JWPR
Journal of World's Poultry Research

2018, Scienceline Publication
J. World Poult. Res. 8(4): 100-104, December 25, 2018
Research Paper, PII: S2322455X1800014-8

Biochemical Alterations in Hypervitaminosis D_3 in Broiler Chicks Concomitantly Challenged with Endotoxin

Rahul Kumar[1]*, Rajinder Singh Brar[2], Harmanjit Singh Banga[2] and Sandip Sodhi[2]

[1]*Assistant Professor, Department of Veterinary Pathology, College of Veterinary Science & AH, DUVASU, Mathura-281001, India*
[2] *Professor Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India*
*Corresponding author's Email: rahulpoultrypatho@gmail.com

Received: 08 Oct 2018
Accepted: 11 Nov 2018

ABSTRACT

Vitamin D_3 is ten times more biologically active than vitamin D_2, over supplementation of vitamin D_3 causes hypercalcemia with deposition of calcium and phosphate as crystals in the visceral organs. Birds are considered more resistant to endotoxin and information on inflammation and homeostasis in birds supplemented with higher dose of vitamin D_3 when suffer endotoxic shock is lacking. The present study was conducted to compare the effect on hemoglobin concentration and biochemical parameters of broiler chicks by administering toxic dose of vitamin D_3 for 21 days concomitantly challenged with endotoxin. The chicks were randomly divided into four groups viz. A, B, C and D. Hemoglobin concentrations of control groups (A and B) and treatment groups (C and D) did not differ significantly ($P<0.05$). Hypercalcemia and hyperphosphatemia was observed in both treatment groups in comparison to the control group. No significant ($P<0.05$) change was observed in the concentrations of total protein and albumin and in the activity of plasma Alanine Aminotransferase, Aspartate Aminotransferase and Alkaline Phosphatase on day 28 of control (A and B) and treatment (C and D) groups. Vitamin D_3 supplementation causes immunomodulation; hence acute endotoxic shock does not incite inflammatory response and disturb the homeostasis in broiler chicks.

Key words: Broiler chicks**,** Hypercalcaemia, Hypervitaminosis D_3, Hyperphosphatemia,

INTRODUCTION

Vitamin D3 is a fat-soluble vitamin, which originates from pro-vitamins ergosterol and 7-dehydrocholesterol based on activity of sun radiation. Vitamin D is added to diets in its crystal form as cholecalciferol (vitamin D_3) which is converted to 25-hydroxycholecalciferol (calcidiol, 25-OH D_3) in the liver and is further converted to the active metabolite 1,25-dihydroxycholecalciferol [1,25-(OH)2 D_3] in the kidneys (Beasley, 1999; Adams, 2010; Kelly et al., 2016). 1,25-dihydroxycholecalciferol (calcitriol) supports calcium and phosphorus absorption in the intestine, affects bone calcification and co-participates in calcium and phosphorus metabolism in the organism (Price et al., 2001; Cheng et al., 2016). In poultry, vitamin D_3 is ten times more biologically activity than vitamin D_2 (Soares et al., 1995; Fritts and Waldroup, 2003; Wideman et al., 2015). Vitamin D_3 is supplemented in the diets of poultry and because the optimum levels of dietary vitamin D_3 are rarely known, there is always a risk of over-supplementation (Nain et al., 2007), which causes hypercalcemiaemia and promotes deposition of calcium and phosphate as crystals in the kidneys, heart and major blood vessels (Cheng et al., 2016; Armstrong et al., 2018).

Lipopolysaccharides (LPS) are cell wall components of Gram-negative bacteria, which cause release of cytokines that regulate different metabolic responses and cause fever, inflammation and cachexia (Abbas et al., 1997). It is considered that birds might be relatively more resistant to endotoxins than mammals (Roeder et al., 1989) and Vitamin D_3 supplementation provide immunomodulation (Bikle, 2010; Schwarz et al., 2012; Shojadoost et al., 2015; Rodriguez-Lecompte et al., 2016; Kelly et al., 2016).

There is a paucity of information on the effects of LPS on cytokines and the acute phase response and their

relationship with inflammation and homeostasis in birds supplemented with higher dose of vitamin D_3, hence, the study is framed to find out the effect of higher dose of vitamin D_3 concomitantly challenged with endotoxin on biochemical parameters of broiler birds.

