (2002), who did not observe changes in the H/L ratios in adult broilers submitted to food restriction. These differences between our data and those obtained by other researchers in birds submitted to stress promoted by fasting are related to fasting duration and type, as well as to bird age during fasting.

There were no effects of sex on differential leukocytes counts. In both sexes, heterophils, lymphocytes, and eosinophils were the most numerous leukocytes in the blood, corresponding to approximately 38, 30, and 15-20% of the total number of immune cells, respectively. Lymphocytes and heterophils were also the most frequent cells in the blood of layers, broilers, and turkeys (Bounous & Steedman, 2000). According to our results, there was an increase in the eosinophil and basophil counts according to bird's age. After hatching, birds are exposed to a high diversity of microorganisms that constitute the natural intestinal microbiote and that

stimulate their immune system (Spencer & Garcia, 1995). This exposure to new agents appears to explain the increase in leukocyte counts after hatching observed in the present study.

Similarly, as verified in the present study, Maxwell *et al.* (1991, 1992) also observed an increase in basophil, heterophil, and eosinophil counts, and H/L ratios in broilers and turkeys submitted to feed restriction, respectively. We observed a reduction in thrombocyte, lymphocyte, and monocyte counts, while a reduction in thrombocyte counts was found only in broilers (Maxwell *et al.*, 1991) and a reduction in thrombocyte counts only in turkeys (Maxwell *et al.*, 1992). Gross & Siegel (1986) also reported an increase in H/L ratio of chicks submitted to fasting. Alternatively, Hocking *et al.* (1993) and De Jong *et al.* (2002) did not find any changes in H/L ratio of broilers caused by feed restriction. These data suggest that the type and the intensity of immune reaction depend on stress type, fasting degree, stress duration and age in which the birds are submitted to stress, and that reduction in monocyte counts are caused by severe stress caused by prolonged fasting.

## CONCLUSION

Post-hatching water and feed fasting for a period longer than 24 hours markedly affects organ growth, blood parameters, and immune system of broilers of both sexes. Water and feed must be offered immediately after hatching to prevent detrimental changes in the physiological functions of broilers.

