Journal of Animal & Plant Sciences, 32(4): 2022, Page: 918-923
ISSN (print): 1018-7081; ISSN (online): 2309-8694
http://doi.org/10.36899/JAPS.2022.4.0493

RELATIONSHIP BETWEEN BLOOD GLUCOSE LEVELS AND *ENTEROCOCCUS CECORUM* FOUND IN KINKY BACK DISEASE IN CHICKENS

K. Özkan[1*]

[1]Ceva Animal Health, Poultry Division, Postal Code; 34453, Maslak, Istanbul, Turkey
[*]Corresponding author's email: kayhanozkan@hotmail.com

ABSTRACT

Kinky back (KB) disease in chickens causes significant economic loss in the commercial poultry industries. The aim of the present study was to determine blood glucose levels in broilers with KB disease and the bacterial agents that affect their organs. The glucose levels were measured and *Enterococcus cecorum* infections were investigated in animals with KB disease. The sixth thoracic vertebrae (T6) and liver samples were taken from 42-d-old Cobb 500 broilers with KB disease from farms located within various geographical areas of Turkey. Ten broilers were selected from each of 10 flocks that showed clinical signs of KB disease (n = 100). Blood samples were taken from the animals in both the control (n = 10) and experimental groups (n = 100) to assess their blood glucose levels. Organ samples were taken from the animals and analyzed using microbiological matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. One-way analysis of variance was used to determine whether there were any statistically significant differences between the means of the control and experimental groups. The bacteriological analyses of the liver and T6 showed that the *E. cecorum* level significantly increased in the experimental group, which was positively correlated with the increasing glucose levels. *Enterococcus cecorum* is one of the most important factors causing KB disease, and the most effective method by which to prevent KB disease is a strong biosecurity program inside the poultry houses.

Keywords: Kinky back, *Enterococcus cecorum,* broiler, glucose, flock.

Published first online January 08. 2022 Published final July 30. 2022

INTRODUCTION

Kinky back (KB) disease was first reported in Great Britain in 1967 (Osbaldiston *et al*., 1967). Clear signs of KB disease, which increases the mortality rate, are observed in breeding broilers and young chickens that are 5–10 weeks old (Jung *et al*., 2014). The incidence of KB disease among male birds ranges from 8 to 30%, depending on the flock (Makrai *et al*., 2011). The causative agent of KB disease is not clear; however, *Enterococcus cecorum*, previously classified as a *Streptococcus* sp., is one of the main bacteria associated with the infection. Field observations have substantiated that *E. cecorum* infections can be carried from the present flock to a new flock, which indicates that a contaminated environment can act as the source of continued infection (De Herdt *et al*., 2008).

Skeletal spondylolisthesis, a condition in which vertebrae placement is altered, is also observed in KB-affected broilers and might be caused by the same factors. *Enterococcus cecorum* might be delivered through bacteremia to the T6 and liver (Goodwin *et al*., 1994). Clinical spondylitis develops during the first week of age and peaks at 3–4 weeks (Dinev, 2012). If the type of litter used is not in compliance with standard practices of health and safety, the chickens become stressed, which can cause *Enterococcus* bacteriemia (Mandal *et al*., 2016). Skeletal spondylolisthesis is caused by this condition, which contributes to immunosuppression of chondronecrosis in the broiler's caput femur (Wideman *et al*., 2013); therefore, broilers with KB disease can be influenced by similar pathogenesis from other stress factors, such as *Enterococcus*. Enterobacteria species and *Lactobacilli* are commonly present in broiler intestinal flora (Proietti *et al*., 2016). If broilers are exposed to stress factors under adverse housing conditions, homeostasis within the cecum, which is part of the intestinal tract, is impaired (Yang *et al*., 2011). Before the chicks are placed into the poultry house, the litter, such as wood chips and rice hulls, is provided as bedding. The animals are in contact with this litter from the first to their last days. Studies have reported that the pathogenic agents in broilers were investigated in samples taken from the litter material (Lu *et al*., 2003) and detected that *Enterococcus* sp. is the main bacteria that cause KB disease. *Enterococcus cecorum* travels within the bloodstream to the T6 vertebra and is freely articulated within the thoracic area of the spine (Behera *et al*., 2019).

