

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

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

Evaluation of Quebracho Red Wood (*Schinopsis lorentzii*) Polyphenolic Vegetable Extract for the Reduction of Coccidiosis in Broiler Chicks

Emanuel Cejas¹, Silvina Pinto², Florencia Prosdócimo¹, Mariano Batallé¹,
Hebe Barrios¹, Guillermo Tellez³ and Mauricio De Franceschi¹

¹Universidad Nacional de Luján

²Facultad de Ciencias Veterinarias (UBA), Argentina

³Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA

Abstract: The effects of a commercially available Polyphenolic Vegetable Extract (PVE) from *Schinopsis lorentzii* (Bioquina[®]) for the reduction of coccidiosis in broiler chicks was evaluated. Day-of-hatch male broiler chicks were obtained from a local hatchery and randomly assigned to three experimental groups consisting of 8 chickens per group with four replicates per treatment: Group 1, PVE 10% - challenged; Group 2, untreated - challenged and Group 3, -untreated, non-challenged. Heated brooder batteries were used for housing and chicks were allowed *ad libitum* access to unmedicated broiler rations and water for the duration of the experiment. On day 21 post-hatch, all birds in groups 1 and 2 were challenged by oral gavage with 3 mL of a mixed suspension of freshly sporulated oocysts of *Eimeria tenella* (50,000 oocyst/mL), *E. maxima* (50,000 oocyst/mL) and *E. avervulina* (50,000 oocyst/mL). Group 3 was sham inoculated with 3 mL of PBS. Body weight gain was obtained seven days after coccidia challenge. Oocyst shedding was performed at 5, 7 and 10 days PI. Intestinal macroscopic lesions and histological morphometric analysis was performed 7 days PI. No significant differences were observed in terms of total mortality or lesion scores. Supplementation of PVE significantly increased body weight gain, crypt:villi ratio and decreased oocyst excretion ($p < 0.05$), suggesting that PVE may have an impact against a mixed subclinical *Eimeria* challenge. Further studies on the potential value of this product as a therapeutic or prophylactic anticoccidial agent are currently being evaluated.

Key words: Chickens, *Schinopsis lorentzii*, polyphenolic vegetable extract, coccidiosis

INTRODUCTION

Avian coccidiosis, a disease caused by *Eimeria* species, an intracellular protozoa belonging to the phylum Apicomplexa has an estimated economic impact of \$800 million worldwide for the poultry industry, due to performance and commercial losses (Williams, 1998; Chapman, 2009). The disease is mainly controlled by the use of chemotherapeutic agents. Nevertheless, due to the increasing emergence of drug-resistant parasite strains, the use of these agents is diminishing and therefore, novel approaches for disease control are urgently needed (Maillard, 2005; Anadon *et al.*, 2006; Boyle *et al.*, 2007; Tacconelli, 2009). Several studies have confirmed that natural dietary supplements to animals enhance their innate defense mechanisms and reduce the severity of several types of infections (Bagchi *et al.*, 2000; Allen and Fetterer, 2002; Arab *et al.*, 2006; Jang *et al.*, 2007; Song *et al.*, 2007; Berezin *et al.*, 2008; Wang *et al.*, 2008; Hamidi *et al.*, 2009). Among them, tannins, natural polyphenols present in virtually all plants, show remarkable biological, physiological and pharmacological properties (Bagchi *et al.*, 2000; Bodet *et al.*, 2006; Haslam, 2007; Liou *et al.*, 2008; Sun *et al.*,

2009; Allen-Hall *et al.*, 2010; Lorrain *et al.*, 2010). Vegetable tannins are complex polyphenolic metabolites of plants based upon two principal structural themes-oligomeric flavan-3-ols (proanthocyanidins) and poly-3,4,5-trihydroxyaroyl esters (gallotannins and ellagitannins), which fundamental structural unit is the phenolic flavan-3-ol ('catechin') nucleus (Haslam, 2007). Condensed proanthocyanidins exist as oligomers (water soluble), containing two to ten or more 'catechin' units and polymers (water insoluble). Hydrolysable tannins have a very restricted taxonomic distribution; they are associated principally with woody and herbaceous dicotyledonous plants (Roux and Paulus, 1961). There has been astonishing progress in the last fifty years in the understanding of the chemistry and biochemistry of tannins.

