Int J Biometeorol
DOI 10.1007/s00484-015-1079-6

ORIGINAL PAPER

Effects of adding aqueous extract of *Tribulus terrestris* to diet on productive performance, egg quality characteristics, and blood biochemical parameters of laying hens reared under low ambient temperature (6.8 ± 3 °C)

Mohsen Akbari[1] · **Mehran Torki**[1]

Received: 31 January 2015 / Revised: 4 October 2015 / Accepted: 6 October 2015

Abstract A study was conducted using 144 laying hens to evaluate the effects of adding aqueous extract of *Tribulus terrestris* to diets on productive performance, egg quality traits, and some blood parameters of laying hens reared under cold stress condition (6.8 ± 3 °C). The birds were randomly assigned to each of four dietary treatments (C, T1, T2, and T3) with six replicate cages of six birds. Diet inclusion of aqueous extract of *T. terrestris* at the rate of 10, 20, and 30 ml/Lit offered to groups T1, T2, and T3, respectively, while group C served as the control diet with no addition. Feed intake (FI), feed conversion ratio (FCR), egg weight (EW), egg production (EP), and egg mass (EM) were evaluated during the 42-day trial period. The EP and EM increased, whereas FCR decreased ($P < 0.001$) in the hens fed the extract-included diet as compared to those fed the basal diet. The serum content of cholesterol decreased and the thickness of egg shell increased in the hens fed the T2 and T3 diet compared to those fed the basal diet. Overall from the results of the present experiment, it can be concluded that diet supplementation with aqueous extract of *T. terrestris* has beneficial effects on productive performance of laying hens reared under cold stress condition.

Keywords Aqueous extract · Cholesterol content · Cold stress · Laying hens · Productive performance · *Tribulus terrestris*

✉ Mehran Torki
torki@razi.ac.ir

[1] Department of Animal Science, Faculty of Agriculture and Natural Resources, Razi University, Imam Avenue, Kermanshah, Kermanshah 6715685418, Iran

Introduction

Animals face a variety of environmental stressors every day. Cold stress occurs when the surrounding temperature decreases below 18 °C. During winter months, the overall ambient temperature ranges from −10 to +5 °C in many regions of the world. The birds are a homeotherm animal that can live comfortably only in a relatively narrow zone of thermoneutrality. In laying hens, such cold stress conditions cause some adverse effects on productive performance including decreased egg production or egg weight and feed efficiency (Sahin et al. 2002). Also, Sahin et al. (2002) observed the negative influence of cold stress (6.8 ± 3 °C) on egg quality traits and some blood parameters of laying hens. In addition, cold stress increased energy requirements and energy consumption of broiler chickens (Blahova et al. 2007). Cold stress compromised intestinal epithelial cell proliferation and induced inflammation in the small intestine through a combined action of nitric oxide, neutrophils, and mast cells (Jing et al. 2013; Zhao et al. 2013). Based on a report by Yang et al. (2011), stress breaks the homeostasis of cecal microflora and default intestinal mucosal immune function in birds (Yang et al. 2011).

It has been reported that the beneficial effects of dietary supplementation by medicinal plants on performance of broiler chickens could be more efficient in birds reared under environmental stress conditions (Hernandez et al. 2004). Most of the antibacterial promoters of poultry productive performance have been banned because the feeding of antibiotics is risky due to not only cross-resistance but also to multiple resistances (Neu 1992). On the other hand, herbs or products containing plant extracts, essential oils, or main components of the aqueous extract are among the alternative growth promoters that are already being used in practice (Lee et al. 2003; Williams and Losa 2001). Regarding animal studies, there are various

Published online: 15 October 2015

records in the literature showing the improving effects of dietary extract of *T. terrestris* on reproductive functions (Grigorova et al. 2007; Kashamov 2008), productivity (Valchev et al. 2008), and health (Grigorova et al. 2008b; Petkova et al. 2009). The positive effect of *T. terrestris* extract on the laying capacity and egg quality in hens has been demonstrated (Grigorova 2008; Grigorova and Kashamov 2007). There are some limited number of studies in which effects of adding phytogenic compounds to diet on performance of poultry have been investigated. For example, in trying to find out the interactions between phytogenic compounds and trace elements, Torki et al. (2014) reported that diet supplementation by the combined form of cinnamon essential oil and zinc has beneficial effects on performance and blood parameters of laying hens reared under cold stress condition (8.8 ± 3 °C). Whether dietary supplementation with plant extracts alleviates the negative effects of cold stress in the laying hens would have been of interest. Regarding the effects of cold stress on gut microflora, it has been reported that medicinal plants have a reducing influence on potentially pathogenic bacteria and shifting the composition of the microflora towards more beneficial bacteria. Bölükbaşi and Erhan (2007) noticed that laying hens fed the thyme-included diet had lower *Escherichia coli* concentration in feces compared to those fed the basal diet.