Birds have a high metabolic rate and body temperature compared with that of mammals with a similar body mass (Goodwin *et al*., 1994). The intestinal wall of a broiler moderates glucose intake by anaerobically converting 30% of the carbohydrates into lactate (Behera *et al*., 2019; Whittow, 1986). Glucose is

the base carbohydrate metabolized by the gastrointestinal tract of avian species (Braun *et al*., 2008) and is formed by two physiological effects—active transport across basolateral membranes and diffusion through intercellular gaps. Active transport studies have proved the stimulation of short-bout currents after glucose is added to a buffer mix that washes the gastrointestinal system of birds under low-salt conditions (Amado *et al*., 2005; Dudas *et al*., 2005), which results from increased oxidative stress and tissue damage (Braun *et al*., 2008; Austad *et al*., 2001). One study has shown that dexamethasone (DX)-induced oxidative stress in a broiler hyperglycemia model results in liver damage. When DX is administered, glycolysis is inhibited as well as the disintegration of protein (Lv *et al*., 2018). On the other hand, an *in vitro* human neuronal model study has found that glucose fluctuations play a role in the damaging effects of diabetes on various organs, including the brain, and that fluctuating glucose levels have an adverse effect on the mechanisms by which neuronal-cell energy is regulated (Russo *et al*., 2012).

Aside from the direct effects of high glucose levels, the accumulation of advanced glycosylation end products has a direct inhibitory effect on the proliferation and differentiation of bone cells (Dyck *et al*., 1999). In addition, neuropathy within the nervous system is a possible adverse effect of hyperglycemia. A series of studies related to this information have reported that the lack of metabolic homeostasis causes injuries to neuronal and Schwann cells, and that microvascular innervation might stimulate nerve ischemia (Dyck *et al*., 1999; Hotta *et al*., 1996). Possible complications that might appear within the broiler's nervous system depend on the localization of harmful pathogens (Noailles *et al*., 2019; Baurhoo *et al*., 2012). The aim of the present study was to demonstrate that *E. cecorum* migrates to the thoracic vertebrae and causes lesions within this area and an increase blood glucose levels.

MATERIALS AND METHODS

Animals and study area: The present study was conducted with the approval of the ethics committee of Abant İzzet Baysal University, Bolu, Turkey (Serial Number: 2019/06).

In this 2019 study, 42-d-old Cobb 500 broilers with KB disease (n = 100) and 10 healthy birds (control group) were used. All animals were given feed and water *ad libitum*. T6 and liver tissue samples were collected from antibiotic-free broiler flocks distributed within different geographic regions in Turkey.

All animals were housed under similar farm conditions within a closed-tunnel ventilation system with a capacity of 511,940 in Sakarya, a major poultry production area in Turkey. All broilers used in the study pattern were from the same breeder. The eggs obtained from these breeders were incubated at the end of the appropriate period and the chicks produced from the same breeder flocks were sent to 11 different poultry houses, each with a capacity of approximately 45,000 (Fig. 1). The animals with symptoms of KB disease were determined for each group on day 39.

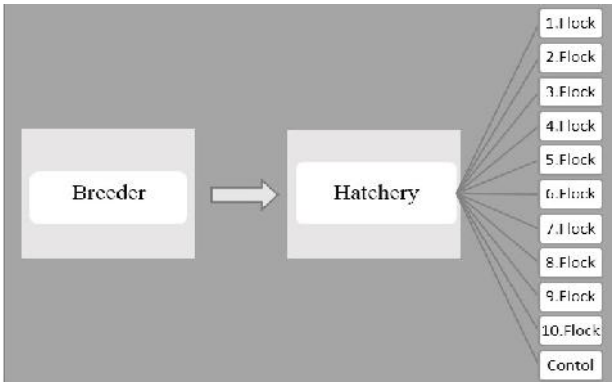

Figure 1. Study pattern of broiler flocks obtained from the same breeder and hatchery.