Recently, a significant number of scientific publications have demonstrated the potential benefit of these phytochemicals against avian experimental coccidial infection (Youn and Noh, 2001; Arab *et al.*, 2006; Jang *et al.*, 2007; Berezin *et al.*, 2008; Naidoo *et al.*, 2008; Wang *et al.*, 2008; Nweze and Obiwulu, 2009; Lorrain *et al.*, 2010).

Schinopsis lorentzii is a hardwood tree, a native of the Paraguayan subtropical area, which forms forests in Gran Chaco region of Argentina, in Paraguay and Bolivia. Some of its common names are coronillo, quebracho Cornillo (Brazil), quebracho chaqueño, quebracho colorado santiagueño, quebracho macho and quebracho bolí (Roux and Paulus, 1961; Salvat *et al.*, 2001). This tree is commercially important due to its extremely hard and durable wood and because of its tannin content. The tanning industry has been exploiting quebracho forests for more than 100 years. This present work investigated the effects of a commercially available Polyphenolic Vegetable Extract (PVE) from *Schinopsis lorentzii* (Bioquina[®]) for the reduction of coccidiosis in broiler chicks.

MATERIALS AND METHODS

Experimental chickens and treatments: Day-of-hatch male broiler chicks (n = 96) were obtained from a local hatchery and randomly assigned to three experimental groups consisting of 8 chickens per group with four replicates per treatment. Group 1 was fed with a standard diet supplemented with 10% PVE and were challenged; Group 2 received an untreated standard diet and were challenged (positive control); Group 3 was composed of untreated and uninfected chickens (negative control). Chicks used were cared for using procedures approved by the National University of Lujan Institutional Animal Care and Use Committee. Heated brooder batteries were used for housing and chicks were allowed *ad libitum* access to unmedicated broiler rations and water for the duration of the experiment.

Compound: Polyphenolic vegetable extract 88% from *Schinopsis lorentzii* (Bioquina[®]) was provided by Porfenc SRL (Buenos Aires, Argentina), active compounds are catechins obtained in a pure state, with 1.2-1.4% ashes.

Eimeria challenge and assessment of fecal oocyst production and body weight changes: On day 21 post-hatch, all birds in groups 1 and 2 were challenged by oral gavage with 3 mL of a mixed suspension of freshly sporulated oocysts of *Eimeria tenella* (50,000 oocyst per milliliter), *E. maxima* (50,000 oocyst per milliliter) and *E. avervulina* (50,000 oocyst per milliliter). Group 3 (untreated and uninfected chickens) were inoculated with 3 mL of PBS.

Body weight was recorded weekly. To evaluate the effect of PVE for the reduction of coccidiosis in broiler chicks, body weight gain after coccidia challenge was evaluated at day 28 (seven days post challenge).

Fecal material was collected from each cage tray (pool of five samples per tray in each replicate cage) at 5, 7 and 10 days post-challenge and the number of oocysts were assessed using a McMaster counting chamber. Total oocyst numbers were calculated using the

following formula: [total number oocysts = oocyst count x dilution factor x (fecal sample volume/counting chamber volume)/number of birds per cage]. Body weights were individually measured every week.

Intestinal macroscopic lesions and histological-morphometric examination: At day 28 of age, 7 days post challenge, all birds were weighed and euthanized by CO₂ inhalation. The gut was examined for the presence of macroscopic lesions in duodenum, jejunum and ileum. The scoring pattern was as follows: 0 = no gross lesions, 1 = mild lesions, 2 = moderate lesions with the presence of petechiae, general redness of mucosa, 3 = severe lesions with numerous petechiae, slime formation, inflammation and 4 = hemorrhagic lesions with increased slime formation, orange reddish color of intestinal contents, severe inflammation.

