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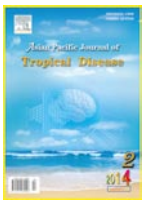

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Efficiency of *Tribulus terrestris* L. as an antibiotic growth promoter substitute on performance and immune responses in broiler chicks

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ABSTRACT

Objective: To evaluate the effect of *Tribulus terrestris* L. (puncture vine) as an antibiotic growth promoter substitute on growth performance, carcass traits, and immune responses in broiler chickens.

Methods: A total of 192 one-day-old as hatched broiler chicks (Ross 308) were randomly allocated to four treatment groups, with 4 replicates of 12 birds. The following treatments were applied: basal diet (control), control+4.5 mg flavophospholipol/kg, or control+1 or 5 g puncture vine powder/kg. Vaccines against newcastle, influenza disease, and sheep red blood cells were administered to immunological stimuli.

Results: Daily feed intake, internal organ weights, and carcass traits were not influenced by the dietary treatments except for carcass yield that increased in broilers fed diet supplemented with 1 g puncture vine powder/kg at 42nd day. Broilers receiving 1 g puncture vine/kg tended to have a lower feed conversion ratio compared to other groups during starter, finisher and entire experimental period ($P>0.05$). Broilers receiving 1 or 5 g puncture vine/kg had higher antibody titer against Newcastle disease virus compared to other groups ($P<0.05$). Broilers receiving 1 g puncture vine/kg had the highest antibody titer against avian influenza virus and sheep red blood cells at 28 and 31 days of age, respectively ($P<0.05$).

Conclusions: In conclusion, the overall results of the current study showed that puncture vine powder seems to have the potential to positively influence growth performance and immune responses of broiler chicks.

1. Introduction

Antibiotics have been used worldwide at subtherapeutic doses in poultry industry to promote growth and prevent poultry pathogens[1-5]. Antibiotic growth promoters were supposed to increase growth rate as a result of improved gut health, resulting in better nutrient utilization and decreased feed conversion ratio[6]. However, the continuous subtherapeutic use of in-feed antibiotics resulted in common problems such as development of antibiotic resistant bacteria[7], drug residues in the body of the birds, and imbalance of normal microflora[8]. As a result, probiotics and prebiotics[9,10], phytogenic and herbal products[11] have received increased attention as possible antibiotic growth promoter substitutions. Aromatic plants and herbal products have received increased attention in recent years because they have been accepted by consumers as natural additives[12].

Puncture vine [*Tribulus terrestris* L. (*T. terrestris*)] is a shrub belonging to the family Zygophyllaceae and it is widely distributed in Turkey, China, Japan, Korea, the western part of Asia, the southern part of Europe and Africa. Puncture vine possesses saponins, flavonoids, alkaloids, glycosides, phytosteroids and other constituents[13-15]. Its main active components are saponins of the furostanol type, termed protodioscin[16]. Most of the plant parts contain compounds with proven antimicrobial, antihypertension, diuretic, antiacetylcholine, haemolytic activity, spermatogenesis and libido enhancer, antitumor activity and effects on cardiovascular system[17].

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Furthermore, Çek *et al.* observed the beneficial influence of puncture vine extract on growth rate of convict cichlids (*Cichlasoma nigrofasciatum*)[18]. Gauthaman *et al.* also reported the positive effect of *T. terrestris* L. extract to rats on body weight, sexual activity and spermatogenesis[19], but later in another study Sahin and Duru failed to monitor any significant effect of puncture vine extract on broiler performance[20]. Tilwari *et al.* observed beneficial influence of alcoholic and aqueous extracts of *T. terrestris* fruits on antibody titers against sheep red blood cell (SRBC) antigen, delayed type hypersensitivity (DTH) response and phagocytic activity in rats[21]. Considering the mentioned pharmaceutical advantages of puncture vine the present study was conducted with the objective to evaluate the impact of two does of *T. terrestris* on growth performance, carcass characteristics and immune responses in broiler chicks.

2. Materials and methods

2.1. Animals and dietary treatments

The experiment included 192 one-day-old newly hatched broiler chicks (Ross 308) from 1 to 42 days of age. Birds were randomly allocated to four treatment groups, with 4 replicates of 12 birds. The following treatments were applied: basal diet (control), control+4.5 mg flavophospholipol/kg, or control+puncture vine powder (1 or 5 g/kg).