The walking ability of each bird was assessed using the gate-scoring method. The protocols that ensured the welfare of the poultry were strictly followed. A bird's gait was graded from 0 (perfect walking) to 5 (unable to move) (Kestin *et al*., 1992; Silvera *et al*., 2017). Ten broilers that showed a symptom score of 5 from each flock (a typical symptom of KB disease) were removed to another chamber to be separated from the others. The affected broilers had difficulty walking and showed symptoms of posterior paralysis. In addition, they sat on their knees and attempted to use their wings to move (Fig. 2A). Spinal nerve compression in T6 was observed (Fig. 2B). The control group of 10 animals that were selected with a gate score of 0 (perfect walking) was put into a chamber separate from the others.

Figure 2. Sitting position of the animal with reduced mobility on the knee, cloaca, and chest as a result of spinal nerve compression. A: Superior view of the disorder of the skeletal system. B: Longitudinal cross-section view with compression of the spine at T6 (arrow).

Glucose levels: Blood glucose levels were measured in each animal in all groups using blood glucose meter strips (Bionime, Taichung, Taiwan). To draw the blood sample, the legs of the chickens were held with one hand and the back was supported with the other hand. The wing vein that was visible between the biceps and triceps was gently punctured, and 1.4–2.5 µL whole blood was applied to the meter strip, which was then inserted into the meter. Within 2–3 sec, the glucose meter displayed the glucose levels in mg/dL. Related studies have reported that this method is the most widely used by clinicians and researchers in both the field and poultry diagnostic laboratories (Goodwin *et al*., 1994; Vale *et al*., 2019).

Bacteriological analyses: The birds' cages were appropriately marked to prevent confusing the study subjects with other poultry on the farms. Animals in all groups were sent to Abant Izzet Baysal University, Experimental Animal Application and Research Center, Bolu, Turkey. The T6 bone and liver samples were taken from the animals and placed into sterile containers. The samples were delivered under cold chain protocols (2–8 °C) to the laboratory.

The bone and liver tissue samples were cultured on Columbia sheep blood agar and Columbia colistin and nalidixic acid (CNA) agar (Oxoid GmbH) at 37 °C for 24 h under microaerophilic conditions. The method used to verify the presence of *E. cecorum* was performed according to the bacteriological analysis method (Dolka *et al*., 2016). A sample was taken from the colony that grew on the agar and was smeared on a matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) plate. Using 300 µL sterile distilled water, a bacterial colony was added to the tubes, after which 1 µL 70% formic acid was added and left to dry at room temperature. After drying, 1 µL each of the matrix (cyano-4-hydroxycinnamic acid) solution was added and left to dry again at room temperature. The plate was then placed in the device and read. The MALDI-TOF/TOF MS spectrometer (Bruker, France) was used for analysis. Measurement results are provided as values between 0 and 3. Accordingly, score values >2 are accepted as correct identification on the basis of genus and species (Jung *et al*., 2018).

Statistical analyses: One-way analysis of variance was used to determine any differences between the KB and control groups using the examined parameters. *Enterococcus cecorum* levels were determined from the livers and T6s. These levels were compared to determine the relationship between the infection and the chicken's blood glucose levels in the experimental and control groups. The colony forming unit (CFU) value of the bacterial amounts detected in the tissues and the blood glucose levels (X) were converted to the Log_{10} base (Y). The data converted on Log_{10} base was as follows:

$$Log_{10}(X) = Y$$

X = *E. cecorum* CFU amount or blood glucose level

Y = converted value

Levene's test was conducted to check the homogeneity of variances and whether homogeneity was violated, and the Games-Howell test was applied (Neveling *et al*., 2019). Post-hoc analysis was conducted using the Games-Howell test. The level of significance of p values < 0.05, < 0.01, and < 0.001 was also determined. The data were analyzed using SPSS ver. 22.0 (IBM Corp., Chicago, IL, USA).