Sections of duodenum and distal ileum was excised from 2 chickens in each replicate (n = 8) at 7 days post *Eimeria* challenge. Each sample of intestine was cut open longitudinally at the antimesenteric attachment. Samples were fixed on dental wax in 0.1 M-phosphate-buffered formalin solution (4%), with the villi on the upper side. After the microscopic study, a 3-mm wide zone from the mesenteric site was cut at right angles to the surface of the mucosa and embedded in paraffin wax. Sections were cut (5 µm) and stained with haematoxylin and eosin (HE staining). From these stained sections, 20 well-oriented villi were selected on each slide. Morphometric indices were randomized, double-blind and determined by computer image analysis (Image-Pro Plus Version 4.0 from Media Cybernetics 8484 Georgia Av. Silver Spring, MD 20910USA.). The morphometric variables analyzed included duodenum and ileum villi height (from the tip of the villi to the villi-crypt junction) and crypt depth (defined as the depth of the invagination between adjacent villi). Crypt:villus ratio, determined as the depth of the mucosal crypt region divided by the length of the villi. Sections were examined under 10x plan objective lens. Prior to image analysis, image brightness was adjusted to standard levels and then, an auto-contrast step was applied to the stored image.

Statistical analysis: Differences in body weights, oocysts excretion, lesion scores and morphometric analysis were analyzed by analysis of variance using the General Linear Models (GLM) procedure. Significant differences (p<0.05) were further separated using Duncan's multiple range test and commercial statistical analysis software V 9.2 (SAS Institute, 2002) and considered significant at p<0.05.

RESULTS

Performance data: The effect of polyphenolic vegetable extract from *Schinopsis lorentzii* on body weight and

Table 1: Effect of Polyphenolic Vegetable Extract (PVE) from *Schinopsis lorentzii* on body weight and body weight gain

	Body weight day 1	Body weight 7 days	Body weight 14 days	Body weight 21 days	Body weight 28 days	Body weight gain
Challenged and treated with PVE	46.4±0.43 ^a	103.5±2.6 ^a	223.5±7.4 ^a	459.7±15.8 ^a	708.5±37.8 ^b	238.0±11.0 ^b
Challenged no treated	46.1±0.44 ^a	106.4±2.2 ^a	225.3±8.0 ^b	427.6±11.8 ^b	665.1±16.6 ^b	190.0±07.7 ^c
No challenged and no treated	46.2±0.43 ^a	106.3±2.1 ^a	223.9±8.1 ^a	390.0±16.8 ^b	818.7±15.5 ^a	347.1±11.0 ^a

Chicks were challenged by oral gavage at 21 days of age with 3 mL of a mixed suspension of *Eimeria tenella* (50,000 oocyst per milliliter), *E. maxima* (50,000 oocyst per milliliter) and *E. avervulina* (50,000 oocyst per milliliter). Body weight gain represents the difference between body weights at day 28 and day 21, seven days after the coccidia challenge.

Data expressed as means ± standard error. Values within columns with different lowercase superscripts differ significantly (p<0.05)

Table 2: Effect of Polyphenolic Vegetable Extract (PVE) from *Schinopsis lorentzii* on the fecal oocyst post challenge output

	Day 5	Day 7	Day 10
Challenged and treated with PVE	780±217.7 ^a	30,625±3732.6 ^b	153,125±46,741.5 ^a
Challenged no treated	3,702±2471.9 ^a	226,250±4131.1 ^a	437,875±319,280 ^a
No challenged and no treated	0±0 ^a	0±0 ^b	0±0 ^a
Percent oocyst excretion Reduction between infected groups	78.9%	86.4%	65%

Chicks were challenged by oral gavage at 21 days of age with 3 mL of a mixed suspension of *Eimeria tenella* (50,000 oocyst per milliliter), *E. maxima* (50,000 oocyst per milliliter) and *E. avervulina* (50,000 oocyst per milliliter).