MATERIALS AND METHODS

The broiler chicks of strain IBL-80 procured from the hatchery, department of animal genetics and breeding (AGB), college of veterinary science, GADVASU, Ludhiana, India were kept for 7 days to acclimatize in laboratory conditions prior to start of sampling protocols. On day 7, the chicks were randomly divided in 2 broad groups (n=16 chicks) viz. treatment group and control group. Treatment group was administered with vitamin D_3 @ 2.5mg/kg body weight (BW) in groundnut oil daily by oral route. On day 28, 8 chicks from the control group were instilled with Normal saline solution (NSS) @ 0.5ml/chick (group A) and other 8 chicks were challenged with Lipopolysaccharide (LPS) @ 0.5ml/chick by intranasal route (group B) and then sacrificed after 12 hours of challenge with endotoxin. Similarly, 8 chicks from the treatment group were challenged with NSS @ 0.5 ml/chick (group C) and other 8 chicks were challenged with LPS @ 0.5 ml/chick by intranasal route (group D) on day 28 of the study. Blood samples (n=32) from wing vein for hemogram from all the broiler chicks of each group were collected 12 hours post endotoxin challenge in heparinized vials following the standard protocols of institutional animal ethical committee.

Plasma was separated and stored in aliquots at -20^{o}C till further use for analysis of various analytes viz. Plasma calcium concentration, plasma albumin etc. and processing for biochemical parameters (mean level of plasma calcium, phosphorous, albumin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were carried out at the end of experiment (day 28). The hemoglobin was estimated by using standard protocol (Benjamin, 1985). The plasma biochemical parameters were estimated using auto analyzer (BIOTRAN BTR-830) and diagnostic reagent kits supplied by Siemens India Limited, Gujarat, India using manufacturer protocols.

Statistical analysis

The statistical analysis was performed using One-Way Analysis of Variance (ANOVA) and all data were analyzed by using SPSS software (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.). The results were presented as mean ± SEM (standard error of mean). Results were considered significant if $P<0.05$.

Ethical approval

The study was conducted without affecting the birds' general wellbeing. Approval was taken from concern authority.

RESULTS

The effect of oral administration of vitamin D_3 and intranasal LPS on biochemical parameters in chicks on day 28 has been summarized and presented in table 1. The level of hemoglobin in treated chicks did not differ significantly ($P<0.05$) from control chicks. The mean plasma calcium and phosphorous concentration in chicks of treatment groups (groups C and D) were significantly ($P<0.05$) higher than chicks in control groups (A and B). The mean plasma total protein, albumin concentration, ALT, AST and ALP in chicks of treatment groups did not differ significantly ($P<0.05$) from control groups.

Table1. Effect of oral administration of vitamin D_3 (2.5mgkg^{-1}day^{-1}) and intranasal lipopolysaccharide (0.5 mg bird^{-1}) on biochemical parameters in broiler chicks on day 28 (Mean±SE)

Parameter	Control groups		Treatment groups	
	Group A (n=8) (Mean±SE)	Group B (n=8) (Mean±SE)	Group C (n=8) (Mean±SE)	Group D (n=8) (Mean±SE)
Hb (g/dL)	12.19±0.33	12.06±0.22	12.40±0.50	12.00±0.20
Calcium (mg/dL)	10.05±0.56^a	9.48±0.31^a	14.41±0.30^b	14.17±0.58^b
Phosphorous (mg/dL)	4.97±0.28^a	4.65±0.16^a	6.03±0.17^b	5.82±0.23^b
Total protein (g/dL)	5.72±0.12	5.56±0.21	5.28±0.18	5.62±0.19
Albumin (g/dL)	3.38±0.18	3.38±0.18	3.38±0.18	3.25±0.16
ALT (U/L)	12.23±0.96	12.55±1.15	10.92±1.56	10.27±1.06
AST (U/L)	226.00±8.44	211.75±10.40	222.62±8.50	240.88±11.19
ALP (U/L)	1278.12±79.18	1216.38±99.13	1151.50±86.07	1101.75±76.32

Group A: control + Normal Saline Solution; Group B: control + LPS; Group C: Vitamin D_3 @ 2.5mg/kg BW + NSS; Group D: Vitamin D_3 @ 2.5mg/kg Body Weight + LipoPolySsaccharide; $^{a, b}$ values within a row lacking a common superscript differ significantly at $P<0.05$.; Hb; haemoglobin, ALT; Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase

DISCUSSION

Administration of vitamin D_3 @ 2.5mg/kg BW for 21 days did not influence the mean hemoglobin concentration. The chicks of the group D which were administered vitamin D_3 @ 2.5mg/kg BW orally daily and challenged with intranasal LPS 12 hours before sacrifice caused no alteration in hemoglobin concentration as compared to control chicks that only challenged with intranasal LPS 12 hours before sacrifice. These results are in agreement with Borissov and Andonova (2000) and Kumar and Mallik (2001) who reported no change in hemoglobin concentration in piglets and calves respectively on endotoxin exposure. However, the results of the present study are in contrast with the findings of Roberson et al. (2000) who conducted vitamin D_3 toxicity in lambs and reported increased hemoglobin and hematocrit values.

Bahman et al. (2011) reported normal plasma/serum calcium in the range of 9-10.5 mg/dL in broiler chicks. In the present study treated groups showed hypercalcemia. The toxic effects of vitamin D_3 are primarily related to the role of vitamin D_3 in the regulation of plasma calcium (Davies and Adams 1978; Reichel and Norman, 1989). The active metabolites of cholecalciferol have been reported to increase the blood calcium level by increased resorption/mobilization of calcium from bone, increased absorption of calcium from intestine and decreased calcium excretion by kidney (Vieth, 1990; Lumeij, 1994; Norman, 1996; Pettifor et al., 1995). Taylor et al., (1968) reported that in chicks given the toxic level of vitamin D_3 there was an increase in plasma calcium, similar results were reported by authors in their studies (Mazumdar et al., 2017; Cheng et al., 2016) whereas no increase in serum calcium level was reported by Cavia et al. (2015) in guinea pigs. Armstrong et al. (2018) reported increased plasma calcium level in dogs prophylactically given calcitriol. Vitamin D_3 toxicity results to higher blood calcium level leading to renal and cardiac failure culminating to death (Beasley, 1999; Radostits et al., 2000; Price et al., 2001) in different species.

Phosphate flux through the gastrointestinal epithelium is enhanced by vitamin D_3. This results from a direct effect of 1,25-dihydroxycholecalciferol, this hormone's action on calcium absorption, the calcium in turn acting as a transport mediator for the phosphate. Vitamin D_3 also increases calcium and phosphate reabsorption by the epithelial cells of the renal tubules, thereby tending to decrease excretion of these substances in the urine (Guyton and Hall, 2001). Similar results were reported by Cavia et al, (2015) in guinea pigs, on the contrary Linda and Aaron (2016) reported decrease in phosphorous concentration as a result of vitamin D_3 supplementation in laying hen. The normal levels of plasma/serum phosphorous in the range of 4-6 mg/dL in apparently healthy broiler chicks (Bahman et al., 2011).

The normal plasma total protein and plasma albumin level in vitamin D_3 toxicity in present study was dissimilar with low total protein level in vitamin D_3 toxicity reported by Roberson et al. (2000) in lambs. The findings in the present study are in contrast with Beasley (1999) who reported clinical signs like proteinuria and hence decrease in plasma total protein concentration in vitamin D_3 toxicity. The non-significant ($P<0.05$) change in the plasma total protein and albumin values may be due to dehydration because of polyuria and hence no change in relative protein concentration of plasma. The pathological changes in the kidneys (mineralization) might have prevented the excretion of plasma proteins and albumin via urine hence, maintaining the concentration of these proteins in the plasma. Bosch et al. (1988) and Al-Dughaym (2004) reported decreased total protein and albumin concentration in endotoxin treated animals. However, in the present study plasma total protein and albumin concentration did not show any significant ($P<0.05$) alteration between the chicks of group B and D exposed to LPS.

The serum transaminases (ALT and AST) are liver specific enzymes and thus their values help in detection of hepatocellular injury (Tenant, 1997). In the present study there was no alteration in their values which might be attributed to normal histoarchitecture of liver of the treatment groups. However, significant ($P<0.05$) increases in serum transaminases have been reported in cross bred calves (Kumar and Mallik, 2001), in rabbits (Yajar et al., 2004) and in pigs (Borissov and Andonova, 2000) challenged with endotoxin. Moreover, it has been opined that damage to any particular organ cannot be cited as cause of increased level of serum transaminases (Kaneko et al., 2008).

