Acta Scientiae Veterinariae, 2018. 46: 1606.

RESEARCH ARTICLE
Pub. 1606

ISSN 1679-9216

Characterization of Immune and Enteric Systems of Broilers after Imunosupression with Dexamethasone

Daiane Carvalho[1], Juliana Inês Herpich[1], Gabriela Zottis Chitolina[1], Marta Sílvia Gava[1], Lucas Brunelli de Moraes[2], Thales Quedi Furian[1], Karen Apellanis Borges[1], Flávia Bornancini Borges Fortes[1], Hamilton Luiz de Souza Moraes[1] & Carlos Tadeu Pippi Salle[1]

ABSTRACT

***Background*:** Bursa of Fabricius (BF) and the thymus are primary lymphoid organs of poultry and play a major role in avian immunity. Enteric system is also involved in immunity. Several pathologic conditions directly impact BF and thymus size, and also affect intestinal parameters. Besides, there are several immune system depressor agents which affect birds. The selection of glucocorticoid as inducer of immunosuppression is applied in many experiments; however there are few studies that are applied to the reality in the field. In this context, the aim of this study was to evaluate the effects of dexamethasone as an inducer of immunosuppression on lymphoid organs and microscopic structures of the jejunum.

***Materials, Methods & Results*:** One-day-old chicks were used as a control group (n = 8) and the treated group (n = 25) received intramuscular dexamethasone on 21, 23, 24 and 26 day-old. Control birds and treated birds were euthanized 8, 16, 24, 32 and 40 h after inoculation; four control birds and six treated birds were euthanized on the eighth day after the last inoculation. Thymus, BF and jejunum were collected during the necropsy. The selected organs were processed, stained with hematoxylin and eosin and photographed. The BF and thymus cuts were evaluated by three histopathologists to determine the depletion score. Ten villi of each jejunum were evaluated for width and length of villi, depth crypt, microvillus length, enterocyte length of each villus, and wall thickness. Treated birds presented a mean weight lower than control group during all the experiment. The mean weight and the relative weight of the BF and thymus of control birds were significantly higher than treated ones. The lymphocyte depletion in BF and thymus scores differed significantly between groups, being higher in the group challenged with dexamethasone. There were no significant differences between groups for depth of crypt, height of core and height of microvilli. The intestines of the control group had higher mean values for length of villi, width of villi, height of enterocyte and thickness of wall. Significant correlation between weight of thymus and weight of BF, between weight of thymus and weight of bird and between weight of BF and bird weight were found.

***Discussion*:** Easy administration, low cost and the absence of suffering during inoculation make the use of glucocorticoids more advantageous to mimic immunosupression in poultry. Treatment with dexamethasone interfered directly in the weight of the birds. Evaluation of immune response of birds can be performed by the ratio of the BF weight in relation to the weight of the bird, as observed in the present study. The relative weight of the BF of untreated animals varied at all ages between 0.21% and 0.29%. It was also observed that the relative weight of BF and thymus in immunosuppressed birds tended to increase according to the end of the treatment. Depletion scores were higher in thymus, suggesting that glucocorticoid promoted more harmful effects on this organ. Dexamethasone had negative influence on length of villus, once mean value was 13% lower than those of control group. Width of villus and height of enterocyte core of treatment group were lower than those observed in control animals. Coefficient of determination greater than 70% was observed for almost all established relationships, except for the length of villi and weight of the thymus. Administration of dexamethasone promotes significant and negative effects on the gain of weight, length of villi, width of villi and height of enterocytes. These finding are useful for future experiments with controlled immunosuppression induction, once it provides significant information of the secondary effects of glucocorticoids administration in poultry.

Keywords: poultry, lymphoid depletion, jejunum, glucocorticoid.

http://dx.doi.org/10.22456/1679-9216.86474

Received: 20 May 2018 Accepted: 18 September 2018 Published: 12 November 2018

[1]Center for Diagnostics and Research in Avian Pathology (CDPA), Faculty of Veterinary, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. [2]Instituto de Pesquisa Veterinária Desidério Finamor (IPVDF), Fundação Estadual de Pesquisa Agropecuária (FEPAGRO), Eldorado do Sul, RS. CORRESPONDENCE: C.T.P. Salle [tadsalle@gmail.com - Fax: +55 (51) 3308-6138]. Center for Diagnostics and Research in Avian Pathology, Federal University of Rio Grande do Sul (UFRGS). Av. Bento Gonçalves n. 8824. CEP 91540-000 Porto Alegre, RS, Brazil.

