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#### ■ Keywords

Aspartate aminotransferase; complete blood count; creatine kinase; lactate; muscle injury.

## Evaluation of Physiological Parameters of Broilers with Dorsal Cranial Myopathy

### ABSTRACT

There are several causes of carcass condemnation in poultry processing plants, including dorsal cranial myopathy (DCM), in which the *anterior latissimus dorsi* (ALD) muscle is affected. DCM etiology has not been elucidated yet, but this lesion impairs the visual quality of carcasses and causes economic losses due to downgrading and condemnation. The effects of this lesion on the systemic health of broilers are still unknown. In this context, the objective of this study was to evaluate muscle injury and systemic health indicators in broilers presenting or not DCM. The following parameters were evaluated: complete blood count (CBC), total protein, albumin, globulin, cholesterol, lactate, and glucose serum levels, aspartate aminotransferase (AST) and creatine kinase (CK) serum activities, and breast muscle pH. Blood samples were collected from 800 42-day-old broilers before feed withdrawal and transportation. In the processing plant, 28 carcasses presented DCM during inspection, and 28 carcasses with no DCM were used as controls. Blood biochemical parameters were not significantly different between broilers with DCM and the controls, except for AST and CK, which activities were higher in the DCM group than in the control group, suggesting that the DCM does not affect the systemic health of the broilers.

### INTRODUCTION

Intensive genetic selection of broilers, aiming at marketing birds at increasingly younger ages, has led to physiological abnormalities, which may cause muscle tissue injuries, such as myopathies (Olivo & Shimokomaki, 2002). The etiology of myopathies, which affect the visual quality of broiler carcasses, has not been elucidated yet (Zimmermann *et al.*, 2012).

The *anterior latissimus dorsi* (ALD) muscle of chickens may present a lesion known as dorsal cranial myopathy (DCM). The affected carcasses present yellowish color among the wings, subcutaneous edema, and an odorless gelatinous citrine-yellow fluid at the affected site. The injured ALD muscle presents hemorrhagic areas, increased density, pallor, adherences to adjacent muscles and, upon sectioning, the affected muscles are thicker than the unaffected ones (Zimmermann, 2008). According to data obtained by the Brazilian Ministry of Agriculture (MAPA), DCM-compatible lesions were first observed in broiler processing inspection lines in 2002, and have since been reported in several processing plants across the country, particularly in the state of Rio Grande do Sul, in southern Brazil. This myopathy causes economic losses due to carcass downgrading and condemnations (Amaral, 2014).

The occurrence of DCM must be notified to the Department of Animal Product Inspection of the state of Rio Grande do Sul as of October, 2010, and, according to MAPA regulations (Brasil, 2010), broiler carcasses presenting DCM must be downgraded or condemned.



In general, carcasses with DCM are trimmed, with the removal of the skin (often from the neck to the cloaca), of the affected and adjacent muscles, as well as of the humerus and of part of the thoracic muscles. Carcasses presenting extensive lesions that compromise their overall visual quality are condemned as a whole (Amaral, 2014).

DCM usually affects apparently healthy heavy broilers. A gradual increase in carcass downgrading and condemnations due to DCM has been detected, particularly in winter, when its incidence may range from 1% (Amaral, 2014) to 6% (Zimmermann *et al.*, 2012). However, as the etiology of DCM has not been elucidated yet, and its effects on the systemic health of broilers are still unknown.

Laboratory tests are used for the diagnosis and assessment of the general health of an animal, but the association between physiological aspects and clinical and laboratory parameters of poultry is not fully elucidated (Schmidt *et al.*, 2007). Blood parameters may be influenced by the bird's nutritional and reproductive status, sex, age, habitat, season of the year, trauma, rearing system, and environmental stress (Campbell, 2004; Thrall, 2004). Blood parameters are used to monitor an animal's general health status, its capacity to fight infectious agents (Voigt, 2003), its physiological status, its reactions to the exposure to different environmental factors (Schmidt *et al.*, 2007), and to diagnose diseases (Noriega, 2000) by evaluating quantitative and qualitative changes in red and white blood cell components (Fudge & Joseph, 2000).