There was no significant decrease ($P<0.05$) in the plasma ALP activity in treatment group C and group D. This may be due to decreased osteoblast cell activity and increased osteoclast cell activity in bones because of high dose of vitamin D_3. Acute doses of vitamin D_3 (>100 times than required level) can result in negative calcium balance because of bone resorption is accelerated as in *Solanum malacoxylon, Cestrum diurnum and Trisetum flavescens* toxicity (Kaneko et al., 2008). Cavia et al. (2015) reported increase in ALP activity in

hypervitaminosis D in guinea pigs. ALP activity of LPS challenged chicks (groups B and D) did not reveal any significant ($P<0.05$) difference from NSS challenged chicks (groups A and C). Similar results were reported was comparable to control group chicks (group A) in crossbred calves (Kumar and Mallik, 2001) and in rats (Bosch et al., 1988) reported similar findings in endotoxemia. This non-significant decrease ($P<0.05$) in ALP activity in treated groups (group C and D) might be due to mineralization causing damage to kidney.

There is no significant change in the hematological and biochemical parameters of broiler chick, this may be due to the immunomodulation provided by Vitamin D_3 fed to the broiler chicks. Different authors in their studies confirmed similar immunomodulatory effects of Vitamin D3 (Bikle, 2010; Schwarz et al., 2012; Shojadoost et al., 2015; Rodriguez-Lecompte et al., 2016).

CONCLUSION

The Vitamin D_3 is an immunomodulator; broiler diet supplemented with Vitamin D_3 causes immunomodulation in broiler birds, hence acute endotoxic shock does not incite inflammatory response and disturb the homeostasis in broiler chicks.

DECLARATIONS

Consent to publish

All authors gave their informed consent prior to their inclusion in the study.

Competing interests

The authors declare that they have no competing interests.

Author`s contribution

All authors have equally contributed in this work.

REFERENCES

Abbas AK, Lichtman AH and Pober JS (1997). Cytokines. In Cellular and Molecular Immunology. WB Saunders, Philadelphia, pp: 250–276.

Adams JS and Hewison M (2010). Update in vitamin D. Journal of Clinical Endocrinology and Metabolism, 95: 471–478. Doi: https://doi.org/10.1210/jc.2009-1773

Al-Dughaym AM (2004). Some endotoxin induced clinical and biochemical changes in plasma of camels (*Camelus dromederius*). Veterinary research communication, 28: 711-18. Doi: https://doi.org/10.1023/B:VERC.0000045956.68656.43.

Armstrong AJ, Hauptman JG, Stanley BJ, Klocke E, Burneko M, Holt DE, Runge JJ and Rubin, JA (2018). Effect of Prophylactic Calcitriol Administration on Serum Ionized Calcium Concentrations after Parathyroidectomy: 78 Cases (2005–2015) J Veterinary Internal Medicine, 32:99–106. Doi: 10.1111/jvim.15028.

Bachmann H, Autzen S, Frey U, Wehr U, Rambeck W, McCormack H and Whitehead C.C (2013). The efficacy of a standardised product from dried leaves of Solanum glaucophyllum as source of 1,25-dihydroxycholecalciferol for poultry. British Poultry Science, 54:5, 642-652, Doi: 10.1080/00071668.2013.825692

Bachmann H, Offord-Cavin E, Phothirath P, Horcajada MN, Romeis P and Mathis GA. (2013). 1,25-Dihydroxyvitamin D3-glycoside of herbal origin exhibits delayed release pharmacokinetics when compared to its synthetic counterpart. The Journal of Steroid Biochemistry and Molecular Biology, 136:333-6. Doi:10.1016/j.jsbmb.2012.09.016.

Bahman Abdi-Hachesoo, Talebi A and Asri-Rezaei S (2011). Comparative Study on Blood Profiles of Indigenous and Ross-308 Broiler Breeders. Global Veterinaria, **7**: 238-241.

Beasley VR (1999). Veterinary Toxicology. International Veterinary Information Service (www.ivis.org), Ithaca, New York.

