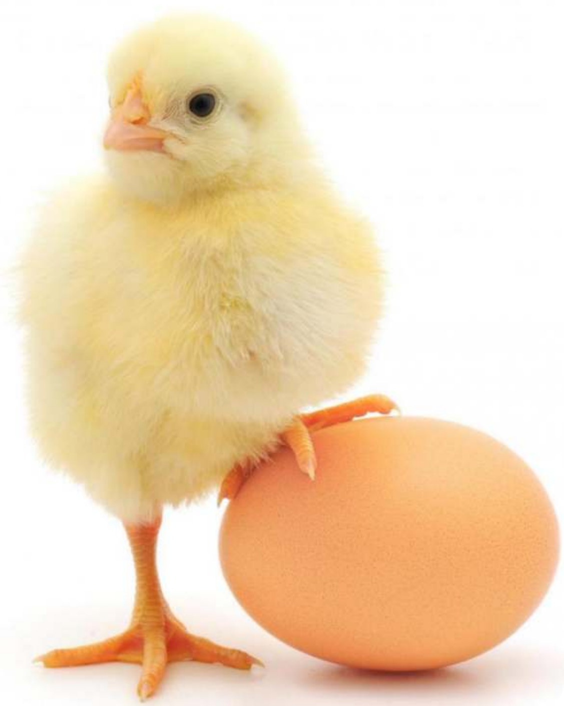
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN: 1682-8356
DOI: 10.3923/ijps.2017.50.55

Research Article

Effect of Tannic Acid Extracted from Cassava (*Manihot esculenta* Crantz) Leaves on Productive Performance, Intestinal Microorganisms and Villi Morphometry in Broilers: A Preliminary Study

Worapol Aengwanich and Thongchai Boonsorn

Stress and Oxidative Stress in Animal Research Unit, Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham 44000, Thailand

Abstract

Background and Objective: Cassava, *Manihot esculenta* Crantz (Euphorbiaceae) leaves contained plentiful of tannic acid. The objective of this study was to investigate the effect of tannic acid extracted from cassava leaves on productive performance and ecology within the small intestines of broilers. **Materials and Methods:** An *in vivo* experiment was conducted to study the effect of tannic acid extracted from cassava leaves on productive performance, intestinal microflora and gut morphology in broilers. Treatments included an antibiotic-free diet (control group), a positive control diet and an antibiotic-free diet with tannic acid extracted from cassava leaves at 10, 20, 30, 40 and 50 mg L^{-1} in drinking water. Body weight, feed intake, average daily gain and feed conversion ratio were investigated for 6 weeks. At week 6, digesta pH, *Lactobacillus* spp., *E. coli* populations and villi height of the duodenum, jejunum and ileum were examined. **Results:** The results revealed the following: The feed intake of broilers fed a positive control diet was lower than that of broilers fed an antibiotic-free diet with tannic acid at 10, 20 and 40 mg L^{-1} in drinking water ($p<0.05$). The body weight and average daily gain of broilers fed an antibiotic-free diet, a positive control diet and an antibiotic-free diet with tannic acid at 10, 40 and 50 mg L^{-1} in drinking water were significantly higher than those of broilers fed an antibiotic-free diet with tannic acid at 20 mg L^{-1} in drinking water ($p<0.05$). The feed conversion ratio of broilers fed an antibiotic-free diet, a positive control diet and an antibiotic-free diet with tannic acid at 10, 30 and 50 mg L^{-1} in drinking water were significantly lower than those of broilers fed an antibiotic-free diet with tannic acid at 20 mg L^{-1} in drinking water ($p<0.05$). The digesta pH in the ileum of broilers fed an antibiotic-free diet with tannic acid at 30 mg L^{-1} in drinking water was lower than that of broilers fed an antibiotic-free diet, a positive control diet, or an antibiotic-free diet with tannic acid at 50 mg L^{-1} in drinking water ($p<0.05$). **Conclusion:** This study showed that the most suitable level of tannic acid extracted from cassava leaves for broiler was 10 mg L^{-1} in drinking water.