The measurement of the activity of the enzyme aspartate aminotransferase (AST) in the blood provides a non-specific marker of hepatocellular injury, and should be measured along with the activity of creatine kinase (CK) to differentiate liver from muscle injuries. Creatine kinase is a muscle-specific enzyme, and its measurement aids the assessment of muscle function in poultry (Lumeij, 1997). Increased CK activity is an indication of muscle lesion, lead poisoning, septicemias, or myopathies caused by vitamin E and selenium deficiencies. A dramatic increase in serum CK activity is associated with capture myopathy or shock disease, in which AST concentrations do not significantly increase (Campbell, 2004). However, muscle and liver lesions may occur simultaneously, and may be triggered by the same or by different pathological processes (Hochleithner, 1994). Moreover, only marked increases in CK serum activity are clinically meaningful, because light muscle lesions, intramuscular injections, moderate transient muscle ischemia, seizures, and muscle jerks also increase CK activity (Lumeij, 1997).

The objective of this study was to determine the effects of DCM on the systemic health of broilers.

## **MATERIALS AND METHODS**

### **Location, experimental period, birds, and management**

The experiment was carried out between July and August 2013 with broilers reared on three broiler farms and slaughtered at the same processing plant belonging to broiler company in the state of Rio Grande do Sul, Brazil. Male Cobb broilers were obtained from the same hatchery and were submitted to the same vaccination program. Approximately 12,000 broilers were housed on each farm in conventional houses with open sides protected with wire mesh and plastic curtains, and 10-cm deep wood-shavings litter. Environmental temperature was maintained between 24 °C and 28 °C. Birds on the three farms were fed *ad libitum* the same feed, containing equal nutrient and energy levels. Birds were reared from one to 42 days of age.

### **Blood sampling**

Sample size calculation for blood collection took into consideration that DCM lesions can only be observed during *postmortem* examination and its incidence is around 1% in winter, especially in heavier broilers. In total, 800 broilers with 42 days of age were selected on the three farms and identified with a numbered tag: 300 broilers with 2.79 kg average body weight (Farm 1), 200 broilers with 2.76 kg average body weight (Farm 2), and 300 broilers with 2.90 kg average body weight (Farm 3). Blood was collected before feed withdrawal and transport to the processing plant. A 3-mL sample was collected from the ulnar vein of each broiler using a 20x22 needle; 1.5 mL was placed in heparinized tubes and 1.5 mL in tubes containing sodium fluoride (NaF). Blood samples were kept under refrigeration and submitted to the Clinical Analysis Laboratory of Passo Fundo University (UPF), state of Rio Grande do Sul. After blood collection, broilers were caught, and transported to the processing plant.

### **Collection of data and tissue samples from broilers with and without DCM at the slaughterhouse.**

Upon arrival at the processing plant, birds were stunned, bled, scalded, plucked. Carcasses were examined after evisceration by trained employees.

During the *postmortem* examination, 28 carcasses with DCM-compatible lesions were identified, being 13 from Farm 1 and 15 from Farm 3. An equal number of carcasses with no DCM lesions (control group) was selected from farm 1 and 3, and all 56 carcasses from each farm were refrigerated until carcass collection



was completed. No DCM-compatible lesions were detected in carcasses from Farm 2.

The pH of the *pectoralis major* muscle of the collected carcasses was measured shortly after slaughter and inspection, immediately after chilling (baseline pH), and 24 hours after refrigeration at  $0 \pm 2$  °C (ultimate pH), according to Bendall (1973), using a buffered sodium iodo acetate solution (5mM/KCl 150mM) to inhibit glycolysis. Sample pH was read using a pH meter (Sentron, model 1001) coupled to a probe (Sentron, LanceFET, model 1074001), whose fine tip was inserted directly into the muscles. Samples of the ALD muscle, heart, liver, gizzard, pancreas, kidneys, and lungs were collected during the late *rigor mortis* period. Tissue samples were fixed in 10% buffered formalin, dehydrated, embedded in paraffin, and stained with hematoxylin-eosin (HE), as proposed by Luna (1968), at the Laboratory of Avian Pathology (Canoas, RS).