All feeds had the same nutritional value, and were formulated to meet or exceed the nutrient requirements of broilers[22], and provided *ad libitum* (Table 1). All the additives were added to the basal diets at the expense of corn. The ambient temperature in the first week was 32 °C and decreased by 3 °C in the second and third weeks, and maintained at 22 °C at the end of the trial. The lighting program was provided as 23 h of light and 1 h of darkness.

2.2. Performance and carcass components

Body weights of chicks were individually determined after their arrival from the hatchery to the experimental farm and at 14, 28, and 42 days of age. Daily weight gain for each dietary treatment was calculated in different periods. Feed consumption was recorded in the course of the whole experiment for each treatment, and the feed conversion ratio (FCR, feed intake/weight gain) was calculated accordingly. Mortality was recorded as it occurred.

At the end of experiment, 8 broilers per treatment were randomly selected, based on the average weight of the group and killed by cervical dislocation. The carcass yield percentage was calculated by dividing eviscerated weight by live weight. The empty proventriculus, empty gizzard, empty small intestine, empty cecum, abdominal fat pad, liver, and heart were excised, weighed and calculated as a percentage of live weight. The gastrointestinal tract was weighed after removal of the content.

Table 1

The ingredient and calculated composition of basal starter, grower, and finisher diets.

Item		Starter	Grower	Finisher
Ingredient (g/kg)	Corn	537.3	533.0	561.5
	Soybean meal	400	396	370
	Soybean oil	20	35	35
	Dicalcium phosphate	19.3	17.1	15.6
	$CaCO_3$	10.5	8.7	8.5
	NaCl	3.5	3.0	3.0
	Trace mineral premix[1]	2.5	2.5	2.5
	Vitamin premix[2]	2.5	2.5	2.5
	DL-methionine	3.1	2.0	1.4
	L-lysine	1.3	–	–
Calculated composition	Metabolizable energy (kcal/kg)	2870	2980	3000
	Crude protein (g/kg)	221	220	210
	Calcium (g/kg)	8.6	7.5	7.0
	Available phosphorus (g/kg)	4.9	4.4	4.1
	Methionine + cysteine (g/kg)	10.1	8.9	8.0
	Lysine (g/kg)	13.3	11.9	11.3
	Threonine (g/kg)	8.3	8.3	6.3
	Tryptophan (g/kg)	3.2	3.2	3.0

[1]: Provided the following per kg of diet: Mg, 56 mg; Fe, 20 mg; Cu, 10 mg; Zn, 50 mg; Co, 125 mg; I, 0.8 mg.

[2]: Provided the following per kg of diet: vitamin A, 10000 IU; vitamin D3, 2000 IU; vitamin E, 5 IU; vitamin K, 2 mg; riboflavin, 4.20 mg; vitamin B12, 0.01 mg; pantothenic acid, 5 mg; nicotinic acid, 20 mg; folic acid, 0.5 mg; choline, 3 mg.

2.3. Immunity

The birds were submitted to immunological stimulus with commercially available oil-adjuvant injectable emulsion against Newcastle disease virus (NDV) and avian influenza virus (AIV, H9N2 subtype) subcutaneously with 0.2 mL per chick at 9 days of age, and SRBC at 25 days of age. Also, at 21 days of life, vaccine against Newcastle disease (Lasota) was administered orally.

SRBC diluted at 1% in sterile phosphate buffered saline solution, was injected at 1 mL dose per bird on the 25 days of the experiment. Plasma were collected 6 days post-SRBC, and specific antibodies against SRBC were enumerated by Bartlett and Smith using hemagglutination technique and expressed in $\log_2$[23]. At 28 days of age, 2 male broilers from each coop were randomly selected, and blood samples were taken by puncture of the brachial vein for analysis of antibody titers against NDV and AIV. Serum antibody titers against NDV and AIV were measured by the hemagglutination inhibition test, and hemagglutination inhibition antibodies were then converted to $\log_2$.

2.4. Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute (2008). Means were compared using Tukey test. Statements of statistical significance were based on $P<0.05$.