INTRODUCTION

Factors that cause immunosuppression are among the major problems currently affecting poultry farms. In this context, studies have been developed to minimize their occurrence in order to enhance the profit in this competitive industry. The lymphoid organs play a major role in avian immunity. Poultry has primary lymphoid organs, the bursa of Fabricius (BF) and the thymus, and secondary lymphoid organs, such as the spleen, bone marrow, gland of Harder, bronchial-associated lymphoid tissue (BALT) and gut-associated lymphoid tissue (GALT) [6,17,23]. The intestine is the site where there are multiple processes that are involved, including immune recognition, immune regulation and the development of immune tolerance. Its barrier function is influenced by a variety of factors such as enzymes, infectious agents, toxins, and hormones [26].

Several pathologic conditions can directly impact BF and thymus size, including microrganisms, micotoxins, chemical substances and medicaments [3]. Besides, there are different immune system depressor agents which affect birds and mammals, including microbiological agent, toxins and also factors that induce physicological stress [7]. Some of theses agents and stress have also often been associated with gastrointestinal disorders [4,19,22]. The use of glucocorticoids as inducers of cellmediated immunosuppression can be applied in many kind of experiments [26]. Although, there are few studies that are applied to the reality in the field. In this context, the aim of this study was to evaluate the effects of dexamethasone as an inducer of immunosuppression on lymphoid organs and microscopic structures of the jejunum.

MATERIALS AND METHODS

Animal experiment

A total of 33 one-day-old chicks were used as a control group (n = 8) and as a treated group (n = 25). Treated birds received intramuscular dexamethasone[1] (Déxium® - 5 mg/kg) on 21, 23, 24 and 26 day-old, according to the protocol previous established [14], with modifications. One control bird and 4 treated birds were randomly selected and euthanized 8, 16, 24, 32 and 40 h after inoculation; 4 control birds and 6 treated birds were euthanized on the eighth day after the last inoculation. The birds were anesthetized with a combination of an intramuscular dose of Ketamine[2] 2% (20 mg/kg) and Xylasine[2] 10% (3 mg/kg), as previously described [25]. Thereafter, the animals were euthanatized through electrocution (110V). Throughout the experimental period, all birds were housed in an acclimatized room, with artificial light and they were distributed into metal brooder cages. The animals were fed with water and feed *ad libitum*.

Organ sampling and preparation of histological sections

Immediately after the sacrifice, the birds were necropsied and the thymus, bursa of Fabricius (BF) and jejunum were collected and placed into a 10% buffered formalin solution. After 24 h fixing, the BFs were cut at the level of their major diameter in order to obtain the largest observation area. Three lobes were selected in each of the thymic chains (6 lobes/bird). The jejunums were cleaved as indicated by a previous study [9]. Thereafter, the organs were identified, processed and stained with hematoxylin and eosin.

Lymphoid depletion evaluation

The evaluation of lymphoid depletion of BF and thymus were performed according to previous studies [2,18]. In both cases, scores range from 1 (less lymphoid depletion) to 5 (more lymphoid depletion). The BF and thymus cuts were evaluated blindly and randomly by three histopathologists and the mode of the score assessment was selected to consider the lymphoid depletion of the organs.

Jejunum measurement

Ten villi of each jejunum were selected according to its integrity (well inserted in the submucosa base, presenting neither discontinuity nor folds, but with simple columnar epithelium at the tip). Width and length of villi, depth crypt, microvillus length, enterocyte length of each villus, as well as wall thickness were measured. Pictures were taken with an Olympus® C-7070[3] camera, coupled to trinocular Olympus® CX40[3] microscopy, with 40x (length of villus), 200x (depth of crypt, thickness of wall) and 400x (width of villus, length of enterocyte, length of microvillus) of magnification. The equipment was calibrated before each use and the Motic® Imagens Advanced (Motic) was used for measurements.

