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CLOSTRIDIUM BUTYRICUM CAN BE USED AS A POTENTIAL ALTERNATIVE FOR THE ANTIBIOTIC IN CHERRY VALLEY DUCKS

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ABSTRACT

The present study was conducted to investigate the effects of *Clostridium butyricum* inclusion, as an alternative for antibiotic, on growth performance, immunity and oxidative status of Cherry Valley ducks. 240 1-day-old Cherry Valley ducks were randomly allocated to 2 groups with 6 replicates of 20ducks, receiving a basal diet supplemented with zinc bacitracin (40 mg/kg diet, Control group) or *Clostridium butyricum* (1.0×10^9cfu/kg diet), for 42 days, respectively. The results indicated that ducks fed diet supplemented with *Clostridium butyricum* achieved similar growth performance to those given zinc bacitracin inclusion diet ($P>0.05$). Likewise, the relative weight of immune organs (thymus, spleen and bursa of Fabricius) in two groupsalso showed a high similarity ($P>0.05$). As expected, *Clostridium butyricum* inclusion significantly increased serumimmune globulin M (IgM) content when compared with the control at 21 d ($P<0.05$). However, this beneficial effect was not observed in the concentrations of serum immune globulin G (IgG), immune globulin A (IgA) and complements ($P>0.05$). The antioxidant capacity in the serum was similar between the two groups. It was suggested that *Clostridium butyricum* can be used as an alternative for the antibiotic, zinc bacitracin, in Cherry Valley ducks, which was well demonstrated by various parameters measured above.

Keywords:*Clostridium butyricum*, Zinc bacitracin, Growth, Immunity, Oxidative status, Cherry Valley ducks.

INTRODUCTION

Antibiotics have been used widely in the poultry production to maintain health and improve production efficiency in the last decades (Rosen, 1995). Antibiotic inclusion at the sub-therapeutic level to poultry diet could decrease the incidence of disease and improve growth rate as well as feed efficiency of birds, especially those grow under overcrowded and unsanitary conditions (Islam *et al*., 2014). It has been estimated that the use of antibiotics in the poultry industry has resulted in a 3-5% increase in growth and feed conversion efficiency (Thomke and Elwinger, 1998).However, the use of antibiotics has resulted in lots of problems such as development of drug-resistant bacteria (Sørum and Sunde, 2001) and drug residues in the body of the birds (Burgat, 1991).The European Union ban on the use of certain antibiotics as growth promoters in broiler production has led to the search for some alternate feed supplements (Yasar *et al*., 2011). As a consequence, the search for alternatives to replace antibiotics has gained increasing interest in animal nutrition in recent years. Probiotics are defined as live and harmless microorganisms and are used widely in farm livestock (Afrc, 1989) since they are associated with reducing clinical disease, increasing growth rates, enhancing antioxidant status and immunity (Capcarova *et al*., 2010; Chen *et al*., 2013a, b; Scharek-Tedin *et al*., 2013). Therefore,probiotics are now usually considered as one of the potential alternatives for antibiotics used in the feed (Yang *et al*., 2009).

*Clostridium butyricum*is a butyric-acid-producing, spore-forming, Gram-positive anaerobe bacterium found in soil and the intestines of healthy animals and humans (Finegold *et al*., 1983; Zhang *et al*., 2011). It has been reported that a probiotic, mainly composed of *Bacillus subtilis* endospores and *Clostridium butyricum*, increased the percentage of blood lymphocytes and relative weight of spleen and bursa of Fabriciusinin broilers when compared with the non-probiotic treatment (Chen *et al*., 2013a). Furthermore, Zhang *et al*. (2014) found that dietary supplementation of *Clostridium butyricum* achieved similar or better effects in promoting growth performance and alleviating the immune stress in *Escherichia coli*K88-challenged broiler chickens as compared with the antibiotic, colistin sulfate. Likewise, Yang *et al*. (2012) observed that the replacement of a dietary antibacterial agent (colistin sulfate) with *Clostridium butyricum* promoted growth performance and immune function and benefited the balance of the intestinal microflora in male Lingnan Yellow broiler chickens. Similar result was also reported by Cao *et al*. (2012) in Lingnan Yellow broiler chickens. Previous studies regarding the application effect of *Clostridium butyricum* inclusion on the poultry mainly focused on broilers. However, there was no data available concerning the application of *Clostridium butyricum*in the ducks production. The current study was therefore conducted to evaluate the effects of *Clostridium butyricum* inclusion, on the growth performance and

immune function as well as antioxidant capacity of the Cherry valley ducks as compared with the antibiotic, zinc bacitracin.