### Blood analyses

The blood in the NaF tubes was centrifuged at 800xg, and the serum was placed in 1.5-mL microtubes, and frozen at -20 °C for determination of lactate (Bioclin, Brazil) and glucose (GOD-PAP; In Vitro, Brazil) serum levels. The blood in the heparinized samples was used for the determination of hematocrit (capillary tube), hemoglobin (cyanmethemoglobin, Labquest, Brazil) levels, and red blood cell, white blood cell and thrombocyte counts (Noro & Wittwer, 2012). Leukocyte differential count was performed in smears dyed with Diff-Quik stain and analyzed under a microscope (1.000 x; Olympus 21CA). The remainder of heparinized blood samples were centrifuged at 800 xg, and the plasma was stored in 1.5-mL microtubes, frozen at -20 °C for later determination of aspartate aminotransferase (AST; Bioclin, Brazil) and creatine kinase (CK, Bioclin, Brazil) activities, and serum concentrations of albumin (BCG, Labtest, Brazil), total protein (Biureto, Laborlab, Brazil) and cholesterol (CHOD, In vitro, Brazil). Biochemical analyses were performed in a semi-automatic analyzer (Mindray BA-88), with a coefficient of variation lower than 5%. Globulin serum level was determined by subtracting albumin level from total protein level, and used to calculate albumin to globulin ratio (A:G ratio). The results obtained in the hematological and biochemical analyses were compared between the groups presenting DCM lesions or not.

### Statistical analysis

Data were analyzed for normality (Shapiro-Wilk test) and homoscedasticity (Levene test), and then submitted

to two-way analysis of variance. The following model was applied:  $Y_{ij} = \mu + G_i + I_j + GI_{ij} + \epsilon_{ij}$ , where:  $Y_{ij}$  = calculated effect,  $\mu$  = mean,  $G_i$  = effect of the  $i^{th}$  group,  $I_j$  = effect of the  $j^{th}$  farm;  $GI_{ij}$  = interaction between group and farm, and  $\epsilon_{ij}$  = residual error. Means were compared by Tukey's test. Histological results were compared by Student's t-test. Statistical analyses were performed using IBM SPSS 19.0, with the significance level set at 95%.

## RESULTS AND DISCUSSION

Blood biochemical parameters were not significantly different between broilers with DCM lesions or not, except for AST and CK, which levels were higher in the DCM group than in the control group ( $p < 0.05$ ; Table 1). These results are consistent with the findings of Kuttappan *et al.* (2013), who verified higher AST and CK activities in broiler carcasses with white striping lesions compared with healthy carcasses. However, those authors reported higher average AST and CK levels, of 1,066 U/L and 123,973 U/L, respectively, in broilers with severe white striping lesions relative to those determined in the present experiment in carcasses presenting DCM lesions. Zimmermann (2008) observed histologic lesions, such as degeneration, necrosis, and lympho-histiocytic infiltrate, in normal anterior *latissimus dorsi* muscle and in other grossly normal muscle groups of broilers; however, the authors did not analyze CK and AST levels. MacRae *et al.* (2006) reported levels of 955 IU/L for CK and 35.1 IU/L for AST in broilers with skeletal muscle fiber growth and growth associated myopathies. In addition to the increase in enzyme activities, those authors reported microscopic lesions, such as necrosis and basophilia, in the *pectoris major* and biceps muscles of 5-week-old broilers with myopathies. However, in the present study, except for the ALD, no other muscles were macroscopically or microscopically evaluated, and therefore, no comparisons with the study of MacRae *et al.* (2006) can be made.