Statistical analysis.

The data were analyzed for normality. Thereafter, parametric tests were applied using SPSS Software and JMP-SAS Software adopting a 5% significance level. The student - *t* test was used to compare the means and Pearson's correlations test was performed to verify the correlation between the variables. The regression coefficient (r^2) was used to verify the degree of explanation of one variable in relation to the other.

RESULTS

Bird weight

The birds submitted to the treatment with dexamethasone presented a mean weight lower than control group ($P < 0.05$) from eight hours after starting treatment until one week after the last inoculation. The weight of the birds increased over time in both groups. It was also possible to verify that birds of control group obtained a weight gain of 26.7% one week after the treatment, whereas birds treated with glucocorticoid obtained only 15.3% of weight gain in the same period.

Bursa of Fabricius and thymus

The mean weight and the relative weight of the BF and thymus of control birds was significantly higher ($P < 0.05$) than treated ones (Table 1). After dexamethasone administration, the organs of the two groups tended to increase the weight over time. The BF and thymus relative weight of the treated group was of 0.1% and 0.8%, respectively, one week after the last inoculation. However, in both cases, the relative weight of control group was superior ($P < 0.05$) in all periods evaluated (Figure 1).

The lymphocyte depletion scores of BF differed significantly ($P < 0.05$) between control and treated groups (Table 1). It was observed epithelial and follicular cysts, hyperplastic epithelium, fibroplasia, hyperemia and edema in the BF of animals inoculated with dexamethasone (Figure 2). Treated birds presented follicles smaller than control birds, although the extreme degree of lymphoid depletion (score 5) was not detected. As observed with the bursa, the depletion scores of the thymus of treated group were higher ($P < 0.05$) in relation to the control group (Table 1). The main histological alterations observed in the thymus of the group challenged with dexamethasone were a reduction in the cortical-medullar ratio and the presence of hemorrhagic areas (Figure 2).