MATERIALS AND METHODS

The animal care and use protocols were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee.

Two hundred and forty 1-day-old Cherry Valley ducks were randomly allocated to 2 groups with 6 replicates of 20ducks, receiving a basal diet (Table 1) supplemented with zinc bacitracin (40mg/kg diet, control group) or *Clostridium butyricum* (1.0×10^9cfu/kg diet, recommended dosage), for 42 days, respectively. The average initial body weight of the ducks in each of the 2 groups was similar. *Clostridium butyricum* was provided by Yuanshan Biotech Co., Ltd (Yancheng, Jiangsu, P. R. China). Ducks were allowed *ad libitum* access to pellet feed and water throughout the experiment. All ducks were placed on a plastic-mesh that was placed 0.4 m above the floor and housed in a room maintained at a brooding temperature of 33 to 35°C for 5 d, the temperature was then decreased by 1°C every other day to 25°C, at which level it was maintained until the end of the experiment. The lighting schedule provided 23 h of light per day throughout the experiment. Body weight (measured at 1 and 42 d after deprivation of feed for 12 h, with water being provided *ad libitum*) and feed consumption during the 42-d experiment were recorded to calculate weight gain, feed intake and feed conversion ratio.

Sample collection: At21 and 42 d of experiment, 6 ducks (1 duck per cage) were randomly selected from each treatment after deprivation of feed for 12 h, weighed and killed by cervical dislocation. Individual blood samples were taken and separated by centrifugation at 4,450×*g* for 10 min at 4°C. Serum samples were frozen at -20°Cuntil analysis for immune and antioxidant parameters. After that, bursa of Fabricius, thymus, and spleen were collected and weighed to obtain the relative organ weights using the following formula: Relative weight of immune organ (g/kg) = immune organ weight (g) / body weight (kg).

Measurement of serum antioxidant parameters: Serum samples were analyzed for glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC) activity, malondialdehyde (MDA) and reduced glutathione (GSH) content using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P. R. China) according to the manufacturer's instructions. Briefly, the activity of SOD was determined by following nitrite method as described by Oyanagui (1984), GSH-Px activity following by dithio-nitro benzene method described by Hafeman *et al*. (1974), MDA content following by thiobarbituric acid method described by Placer *et al*. (1966), GSH content following modified Beutler method described by Zhang and Meng (1989). One unit of SOD was defined as the amount of SOD required to produce 50% inhibition of the rate of nitrite production at 37°C. One unit of GSH-Px activity was defined as the amount of enzyme that would catalyze the conversion of 1 µmol/L of GSH to oxidized GSH at 37°C in 5 min. MDA and GSH were expressed as nmol/L andmg/mL in serum, respectively. The spectrometric method was applied to evaluate T-AOC. In the reaction mixture, ferric ionwas reduced by antioxidant reducing agents and blue complex Fe^{2+}-TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) was produced. This blue complex then reacted with phenanthroline to generate a stable complex which could be monitored by the absorbance at 520 nm. One unit of T-AOC was defined as 0.01increase in absorbance value at 37°C per min.

Determination of serum immune parameters: Serum immunoglobulin A (IgA),immunoglobulin G (IgG), complement component 3 (C3) and complement component 4 (C4) concentration were determined inappropriately diluted serum samples by a sandwichenzyme-linked immunosorbent assay (ELISA) as described by Gaça *et al*. (1999) using microtiter plates and chicken-specific IgA, IgG, C3 and C4 ELISA quantitation kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P. R. China). Immuno turbidimetric method was used to measure serum immunoglobulin M (IgM) content using the kits provided by same company mentioned above.

Statistical analysis: All data were analyzed as a completely randomized design using one-way ANOVA (SPSS, 2008). The differences were considered to be significant at $P<0.05$. Results were expressed as means ± standard error.