RESULTS

Identification using the MALDI-TOF/TOF MS (Bruker) yielded *E. cecorum* and determined that this bacteria caused the disease. Spectral similarity was performed using the Vitek MS IVD V2, throughput system MS-CE ver. CLI 2.0.0 (Bruker). The identification of the causative agent was later verified by sequencing of the VITEK 2 GP card as well as the 16S rRNA gene of *E. cecorum* with 97% similarity. For molecular identification of the ≈500 bp *E. Cecorum*, a polymerase chain reaction amplification of the 16S rRNA gene was conducted to using the primers 27F (Microsynth/Seqlab, Goettingen, Germany) (Frank *et al*., 2008; Singhal *et al*., 2015). This application matched a 468-bp sequence and showed 99% congenerical with the *E. cecorum* 16S rRNA gene (GenBank accession number: LDED00000000) (Dolka *et al*., 2015).

Table 1 shows the confirmed CFU amount and glucose levels from the liver and T6 vertebrae samples taken from the experimental and control groups. According to the post-hoc results, there were significant relationships between both bacteria (CFU Log_{10}) and glucose levels (ng/dL Log_{10}) in the broilers. There was a significant difference between the glucose levels in the experimental (with KB) and control groups (without KB) $p < 0.05$).

Table 1. Bacterial load in the liver and T6 vertebrae between experimental and control groups as it is related to glucose levels ($p < 0.05$).

Group		Sum of Squares	df	Mean Square	F	Sig.	SEM
Experimental	Between groups	392.621	7	56.089	94.550	0.000	0.262245
	Within groups	42.712	72AAW	0.593			
	Total	435.332	79				
Control	Between groups	68.081	7	9.726	209.044	0.000	0.10631
	Within groups	3.350	72	0.47			
	Total	71,430	79				

As seen in Fig. 3, the bacterial mean CFU levels of all KB broilers increased in direct proportion to their mean glucose levels (ng/dL). The linear increase was observed in all samples except for the T6 *E. cecorum* levels of animals in the second flock.

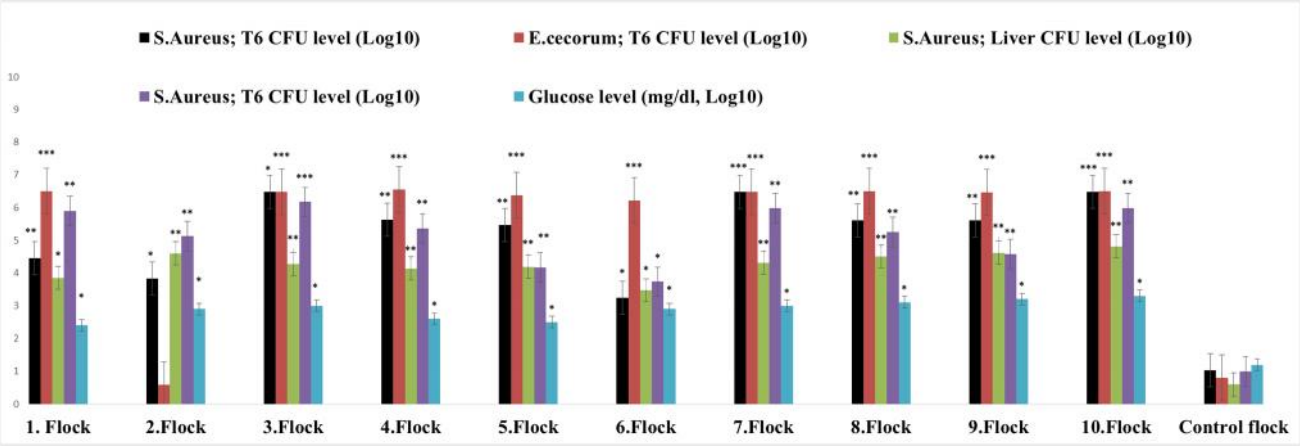

Figure 3. The mean bacterial colony forming units (CFUs) and glucose levels in the groups. According to post-hoc analysis, *Enterococcus cecorum* CFU-Log_{10} values increased in direct proportion to glucose levels in the experimental group compared to that in the control group ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

DISCUSSION

The present study represents the relationship between blood glucose levels and bacterial load in the liver and T6 of the animals with KB disease. Our findings indicated that the bacterial causative agent for KB disease, *E. cecorum*, was associated with hyperglycemia in the broilers.