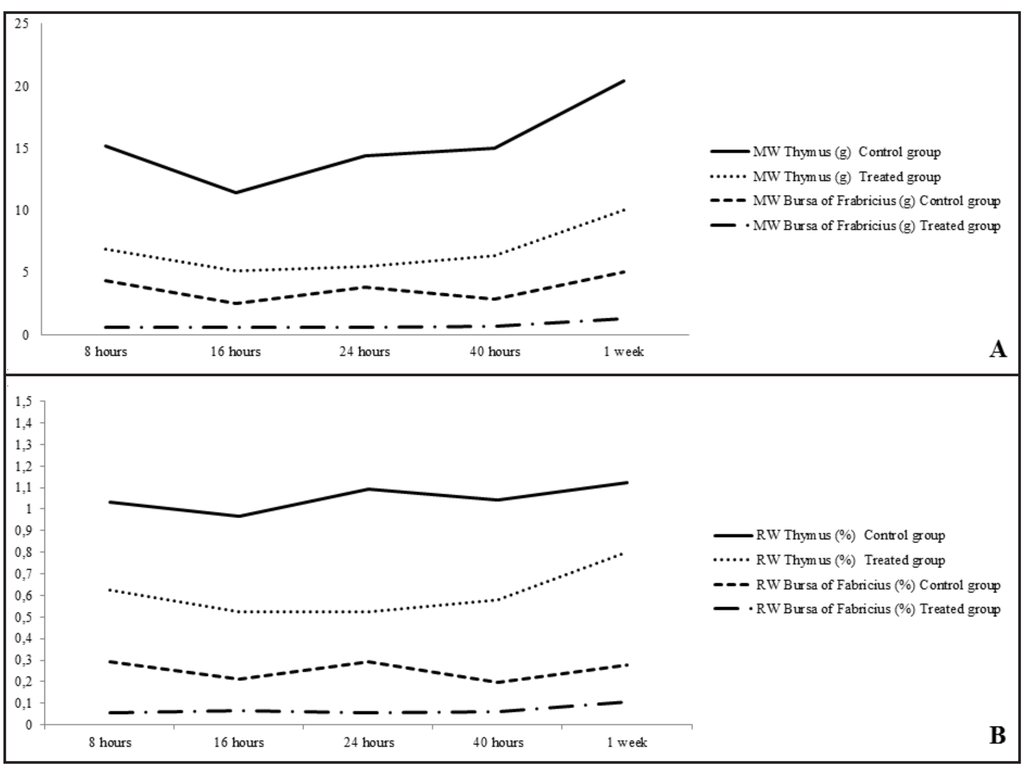

Figure 1. Mean weight (A) and relative weight (%) (B) of thymus and BF over time in animals of control and treated groups.MW: Mean Weight, RW: Relative Weight.

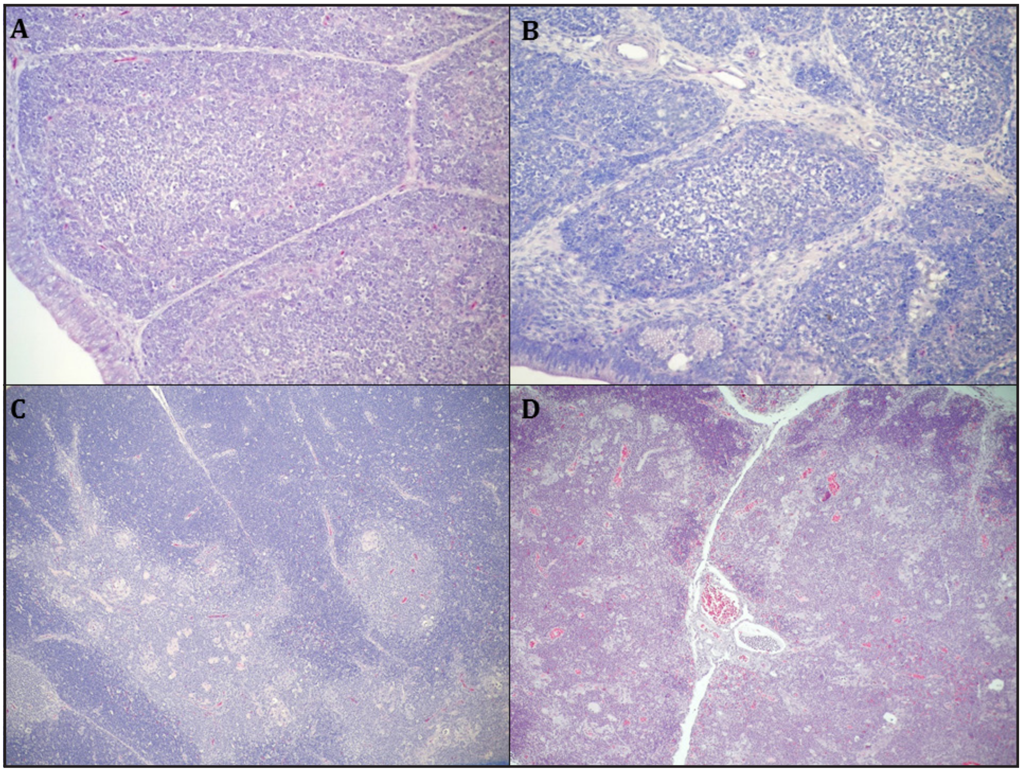

Figure 2. A- BF with intact follicles and without histological alterations (control group) [20x magnification]. B- BF with reduction of lymphoid follicles (depletion) and increase of interfollicular space (treated group) [20x]; C- thymus without histological alterations (control group) [10x]; D- thymus with lymphocyte depletion, reduction of cortical-medullar ratio and presence of hemorrhagic areas (treated group) [10x].

Table 1. Mean weight (MW), relative weight (RW), mode and mean depletion score of the BF and thymus of control and treated groups.

	Mean weight (g) ± standard deviation	Relative weight (%)	Mode depletion score	Mean depletion score
BF control	4.20 ± 1.8[a]	0.26[a]	1[a]	1.38[a]
BF Treated	0.70 ± 0.29[b]	0.06[b]	3[b]	2.65[b]
Thymus control	17.18 ± 4.06[a]	1.08[a]	2[a]	1.63[a]
Thymus treated	6.3 ± 1.7[b]	0.5[b]	3[b]	3.56[b]

Values referring to the mean of the five collections. Values with different letters in the same column for the same organ differ significantly ($P < 0.05$).