Data expressed as means ± standard error. Values within columns with different lowercase superscripts differ significantly (p<0.05)

Table 3: Effect of Polyphenolic Vegetable Extract (PVE) from *Schinopsis lorentzii* on intestinal macroscopic lesion scores at 7 days post *Eimeria* challenge

	Duodenum	Jejunum	Ileum
Challenged and treated with PVE	2.37±0.25 ^a	1.68±0.15 ^a	2.65±0.19 ^a
Challenged no treated	2.89±0.20 ^a	1.96±0.20 ^a	3.03±0.26 ^a
No challenged and no treated	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Chicks were challenged by oral gavage at 21 days of age with 3 mL of a mixed suspension of *Eimeria tenella* (50,000 oocyst per milliliter), *E. maxima* (50,000 oocyst per milliliter) and *E. avervulina* (50,000 oocyst per milliliter).

At 7 dpi, birds were weighed and euthanized by CO₂ inhalation. The gut was examined for the presence of macroscopic lesions and the scoring pattern was as follows: 0 = no gross lesions, 1 = mild lesions, 2 = moderate lesions with the presence of petechiae, general redness of mucosa, 3 = severe lesions with numerous petechiae, slime formation, inflammation and 4 = hemorrhagic lesions with increased slime formation, orange reddish color of intestinal contents, severe inflammation.

Data expressed as means ± standard error. Values within columns with same lowercase superscripts do not differ significantly (p>0.05)

body weight gain is summarized in Table 1. There were no significant differences on body weight at day 1, 7 and 14 days between the three experimental groups. However, it was interesting to observe that by day 21 (day of oral gavage with the mixed suspension of *E. tenella*, *E. maxima* and *E. acervulina*), chickens that received dietary supplementation of PVE had a significant increase in body weight when compared with the other two groups. Nevertheless, at seven days post challenge, the control group (untreated, non infected) showed a significant increase in body weight compared with the *Eimeria* challenged groups. Although no significant differences in body weight at day 28 were observed between the infected groups, group 1 (treated with PVE) was 43.4 grams heavier (p>0.05). Significant differences in body weight gain, seven days after coccidia challenge were observed between all groups. This time however, chickens that received dietary PVE were 48 grams heavier (p<0.05) than the chickens in the infected non treated group (Table 1). No significant differences on total mortality were observed in the chickens challenge with the mixed culture of *Eimeria* compared with the control non-challenged group (data not shown).

Fecal oocyst post challenge output: The effect of polyphenolic vegetable extract from *Schinopsis lorentzii* on the fecal oocyst post challenge output is shown in Table 2. At five days post *Eimeria* challenge, a numerical reduction in oocyst shedding (78.9%) was observed between group 1 and group 2 (infected non-treated). However, at seven days post challenge, a significant reduction (86.4%) of total oocyst excretion was observed between group 1 and group 2. Oocyst shedding was also numerically reduced (65%) at 10 days post challenge (Table 2).

Intestinal macroscopic lesions: Table 3 summarizes the effect of polyphenolic vegetable extract from *Schinopsis lorentzii* on intestinal macroscopic lesion scores. No significant differences on lesions scores were observed between the three groups (Table 3).

Histological-morphometric examination: The histological examination of intestinal tissues showed developing oocysts in each of the coccidia challenged chicks only. Dietary PVE did not increase duodenum or ileum villi height. However, dietary PVE significantly increased crypt:villi ratio in duodenum when compared

Table 4: Effect of Polyphenolic Vegetable Extract (PVE) from *Schinopsis lorentzii* on morphometric examination of duodenum and ileum tissue at 7 days post *Eimeria* challenge

	Duodenum villi height	Duodenum Crypt	Duod. Crypt: villi ratio	Ileum villi height	Ileum Crypt	Ileum Crypt: villi ratio
Challenged and treated with PVE	1,500±250 ^b	390±125 ^a	0.260±0.13 ^a	750±136 ^b	190±50 ^a	0.25±0.18 ^a
Challenged no treated	1,600±150 ^b	200±50 ^b	0.125±0.59 ^b	950±260 ^b	160±65 ^a	0.17±0.69 ^b
No challenged and no treated	2,000±175 ^a	270±134 ^b	0.135±0.70 ^b	1,100±175 ^a	220±73 ^a	0.20±0.40 ^a