Key words: Cassava leaves, tannic acid, productive performance, digesta, *Lactobacillus* spp., *Escherichia coli,* villi height

Received: June 16, 2016 Accepted: November 26, 2016 Published: January 15, 2017

Citation: Worapol Aengwanich and Thongchai Boonsorn, 2017. Effect of tannic acid extracted from cassava (*Manihot esculenta* Crantz) leaves on productive performance, intestinal microorganisms and villi morphometry in broilers: A preliminary study. Int. J. Poult. Sci., 16: 50-55.

Corresponding Author: Worapol Aengwanich, Stress and Oxidative Stress in Animal Research Unit, Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham 44000, Thailand Tel/Fax: +66-043-742823

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cassava, *Manihot esculenta* Crantz (Euphorbiaceae) is a perennial shrub belonging to the Euphorbiaceae family. The *Manihot* genus is comprised of 98 species, of which *M. esculenta* is the most widely cultivated member. It originated in South America and has subsequently been distributed throughout tropical and subtropical regions of Africa and Asia[1]. In Thailand, cassava is considered one of the most economically important crops, with an annual production of around 25 million tons[2]. Cassava is important to Thailand because Thailand was the first major cassava exporter. Even though the major producers of cassava worldwide are Nigeria, Brazil, Indonesia, Thailand and Congo, most of these nations produce cassava for domestic consumption. In Thailand, 68% of cassava products are intended for export[3]. Cassava leaves, depending on variety, are rich in cyanide[4], tannin[4,5] and phytate. Cassava leaves contain condensed tannins, about 30-50 g kg^{-1} on a dry-matter basis[5], which consist of anthocyanidins; among these, two have been identified as cyanidin and delphinidin[6]. Tannins are a complex group of polyphenolic compounds. They possess the capacity to form reversible and irreversible complexes with proteins, polysaccharides, alkaloids, nucleic acids and minerals. Different forms of tannins have diverse biological activities: Anti-tumour, anti-mutagenic, anti-diabetic, anti-proliferative, anti-bacterial and anti-mycotic[7,8]. This compound has the capability to lower protein digestibility and amino acid availability, either by forming indigestibility complexes with dietary protein or via the inactivation of proteolytic enzyme[4,5,8]. Generally, tannin reduces feed palatability, feed efficiency and growth rate and increased endogenous losses of minerals and amino acids in non-ruminants. Moreover, tannins have shown bacteriostatic and bactericidal properties *in vitro* against pathogenic bacteria, including *Escherichia coli*, *Salmonella* spp., *Proteus* spp. and *Clostridium* spp.[9]. Tannic acid is a synonym for hydrolyzable tannins[10]. Tannic acid is a gallotannin consisting of 8-10 molecules of gallic acid per molecule of glucose[9]. It is astringent in taste. Tannic acid is a low-molecular-weight polymer of gallic acid and 3-galloylgallic acid esterified with glucose of variable composition[10]. Fasuyi[11] reported that cassava leaves contained tannic acid (polyphenols) on about a 9.7/100 g dry-matter basis. However, information about the use of tannic acid from cassava leaves in broilers is limited. Therefore, the objective of this study was to investigate the effect of tannic acid extracted from cassava leaves on productive performance and ecology within the small intestines of broilers. In future, this study will create basic knowledge regarding the using tannic acid extracted from cassava leaves in the animal production industry or in humans.

MATERIALS AND METHODS

Collection and processing of cassava leaves and extraction: Cassava leaves were freshly collected from the hectares of cassava farmers in Maha Sarakham province, Thailand and transported in plastic bags. The harvested leaves were weighed and washed. The cassava leaves were dried at 60°C in a hot-air oven (Memmert® Model 30-750). Then, the dried cassava leaves were ground. Tannic acid was extracted from the cassava leaf powder by using a water bath filled with tap water at 80°C. The pH of the extract was adjusted by using acetic acid. Large-molecular-weight tannin was left via the sedimentation method for 24 h. The supernatant of the extract was collected and the level of tannic acid was investigated via Pholin-Ciocalteu assay, using tannic acid as a standard solution.