RESULTS AND DISCUSSION

The mortality rate was similar between the two groups (data not shown). As indicated in the Table 2, ducks fed *Clostridium butyricum* inclusion diet had similar final weight, body weight gain, feed intake as well as feed conversion ratio to those given diet containing the antibiotic ($P>0.05$), suggesting that the replacement of antibiotic with *Clostridium butyricum* was feasible in the present study when considering similar growth performance of ducks. Our result was consistent with the results by Cao *et al*. (2012) and Yang *et al*. (2012) who reported that Lingnan Yellow broilers fed *Clostridium butyricum* inclusion (from 2.5×10^7 to 1×10^8cfu/kg diet and 1.0×10^7 to 3.0×10^7cfu/kg diet, respectively) diets had similar growth performance to those given diet containing colistin sulfate. Similarly, Zhang *et al*. (2014) also found that supplementation of

Clostridium butyricum (2.0×10^7cfu/kg diet) achieved similar effect to the antibiotic (colistin sulfate) in promoting growth performance of *Escherichia coli*K88-challenged broiler chickens. It has been reported that *Clostridium butyricum* supplementation improved intestinal morphological structure, increased nutrients digestion, balanced intestinal microflora, enhanced immune function of animals (Murayama *et al*., 1995; Zhang *et al*., 2011; Cao *et al*., 2012; Yang *et al*., 2012; Chen *et al*., 2013a), which may be the reasons why ducks in the *Clostridium butyricum* group reached similar growth performance to those in the antibiotic group.

Primary and secondary lymphoid organs in the poultry provide the sites for maturation of lymphocytes and the interaction between lymphocytes and antigens (Galal, 2008), which are usually weighed as a measure of the development and function of the immune system (Kwak *et al*., 1999). In the present study, we found that ducks received diet containing *Clostridium butyricum* had similar relative immune organs weight to those (Table 3) in the antibiotic group ($P>0.05$), and it indicated that the growth and development of the immune organs showed a general similarity between the two groups. Similarly, Awad *et al*. (2009) found that dietary *Lactobacillus* inclusion did not improve the absolute and relative weight of spleen, thymus as well as bursa of Fabricius in broiler chickens (the control diet was an antibiotic-free diet). However, Chen *et al*. (2013a) reported that probiotic, mainly composed of *Bacillus subtilis* endospores and *Clostridium butyricum*, increased relative weight of spleen and bursa of Fabricius of broilers fed either low or high nutrient density diet. This discrepancy may be due to diet composition, antibiotic presence, kinds of probiotics, animal species and management. The majority of immunoglobulin during a primary immune response is of the class IgM, which has the ability to easily agglutinate large antigens and therefore to cause the precipitation of soluble antigens, resulting enhanced immune system's ability to remove antigens through phagocytosis (Dibner *et al*., 1998).A switch from IgM to IgG or IgA can be found at the end of a primary immune response, however, immunoglobulin class switching to IgG or IgA is mostly apparent during a second exposure to the same antigen (Erf and Bottje, 1996). In our study, there was no significant difference (Table 4) in the concentration of serum IgG, IgA, C3 and C4 ($P>0.05$) between the two groups throughout the experiment, whereas *Clostridium butyricum* inclusion significantly increased serum IgM content at 21 d ($P<0.05$) when compared with the antibiotic group, indicating that the ducks fed diet containing *Clostridium butyricum* exhibited a more favorable immune status than those given antibiotic supplementation diet, which may be due to that *Clostridium butyricum* can act as a mitogen resulting in proliferation and transformation of B cells, which would mature after homing and class-switching to immunoglobulin secretion (Murayama *et al*., 1995). Murayama *et al*. (1995) found that the oral administration of *Clostridium butyricum* MIYAIRI 588 stimulated polyclonal mucosal immune activity in mice through enhancing the production of polyclonal IgA in the small intestine and the production of IgA, IgM and IgG in the Peyer's patch cell culture. In the contrast, Zhang *et al*. (2014) found that *Escherichia coli*K88-challenged broilers fed *Clostridium butyricum* or antibiotic inclusion diet had similar serum concentrations of IgM, IgY and IgA and complement components. Similar result was also reported by Yang *et al*. (2012) in which birds fed diets supplemented with different levels of *Clostridium butyricum*or colistin sulfate had similar the contents of IgM, IgY and IgA and complement components in the serum. In addition, Zhang and Kim (2014) observed that probiotic composed of *Lactobacillus acidophilus*, *Bacillus subtilis* and *Clostridium butyricum* exerted no beneficial effect on serum IgA and IgM content as compared with avilamycin group despite which in the probiotic group were significantly higher than that in the antibiotic-free group. The inconsistency above may result from probiotics formulation, animal species, animals' physiological state, and management.