Chicks were challenged by oral gavage at 21 days of age with 3 mL of a mixed suspension of *Eimeria tenella* (50,000 oocyst per milliliter), *E. maxima* (50,000 oocyst per milliliter) and *E. avervulina* (50,000 oocyst per milliliter). Duod. = Duodenum

Data expressed as means ± standard error. Values within columns with different lowercase superscripts differ significantly (p<0.05)

with group 2 (infected non-treated) and group 3 (control). Ileum crypt:villi ratio in the chicks that were supplemented with dietary PVE was significantly increased when compared with group 2 and numerically higher when compared with the chickens in the control group (Table 4).

DISCUSSION

Due to international regulations and consumer pressures, there is a need to develop alternatives for antibiotic growth promoters in animal and poultry feeding. In addition to probiotics and prebiotics, feed ingredients containing tannin are attracting more interest to control or prevent subclinical diseases and to maximize growth performance and economic viability. Different plant compounds (i.e., herbs, organic acids, essential oils) seem to be candidates of interest as alternatives to antibiotic growth promoters, because they have been shown to reduce gastrointestinal infections and increase performance (Sandoval-Chacon *et al.*, 1998; Giannenas *et al.*, 2003; Naidoo *et al.*, 2008; Schiavone *et al.*, 2008; Wang *et al.*, 2008; Lupini *et al.*, 2009; Aengwanich and Suttajit, 2010). Substantial accumulations of vegetable tannins may occur in almost any part of a plant-seeds, fruit, leaves, wood, bark, root. Tannins represent one of several categories of useful antimicrobial phytochemicals and tannins of different plant species have specific physical and chemical properties; thus, they have very different biological activities and detailed study is required to characterize their potential beneficial effects (Berkelhammer, 2003; Haslam, 2007). Nevertheless, for decades, scientists have studied the chemistry and biochemistry of these complex vegetable polyphenolic metabolites, which have a remarkable impact in health and disease (Sandoval-Chacon *et al.*, 1998; Cardoso *et al.*, 2005; Navarro-Peran *et al.*, 2005; Song *et al.*, 2005; Bodet *et al.*, 2006; Jang *et al.*, 2007; Wang *et al.*, 2008; Allen-Hall *et al.*, 2010).

Chickens that received dietary supplementation of PVE for twenty one days had a significant increase in body weight compared with the other two groups, suggesting that PVE may have some growth promoting or prebiotic activities. In the present study, body weight gain data of the chicks was used to check the virulence of the *Eimeria* mixed culture challenge used. The coccidial challenge did reduce weight gain in both infected groups

as compared with the control group. Nevertheless, a significant increase in body weight gain (48 grams), 7 days post coccidia challenge was observed in the group that received dietary supplementation of PVE when compared with the infected non treated group. This observation was associated with a significant increases in crypt:villi ratio in both, duodenum and ileum. *E. acervulina* proliferates in duodenum and *E. maxima* proliferates primarily in the distal and proximal ileum, often severely damaging the structure of the intestinal mucosa. The epithelial damage can reduce the nutrient absorption, which is partly compensated by the increase in gut length (Ruff and Edgar, 1982), as was also seen in the present trial. The histological preparations showed that all the coccidia challenge birds carried developing oocysts in the duodenum and ileum, but the epithelial damage in general was not severe, as was also supported by the gross lesions and the low mortality rate, indicating that the challenge was mild (subclinical). However, the intestinal infection increased the crypt:villi ratio. This data supported the hypothesis that dietary PVE supplementation would improve the crypt-villus ratio. It was surprising, however, that the crypt-villus ratio was decreased not only in the infected non treated group, but also in the control group. This suggests that dietary PVE alleviates the parasite challenge of coccidia-challenged birds and also stabilizes the mucosal structure. Perhaps, the protective effect of dietary PVE observed in the present study, may be at least partly due to its antioxidant properties that has contributed to the observed effect of PVE to the epithelial crypt-villus ratio in duodenum and ileum. It is well documented that during chicken coccidiosis, the generation of proinflammatory mediators, together with the oxidative and Nitrous Oxide (NO) species, contribute principally to inflammatory injury, diarrhea, mortality and weight loss (Allen, 1997; Sandoval-Chacon *et al.*, 1998; Allen and Fetterer, 2002; Bodet *et al.*, 2006; Brenes *et al.*, 2008; Naidoo *et al.*, 2008; Hamidi *et al.*, 2009; Allen-Hall *et al.*, 2010). Therefore, substances that generate oxidative stress or have antioxidant properties, such as n-3 fatty acids, γ -tocopherol, curcumin, essential oil blends and green tea extracts, demonstrated certain coccidiostat effects (Kurkure *et al.*, 2006; Oviedo-Rondon *et al.*, 2006; Jang *et al.*, 2007; Brenes *et al.*, 2008; Wang *et al.*, 2008; Betti *et al.*, 2009). It seems that after parasite

