Effects of post-hatching feeding management (fasting) on blood parameters of broilers

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A number of 200 fertile eggs (57.34 ±0.81 g) from 29-week-old Cobb broiler breeders were incubated. After hatching, chicks were sexed and weighed. Three sexed groups were designated to different post-hatching feeding management: feed and water ad libitum, 24 hours fasting, and 48 hours fasting. Feed and water were offered ad libitum to the chicks in treatments 2 and 3 after fasting period until 120 hours of age. Statistical analysis included analysis of variance followed by least squares regression, and means were compared by F Test at 5% significance level. For each age period, 4 birds per group were weighed and euthanized. Blood was collected in Eppendorf tubes containing a drop of anticoagulant. Fit equations were determined for each blood parameter tested: plasma glucose concentration (PGC, mg/dL), plasma protein concentration (PPC, µg/dL), total red blood cell count (RBC, x10⁶/µL), hematocrit volume (HCT, %), mean corpuscular volume (MCV, fL), hemoglobin concentration (HGB, g/dL), and specific lymphocytes count (L, %) and heterophil (H, %) to determine heterophil/lymphocyte ratio (H/L).

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\begin{align*}
\text{PGC} &= 191.22 - 1.91271 T + 0.03504 T^2 + 0.62285 A (R^2 = 0.2675); \\
\text{PPC (females)} &= 1.22 + 0.00480 T + 0.02558 A - 0.00015 A^2 (R^2 = 0.2488); \\
\text{PPC (males)} &= 2.78 - 0.00364 T + 0.00096T^2 - 0.00677 A + 0.00009 A^2 - 0.00055 TA (R^2 = 0.5397); \\
\text{RBC} &= 1.17 + 0.01355 T + 0.02953 A - 0.00016 A^2 - 0.00022 TA (R^2 = 0.2488); \\
\text{HCT} &= 15.25 + 0.14548 T + 0.28015 A - 0.00157 A^2 - 0.00248 TA (R^2 = 0.1135); \\
\text{MCV (females)} &= 0.00011 - 0.04507 T - 0.13893 A + 0.00145 A^2 (R^2 = 0.4164); \\
\text{MCV (male)} &= 109.07 + 0.71290 A (R^2 = 0.2761); \\
\text{HGB} &= 6.61 + 0.10260 T + 0.11182 A - 0.00054 A^2 - 0.00146 TA (R^2 = 0.1182); \\
\text{H} &= 40.81 - 0.15528 T + 0.71576 A - 0.00719 A^2 (R^2 = 0.4485); \\
\text{L} &= 92.12 - 2.79 T + 0.04134 T^2 - 0.00016 T^3 (R^2 = 0.3726); \\
\text{H/L} &= -2.41 + 0.23095 T + 0.00354 T^2 + 0.00001 T^3 (R^2 = 0.3404), \\
\end{align*}
\]

where T is fasting time (in hours), and A is age (in hours). Statistical differences were observed between sexes for PPC and MCV, and these were analyzed separately. Fasting time affected L and H/L ratio, and age (A) affected MVC (males). TxA interaction significantly affected PPC (males), RBC, HCT, and HGB. Fasting time (T) and Age (A) affected PGC, PPC (females), MCV (females), and H, but their interaction was not significant.

Key-words: chick, fasting, hatch, hematology, poultry
Introduction

Studies on the effects of fasting between chick hatching and housing on production parameters evidence that access to water and feed immediately after hatching is particularly important for the bird’s subsequent performance (Moran, 1990). However, there is not detailed information in literature on the effects of post-hatching fasting, followed or not by feeding, on chick blood parameters, or if males and females present the same response.

This study aimed at analyzing the hematological profile of female and male broiler chicks submitted to post-hatching fasting followed by provision of water and feed ad libitum.

Material and methods

Fertile eggs (57.34±0.81g) from 29-week-old COBB 500 broiler breeders were incubated at 37.8°C and 60% RH, and turning every 2 hours.