3. Results

3.1. Performance and carcass traits

Data on performance indices are summarized in Table 2. Treatments failed to induce any marked effect on feed consumption. Supplementing 1 g puncture vine/kg markedly enhanced body weight at 14 days of age ($P<0.05$). Broilers receiving puncture vine (1 g/kg) or basal diet had higher body weight compared to other groups at 42 days of age ($P<0.05$). Antibiotic group tended to have a higher body weight at 28 days of age although the differences did not reach statistical significance. No differences because of treatment effects were observed on mortality. Broilers receiving flavophospholipol had lower FCR compared to other groups during growing period, although the differences did not reach statistical significance. Broilers receiving puncture vine at 1 g/kg tended to have a lower FCR compared to other groups during starter, finisher and entire experimental period ($P>0.05$).

Table 2

Effects of *T. terrestris* L. and antibiotic on performance indices of broilers at different ages.

Dietary treatments	Body weight (g)			Daily feed intake (g/d)				Feed: gain (g:g)			
	14 d	28 d	42 d	0–14 d	14–28 d	28–42 d	0–42 d	0–14 d	14–28 d	28–42 d	0–42 d
Control	261.50^{b}	1030.30	2210.60^{a}	25.92	95.15	160.75	91.75	1.49	1.88	2.07	1.82
Antibiotic	274.50^{ab}	1062.00	2090.50^{b}	26.17	94.27	159.72	91.12	1.47	1.80	2.14	1.90
TT (1 g /kg)	283.20^{a}	1033.00	2213.00^{a}	26.05	93.02	159.00	90.52	1.40	1.86	2.02	1.79
TT (5 g/kg)	266.00^{ab}	994.20	2088.00^{b}	24.55	90.77	156.25	88.05	1.42	1.88	2.56	1.85
SEM	5.37	25.94	48.41	0.53	1.58	3.08	1.40	0.65	1.07	2.23	0.83

TT: *T. terrestris* L. ^{a,b}: Values in the same column not sharing a common superscript differ significantly ($P<0.05$).

Table 3 shows carcass, abdominal fat, and relative organ weights as a percentage of live weight at slaughter, and absolute small intestine and cecum lengths. The percentage of carcass was statistically improved by the dietary treatments. The weight of internal organs, were not markedly affected and no pathological lesions and damages were noticed for proventriculus, heart, gizzard, liver, cecum and small intestine.

Table 3

Effects of *T. terrestris* L. and antibiotic on carcass yield and internal relative organ weight of broilers at 42 days of experiment.

Relative organ weight	Dietary treatments				
	Control	antibiotic	TT (1 g/kg)	TT (5 g/kg)	SEM
Carcass (%)	70.63^{ab}	70.52^{b}	72.41^{a}	72.28^{ab}	0.76
Abdominal fat (%)	1.58	1.64	1.57	1.44	0.11
Liver (%)	2.44	2.57	2.27	2.28	0.10
Gizzard (%)	2.61	2.76	2.46	2.37	0.16
Heart (%)	0.50	0.55	0.53	0.50	0.03
Proventriculus (%)	0.51	0.43	0.51	0.53	0.03
Small intestine (%)	4.56	4.60	4.46	4.55	0.30
Small intestine (cm)	195.12	205.35	195.00	199.50	4.8
Cecum (%)	0.63	0.64	0.68	0.71	0.04
Cecum (cm)	38.87	40.12	38.62	41.62	1.29

TT: *T. terrestris* L. ^{a,b}: Values in the same row not sharing a common superscript differ significantly ($P<0.05$).

3.2. Immune responses

The effect of experimental diets on immune responses is presented in Table 4. Broilers receiving at 1 or 5 g/kg had higher antibody titer against AIV compared to control groups ($P<0.05$). Broilers receiving puncture vine at 5 g/kg had the highest antibody titer against NDV at 28 days of age ($P<0.05$). Antibody titer against SRBC increased in the group treated with puncture vine 1 g/kg diet compared with those fed basal diet ($P<0.05$).

Table 4

Effects of *T. terrestris* L. and antibiotic on antibody titers against Newcastle and Influenza viruses at d 28 and sheep red blood cells (SRBC) at d 31.

Dietary treatments	New castle (log_2)	Influenza (log_2)	SRBC (log_2)
Control	5.57^{a}	3.37^{a}	6.71^{a}
Antibiotic	6.37^{abc}	4.25^{ab}	7.25^{ab}
TT (1 g/kg)	6.00^{ab}	6.62^{d}	8.28^{bc}
TT (5 g/kg)	7.42^{c}	6.00^{cd}	7.14^{ab}
SEM	0.41	0.17	0.57

TT: *T. terrestris* L. ^{a,b,c,d}: Values in the same column not sharing a common superscript differ significantly ($P<0.05$).