invasion, free radicals, together with high levels of NO production, are the major factors that compromise the cellular antioxidant defense system. Compounds that are meeting the demands of antioxidant defense system or directly interfere with free radicals, such as tannins, may restore the balance of oxidants/antioxidants, leading to improvement in intestinal integrity and performance during subclinical coccidiosis (Allen, 1997; Bagchi *et al.*, 2000; Allen and Fetterer, 2002; Naidoo *et al.*, 2008).

Dietary administration of PVE also reduced (numerically) oocyst shedding at five days post coccidia challenge (78.9%) when compared with the infected non treated group. However, at seven days post challenge, a significant reduction (86.4%) of total oocysts excretion was observed between both challenged groups. Oocyst shedding was also numerically reduced (65%) at 10 days post challenge in the dietary PVE group. These findings are in agreement with other works, where the use of different plant extracts have shown to have potential benefits in reducing coccidial infections (Jang *et al.*, 2007; Wang *et al.*, 2008; Nweze and Obiwulu, 2009).

Supplementation of PVE significantly increased body weight gain, crypt:villi ratio and decreased oocyst excretion, suggesting that the proanthocyanidins in the PVE have potential impact against a mixed subclinical *Eimeria* challenge.

From this study we conclude that the PVE used, have potential benefits in treating coccidial challenges. Even though the role of tannins in poultry nutrition requires further investigation, the promising results obtained with the PVE from *Schinopsis lorentzii* (Bioquina®) justify further studies on the potential value of this product as a therapeutic or prophylactic anticoccidial agent.