Reactive oxygen species (ROS) are produced during normal metabolism in cells, but increased ROS overwhelming the cell's antioxidant protection can result in indiscriminate damages to DNA, proteins and membrane lipids (Yu, 1994). SOD is generally regarded as one of the main antioxidant enzymes in scavenging the oxygen free radical (McCord, 1979). Increased SOD activity is generally considered as improved antioxidant capacity to wipe out oxygen free radicals. MDA is the main end product of lipid peroxidation by ROS and increased MDA level is an important indication of lipid peroxidation (Satoshi*et al*., 1989). GSH is one of the sensitive indicators of the oxidative stress among the components of glutathione system (Troncoso *et al*., 1997). GSH-Px, the component of glutathione system, catalyzes the decomposition of lipid hydroperoxides and other ROS in the presence of GSH which acts as free radical scavenger, while GSH is oxidized (Hornsby and Crivello, 1983). T-AOC, as the reflection of serum ferric reducing/antioxidant power, is usually used to evaluate the antioxidant capacity in cells (Swennen *et al*., 2011). Commensal intestinal bacteria can produce certain factors which can act as free radicals scavengers (Lin and Yen, 1999). Lin and Yen (1999) demonstrated a very good antioxidant effect of these probiotic bacteria in inhibiting lipid peroxidation in an *in vivo* study using intact cells and intracellular cell-free extracts of *Bifidobacterium* and *Lactobacillus*. Likewise, probiotics as *Lactobacillus* bacteria could inhibit the accumulation of ROS in animal cells and therefore could be supplemented to broilers under stress to alleviate oxidative damages (Madsen *et al*., 1999; Bengmark and Martindale, 2005; Peran *et al*.,

2006, 2007). There were no reports regarding the effect of *Clostridium butyricum* inclusion on the antioxidant capacity on ducks at the present. In the current study, we unfortunately found that there was no significant difference in the serum antioxidant parameters (Table 5) between the two groups (P>0.05) despite the fact that numerically higher GSH-Px, SOD and T-AOC activity and GSH content as well as lower MDA concentration were always observed in the ducks fed diet containing *Clostridium butyricum* during the entire experiment. The unaffected serum antioxidant status of ducks may be because that the ducks are well prepared to deal with various oxidative stresses under normal conditions and strict management in the experiment and therefore it was hard to detect any affected changes.

Table 1.Ingredient and nutrient composition of basal diet (%, as-fed basis unless otherwise stated)

Ingredients	1-21d	22-42d
Corn	50.0	52.0
Wheat	3.0	10.0
Wheat middling	11.0	8.0
cottonseed meal	5.0	6.0
Soybean meal	8.0	5.0
Corn gluten meal	10.0	7.0
Meat and bone meal	3.0	3.0
Rice bran	6.5	5.1
L-Lysine	0.15	0.2
DL-Methionine	0.15	0.2
Limestone	1.4	1.2
Dicalcium phosphate	0.5	1.0
Salt (NaCl)	0.3	0.3
Premix†	1.0	1.0
Total	100.0	100.0
Nutrient levels		
ME§, (MJ/kg)	12.13	12.34
Crude Protein	19.38	17.19
Calcium	0.95	1.00
Available phosphorus	0.42	0.30
Lysine	0.80	0.48
Methionine + Cysteine	0.74	0.48

†Premix provided per kg of diet: VitaminA, 10000 IU; Vitamin D_3, 2000 IU; Vitamin E, 20 IU; thiamin, 3 mg; riboflavin, 4 mg; nicotinamide, 60 mg; choline chloride, 600 mg; calcium pantothenate, 11 mg; pyridoxine·HCl, 2.5 mg; biotin, 0.2 mg; folic acid, 0.6 mg; vitamin B_{12} (cobalamin), 0.012 mg; Fe (ferrous sulphate), 80 mg; Mn (manganese sulphate), 80 mg; Zn (zinc oxide), 60 mg; I (calcium iodate), 0.4 mg; Se (sodium selenite), 0.2 mg.