4. Discussion

As puncture vine has been reported to have antibacterial and increasing testosterone availability activities[24,25], an increase in performance criteria of broilers was anticipated. Theoretically, moderately increasing testosterone availability

during training may promote greater gains in strength and muscle mass[24]. Sahin and Forbes indicated the favorable influence of dehydroepiandrosterone on modulation of corticosterone administration effects and it resulted in increase of muscle mass[25]. Similar to our results Çek *et al.*[18] reported the beneficial influence of *T. terrestris* extract on growth rate of convict cichlids (*Cichlasoma nigrofasciatum*) which is in agreement with the findings of Dimitrov *et al.*[26] who reported that rams with sexual disorders given *T. terrestris* extract had higher average daily gain, sexual activity and spermatogenesis. However, Sahin and Duru did not notice any significant differences in performance parameters for broilers supplemented with *T. terrestris* extract[20]. In the present study, broilers receiving flavophospholipol had lower FCR compared to other groups during grower period, although the differences did not reach statistical significance. As antibiotics, herbs and phytogenic products could control and limit the growth and colonization of numerous pathogenic and nonpathogenic species of bacteria in chicks′ gut. This may lead to a greater efficiency in digestibility and utilization of feed, resulting in an enhanced growth and improved FCR[27]. A higher dosage of *T. terrestris* in the diet may have had an adverse effect on some beneficial microbial populations such as lactobacillus, preventing the herb from exhibiting its positive influence on performance and resulting in a poorer FCR. Similarly in our another trial use of *E. purpurea* L. (5 g/kg) diet had positive effect on performance and humoral immune responses of broiler chicks and supplementation of 10 g/kg suppressed performance and humoral immune responses of broilers[28].

In the present study carcass percentage was statistically improved by the dietary treatments. The weight of internal organs, were not markedly affected and no pathological lesions and damages were noticed for proventriculus, heart, gizzard, liver, cecum and small intestine. These results are consistent with those reported by Sahin and Duru who did not find any differences among the control treatment and those containing *T. terrestris* extract on relative weight of heart, abdominal fat pad, crop, proventriculus, gizzard, pancreas, ceca and colon[20]. In another study, carcass traits of broilers fed diets supplemented with *Azadirachta indica* or antibiotic were not affected by dietary treatments[29].

Tilwari *et al.*[21] observed beneficial influence of alcoholic and aqueous extracts of fruits of *T. terrestris* on antibody titers against SRBC antigen, DTH response and phagocytic activity in Wistar rats. In other trial with Wistar rats, Tilwari *et al.*[30] reported that, *T. terrestris* showed significant dose dependent increase in the ′humoral antibody titre and DTH response′ as indicated by increase in footpad thickness. *T. terrestris* contains different types of active components such as saponins and phenolic compounds, alkaloids and glycosides which can be responsible for the immunomodulatory activity[21]. Mungantiwar *et al.* showed that alkaloidal fraction of *Boerhaavia diffusa* might modulate the immune responses in mice[31]. Also, Singh *et al.* reported that, saponins enhances the immunity by increasing the proliferation of cells by concanavalin A, increasing humoral response to SRBC and macrophage migration inhibition[32].

Use of antibiotic, flavophospholipol, failed to have any effect on antibody titer against AIV, NDV, and SRBC in comparison with control groups. Landy *et al.* reported slight humoral immune responses of broilers supplemented with flavophospholipol[29]. Belay *et al.* reported no effect of dietary virginiamycin on the total IgG, IgM antibody titers in 7 week old broiler chickens[33]. Therefore, the influence of enteric conditioners and their effects on gut microflora could be mainly limited to the mucosal immune complement and not the systemic portion of the immune system. Although the contribution of gut microflora to the development and physiological status of the humoral and cellular mucosal immune system is well understood[34,35], the effects on the systemic immune complement may be less dominant.

In conclusion, the overall results of the current study showed that puncture vine powder seems to have the potential to positively influence growth performance and immune responses of broiler chicks. Thus, it could be considered as a natural growth promoter.

Conflict of interest statement

We declare that we have no conflict of interest.

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