REFERENCES

- 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.
- Allen, P.C., 1997. Production of free radical species during *Eimeria maxima* infections in chickens. *Poult. Sci.*, 76: 814-821.
- Allen, P.C. and R.H. Fetterer, 2002. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clin. Microbiol. Rev.*, 15: 58-65.
- Allen-Hall, L., J.T. Arnason, P. Cano and R.M. Lafrenie, 2010. *Uncaria tomentosa* acts as a potent TNF-alpha inhibitor through NF-kappaB. *J. Ethnopharmacol.*, 127: 685-693.
- Anadon, A., M.R. Martinez-Larranaga and M. Aranzazu Martinez, 2006. Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regul. Toxicol. Pharmacol.*, 45: 91-95.
- Arab, H.A., S. Rahbari, A. Rassouli, M.H. Moslemi and F. Khosravirad, 2006. Determination of artemisinin in *Artemisia sieberi* and anticoccidial effects of the plant extract in broiler chickens. *Trop. Anim. Health Prod.*, 38: 497-503.
- Bagchi, D., M. Bagchi, S.J. Stohs, D.K. Das, S.D. Ray, C.A. Kuszynski, S.S. Joshi and H.G. Pruess, 2000. Free radicals and grape seed proanthocyanidin extract: Importance in human health and disease prevention. *Toxicology*, 148: 187-197.
- Berezin, V.E., A.P. Bogoyavlenskiy, V.P. Tolmacheva, N.R. Makhmudova, S.S. Khudyakova, S.V. Levandovskaya, E.S. Omirtaeva, I.A. Zaitceva, G.B. Tustikbaeva, O.S. Ermakova, P.G. Alekseyuk, R.C. Barfield, H.D. Danforth and R.H. Fetterer, 2008. Immunostimulating complexes incorporating *Eimeria tenella* antigens and plant saponins as effective delivery system for coccidia vaccine immunization. *J. Parasitol.*, 94: 381-385.
- Berkelhammer, G., 2003. A medical compound derived from herbs. *Science*, 299: 1985.
- Betti, M., T.I. Perez, M.J. Zuidhof and R.A. Renema, 2009. Omega-3-enriched broiler meat: 3. Fatty acid distribution between triacylglycerol and phospholipid classes. *Poult. Sci.*, 88: 1740-1754.
- Bodet, C., F. Chandad and D. Grenier, 2006. Anti-inflammatory activity of a high-molecular-weight cranberry fraction on macrophages stimulated by lipopolysaccharides from periodontopathogens. *J. Dent. Res.*, 85: 235-239.
- Boyle, E.C., J.L. Bishop, G.A. Grassl and B.B. Finlay, 2007. *Salmonella*: From pathogenesis to therapeutics. *J. Bacteriol.*, 189: 1489-1495.
- Brenes, A., A. Viveros, I. Goni, C. Centeno, S.G. Sayago-Ayerdy, I. Arijia and F. Saura-Calixto, 2008. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poult. Sci.*, 87: 307-316.
- Cardoso, M.P., J.M. David and J.P. David, 2005. A new alkyl phenol from *Schinopsis brasiliensis*. *Nat. Prod. Res.*, 19: 431-433.
- Chapman, H.D., 2009. A landmark contribution to poultry science - prophylactic control of coccidiosis in poultry. *Poult. Sci.*, 88: 813-815.
- Giannenas, I., P. Florou-Paneri, M. Papazahariadou, E. Christaki, N.A. Botsoglou and A.B. Spais, 2003. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. *Arch. Tierernahr.*, 57: 99-106.