**Table 1** – Means and standard deviation of serum biochemical parameters for broilers with and without DCM.

Parameter	DCM group	Control group	p value
AST (IU/L)	409 ± 161	320 ± 94	0.0134
CK (IU/L)	54.091 ± 38.425	35.203 ± 28.613	0.0364
Albumin (g/L)	22.8 ± 0.16	22.11 ± 0.18	0.0844
Globulin (g/L)	12.9 ± 0.33	14.8 ± 0.39	0.0268
Albumin:Globulin	1.91 ± 0.73	1.60 ± 0.60	0.0401
Cholesterol (mg/dL)	153 ± 11.9	149 ± 15.2	0.2539
Glucose (mg/dL)	241 ± 30.1	242 ± 30.5	0.9069
Lactate (mg/dL)	30.6 ± 7.4	31.3 ± 8.9	0.6831





The histological analyses of the ALD muscle of broilers presenting DCM confirmed the presence of fibers with moderate to severe floccular necrosis, hyalinization, and moderate to severe coagulative necrosis of muscle fibers, with no adipocyte changes in the affected fibers, mild to moderate multifocal mononuclear infiltration, and diffuse edema. The liver presented moderate to diffuse hepatocyte vacuolization, cholangitis, cholestasis, and bile duct hyperplasia. These findings are consistent with those of Zimmermann (2008), who detected similar histological muscle lesions as well as moderate bile duct proliferation and hepatocyte vacuolization in broiler carcasses presenting DCM. These liver findings, along with the muscle lesions mentioned earlier, explain the increase in AST activity. In the lung, there was moderate multifocal interstitial bronchopneumonia. The samples obtained from the muscles and viscera of carcasses with no DCM did not show any important histological lesions.

No differences in erythrocyte and thrombocyte counts, hematocrit values, or hemoglobin levels were detected between DCM and control carcasses ( $p > 0.05$ , Table 2). Total erythrocyte counts allow diagnosing anemia or hemoconcentration (Noriega, 2000), and its levels vary across bird species, age, sex, and are influenced by hormones and environmental factors (Campbell, 2004).

**Table 2** – Hematological parameters of broilers with DCM and without DCM (means and standard deviation) and reference values for *Gallus gallus domesticus*.

Parameter	DCM group	Control group	p value
Erythrocytes ( $\times 10^6/\mu\text{L}$ )	$2.25 \pm 0.34$	$2.39 \pm 0.34$	0.1561
Hematocrit (%)	$30.9 \pm 2.69$	$31.0 \pm 3.02$	0.9206
Hemoglobin (g/L)	$100 \pm 22.2$	$96.5 \pm 8.69$	0.4386
Thrombocytes ( $\mu\text{L}$ )	$12,924 \pm 5,798$	$12,337 \pm 5,594$	0.6046
Leukocytes ( $\mu\text{L}$ )	$8,041 \pm 4,100$	$7,784 \pm 4,027$	0.7574
Heterophils ( $\mu\text{L}$ )	$3,326 \pm 2,189$	$2,644 \pm 2,029$	0.2345
Lymphocytes ( $\mu\text{L}$ )	$3,887 \pm 2,879$	$4,431 \pm 2,397$	0.4619
Monocytes ( $\mu\text{L}$ )	$503 \pm 574$	$608 \pm 538$	0.5340
Eosinophils ( $\mu\text{L}$ )	$153 \pm 228$	$173 \pm 244$	0.6863

Total differential and absolute leukocyte counts were statistically similar between DCM and control broiler carcasses ( $p > 0.05$ , Table 2). Kuttappan *et al.* (2013) did not identify any hematological differences between broilers with and without white-stripping lesions either. Leukocyte counts are associated with the inflammatory response (Kokosharov, 1998), and differential leucocyte counts allows identifying the viral or bacterial nature of an infection and the general health status of an animal (Noriega, 2000; Campbell, 2004). In general, white blood cell count in peripheral blood increases in of stress situations

and inflammatory conditions due to generalized or localized infections, trauma, toxicity, neoplasms. However, in poultry, total leukocyte and absolute lymphocyte counts maybe abnormal owing to the fact that activated thrombocytes may be mistaken for lymphocytes, resulting in an overestimation of lymphocyte counts. Therefore, total and differential leukocyte counts in birds, fishes, reptiles, and amphibians must be performed by an experienced technician (Noro & Wittwer, 2012).