The present study showed that bacterial infection was caused by co-infestation of *E. cecorum* in the animals with KB symptoms and disorders in their blood glucose levels, which might contribute to the degeneration of the inner intestinal wall. To regulate glucose metabolism, broilers have alfa, beta, delta, and islet cells in the pancreas, which play roles in regulating blood glucose levels (Braun *et al*., 2008; Chassaing *et al*., 2017). Any fluctuation in glucose metabolism results in the destruction of the nervous system. Within this context, osteoblast damage within the vertebral bones in animals showing symptoms of lameness is a result of an inconsistency in glucose levels (Ghodsi *et al*., 2016).

During acute inflammation, blood glucose levels rise to meet the energy needs of the body (Zaefarian *et al*., 2019). Within this context, catabolic activity damages broiler bone tissue and may create an opportunity for bacteria to settle and thrive. In addition, microorganisms in the chicken litter play an important role in the pathogenesis of KB disease.

Dolka *et al*. (2016) have reported that intestinal colonization, bacteremia, and osteochondrosis dissecans of the free thoracic vertebrae in early life are crucial to the pathogenesis of enterococcal spondylitis. Quamar *et al*. (2021) have shown that the microbial load of the litter can be controlled with diet to prevent lameness and skeletal disorders; however, with a holistic approach, biosecurity, well-maintained litter, and correct poultry management should be implemented to reduce the number of these bacteria in the litter and flora of the gastrointestinal system. Feed additives can be prepared to reduce blood glucose levels when bacteria are present during the growth period of the animals. In addition, Ebrahimi *et al*. (2016) have reported that adding dried orange peel to feed meal decreases glucose levels until the animals are of slaughter age.

Conclusion: The results of the present study indicated that a statistically significant level of *E. cecorum* was

detected in animals with KB disease compared to that in the control group. An increase in blood glucose levels in the sick animals caused an increase in the number of bacteria in the tissues. Current results showed that *E. cecorum* might be an important causative factor in KB disease. Hyperglycemia can increase the destructive ability of these bacteria after they enter the body. This finding indicates that other pathogens might play a major role in the occurrence of the disease from the bacterial load in the litter. If some of these pathogens are prevented from entering the body, the synergistic effect for developing various diseases can be eliminated. Once the animals with KB disease have been sent to the slaughterhouse, the poultry houses should be kept empty for suitable periods of time and effective disinfectants should be used to decrease the levels of disease-causing agents for the next broiler-production period. For early diagnosis of skeletal-system disorders, blood samples can be taken from 1-week-old chick and evaluated using biochemical parameters to help prevent possible diseases that could be encountered in later ages. Future studies can reveal the relationship between these diseases in broilers and breeding animals.

Acknowledgements: We thank the poultry-production farmers in the Marmara region for providing their assistance in the present study.

REFERENCES

Amado, G., M.A.D Castillo, J.R.E. Perez, and M.G.D. Bello (2005). Intestinal D-glucose and L-alanine transport in Japanese quail (Coturnix coturnix). Poult. Sci. 84: 947–950.

Austad, S.N., D.J. Holmes and R. Fluckiger (2001). Comparative biology of aging in birds: an update. Exp. Gerontol. 36:869–883.

Baurhoo, B., P.Ferket, C.M. Ashwell, D.J. Oliviera, and X. Zhao (2012). Cell walls of saccharomyces cerevisiae differentially modulated innate immunity and glucose metabolism during late systemic inflammation. PLos One. 7(1): 30323.

Behera, D.P., A.P.S. Sethi, C. Singh , U. Singh and M. Wadhwa (2019). Effect of citrus waste on blood parameters of broiler birds with and without cocktail of enzymes. Vet. World. 12(4):483-488.

Braun, E.J. and K.L. Sweazea (2008). Glucose regulation in birds. Comp. Biochem. Physiol. B. Bioc. 151(1):1–9.