A completely randomized experimental design was used, and each sex was designated to a 3 x 5 factorial design with three treatments (ad libitum feed and water supply; feed and water fasting for 24 hours, followed by ad libitum feed and water supply; feed and water fasting for 48 hours, followed by ad libitum feed and water supply) and five ages (24, 48, 72, 96, and 120 h of age). Chicks were housed in brooders, and fed with a pre-starter feed based on corn and soybeans, containing 22% CP and 2900 Kcal ME/Kg. At the different ages, four birds per treatment per sex were euthanized by decapitation for blood collection.

Blood samples of 20µL per bird were used to evaluate hematocrit rate (HCT, %), hemoglobin concentration (HGB, g/dL), mean corpuscular volume (MCV, fL), and global red blood cell count (RBC, x10⁶/µL). The Kit Glucose PAP liquiform (Labtest, 500ml, cat. 84) was used to determine plasmatic glucose dosage. Two spectrophotometer readings, using 505nm wavelength were performed for each bird. Plasmatic protein concentration was determined by conventional biochemical assay of Bradford (Bradford, 1976). Plasma samples were prepared using 15µL of plasma added to 20 µL of water and 1mL of Bradford solution (50mg Coomassie Brilliant Blue G + 25ml Ethanol 95% + 50ml orthophosphoric acid at 85% + 400ml bi-distilled water). Readings (two per bird) were performed in spectrophotometer at 595nm wavelength.

Differential leukocyte count used blood smears individually stained with modified Rosenfeld dye (0,97g Giemsa + 054g May-Grünwald + 1L Methanol + 0,5g Wright). One hundred leukocytes in the blood smears were analyzed for specific lymphocytes and heterophils count in order to determine heterophil/lymphocyte ratio (H/L) (according to Gonzales et al., 2003).

Results were submitted to analysis of variance and regression analysis using least square methods, and evaluated using the F Test at a 5% significance level.

Results and discussion

Glucose plasma concentration (GPC) was not influenced by sex (p>0.05). Within the analyzed fasting duration and age, GPC was described by a quadratic effect as a function of fasting and age ($\hat{Y} = 191.22 - 1.91271T + 0.03504T^2 + 0.62285I$; $R^2 = 0.2675$). Independent of fasting duration, GPC increased with age. However, chicks submitted to fasting continued to present lower GPC values as compared to chicks fed ad libitum even after being fed for 72h. According to Rupley (1999), the decrease in glucose values may be associated to starvation, hepatopathy, septicemia, neoplasia, aspergillosis, endocrinopathy,
and malnutrition, as happened in our experiment. Despite this decrease, chicks never presented hypoglycemia, which is characterized by glucose levels lower than 150 mg/dL (Rupley, 1999).

Plasmatic protein concentration (PPC) was influenced by sex (p<0.05). In females and males, within the analyzed fasting durations and ages, it was described by a quadratic effect as a function of fasting and age \( \hat{Y} = 1.22 + 0.00480 T + 0.02558 I - 0.00015 I^2; R^2 = 0.2488; \)
\[ \hat{Y} = 2.78 - 0.00364 T + 0.00096 T^2 - 0.00677 I + 0.00009 I^2 - 0.00055 TI; R^2 = 0.5397; \] (respectively). Post-hatching fasting followed by feeding did not change female PPC profile; however, it caused an increase of PPC values as compared to females fed ad libitum, independent of age. In all treatments, females presented higher PPC values at 96 h of age. PPC values recorded for males were different from those of females. Chicks fed after 24-h fasting presented lower values as compared to those fed. Although chick submitted to 48-hour fasting initially presented higher PPC as compared to the fed birds, PPC decreased with post-fasting feeding, presenting significantly lower values as compared to fed chicks and those submitted to 24-h fasting.