Benjamin MM (1985). Outline of Veterinary clinical Pathology.3rd edition. Kalyani Publishers, Ludhiana, India 25,48,60.

Bikle DD (2010). Vitamin D: newly discovered actions require reconsideration of physiologic requirements. Trends Endocrinology Metabolism, 21: 375–384. Doi: https://doi.org/10.1016/j.tem.2010.01.003

Borissov I and Andonova M (2000). Escherichia coli lipopolysaccharide induced experimental infection in piglets: clinical and laboratory findings. Revue de Medicine Veterinaire, 151: 931-936.

Bosch MA, Gracia R, Pagani R, Portoles MT, Diaz-Laviada I, Abarca S, Ainaga MJ, Risco C and Municio AM (1988). Induction of reversible shock by *Escherichia coli* lipopolysaccharide in rats: Changes in serum and cell membrane parameters. British Journal of Experimental Pathology, 69: 805-812.

Cavia P, Holcombe, H, Parry, NM, Rick, M, Brown, DE, Albers, TM Refsal, KR Morris, J, Kelly, R and Marko, ST (2015). Hypervitaminosis D and Metastatic Calcification in a Colony of Inbred Strain 13 Guinea Pigs, Veterinary Pathology, Vol. 52(4) 741-751

Cheng G, Yi F, Yanhui L, Xu Z, Lu Z, Fang Y, Susanna SX, Qingbo X, Yi Z, Youfei G, Xian W and Wei K (2016).. Microsomal Prostaglandin E Synthase-1–Derived PGE2 Inhibits Vascular Smooth Muscle Cell Calcification Arteriosclerosis, Thrombosis, and Vascular Biology, 36:108-121. DOI: 10.1161/ATVBAHA.115.306642.

Davies M and Adams PH (1978). The continuing risk of vitamin-D intoxication. Lancet, 2: 621-623. https://doi.org/10.1016/S0140-6736(78)92838-6

Fritts CA and Waldroup PW (2003). Effect of source and level of vitamin D on live performance and bone development in growing broilers. Journal of Applied Poultry Research, 12(1):45-52. https://doi.org/10.1093/japr/12.1.45

Guyton AC and Hall JE (2011). *Guyton & Hall Textbook of medical physiology* (11 edition). Philadelphia, Pensylvania. Saunders/Elsevier; 985. **doi**: 10.4103/sni.sni_327_17

Kaneko JJ, Harvey JW and Bruss ML (2008). In: Clinical biochemistry of domestic animals (6th ed.). Academic press Inc., San Diego, 364-97: 706. https://doi.org/10.1111/j.1939-165X.2009.00202.x

Kelly A T, Jonathan WB, Luis MS, Olivia BY, Joshua VP, J, MC, Nicholas RS, Daniel DB, and Leggy AA (2016). Synthesis and Evaluation of Vitamin D Receptor-Mediated Activities of Cholesterol and Vitamin D Metabolites. European Journal of Medicinal Chemistry, 109: 238–246. Doi:10.1016/j.ejmech.2016.01.002.

Kumar R and Mallik JK (2001). Effect of multiple injections of Escherichia coli endotoxin on the pharmacokinetics and dosage regimens of a long-acting formulation of oxytetracycline (OTC-LA) in crossbreed calves. Veterinarski arhiv, 71: 245-263.

Linda CB and Aaron JC (2015). Interactive effects of vitamin D_3 and strontium on performance, nutrient retention and bone mineral composition in laying hens. The Journal of the Science of Food and Agriculture, 95: 1080–1087

Lumeij JT (1994). In ―Avian medicine: Principles and Applications (B W Ritchiie,G H Harrison and L R Harrision, Eds), Wingers, Lake Worht, FL, pp: 582-606.

Mazumdar I, Goswami K and Ali MS (2017). Status of Serum Calcium, Vitamin D and Parathyroid Hormone and Hematological Indices Among Lead Exposed Jewelry Workers in Dhaka, Bangladesh. Indian Journal of Clinical Biochemistry. 32(1):110-116. Doi: 10.1007/s12291-016-0582-9.

Nain S, Laarveld B, Wojnarowicz C and Olkowski AA (2007). Excessive dietary vitamin D supplementation as a risk factor for sudden death syndrome in fast growing commercial broilers. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 148: 828-833. Doi:https://doi.org/10.1016/j.cbpa.2007.08.023.