Chassaing, B., S.M. Raja, J.D. Lewis, S. Srinivasan and A.T. Gewirtz (2017). Colonic Microbiota Encroachment Correlates With Dysglycemia in Humans. CMGH. 4(2): 205–221.

De Herdt, P., P. Defoort, J. Van Steelant, H. Swam, L. Tanghe, S. Van Goethem and M. Vanrobaeys (2008). Enterococcus cecorum osteomyelitis and arthritis in broiler chickens. Vlaams. Diergeneeskd. Tijdschr. 78:44–48.

Dinev, I. (2012). Pathomorphological investigations on the incidence of clinical spondylolisthesis (kinky back) in different commercial broiler strains. Rev. Med. Vet. 163(11):511–515.

Dolka, B., O.R. Heideman, T. Ida and C.J. Peter (2015). Draft genome sequences of five clinical Enterococcus cecorum strains isolated from different poultry species in Poland. Genome Announc. 3 (5): e01085-15.

Dolka, B., D.C. Chmiel, L. Makrai and P. Szeleszczuk (2016). Phenotypic and genotypic characterization of Enterococcus cecorum strains associated with infections in poultry. BMC Vet. Res. 12(1):1–13.

Dudas, P.L., R.M. Pelis, E.J. Braun and J.L. Renfro (2005). Transepithelial urate transport by avian renal proximal tubule epithelium in primary culture. J. Exp. Biol. 208:4305–4315.

Dyck, P.J., J.L. Davies, D.M. Wilson, F.J. Service, L.J. Melton and P.C. O'Brien (1999). Risk factors for severity of diabetic polyneuropathy: intensive longitudinal assessment of the Rochester diabetic neuropathy study cohort. Diabetes Care. 22:1479–1486.

Ebrahimi, A., A.A.A. Qotbi, A. Seidavi, F.W. Edens, V. Laudadio and V. Tufarelli (2016). Selected plasma constituents of broiler chickens fed different levels of dried sweet orange (Citrus Sinensis) peels. J. Anim. Plant. Sci. 26 (4): 949-955.

Frank, J.A., C.I. Reich, S. Sharma, J.S. Weisbaum, B.A. Wilson and G.J. Olsen (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl. Environ. Microbiol. 74(8):2461-2470.

Ghodsi, M., B. Larijani, A.A. Keshtkar, E.N. Esfahani, S. Alatab and M.R.M. Tehrani (2016). Mechanisms involved in altered bone metabolism in diabetes: A narrative review. J. Diabetes Metab. Disord. 15(1):1–9.

Goodwin, M.A., D.I. Bounous, J. Brown, B.L. McMurray, W.L. Ricken and D.L. Magee (1994). Blood glucose values and definitions for hypoglycemia and hyperglycemia in clinically normal broiler chicks. Avian Dis. 38(4):861-5.

Hotta, N., N. Koh, F. Sakakibara, J. Nakamura, Y. Hamara, T. Hara, E. Nakashima, H. Sasaki, H. Fukasawa, H. Kakuta and N. Sakamoto (1996). Effects of propionyl-L-carnitine and insulin on the electroretinogram, nerve conduction and nerve blood flow in rats with streptozotocin-induced diabetes. Pflugers Arch. 431:564–570.

Jung, A. and S. Rautenschlein (2014). Comprehensive

report of an *Enterococcus cecorum* infection in a broiler flock in Northern Germany. BMC Vet. Res. 10(1): 1–8.

Jung, A., L.R. Chen, M.M. Suyemoto, H.J. Barnes and L.B. Borst (2018). A review of Enterococcus cecorum infection in poultry. Avian Dis. 62(3):261-271.

Kestin, S.C., T.G. Knowles, A.E. Tinch and N.G. Gregory (1992). Prevalence of leg weakness in broiler chickens and its relationship with genotype. Vet. Rec. 131(9):190–194

Lu, J., S. Sanchez, C. Hofacre, J.J. Maurer, B.G. Harmon and M.D. Lee (2003). Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. Appl. Environ. Microbiol. 69(2):901–908.