Jejunum

Thirty images were analyzed and seventy measurements were taken in each of the evaluated jejunum. The mean and standard deviation for the variables length of villi, depth of crypt, thickness of wall, width of villi, height of core, height of microvillus and height of enterocyte were established (Table 2). The intestines of the control group presented higher ($P < 0.05$) mean values for length of villi, width of villi, height of enterocyte and thickness of wall.

Statistical associations

Significant correlation between weight of thymus and weight of BF ($r^2 = 0.867$; $P < 0.05$), between weight of thymus and weight of bird ($r^2 = 0.864$; $P < 0.05$) and between weight of BF and bird weight ($r^2 = 0.718$; $P < 0.05$) were observed (Figure 3).

Table 2. Mean and standard deviation (SD) of analyzed variables in jejune of control and treated groups.

	Length of villi (µm) ± SD	Depth of crypt (µm) ± SD	Thickness of wall (µm) ± SD	Width of villi (µm) ± SD	Height of core (µm) ± SD	Height of microvillus (µm) ± SD	Height of enterocyte (µm) ± SD
Control	11868.78 ± 1310.14^{a}	633.66 ± 49.40^{a}	14378.50 ± 1338.02^{a}	658.03 ± 79.57^{a}	50.53 ± 4.95^{a}	20.81 ± 1.90^{a}	193.45 ± 11.56^{a}
Treated	10502.42 ± 1306.01^{b}	596.43 ± 71.77^{a}	12501.50 ± 1504.35^{b}	599.63 ± 55.00^{b}	51.69 ± 3.97^{a}	19.46 ± 1.92^{a}	181.17 ± 11.45^{b}

Values with different letters in the same column differ significantly ($P < 0.05$).

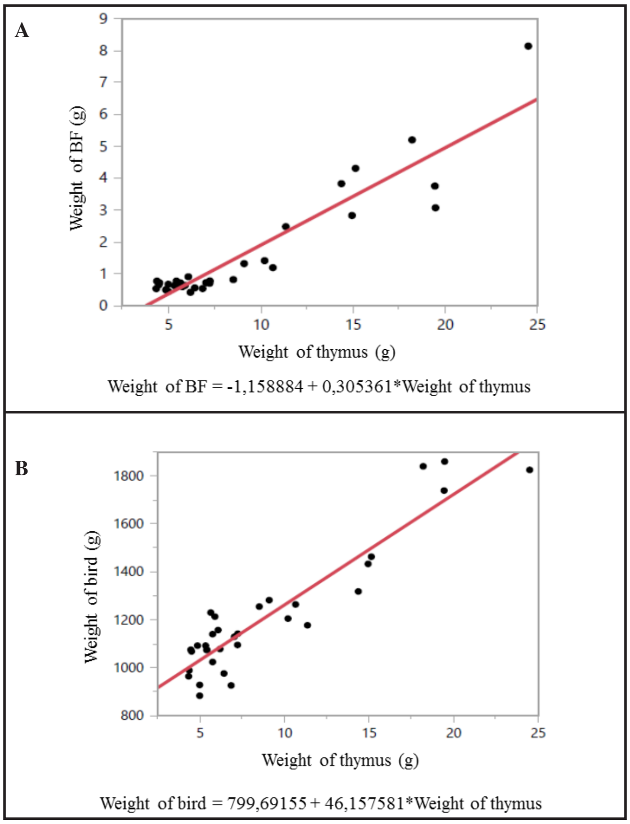

Figure 3. Regression model obtained between variables "weight of BF" and "weight of thymus" (A) and between variables "weight of bird" and "weight of thymus" (B).