§ME: Metabolizable energy was calculated by China feed data.

In conclusion, ducks fed diet, in which the antibiotic, zinc bacitracin, was replaced by *Clostridium butyricum*,achieved similar growth performance and antioxidant capacity, and more favorable immune status (evidenced by enhanced serum IgM content at 21 d) when compared with those given diet supplemented with antibiotic, indicating the *Clostridium butyricum* could be used as an alternative for the antibiotic in Cherry Valley ducks.

Table 2.Effect of the replacement of antibiotic by *Clostridium butyricum* on the growth performance of Cherry Valley ducks

Items	Control	*Clostridium butyricum*
Body weight (42 d), kg	3.09±0.01	3.08±0.01
Average feed intake, g/d	153.87±2.12	152.01±1.63
Average weight gain, g/d	72.10±0.22	72.04±0.13
Feed conversion ratio	2.13±0.01	2.11±0.01

Table 3.Effect of the replacement of antibiotic by *Clostridium butyricum* on the immune organ weight of Cherry Valley ducks (g/kg)

Items	Control	*Clostridium butyricum*
Thymus		
21 d	2.44±0.06	2.51±0.07
42 d	1.62±0.18	1.82±0.12
Bursa of Fabricius		
21 d	1.23±0.05	1.44±0.02
42 d	0.71±0.01	0.78±0.04
Spleen		
21 d	1.04±0.06	1.03±0.07
42 d	0.54±0.02	0.66±0.04

Table 4. Effect of the replacement of antibiotic by *Clostridium butyricum* on the serum immune parameters of Cherry Valley ducks

Items§	Control	*Clostridium butyricum*
IgM, μg/mL		
21 d	327.15±24.88	463.01±21.66*
42 d	243.04±32.11	191.94±23.02
IgG, mg/mL		
21 d	3.88±0.29	4.04±0.22
42 d	3.82±0.29	4.11±0.30
IgA, μg/mL		
21 d	131.81±5.26	136.57±2.99
42 d	136.96±4.56	136.19±23.0
C3, μg/mL		
21 d	204.79±7.67	221.91±8.31
42 d	237.88±4.83	245.59±11.14
C4, μg/mL		
21 d	120.66±4.33	121.79±2.49
42 d	121.64±3.65	126.94±4.47

§IgM, immunoglobulin M; IgG, immunoglobulin G; IgA, immunoglobulin A; C3, complement-3; C4, complement-4.

*Means within a row differsignificantly (P<0.05).

Table 5. Effect of the replacement of antibiotic by *Clostridium butyricum* on the serum antioxidant capacity of Cherry Valley ducks

Items[§]	Control	*Clostridium butyricum*
SOD, U/L		
21 d	86.15±5.16	97.14±0.86
42 d	98.71±1.62	99.79±3.77
MDA, nmol/mL		
21 d	4.25±0.06	3.85±0.36
42 d	2.92±0.19	2.72±0.08
GSH, mg/mL		
21 d	5.76±0.17	6.14±0.26
42 d	8.47±0.48	8.98±0.27
GSH-Px, U/mL		
21 d	376.00±17.02	386.30±9.72
42 d	326.12±38.47	334.87±12.23
T-AOC, U/L		
21 d	12.37±0.34	12.33±0.98
42 d	13.12±0.84	14.09±0.78

[§]SOD, superoxide dismutase; MDA, malondialdehyde; GSH, reduced glutathione; GSH-Px,glutathione peroxidase; T-AOC, total antioxidant capacity.

REFERENCES

Afrc, R. F. (1989). Probiotics in man and animals. J. Appl. Bacteriol., 66: 365-378.

Awad, W. A., K. Ghareeb, S. Abdel-Raheem, and J. Böhm (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult. Sci., 88: 49-56.

Bengmark, S.and R. Martindale (2005). Prebiotics and synbiotics in clinical medicine. Nutr. Clin. Pract., 20: 244-261.

Burgat, V. (1991). Residues of drugs of veterinary use in food. La Rev. du Prat., 41: 985-990.

Gaça, M. D. A ., J. A. Pickering, M. J. P. Arthur and R. C. Benyon (1999). Human and rat hepatic stellate cells produce stem cell factor: a possible mechanism for mast cell recruitment in liver fibrosis. J. Hepatol., 30:850-858.