RBC, HGB, and HCT were not influenced by sex (p>0.05), which is not consistent with data found by Herbert et al. (1989) for ducks. In that study, male ducks had higher RBC, HGB, and HCT than females. These parameters, within the analyzed fasting durations and ages, were described by a quadratic effect as a function of fasting and age \( \hat{Y} = 1.17 + 0.01355 T + 0.02953 I - 0.00016 I^2 - 0.00022 TI; R^2 = 0.2488; \)
\[ \hat{Y} = 15.25 + 0.14548 T + 0.28015 I - 0.00157 I^2 - 0.00248 TI; R^2 = 0.1135; \]
\[ \hat{Y} = 6.61 + 0.10260 T + 0.11182 I - 0.00054 I^2 - 0.00146 TI; R^2 = 0.1182, \] respectively. Chicks submitted to feed and water fasting presented higher RBC, HGB, and HCT values than fed chicks; however, after fasted birds were fed, the values of these parameters were significantly lower as compared to fed chicks. These lower values may be related with the lower plasmatic volume caused by water fasting, as well as the increase in these values is possibly related to the large volume of water ingested after fasting. An increase in HCT values resulting from feed and water fasting was also found in turkeys (Augustine, 1982) and broilers (Knowles et al., 1995), with 2 and 7 weeks of age, respectively. As to age, in the present study, fed chicks presented an increase in RBC, HGB, and HCT values during the first week of age, which was also observed in ducks by Herbert et al. (1989).

MCV was influenced by sex (p<0.05). In females, within the analyzed fasting durations and ages, it was described by a quadratic effect as a function of fasting and age \( \hat{Y} = 0.00011 - 0.04507 T - 0.13893 I + 0.00145 I^2; R^2 = 0.4164. \) Independent fasting duration, MCV increased with age. Independent of age, MCV decreased females fed after fasting as compared to those immediately fed. These results were also observed by Maxwell et al. (1990b) in broilers submitted to feed restriction. In males, MCV is described by a linear effect as a function of age \( \hat{Y} = 109.07 + 0.71290 I; R^2 = 0.2761. \) There was no influence of fasting duration on MCV values, which increased with age.

According to Thrall et al. (2004), similarly to mammals, bird HCT is affected by changes in the plasmatic volume, as well as by erythrocyte number and size. In the present study, fed chicks increased RBC, HCT, HGB, and MCV values as they aged, indicating a relationship between these parameters and age. However, when feed and water were offered after fasting, RBC, HTC, and HGB values dropped, probably due to the high water intake, changing the ratio between these parameters and MCV, as mentioned above.

The number of heterophils (H) was not influenced by sex (p>0.05). Within the analyzed fasting durations and ages, it was described by a quadratic effect as a function of fasting and age \( \hat{Y} = 40.81 - 0.15528 T + 0.71576 I - 0.00719 I^2; R^2 = 0.4485. \) H number decreased as fasting duration and age increased.

The number of lymphocytes (L) was not influenced by sex (p>0.05) and was described by a cubic effect as a function of fasting duration jejum \( \hat{Y} = 92.12 - 2.79 T + 0.04134 T^2 - 0.00016 T^3; R^2 = 0.3726. \) L numbers markedly decreased as fasting duration increased, but remained constant with age, independent from fasting. A reduction in L numbers was also observed in broilers submitted to feed restriction at 4-12 and 13-20 weeks of age (Maxwell et al., 1990a). These authors also found difference between sexes in this parameter, but this
difference was not observed in the chicks of the present study. The reduction in L and H numbers indicate that post-hatching fasting depresses the cell-mediated immune system of chicks.

Heterophil/lymphocyte ratio (H/L) was not influenced by sex (p>0.05), which is consistent with the findings of Gonzales et al. (2003), and was described by a cubic effect as a function of fasting duration \( \bar{Y} = -2.41 + 0.23095 T + 0.00354 T^2 + 0.00001 T^3; R^2 = 0.3404 \). H/L ratio increased as a function of fasting duration. In addition, independent of fasting, H/L ratio remained constant as a function of age. An increase in H/L ratio was also recorded by Gross & Siegel (1986) in chicks submitted to fasting.

According to Gross & Siegel (1983) and Thrall et al. (2004), the increase in H/L ratio can be used as an indication of stress. Therefore, our data suggest that post-hatching fasting induce blood changes indicating stress, and therefore, depression of the immune system os chicks.

Our results also indicate that feeding after up to 4 days of fasting do not allow the recovery of GPC, PPC, RBC, HCT, HGB, and MCV values in female broiler chicks, as well as of H number and H/L ratio of broiler chicks submitted to fasting.

Other possibly indirect factors, in addition of age and fasting duration, may affect the analyzed parameters, particularly GPC and PPC in females, and RBC, HGB, HCT, and MCV in males.

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References