The obtained glucose and cholesterol levels are consistent with the reference values proposed by Lumeij (1997) and Campbell (2004) values for clinically-healthy broilers, and were not different between the evaluated groups ( $p > 0.05$ ; Table 1). Hyperglycemia is associated with acute stress, and blood glucose levels increases due to pre-slaughter fasting (Puvadolpirod & Thaxton, 2000). Also, no significant differences in the serum levels of lactate were determined between the DCM and the control groups ( $p > 0.05$ ; Table 2). Lactate is an end product of anaerobic glycolysis and it is used as stress indicator in animals at the time of slaughter (Bertolini *et al.*, 2006); however, this does not apply to the present study because blood samples were collected prior to fasting.

The DCM and the control groups presented average albumin:globulin ratios of 1.91 and 1.60, respectively. These ratios are higher than that reported by Lumeij (1997), of 0.8 for broilers. Although globulin values and albumin:globulin ratios were different between the MDC and control groups, no differences in total protein values were observed. According to Lumeij (1997), in both chronic and acute inflammatory processes, total plasma protein levels increase due to an increase in globulin levels and an occasional decrease in albumin levels, resulting in reduced albumin/globulin ratios. Differently from Lumeij (1997), both groups evaluated in the present study presented increased globulin levels, but changes in total protein levels.

No pH differences between MDC and control carcasses were detected in hot or chilled carcasses. However, after refrigeration, the pH value of 5.9 measured in carcasses from farm 1 with MDC lesions was significantly different of the pH of 5.7 obtained in the control carcasses ( $p < 0.05$ ; Table 3). In addition, no gross meat quality changes were detected in among the groups (data not shown). Chicken breast meat pH is 5.7 to 5.9, on average (Mendes, 2001), and normal pH values, at the end of the *postmortem* examination, should range between 5.60 and 5.86 (Sams & Mills, 1993).



**Table 3** – Means and standard deviation of *pectoralis major* muscle pH in broilers from the DCM and control groups.

pH	DCM group	Control group	p value
Shortly after slaughter	6.37 ± 0.12	6.37 ± 0.20	0.9336
After chilling (baseline pH)	6.15 ± 0.12	6.11 ± 0.10	0.1493
After refrigeration (0 ± 2 °C, final pH)	5.88 ± 0.16	5.82 ± 0.14	0.0376

The DCM group present significantly higher ( $p < 0.05$ ) serum activities of AST (409 U/L) and CK (54.091 U/L) than the control group (320 U/L and 35.203 U/L for AST and CK, respectively), which, together with the histological findings in the ALD muscle, show that the presence of DCM alone may increase the levels of enzyme markers of muscle damage.

The results of the present study showed that the AST and CK serum activities both in the DCM and control groups were abnormal compared with the average value 209 U/L determined in clinically healthy 42-day-old broilers by Borsa *et al.* (2006). In addition, the obtained AST and CK values of both evaluated groups were higher than those reported by Dickel (1992), 222 U/L for broilers with ascites, and those of Fernandez *et al.* (1994), of 198-225 U/L in layer hens fed a diet contaminated with aflatoxin.

The CK activity in the DCM carcasses determined in the present study was higher than those obtained by Lumeij (1997), who describes 0-350 U/L in clinically healthy broilers, as well as those reported by Dickel (1992), of 340 U/L in broilers with ascites. Bridi & Silva (2012) determined CK values of 18.962 U/L and 17.705 U/L in broilers slaughtered by Halal and conventional methods, respectively.

It should be noted, however, that healthy broilers (control group) may present lesions in other muscle groups, which may not be grossly detectable. As the other evaluated hematological and biochemical parameters, as well as the pH of *pectoralis major* muscle were not significantly different from the reference values or between the DCM and the control groups, it may be inferred that broilers presenting or not DCM are physiologically similar, except for the ALD muscle lesion.

## CONCLUSION

Despite the observed higher muscle enzyme activities, broilers presenting dorsal-cranial myopathy did not show any abnormal hematological, biochemical or muscle pH values, indicating that the presence of this lesion does not affect their overall health status, and therefore, any health risks for consumers.

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