Birds and diets: Eighty four broilers, Arber Acres chicks that were 1 day old, were obtained from a commercial farm in Roi-Et province, in the Northeast part of Thailand. They were reared in open housing at Mahasarakham College of Agriculture and Technology. The chicks were allocated to 21 pens, with each pen containing four chicks and received 7 treatments, i.e., an antibiotic-free diet (control group), a positive control diet (flavophospholipol at 2 mg kg^{-1} in diet) and an antibiotic-free diet with tannic acid extracted from cassava leaves at 10, 20, 30, 40 and 50 mg L^{-1} in drinking water, with three replicates of each treatment for 6 weeks. Diet and water were provided for *ad libitum* consumption. All diets were formulated so as to meet NRC[12] requirements for broiler chickens. The ingredients and nutrient composition of the diets are shown in Table 1.

***In vivo* study:** At 42 days of age, three broilers from each replicate were killed via cervical dislocation and their intestinal tracts were removed. The duodenum, jejunum and ileum were divided into three parts for the investigation of the digesta pH, microflora (*Lactobacillus* spp. and *E. coli* populations) and villi height. In part 1, fresh digesta were collected from the duodenum, jejunum and ileum and then transferred to plastic tubes. The pH of each sample was investigated by using a pH meter (Consort® C860). In part 2, fresh digesta of the duodenum, jejunum and ileum were collected aseptically in sterilized plastic tubes. The samples

Table 1: Chemical composition of ingredients used in diet formulation

	Diets (%)	
Ingredients	0-3 weeks	3-6 weeks
Corn	51.70	60.20
Soybean meal (44% CP)	32.00	27.70
Fish meal (60% CP)	7.50	4.50
Soybean oil	6.50	5.25
Dicalcium phosphate (16% P)	0.70	0.60
Oyster shell meal	0.95	1.15
DL-methionine	0.15	0.10
Salt	0.25	0.25
*Vitamin/mineral mix	0.25	0.25
Total	100.00	100.00
Chemical composition		
ME (kcal kg^{-1})	3,201.10	3,200.74
CP (%)	22.97	20.01
Fat (%)	9.36	8.13
Fiber (%)	3.43	3.29
Calcium (%)	1.07	0.95
Available phosphorus (%)	0.46	0.35
Digestible lysine (%)	1.33	1.10
Digestible methionine (%)	0.56	0.45
Digestible methionine+cystine (%)	0.89	0.76

*Vitamin/mineral mixed (per 1 kg diet) : Vit A 10,000 IU, Vit D3 2,000 IU, Vit E 11.00 mg, Vit K3 1.50 mg, Vit B1 1.50 mg, Vit B2 4.00 mg, Vit B6 4.00 mg, Vit B12 0.40 mg, pantothenic acid 10.00 mg, niacin 22.00 mg, folic acid 0.40 mg, biotin 0.10 mg, Se 0.10 mg, Fe 60.00 mg, Mn 70.00 mg, Zn 50.00 mg, Cu 8.00 mg, Co 0.50 and 10.70 mg