- Hamidi, H., J. Pourreza and H. Rahimi, 2009. Dietary betaine affect duodenal histology of broilers challenged with a mixed coccidial infection. Pak. J. Biol. Sci., 12: 291-295.
- Haslam, E., 2007. Vegetable tannins-lessons of a phytochemical lifetime. Phytochemistry, 68: 2713-2721.
- Jang, S.I., M.H. Jun, H.S. Lillehoj, R.A. Dalloul, I.K. Kong, S. Kim and W. Min, 2007. Anticoccidial effect of green tea-based diets against *Eimeria maxima*. Vet. Parasitol., 144: 172-175.
- Kurkure, N.V., S.W. Kolte, A.G. Bhandarkar and D.R. Kalorey, 2006. Evaluation of herbal coccidiostat 'Coxynil' in broiler. In. J. Exp. Biol., 44: 740-744.
- Liou, Y.M., S.C. Kuo and S.R. Hsieh, 2008. Differential effects of a green tea-derived polyphenol (-)-epigallocatechin-3-gallate on the acidosis-induced decrease in the Ca(2+) sensitivity of cardiac and skeletal muscle. Pflugers Arch., 456: 787-800.
- Lorrain, B., O. Dangles, C. Genot and C. Dufour, 2010. Chemical modeling of heme-induced lipid oxidation in gastric conditions and inhibition by dietary polyphenols. J. Agric. Food Chem., 58: 676-683.
- Lupini, C., M. Cecchinato, A. Scagliarini, R. Graziani and E. Catelli, 2009. *In vitro* antiviral activity of chestnut and quebracho woods extracts against avian reovirus and metapneumovirus. Res. Vet. Sci., 87: 482-487.
- Maillard, J.Y., 2005. Antimicrobial biocides in the healthcare environment: Efficacy, usage, policies and perceived problems. Ther. Clin. Risk Manag., 1: 307-320.
- Naidoo, V., L.J. McGaw, S.P. Bisschop, N. Duncan and J.N. Eloff, 2008. The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. Vet. Parasitol., 153: 214-219.
- Navarro-Peran, E., J. Cabezas-Herrera, F. Garcia-Canovas, M.C. Durrant, R.N. Thorneley and J.N. Rodriguez-Lopez, 2005. The antifolate activity of tea catechins. Cancer Res., 65: 2059-2064.
- Nweze, N.E. and I.S. Obiwulu, 2009. Anticoccidial effects of *Ageratum conyzoides*. J. Ethnopharmacol., 122: 6-9.
- Oviedo-Rondon, E.O., M.E. Hume, C. Hernandez and S. Clemente-Hernandez, 2006. Intestinal microbial ecology of broilers vaccinated and challenged with mixed *Eimeria* species and supplemented with essential oil blends. Poult. Sci., 85: 854-860.
- Roux, D.G. and E. Paulus, 1961. Condensed tannins. 8. The isolation and distribution of interrelated heartwood components of *Schinopsis* sp. Biochem. J., 78: 785-789.
- Ruff, M.D. and S.A. Edgar, 1982. Reduced intestinal absorption in broilers during *Eimeria mitis* infection. Am. J. Vet. Res., 43: 507-509.
- Salvat, A., L. Antonnacci, R.H. Fortunato, E.Y. Suarez and H.M. Godoy, 2001. Screening of some plants from Northern Argentina for their antimicrobial activity. Lett. Appl. Microbiol., 32: 293-297.
- Sandoval-Chacon, M., J.H. Thompson, X.J. Zhang, X. Liu, E.E. Mannick, H. Sadowska-Krowicka, R.M. Charbonnet, D.A. Clark and M.J. Miller, 1998. Antiinflammatory actions of cat's claw: The role of NF-kappaB. Aliment. Pharmacol. Ther., 12: 1279-1289.
- SAS Institute Inc., 2002. SAS user's guide: Statistics. SAS Institute Inc., Cary, NC.
- Schiavone, A., K. Guo, S. Tassone, L. Gasco, E. Hernandez, R. Denti and I. Zoccarato, 2008. Effects of a natural extract of chestnut wood on digestibility, performance traits and nitrogen balance of broiler chicks. Poult. Sci., 87: 521-527.
- Song, J.M., K.H. Lee and B.L. Seong, 2005. Antiviral effect of catechins in green tea on influenza virus. Antiviral Res., 68: 66-74.
- Song, J.M., K.D. Park, K.H. Lee, Y.H. Byun, J.H. Park, S.H. Kim, J.H. Kim and B.L. Seong, 2007. Biological evaluation of anti-influenza viral activity of semi-synthetic catechin derivatives. Antiviral Res., 76: 178-185.
- Sun, Y., W.C. Hung, F.Y. Chen, C.C. Lee and H.W. Huang, 2009. Interaction of tea catechin (-)-epigallocatechin gallate with lipid bilayers. Biophys. J., 96: 1026-1035.
- Tacconelli, E., 2009. Antimicrobial use: Risk driver of multidrug resistant microorganisms in healthcare settings. Curr. Opin. Infect. Dis., 22: 352-358.
- Wang, M.L., X. Suo, J.H. Gu, W.W. Zhang, Q. Fang and X. Wang, 2008. Influence of grape seed proanthocyanidin extract in broiler chickens: Effect on chicken coccidiosis and antioxidant status. Poult. Sci., 87: 2273-2280.
- Williams, R.B., 1998. Epidemiological aspects of the use of live anticoccidial vaccines for chickens. Int. J. Parasitol., 28: 1089-1098.
- Youn, H.J. and J.W. Noh, 2001. Screening of the anticoccidial effects of herb extracts against *Eimeria tenella*. Vet. Parasitol., 96: 257-263.