Lv, Z.P., Y.Z. Peng, B.B. Zhang, H. Fan, D. Liu and Y.M. Guo (2018). Glucose and lipid metabolism disorders in the chickens with dexamethasone-induced oxidative stress. J. Anim. Physiol. Anim. Nutr. 102(2):706–717.

Makrai, L., C. Nemes, A. Simon, E. Ivanics, Z Dudas, L. Fodor and R. Glavits (2011). Association of *Enterococcus cecorum* with vertebral osteomyelitis and spondylolisthesis in broiler parent chicks. Acta Vet. Hung. 59(1): 11–21.

Mandal, R.K., T. Jiang, A.A. Rubaye, D.D. Rhoads, RF Wideman, J. Zhao, I. Pevziner and Y.M. Kwon (2016). An investigation into blood microbiota and its potential association with bacterial chondronecrosis with osteomyelitis (BCO) in Broilers. Sci. Rep. 6(25882):1–11.

Neveling, D.P., L.V. Emmenes, J.J. Ahire, E. Pieterse, C. Smith, L.M.T. Dicks (2019). Effect of a multi-species probiotic on the colonisation of Salmonella in broilers. Probiotics & Antimicro. Prot. 12: 896–905.

Noailles, A., O. Kutsyr, V. Maneu, I. Ortuño-Lizarán, L. Campello, E. Juan, V.G. Vicente, N. Cuenca and P. Lax (2019). The Absence of Toll-Like Receptor 4 Mildly Affects the Structure and Function in the Adult Mouse Retina. Front. Cell. Neurosci. 13: 59.

Osbaldiston, G.M. and D.R. Wise (1967). Spondylolisthesis and leg weak-ness in the chicken – a common etiol ogy. Vet. Rec. 80:320-322.

Proietti, P.C., A.D. Bosco, F. Hilbert, M.P. Franciosini and C. Castellini (2016). Evaluation of intestinal bacterial flora of conventional and organic broilers using culture-based methods. Ital. J. Anim. Sci. 8(1):51–63.

Quamar, A., J. Waheed, A. Hamza, S.G. Mohyuddin, Z. Lu, Z. Namula, Z. Chen and J.J. Chen (2021). The role of intestinal microbiota in chicken health, intestinal physiology and immunity. J. Anim. Plant. Sci. 31(2): 342-351.

Russo, V.C., S. Higgins, G.A. Werther and F.J. Cameron (2012). Effects of fluctuating glucose levels on neuronal cells in vitro. J. Neurochem. 37(8):1768–1782.

Silvera, A.M., T.G. Knowles, A. Butterworth, D. Berckmans, E. Vranken and H.J. Blokhuis (2017). Lameness assessment with automatic monitoring of activity in commercial broiler flocks. Poult. Sci. 96(7):2013–2017.

Singhal, N., M. Kumar, P.K. Kanaujia and J.S. Virdi (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front. Microbiol. 6:791.

Vale, M.M., D.R. Klein, T.Branco and M.P. Santos (2019). Glycemic response of poultries in different feeding systems. Acta Sci. Anim. Sci. 41(1):43148.

Whittow, G.C. (1986). Energy Metabolism in Avian Physiology. Ed PD Sturkie 4th ed, 110-120, Spinger Verlag, Newyork

Wideman, R.F., A. Al-Rubaye, A. Gilley, D. Reynolds, H. Lester, D. Yoho, J.M. Hughes and I. Pevzner (2013). Susceptibility of 4 commercial broiler crosses to lameness attributable to bacterial chondronecrosis with osteomyelitis. Poult. Sci. 92(9):2311–2325.

Yang X.J., W.L. Li, Y. Feng and H. Yao (2011). Effects of immune stress on growth performance, immunity, and cecal microflora in chickens. Poult. Sci. 90(12):2740–2746.

Zaefarian, F., M.R. Abdollahi, A. Cowieson and V. Ravindran (2019). Avian liver: the forgotten organ. Animals; 9(2):63.