were weighed and diluted in peptone water to an initial 10^{-1} dilution. Microbial populations were determined via serial dilution (10^{-1} to 10^{-7}) of samples in PBS before inoculation onto petri dishes of sterile agar. The selective media for *Lactobacillus* spp. and *E. coli* were prepared. *Lactobacillus* and *E. coli* was grown on MRS agar and VRBA, respectively. The plates were incubated at 37°C, anaerobically (73% N, 20% CO_2, 7% H_2) for *Lactobacillus* and aerobically for *E. coli*. The plates were counted between 24 and 48 h after inoculation. Colony-forming units were defined as distinct colonies measuring at least 1 mm in diameter. In part 3, approximately 2 cm of the duodenum, jejunum and ileum was collected. The samples were gently flushed twice with physiological saline (1% NaCl) to remove the intestinal contents and placed in 10% formalin for fixation. The samples were processed for 24 h in a tissue processor with ethanol for dehydration and the samples were embedded in paraffin. Sections (5 µm) were made from the tissue and stained with hematoxylin-eosin. Villus height (µm) was measured from the tip of the villus to the villus crypt junction by using a light microscope (Nikon® Eclipse E200) with a camera (Nikon® DS-Fi2).

Statistical analysis: Data were analyzed via one-way ANOVA. The treatment means were separated using Duncan's Multiple Range Test (DMRT). Statistical significance was set at $p<0.05$.

RESULTS

Productive performance: The feed intake of broilers fed a positive control diet was lower than those of broilers fed an antibiotic-free diet with tannic acid at 10, 20 and 40 mg L^{-1} in drinking water ($p<0.05$) but not significantly different from broilers fed an antibiotic-free diet with tannic acid at 30 and 50 mg L^{-1} in drinking water ($p>0.05$). The body weight and average daily gain of broilers fed an antibiotic-free diet, a positive control diet and an antibiotic-free diet with tannic acid at 10, 40 and 50 mg L^{-1} in drinking water were significantly higher than those of broilers fed an antibiotic-free diet with tannic acid at 20 mg L^{-1} in drinking water ($p<0.05$). The feed conversion ratio of broilers fed an antibiotic-free diet, a positive control diet and an antibiotic-free diet with tannic acid at 10, 30 and 50 mg L^{-1} in drinking water were significantly lower than those of broilers fed an antibiotic-free diet with tannic acid at 20 mg L^{-1} in drinking water ($p<0.05$).

Digesta pH, microflora and villi height: The digesta pH within the ilea of broilers fed an antibiotic-free diet with tannic acid at 30 mg L^{-1} in drinking water was significantly lower than those of broilers fed an antibiotic-free diet, a positive control diet and an antibiotic-free diet with tannic acid at 50 mg L^{-1} in drinking water ($p<0.05$). The populations of *Lactobacillus* spp., *E. coli* and villi height within the duodenum, jejunum and ileum for all groups were not significantly different ($p>0.05$) (Table 2).

DISCUSSION

Traditional concepts of poultry nutrition hold tannins to be anti-nutritional factors. Tannins are generally considered undesirable in simple stomached animals feed. In monogastric farm animals, it is commonly accepted that dietary tannins reduce digestibility (in particular that of crude protein) and consequently growth performance[13]. Results of the present study showed that the feed intake of broilers fed an antibiotic-free diet with tannic acid at 30 and 50 mg L^{-1} in drinking water were not different from that of a positive control group. Any difference was not observed in the body weight and average daily gain of the control group, a positive control group and groups of broilers fed an antibiotic-free diet with tannic acid at 10, 40 and 50 mg L^{-1} in drinking water. Moreover, there was not any difference in the feed conversion rate of the control group, a positive control group and group of broilers fed an antibiotic-free diet with tannic

Table 2: Effect of an antibiotic diet and tannic acid extracted from cassava leaves at 10, 20, 30, 40 and 50 mg L^{-1} in drinking water on productive performance and digesta pH, population of *Lactobacillus* spp., *E. coli* and villi height in the duodenum, jejunum and ileum of broilers