DISCUSSION

Broilers are constantly subjected to several potentially immunosuppressive stimuli over the raising period [7]. Controled immunosuppression in birds can be induced by the inoculation of agents with tropism to the immune system or through food contaminated with mycotoxins. However, easy administration, low cost and the absence of suffering during inoculation make the use of glucocorticoids more advantageous in comparison to other methods [13]. These drugs act by decreasing the population of both B and T lymphocytes, promoting a considerable immunodepression, according to the dose and routes of administration. Also, administration of dexamethasone may be a satisfactory manner to mimic stress and provide a way to study the effects of stress conditions on enteric mucosa integrity [26].

Treatment with dexamethasone interfered directly in the weight of the birds. The gain was approximately 30% greater in birds from control group. Considerable lower weight in birds treated with dexamethasone compared to untreated birds are cited in the literature [8,15]. Once dexamethasone promotes changes in intestinal permeability [26], it is possible that there is less absorption and consequently a lower gain of weight. This finding is important, since dexamethasone may mimic pathological conditions of immunodepression and stress, which can be devastate in the field and cause similar injury.

The BF of treated group presented follicular atrophy and moderate intrafollicular lymphocyte depletion. Futhermore, it was observed the presence of epithelial and follicular cysts, hyperplastic epithelium, fibroplasia, hyperemia and edema in the BF of animals inoculated with dexamethasone. The mean weight of the BF of health animals was 4.2 g, however with a high standard deviation (1.81 g). This considerable organ weight variability was also addressed in other studies [3,10]. Even with the continuous and intense genetic selection, in order to optimize poultry production and provide greater uniformity in the carcass over the last 50 years, the individual variability in the biological parameters of birds was not lost [3]. In the present study, the BF of control group presented lower lymphoid depletion scores and 5.7 times higher weights compared to animals submitted to glucocorticoid treatment. Huff *et al*. [11] also observed reduction in BF size and weight of turkeys submitted to dexamethasone treatment.

Evaluation of immune response of birds can be performed by the ratio of the weight of BF in relation to the weight of the bird [5,8,20], as described in the present study. Cazabán *et al*. [3] found a relative BF weight varying between 0.11-0.13% in birds with normal immunological status, suggesting that a proportion equal or above 0.11% in birds from 7 to 42 day-old it would be ideal. This value proposed by the authors did not reflected what was found in the present study, in which the relative weight of the BF of group control varied at all ages between 0.21% and 0.29%. On the other hand, McMullin [16] observed approximately the same value, proposing 0.3% for healthy birds.

The maximum depletion score 5 was not found in the birds of the treated group. On the other hand, the thymus depletion score of the same group varied between 2 and 5. These data suggest that glucocorticoid promoted more harmful effects on the thymus than on BF. The thymus is the organ most affected when animals inoculated with glucocorticoid were challenged with *Candida albicans* [1]. As well, more severe effects on the thymus in Wistar rats submitted to dexamethasone treatment are observed when compared to other lymphoid organs [12]. These authors also found that one week after the end of treatment, there was reversibility of the organ's condition. Nevertheless, the data of the present study showed that the partial recovery of the organ did not reach the same level of the birds of the control group.

The structure of the enteric mucosa may reveal information about the absorption capacity of the intestine and it is associated with the performance of the animals. Depth of crypt is an indicator of maturation of the intestinal epithelium, in other words, deeper crypt indicates a more mature intestinal epithelium [15]. The mean depth of crypt in animals from the control and treated groups did not differ statistically. However, very high standard deviation in both groups was observed. Differently from the data reported in this work, Li *et al*. [15] found increase in the depth of jejunum crypts in broilers submitted to glucocorticoid treatment. Likewise, rats under stress conditions had greater depth of crypt and smaller villi than normal ones [24].

Dexamethasone and other stress factors have negative influence on length of villus [15,21,24]. In the present study, the intestines of birds submitted to treatment with glucocorticoid birds presented mean value of length of villus 13% lower than those of control group. Height of villi is one of the apparent indicators of the ability of the intestine to absorb nutrients [15], which may explain the lower gain of weight of these animals during treatement. Similar results were found by researchers that compared the immune response and the integrity of duodenum of birds submitted to diets with addition of mycotoxins for two weeks [21]. In the present study, the width of villus and height of enterocyte core in birds treated with glucocorticoids were lower than those observed in control animals. On the other hand, Li *et al*. [15] did not detect difference of width of villus between healthy animals and broilers inoculated with different doses of dexamethasone.