Norman AW (1996). Vitamin D. In: E.E. Ziegler, L.J. Filer Jr. (Eds.) Present knowledge in nutrition. 7th ed. ILSI Press, Washington, DC; 120–129.

Pettifor JM, Bikle DD, Cavaleros M, Zachen D, Kamdar MC and Ross FP (1995). Serum levels of free 1,25-dihydroxyvitamin D in vitamin D toxicity. Annals of Internal Medicine, 122: 511–513. Doi: 10.7326/0003-4819-122-7-199504010-00006

Price PA, Buckley JR and William MK (2001). The amino bisphosphonate ibandronate prevents vitamin D induced calcification of arteries, cartilage, lungs and kidneys in rats. Journal of Nutrition, 131: 2910-2915. https://doi.org/10.1093/jn/131.11.2910

Radostits OM, Gay CC, Blood DC and Hinchcliff KW (2000). Veterinary Medicine-A textbook of the diseases of cattle, sheep, pigs, goats and horses.9th Edn. Book Power (formerly ELST), UK. 561, 563, 1543, 1642.

Reichel H,Koeffler HP and Norman AW (1989). The role of the vitamin D endocrine system in health and disease. New England Journal of Medicine, 320:980–91. DOI: 10.1056/NEJM198904133201506.

Roberson RJ, Swecker WS and Hullender LL (2000). Hypercalcemia and hypervitaminosis D in two lambs. JAVMA, 216: 1115-1118. https://doi.org/10.2460/javma.2000.216.1115.

Rodriguez-Lecompte JC, Yitbarek A, Cuperus T, Echeverry H and Van Dijk A (2016). The immunomodulatory effect of vitamin D in chickens is dose-dependent and influenced by calcium and phosphorus levels Poultry Science, 95(11):, 2547–2556. Doi: doi.org/10.3382/ps/pew186

Roeder DJ, Lei M and Morrison DC (1989). Endotoxic-lipopolysaccharide-specific binding protein on lymphoid cells of various animal species: Association with endotoxin susceptibility. Infection and Immunity, 57: 1054–1058. Doi: Doi.org/ 0019-9567/89/041054-05$02.00/0.

Schwarz, A., Navid, F., Sparwasser, T., Clausen, B.E. and Schwarz, T., 2012. 1, 25-dihydroxyvitamin D exerts similar immunosuppressive effects as UVR but is dispensable for local UVR-induced immunosuppression. Journal of Investigative Dermatology, 132(12): 2762-2769. Doi: 10.1038/jid.2012.238.

Shojadoost, B., Behboudi, S., Villanueva, A.I., Brisbin, J.T., Ashkar, A.A. and Sharif, S., 2015. Vitamin D3 modulates the function of chicken macrophages. Research in veterinary science, 100: 45-51. Doi: 10.1016/j.rvsc.2015.03.009.

Soares JH, Kerr JM and Gray RW (1995). 25-hydroxycholecalciferol in poultry nutrition. Poultry Science, 74: 1919-1934. Doi:https://doi.org/10.3382/ps.0741919.

Taylor TG, Morris KML and Kirkley J (1968). Effects of dietary excesses of vitamins A and D on some constituents of the blood of chicks. British Journal of Nutrition, 22(4): 713-721. Doi: https://doi.org/10.1079/BJN19680081.

Tenant BC (1997). Hepatic function. In-Clinical biochemistry of domestic animals Kaneko JJ, Harvey JW and Bruss ML (ed). 5th Edition. Academic press. San Diego, 327-352.

Vieth R (1990). The mechanisms of vitamin D toxicity. Bone Miner, 11: 267–272. Doi:https://doi.org/10.1016/0169-6009(90)90023-9.

Wideman, RF Jr., Blankenship, J, Pevzner IY and Turner, BJ (2015). Efficacy of 25-OH Vitamin D 3 prophylactic administration for reducing lameness in broilers grown on wire flooring. Poultry Science 94:1821–1827. Doi: doi.org/10.3382/ps/pev160.

Yajar E, Col R, Uney K, Atalay B, Eelmas M and Tras B (2004). Effect of pentoxifylline on biochemical parameters in endotoxaemic New Zealand white rabbits. Bulletin of Veterinary Institute in Pulawy B, 297-299.