			Tannic acid				
Parameters	Control	Antibiotic*	10 mg L^{-1}	20 mg L^{-1}	30 mg L^{-1}	40 mg L^{-1}	50 mg L^{-1}
Productive performances							
Feed intake (g day^{-1})	67.76 ± 2.11^{ab}	64.58 ± 5.090^{b}	70.44 ± 2.31^{a}	71.73 ± 1.300^{a}	67.66 ± 1.9100^{ab}	72.02 ± 1.030^{a}	69.05 ± 0.600^{ab}
Body weight (g $head^{-1}$)	1770.50 ± 85.95^{a}	17795.67 ± 50.00^{a}	1826.64 ± 76.04^{a}	1604.67 ± 58.32^{b}	1729.67 ± 123.47^{ab}	1762.42 ± 80.56^{a}	1858.92 ± 33.59^{a}
Average daily gain (g day^{-1})	42.16 ± 2.05^{a}	42.75 ± 1.19^{a}	43.49 ± 1.81^{a}	38.21 ± 1.390^{b}	41.18 ± 2.9400^{ab}	41.96 ± 1.920^{a}	44.26 ± 0.800^{a}
Feed conversion ratio	1.61 ± 0.10^{bc}	1.51 ± 0.15^{c}	1.62 ± 0.09^{bc}	1.88 ± 0.100^{a}	1.65 ± 0.1000^{bc}	1.72 ± 0.050^{ab}	1.56 ± 0.040^{bc}
Digesta pH							
Duodenum	5.90±0.16	5.84±0.29	5.82±0.26	5.72±0.32	5.73±0.16	5.79±0.41	5.64±0.16
Jejunum	5.93±0.20	5.77±0.41	5.58±0.32	5.63±0.41	5.37±0.13	5.44±0.29	5.37±0.25
Ileum	6.41 ± 0.15^{a}	6.28 ± 0.55^{a}	5.91 ± 0.22^{ab}	5.94 ± 0.50^{ab}	5.44 ± 0.07^{b}	5.97 ± 0.28^{ab}	6.28 ± 0.24^{a}
***Lactobacillus* spp.**							
Duodenum ($\times 10^4$ CFU g^{-1})	0.40±0.680	0.86±0.860	0.93±1.54	0.69±11.08	3.52±0.5300	2.39±3.7200	0.870±1.0700
Jejunum ($\times 10^4$ CFU g^{-1})	0.25±0.430	1.06±1.830	0.05±0.06	0.61±0.540	0.25±0.2000	0.14±0.2000	0.002±0.0039
Ileum ($\times 10^4$ CFU g^{-1})	7.06±11.96	97.25±84.18	275.71±46.92	241.25±36.92	109.93±167.96	135.58±104.97	45.100±67.480
E. coli							
Duodenum (CFU g^{-1})	0.00±0.000	1.11±1.92	0.00±0.00	10.00±17.32	3.33±5.77	6.67±8.82	3.33±5.77
Jejunum (CFU g^{-1})	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00
Ileum (CFU g^{-1})	6.67±11.55	0.00±0.00	0.00±0.00	0.00±0.000	0.00±0.00	3.33±5.77	0.00±0.00
Villi height							
Duodenum (μm)	1109.67±176.68	1023.55±75.920	1259.29±212.35	1126.29±205.81	1212.48±121.70	915.59±170.29	190.79±161.43
Jejunum (μm)	1037.13±74.910	1080.20±1014.6	1014.60±219.58	960.63±321.67	729.55±137.18	989.07±72.240	797.19±69.380
Ileum (μm)	639.15±48.240	810.48±277.49	800.47±400.40	604.64±81.490	520.35±64.040	640.02±127.82	546.42±56.990

[a-c]Letters within row, mean with no common superscript differ significantly ($p<0.05$), *Flavophospholipol at 2 mg kg^{-1} in diet

acid at 10, 30 and 50 mg L^{-1} in drinking water. This indicates that some levels of tannic acid extracted from cassava leaves had no effect on the productive performance of broilers when compared with the control or positive control group. Results of the present study are in accordance with the report of the EFSA FEEDAP Panel[10], it is found that tannic acid prepared from chestnut wood at concentrations of up to 2,000 mg had no adverse effects on the growth or feed intake of chickens for 42 days. However, other reports have shown that growth and feed intake were reduced in a 41 days trial at concentrations greater than 1,000 mg of tannic acid prepared from chestnut wood.