## REFERENCES



- Almeida JG. Efeito do intervalo de tempo entre o nascimento e o alojamento no desempenho, características de carcaça e viscerais de frangos de corte provenientes de matrizes de diferentes idades [dissertação]. Jaboticabal (SP): Universidade Estadual Paulista; 2002.
- Baião NC, Cançado SV. Efeito do intervalo entre nascimento e o alojamento de pintos sobre o desempenho dos frangos. *Arquivo Brasileiro de Medicina Veterinária e Zootenia* 1998; 50:191-194.
- Bounous DI, Stedman NL. Normal avian hematology: chicken and turkey. In: Feldman, BF, et al. *Schalm's veterinary hematology*. 5<sup>th</sup> ed. Philadelphia: Lippencolt; 2000. p.1147-1154.
- Bradford MM. A rapid and sensitive method for the quantitation of microgran quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 1976; 72:248-254.
- Bruno LDG, Macari M. Ingestão de água: mecanismos regulatórios. In: Macari M, Gonzales E, editores. *Fisiologia aviária aplicada a frangos de corte*. Jaboticabal: Facta; 2002. p.201-208.
- De Jong IC, Van Voorst S, Ehlhardt DA, Blokhuis HJ. Effects of restricted feeding on physiological stress parameters in growing broiler breeders. *British Poultry Science* 2002; 43:157-168.
- Donaldson WE, Christensen VL. Dietary carbohydrate level and glucose metabolism in turkey poults. *Comparative Biochemistry and Physiology* 1991; 98(A):347-350.
- Engku Azahan EA, Forbes JM. Growth, food intake and energy balance of layer and broiler chickens offered glucose in the drinking water and the effect of dietary protein content. *British Poultry Science* 1989; 30(4):907-917.
- Gonzales E, Kondo N, Saldaña ES, Loddy MM, Careghi C, Decuypere E. Performance and physiological parameters of broiler chickens subjected to fasting on the neonatal period. *Poultry Science* 2003; 82:1250-1256.
- Gomide Junior MH, Sterzo EV, Pires DL, Boleli IC. Efeitos da injeção de ácido ascórbico in ovo sobre a densidade e integridade dos vilos intestinais de frangos fêmeas submetidos a jejum pós-eclosão. 11º Simpósio Internacional de Iniciação Científica da USP; 2004; Piracicaba, São Paulo. Brasil.
- Gross WB, Siegel PB. Effect of initial and second periods of fasting on heterophil/lymphocyte ratios and body weight. *Avian Disease* 1986; 30:345-346.
- Hocking PM, Maxwell MH, Mitchell MA. Welfare assessment of broiler breeder and layer females subjected to food restriction and limited access to water during rearing. *British Poultry Science* 1993; 34:443-458.
- Katanbaf MN, Jones DE, Dunnington EA, Gross WB, Siegel PB. Anatomical and physiological responses of early and late feathering broiler chickens to various feeding regimes. *Archives Geflügelkeit* 1988; 52:119-126.
- Knowles TG, Warriss PD, Brown SN, Edwards JE, Mitchell MA. Response of broilers to deprivation of food and water for 24 hours. *British Veterinary Journal* 1995; 151(2):197-202.
- Lilburn MS. Practical aspects of early nutrition for poultry. *Journal of Applied Poultry Research* 1998; 7:420-424.
- Maiorka A. Desenvolvimento do trato gastrointestinal de frangos [dissertação]. Jaboticabal (SP): Universidade Estadual Paulista; 2002.
- Maiorka A, Santin E, Dahlke F, Boleli IC, Furlan RL, Macari M. Posthatching water and feed deprivation affect the gastrointestinal tract and intestinal mucosa development of broiler chicks. *Journal of Applied Poultry Research* 2003; 12:483-489.
- Maxwell MH, Robertson GW, Spence S, Mccorquodale CC. Comparison of haematological values in restricted –and ad libitum-fed domestic fowls: red blood cell characteristics. *Poultry Science* 1990; 31:407-13.
- Maxwell MH, Robertson GW, Anderson IA, Dick LA, Lynch M. Haematology and histopathology of seven-week-old broilers after early food restriction. *Research in Veterinary Science* 1991; 50:290-297.
- Maxwell MH, Hocking PM, Robertson GW. Differential leucocyte responses to various degree of food restriction broilers, turkeys and ducks. *British Poultry Science* 1992; 33(1):177-187.
- Noy Y, Sklan D. The effect of different types of early feeding on performance in chicks and poults. *Journal of Applied Poultry Research* 1999; 8:16-24.
- Pires DL, Boleli IC, Gomide Junior MH, Sterzo EV. Efeito da injeção de ácido ascórbico in ovo sobre a concentração de proteína plasmática em pintos submetidos ou não a jejum pós-eclosão [CD-ROM]. Congresso de Iniciação Científica da UNESP; 2003; Marília, São Paulo. Brasil.
- SAS Institute. Software and services: system for windows. Version 8.0 software. Cary, USA; 2002.
- Spencer JL, Garcia MM. Resistance of chicks and poults fed vermicompost to ceecal colonization by *Salmonella*. *Avian Pathology* 1995; 24:157-70.
- Sterzo EV, Boleli IC, Gomide Junior MH, Pires DL, Satake FM. Efeito da injeção de ácido ascórbico in ovo sobre a integridade dos vilos intestinais de frangos machos submetidos a jejum pós-eclosão [CD-ROM]. XV Congresso de Iniciação Científica da UNESP; 2003; Marília, São Paulo. Brasil.
- Swenson MJ. Propriedades fisiológicas e constituintes químicos e celulares do sangue. In: Swenson M.J, Reece WO, editors. *Dukes: fisiologia dos animais domésticos*. 11<sup>th</sup> ed. Rio de Janeiro: Guanabara Koogan; 1996. 853p.
- Verma D, Malik MR, Parmar ML, Taluja JS. Morphogenesis of bursa of fabricius in chick. *Indian Journal of Animal Sciences* 1999; 69(5):295-297.
- Warriss PD, Kestin SC, Edwards JE. Response of newly hatched chicks to inanition. *Veterinary Records* 1992; 130(3):49-53.
- Woods HA. Egg-mass size and cell size: effects of temperature on oxygen distribution. *American Zoology* 1999; 39:244-252.



Pires DL, Malheiros EB, Boleli IC



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Yamaushi K, Kamisoyama H, Isshiki Y. Effects of fasting and refeeding on structures of the intestinal villi and epithelial cells in White Leghorn hens. *British Poultry Science* 1996; 37:909-921.