Coefficient of determination (r^2) greater than 70% was observed for almost all established relationships, except for the length of villi and weight of the thymus. The weight of the birds is related to the weight of the BF. However, the bursal growth is faster than the animal's growth in the first three weeks after hatching [10]. Cazabán *et al*. [3] also found a positive correlation between broiler and BF weight, obtaining $r^2 = 0.87$. However, the authors affirmed that the correlation decreases over time, because the influence of external factors increases.

CONCLUSION

Administration of dexamethasone promotes significant and negative effects on the gain of weight, length of villi, width of villi and height of enterocytes in birds. These finding are useful for future experiments with controlled immunosuppression induction, once it provides significant information of the secondary effects of glucocorticoids administration in poultry.

MANUFACTURERS

[1]Chemitec Agro-Veterinária. São Paulo, SP, Brazil.

[2]Biolab Searle. São Paulo, SP, Brazil.

[3]Olympus Corporation. Tokyo, Japan.

Ethical approval. The research Project is registered at the Animal Care Committee of the Veterinary Research Institute Desidério Finamor (CEUA-IPVDF). Registration number: 21/2012.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

REFERENCES

1 **Brandão A.A.H. 1991.** Imunossupressão experimental em camundongos. Aspectos histológicos dos órgãos linfóides após tratamento com dexametasona e inoculação de *Candida albicans*. *Revista de Odontologia da UNESP*. 20: 9-16.

2 **Carvalho D., Moraes L.B., Chitolina G.Z., Herpich J.I., Osório F.S., Fallavena L.C.B., Moraes H.L.S. & Salle C.T.P. 2016.** Evaluation of thymic lymphocyte loss of broiler using Digital Analysis of the Lymphoid Depletion System (ADDL). *Pesquisa Veterinária Brasileira*. 36(7): 652-656.

3 **Cazabán C., Majo Masferrer N., Dolz Pascual R., Nofrarias Espadamala M., Costa T. & Gardin Y. 2015.** Proposed bursa of Fabricius weight to body weight ratio standard in commercial broilers. *Poultry Science*. 94(9): 2088-2093.

4 **Cera K.R., Mahan D.C., Cross R.F., Reinhart G.A. & Whitmoyer R.E. 1988.** Effect of age, weaning and post weaning diet on small intestinal growth and jejunal morphology in young swine. *Journal of Animal Science*. 66(2): 574-584.

5 **Corrier D.E., Elissalde M.H., Ziprin R.L. & Deloach J.R. 1991.** Effect of Immunosuppression with cyclophosphamide, Cyclosporin, or Dexamethasone on *Salmonella* Colonozation of Broiler Chicks. *Avian Disease*. 35(1): 40-45.

6 **Fletcher O.J., Abdul-Aziz T. & Barnes H.J. 2008.** Lymphoid system. In: Fletcherand O.J. & Abdul-Aziz T. (Eds). *Avian histopathology*. 3rd edn. Jacksonville: The American Association of Avian Pathologists, pp.24-57.

7 **Galha V., Bondan E.F. & Lallo M.A. 2008.** Relação entre imunossupressão e coccidiose clínica em frangos de corte criados comercialmente. *Journal of the Health Sciences Institute*. 26(4): 432-437.

8 **Galha V., Bondan E.F., Bonamin L.V. & Lallo M.A. 2010.** Coccidiose clínica em frangos de corte infectados naturalmente e imunossuprimidos com dexametasona. *Arquivos do Instituto Biológico*. 77(1): 25-31.

9 **Gava M.S., Moraes L.B., Carvalho D., Chitolina G.Z., Fallavena L.C.B., Moraes H.L.S., Herpich J.I. & Salle C.T.P. 2015.** Determining the best sectioning method and intestinal segment for morphometric analysis in broilers. *Brazilian Journal of Poultry Science.* 17(2): 145-150.

10 **Glick B. 1956.** Normal Growth of the Bursa of Fabricius in Chickens. *Poultry Science*. 35(4): 843-851.