Cao, G. T., Y. P. Xiao, C. M. Yang, A. G. Chen, T. T. Liu, L. Zhou, L. Zhang, and P. R. Ferket (2012). Effects of *Clostridiumbutyricum*on growth performance, nitrogen metabolism, intestinal morphology and cecal microflora in broiler chickens. J. Anim. Vet. Adv., 11: 2665-2671.

Capcarova, M., J. Weiss, C. Hrncar, A.Kolesarova, and G. Pal (2010). Effect of *Lactobacillus fermentum* and *Enterococcus faecium* strains on internal milieu, antioxidant status and body weight of broiler chickens. J. Anim. Physiol. Anim. Nutr., 94: 215-224.

Chen, W., J. P. Wang, L. Yan, and Y. Q. Huang (2013a). Evaluation of probiotics in diets with different nutrient densities on growth performance, blood characteristics, relative organ weight and breast meat characteristics in broilers. Br. Poult. Sci. 54: 635-641.

Chen, W., X. Z. Zhu, J. P. Wang,Z. X. Wang, and Y. Q. Huang (2013b). Effects of*Bacillus subtilis*var. *Natto* and *Saccharomyces cerevisiae* fermented liquid feed on growth performance, relative organ weight, intestinal microflora, and organ antioxidant status in Landes geese. J. Anim. Sci., 91: 978-985.

Dibner, J. J., C. D. Knight, M. L.Kitchell, C. A. Atwell, A. C. Downs, and F. J.Ivey (1998). Early feeding and development of the immune system in neonatal poultry. J. Appl. Poult. Res., 7: 425-436.

Erf, G. F.and W. G.Bottje (1996). Nutrition and immune function in chickens: Benefits of dietary vitamin E supplementation. Proc. Arkansas Nutr. Conf., Fayetteville, AR., 113-130 p.

Finegold, S. M., V. L. Sutter and G. E. Mathisen (1983). Normal indigenous intestinal florain: human intestinal microflora in health and disease.London, UK., 3-31p.

Galal, A. (2008). Immunocompetence and some hematological parameters of naked neck and normally feathered chicken. J. Poult. Sci., 45: 89-95.

Hafeman, D. G., R. A.Sunde, and W. G. Hoekstra (1974). Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr., 104: 580-587.

Hornsby, P. J. and J. F.Crivello (1983). The role of lipid peroxidation and biological antioxidants in the function of the adrenal cortex. Part.1:A background review. Mol. Cell. Endocrinol., 30: 1-20.

Islam, M. M., S. T. Ahmed, Y. J. Kim, H. S. Mun, Y. J. Kim, and C. J. Yang (2014). Effect of sea tangle (*Laminaria japonica*) and charcoal supplementation as alternatives to antibiotics on growth performance and meat quality of ducks. Asian-Australas J. Anim. Sci.,27: 217-224.

Kwak, H., R. E.Austic, and R. R.Dietert (1999). Influence of dietary arginine concentration on lymphoid organ growth in chickens. Poult. Sci., 78: 1536-1541.

Lin, M. Y. and C. L. Yen (1999).Antioxidative ability of lactic acid bacteria. J. Agric. Food Chem., 47: 1460-1466.

Madsen, K. L., J. S. Doyle, L. D. Jewell, M. M. Tavernini, and R. N. Fedorak (1999). *Lactobacillus* species prevents colitis in

interleukin 10 gene-deficient mice. Gastroenterology, 116: 1107-1114.

McCord, J. M. (1979). Superoxide, superoxide dismutase andoxygen toxicity. Rev. Biochem.Toxicol., 1: 109-124.

Murayama, T., N.Mita, M. Tanaka, T.Kitajo, T. Asano, K. Mizuochi, and K. Kaneko (1995). Effectsoforally administered *Clostridium butyricum* MIYAIRI 588 on mucosal immunity in mice. Vet.Immunol.Immunopathol., 48: 333-342.

Oyanagui, Y.(1984). Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. Anal.Biochem., 142: 290-296.