Sugiharto[14] reported that organic acids, such as lactic, acetic, fumaric, propionic caprylic and tannic acids have been shown to exhibit beneficial effects on the intestinal health and performance of birds. Dietary organic acid supplementation increased Lactic Acid Bacteria (LAB) counts in the ilea and caeca of broiler chickens. The antimicrobial properties of acids have been suggested to play a crucial role in controlling the population of pathogenic bacteria in the guts of birds. Organic acids can freely diffuse through the semi-permeable membrane of the bacteria into the cell cytoplasm. Once in the cell, where the pH is maintained near 7, the acid will dissociate and suppress bacterial cell enzymes (e.g., decarboxylases and catalases) and nutrient transport systems. The reduction of pathogenic intestinal bacteria, which produce toxins that cause damage to the intestinal villi and crypt structure is associated with improved gut structure in chickens. In concert with the antimicrobial effect, the inclusion of organic acids seems to have direct effects on the histomorphology of the gut by increasing the height of the villi. The potential of organic acids to lower chyme pH is another property of these compounds that support growth. However, in this study it was observed that the populations of *Lactobacillus* spp., *E. coli* and villi height within the duodenum, jejunum and ileum of all groups were not different. This phenomenon indicated that tannic acid extracted from cassava leaves had no effect on the ecology within the guts of broilers. The results of this study differed from the study of Samanta *et al.*[15], who found that the ingestion of tannic acid by male Albino rats reduced the bacterial population in 6 days and, after that, increased the bacterial population. The changes in microbial population indicated that tannic acid may impair the ecological balance of the gastrointestinal flora. However, the bacteria population was not determined at the early stage of this experiment. Thus, the trend of bacterial change was not found during these stages. Another result was that the digesta pH in the ilea of the group that received tannic acid at 30 mg L^{-1} in drinking water was lower than those of the control and the positive control group. Given this phenomenon, It could not be explained that why the digesta pH of this group was lower than those of the two control groups, because productive performance and other parameters regarding the small intestines of broilers, such as the lactic acid bacteria (*Lactobacillus* spp., population) were not different. Aengwanich and Suttajit[16,17] reported that polyphenols extracted from tamarind seed cost could improve productive performance and reduced both stress and oxidative stress in broilers under chronic heat stress. Therefore, the results of this study may be seen as fundamental knowledge regarding the use of tannin from cassava leaves in poultry production in hot climates.

CONCLUSION

This study has shown that tannic acid extracted from cassava leaves some levels had an effect on feed intake, body weight and average daily gain and the feed conversion ratio of broilers. Whereas, this extract has no effect on the ecology within the small intestines of broilers. The recommended level of tannic acid extracted from cassava leaves for broilers was 10 mg L^{-1} in drinking water.

SIGNIFICANCE STATEMENTS

- Cassava, *Manihot esculenta* Crantz (Euphorbiaceae) leaves contained tannic acid (polyphenols) on about a 9.7/100 g dry-matter basis
- Any difference was not observed in the body weight and average daily gain of the control group, a positive control group and groups of broilers fed an antibiotic-free diet with tannic acid at 10, 40 and 50 mg L^{-1} in drinking water
- Tannic acid extracted from cassava leaves had no effect on the ecology within the guts of broilers
- The most suitable level of tannic acid extracted from cassava leaves for broiler was 10 mg L^{-1} in drinking water

ACKNOWLEDGMENTS

This study was funded and supported by Faculty of Veterinary Sciences, Mahasarakham University, Thailand. We would like to thank to Mr. Pongsak Pooponpan, Mr. Anan Srikueaklin and Mr. Jeerasak Viengpraving for laboratory investigation. We also would like to thank Mahasarakham College of Agriculture and Technology for housing supported.