11 **Huff G.R., Huff W.E., Balog J.M. & Rath N.C. 1998.** The effects of dexamethasone immunosuppression on turkey osteomyelitis complex in an experimental *Escherichia coli* respiratory infection. *Poultry Science*. 77(5): 654-661.

12 **Hussar P., Tokin I., Filimonova G., Tokin I. & Hussar U. 2011.** Dexamethasone induced T lymphocyte apoptosis in different lymphoid organs. *Papers on Anthropology*. 20: 117-127.

13 **Isobe T. & Lillehoj H.S. 1993.** Dexamethasone suppresses T cell-mediated immunity and enhances diseases suceptibility to *Eimeria mivati* infection. *Veterinary Immunology and Immunopathology*. 39(4): 431-445.

14 **Kong F.K., Chen C.H. & Cooper M.D. 2002.** Reversible disruption of thymic function by steroid treatment. *The Journal of Immunology*. 168(12): 6500-6505.

15 **Li Y., Cai H.Y., Liu G.H., Dong X.L., Chang W.H., Zhang S., Zheng A.J. & Chen G.L. 2008.** Effects of stress simulated by dexamethasone on jejunal glucose transport in broilers. *Poultry Science*. 88(2): 330-337.

16 **Mcmullin P. 2004.** Infectious bursal disease, IBD, Gumboro. In: *A pocked guide to poultry health and disease*. Sheffield: 5M Enterprises Limited, pp.144-146.

17 **Montassier H.J. 2009.** Fisiopatologia do sistema imune. In: Berchieri Junior A., Silva E.N., Di Fábio O.J., Sesti L. & Zuanaze M.A.F. (Eds). *Doença das aves*. 2.ed. Campinas: Fundação Apinco, pp.391-434.

18 **Muskett J.C., Hopkins I.G., Edwards K.R. & Thornton D.H. 1979.** Comparison of two infectious bursal disease vaccine strains: efficacy a potential hazards in susceptible and maternally immune birds. *Veterinary Record*. 104(15): 332-334.

19 **Olsen R.E., Sundell K., Mayhew T.M., Myklebust R. & Ringo E. 2005.** Acute stress alters intestinal function of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture*. 250(1-2): 480-495.

20 **Pope C.R. 1991.** Pathology of lymphoid organs with emphasis on immunosuppression. *Veterinary Immunology and Immunopathology*. 30(1): 31-44.

21 **Revajová V., Slaminková Z., Gresáková L. & Levkut M. 2013.** Duodenal morphology and immune responses of broiler chickens fed low doses of deoxynivalenol. *Acta Veterinaria Brno*. 82: 337-342.

22 **Saunders P.R., Kosecka U., Mckay D.M. & Perdue M.H. 1994.** Acute stressors stimulation secretion and increase epithelial permeability in rat intestine. *American Journal of Physiology*. 267: G794-G799.

23 **Schat K.A. & Skinner M.A. 2014.** Avian immunosuppressive diseases and immune evasion. In: Schat K.A., Kaspers B. & Kaiser P. (Eds). *Avian Immunology*. 2nd edn. San Diego: Elsevier, pp.275-297.

24 **Tannuri U., Carrazza F.R. & Iriya K. 2000.** The effects of glutamine-supplemented diet on the intestinal mucosa of the malnourished growing rat. *Revista do Hospital das Clínicas da Faculdade de Medicina de São Paulo*. 55(3): 87-92.

25 **Tranquilim M.V., Botelho G.G.K., Bortolini Z., Lehmkuhl R.C. & Lang R.R. 2007.** Osteossíntese de úmero em *Ramphastos dicolorus* (tucano de bico verde): relato de caso. *Ambiência*. 3(2): 261-266.

26 **Vicuña E.A., Kuttappan V.A., Galarza-Seeber R., Latorre J.D., Faulkner O.B., Hargis B.M., Tellez G. & Bielke L.R. 2015.** Effect of dexamethasone in feed on intestinal permeability, differential white blood cell counts, and immune organs in broiler chicks. *Poultry Science*. 94(9): 2075-2080.

1606

http://dx.doi.org/10.22456/1679-9216.86474