Peran, L., D. Camuesco., M. Comalada, A. Nieto, A. Concha, J. L. Adrio, M. Olivares, J. Xaus, A. Zarzuelo, and J.Gálvez (2006). *Lactobacillus fermentum*, a probiotic capable to release glutathione, prevents colonic inflammation in the TNBS model of rat colitis. Int. J. Colorectal. Dis., 21: 737-746.

Peran, L., S. Sierra., M.Comalada, F. Lara-Villoslada, E. Bailón, A. Nieto, A. Concha, M. Olivares, A. Zarzuelo, J. Xaus, and J. Gálvez (2007). A comparative study of the preventative effects exerted by two probiotics, *Lactobacillus reuteri* and *Lactobacillus fermentum*, in the trinitrobenzene sulfonic acid model of rat colitis. Br. J. Nutr., 97: 96-103.

Placer, Z. A., L. L. Cushman, and B. C. Johnson (1966). Estimation of production of lipid peroxidation (malonyldialdehyde) in biochemical systems. Anal. Biochem., 16: 359-364.

Rosen, G. D. (1995). Antibacterials in poultry and pig nutrition. Biotechnology in Animal Feeds and animal Feeding;Weinheim (Germany).143-172p.

Satoshi, S., T. Kiyoji, K. Hiroyo, and N. Fumio (1989).Exercise-induced lipid peroxidation and leakage of enzyme before and after vitamin E supplementation. Int. J. Biochem., 21: 835-838.

Scharek-Tedin, L., R. Pieper, W. Vahjen, K. Tedin, K. Neumann, and J. Zentek (2013). *Bacillus cereus* var. Toyoi modulates the immune reaction and reduces the occurrence of diarrhea in piglets challenged with *Salmonella Typhimurium* DT104. J. Anim. Sci., 91: 5696-5704.

Sørum, H. and M.Sunde (2001). Resistance to antibiotics in the normal flora of animals. Vet. Res., 32: 227-241.

Swennen, Q., P. A. Geraert, Y. Mercier, N. Everaert, A. Stinckens, H. Willemsen, Y. Li, E. Decuypere, and J. Buyse (2011). Effects of dietary protein content and 2-hydroxy-4-methylthiobutanoic acid or DL-methionine supplementation on performance and oxidativestatus of broiler chickens. Br. J. Nutr., 106: 1845-1854.

Thomke, S.and K. Elwinger(1998).Growth promotants in feeding pigs and poultry. II. Mode of action of antibiotic growth promotants. Ann Zootech., 47: 153-167.

Troncoso, P., G. Smok, and L. A.Videla (1997). Potentiation of ischemia-reperfusion liver injury by hyperthyroidism in the rat. Free Radic. Biol. Med., 23:19-25.

Yang, C. M.,G. T. Cao, P. R.Ferket, T. T. Liu, L. Zhou,L. Zhang,Y. P. Xiaoand A. G. Chen (2012). Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens.Poult. Sci., 91: 2121-2129.

Yang, Y., P. A. Iji, and M. Choct (2009). Dietary modulation of gut microflora inbroiler chickens: a review of the role ofsix kinds of alternatives to in-feedantibiotics. World's Poult. Sci. J., 65: 97-114.

Yasar, S., D. Namik, G. Fatih, A. Gokcimen, and K. Selcuk (2011). Effects of inclusion of aeriel dried parts of some herbs in broiler diets. J. Anim. Plant Sci., 21: 465-476.

Yu, B. P.(1994).Cellular defenses against damage from reactive oxygen species. Physiol. Rev., 74: 139-162.

Zhang, B. K., X. Yang, Y. M. Guo, and F. Y. Long (2011).Effects of dietary lipids and *Clostridium butyricum* on the performance and the digestive tract of broiler chickens. Arch. Anim. Nutr., 65: 329-339.

Zhang, L., G. T. Cao, X. F. Zeng, L. Zhou, P. R. Ferket, Y. P. Xiao, A. G. Chen, and C. M. Yang (2014). Effects of *Clostridium butyricum*on growth performance, immune function, and cecal microflora in broiler chickens challenged with *Escherichia coli*K88. Poult. Sci., 93: 46-53.

Zhang, P. and X. J. Meng (1989).Determination of reduced glutathione in different tissues of rat by spectrophotometry method. Chin. J. Exp.Surg., 6: 141-142.

Zhang, Z. F. and I. H. Kim (2014). Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers.Poult. Sci., 93: 364-370.