REFERENCES

1. Blagbrough, I.S., S.A.L. Bayoumi, M.G. Rowan and J.R. Beeching, 2010. Cassava: An appraisal of its phytochemistry and its biotechnological prospects. Phytochemistry, 71: 1940-1951.
2. Piyachomkwan, K. and M. Tanticharoen, 2011. Cassava industry in Thailand: Prospects. J. Royal Inst. Thailand, 3: 160-170.
3. Poramacom, N., A. Ungsuratana, P. Ungsuratana and P. Supavititpattana, 2013. Cassava production, prices and related policy in Thailand. Am. Int. J. Contemp. Res., 3: 43-51.
4. Oresegun, A., O.A. Fagbenro, P. Ilona and E. Bernard, 2016. Nutritional and anti-nutritional composition of cassava leaf protein concentrate from six cassava varieties for use in aqua feed. Cogent Food Agric., Vol. 2. 10.1080/23311932.2016.1147323
5. Ravindran, V., 1993. Cassava leaves as animal feed: Potential and limitations. J. Sci. Food Agric., 61: 141-150.
6. Latif, S. and J. Muller, 2015. Potential of cassava leaves in human nutrition: A review. Trends Food Sci. Technol., 44: 147-158.
7. Masek, T., K Starcevic and Z. Mikulec, 2014. The influence of the addition of thymol, tannic acid or gallic acid to broiler diet on growth performance, serum malondialdehyde value and cecal fermentation. Eur. Poult. Sci., Vol. 78. 10.1399/eps.2014.64
8. Ye, M.H., Y.L. Nan, M.M. Ding, J.B. Hu, Q. Liu, W.H. Wei and S.M. Yang, 2016. Effects of dietary tannic acid on the growth, hepatic gene expression and antioxidant enzyme activity in Brandt's voles (*Microtus brandti*). Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol., 196: 19-26.
9. Lee, S.H., P.L. Shinde, J.Y. Choi, I.K. Kwon and J.K. Lee *et al*., 2010. Effects of tannic acid supplementation on growth performance, blood hematology, iron status and faecal microflora in weanling pigs. Livest. Sci., 131: 281-286.
10. EFSA., PAPSAF and FEEDAP., 2014. Scientific opinion on the safety and efficacy of tannic acid when used as feed flavouring for all animal species. EFSA J., Vol. 12. 10.2903/j.efsa.20 14.3828
11. Fasuyi, A.O., 2005. Nutrient composition and processing effects on cassava leaf (*Manihot esculenta*, Crantz) antinutrients. Pak. J. Nutr., 4: 37-42.
12. NRC., 1994. Nutrient Requirements of Poultry. 9th Edn., National Academy Press, Washington, DC., USA., ISBN-13: 9780309048927, Pages: 155.
13. Redondo, L.M., P.A. Chacana, J.E. Dominguez and M.E.F. Miyakawa, 2007. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Front. Microbiol., Vol. 5. 10.3389/fmicb.2014.00118
14. Sugiharto, S., 2014. Role of nutraceuticals in gut health and growth performance of poultry. J. Saudi Soc. Agric. Sci. 10.1016/j.jssas.2014.06.001
15. Samanta, S., S. Giri, S. Parua, D.K. Nandi, B.R. Pati and K.C. Mondal, 2004. Impact of tannic acid on the gastrointestinal microflora. Microb. Ecol. Health Dis., 16: 32-34.
16. Aengwanich, W. and M. Suttajit, 2010. Effect of polyphenols extracted from Tamarind (*Tamarindus indica* L.) seed coat on physiological changes, heterophil/lymphocyte ratio, oxidative stress and body weight of broilers (*Gallus domesticus*) under chronic heat stress. Anim. Sci. J., 81: 264-270.
17. Aengwanich, W. and M. Suttajit, 2013. Effect of polyphenols extracted from tamarind (*Tamarindus indica* L.) seed coat on pathophysiological changes and red blood cell glutathione peroxidase activity in heat-stressed broilers. Int. J. Biometeorol., 57: 137-143.