Keeping in view the mentioned advantages of *T. terrestris*, a research study was conducted with the objective of evaluating the impact of different levels of dietary supplemental aqueous extract of *T. terrestris* on productive performance, egg quality characteristics, and some of blood biochemical parameters in laying hens reared under low ambient temperature.

Material and methods

Animals and diets

All experimental protocols were adhered and approved by the guidelines of the Animal Ethics Committee of Razi University (Kermanshah, Iran). One hundred and forty-four Lohmann LSL-Lite laying hens (40 weeks age, with mean body weight of 1410 ± 140 g) were allocated to 24 cages of six birds. The birds were randomly assigned to each of four dietary treatments with six replicate cages of six birds (six cages per dietary treatment and six birds per cage). Body weights were recorded at the beginning and at the end of the study to determine body weight changes. Hens in each six cages were randomly allotted to one of the four dietary treatments. A period of 14 days was used for bird adaptation to the experimental diets. The experimental period lasted 6 weeks started with the 42-week-old birds. The birds were placed into the cages and kept under 16 h of light/8 h dark cycle. The temperature was kept as close as to a constant (6.8 ± 3 °C). Average ambient relative humidity inside the rearing house was 64.4 ± 6.5 %. Feed was offered on the basis of catalogue (110 g/hen/day) and water (18 ± 4 °C) was supplied ad libitum. A corn-soybean meal basal diet was formulated to contain adequate levels of all nutrients as recommended by the Lohmann LSL-Lite catalogue (Lohmann LSL-Classic International, 2011); (metabolisable energy, ME = 2800 kcal/kg and crude protein, CP = 14.95 g/100 g diet), and the four following experimental diets were created (basal diet with no additive as the control diet (C group); treatment groups of 1 to 3 were diets included 10, 20, and 30 ml *T. terrestris*/Lit water, respectively).

Preparation of *T. terrestris* extract

Plant was identified and authenticated by plant taxonomist. *T. terrestris* extract prepared as described by Xue et al. (2007). About 100 g of thoroughly washed leaves of *T. terrestris* were macerated with 100 ml of sterile distilled water in a blender for 30 min. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000*g* for 40 min. The supernatant was filtered through Whatman No. 1 filter paper and sterilized at 1000 °C for 20 min. The extract was preserved aseptically in bottles at 4 °C until further use.

Productive performance and egg quality traits

The productive performance of laying hens including egg production (EP), feed intake (FI), and egg weight (EW) was recorded daily from each cage, and feed conversion ratio (FCR: g feed: g egg) and egg mass (EM) were calculated. Egg mass and feed conversion ratio were measured weekly. Random samples of 18 eggs from each treatment were collected (biweekly) to measure egg quality in terms of yolk color, eggshell thickness, eggshell weight (%), egg index, and Haugh unit. The Haugh unit, based on the height of albumen which in turn is determined by a micrometer, was calculated by the following formula presented by Eisen et al. (1962) ($HU = 100 \log [H + 7.57–1.7 W^{.37}]$. The variables in the formula are H = height of egg white in mm, and W = weight of the egg in grams). The egg shell thickness was a mean value of measurement at three locations on the egg (air cell, equator, and sharp end) by using a dial, pipe gage. Egg yolk color was measured using the Roche fan scale.

Determination of blood biochemical parameters

After a 15-h fasting, blood samples were collected in non-heparinized tubes at day 42 of trial from two birds per each cage (3 ml per bird) by puncturing the brachial vein and centrifuged at 2500×*g* for 10 min to obtain sera (SIGMA 4-15 Lab Centrifuge, Germany). Individual serum samples were analyzed for glucose, albumin, cholesterol, uric acid, and triglycerides by

spectrophotometer (Shimadzu UV-1700) using Pars Azmoon kit package (Pars Azmoon Co; Tehran, Iran).

Statistical analysis

Bird's performance, egg quality characteristics, and blood parameters data were subjected to statistical analysis using analysis of variance (ANOVA) appropriate for a completely randomized design. When significant effects were detected by ANOVA, treatment means were compared using Duncan's multiple range test. All statistical analyses were performed with SAS (SAS, 2001). Differences were considered significant at $P < 0.05$. All of the parameters were analyzed as follows: $Yij = \mu + Ti + eij$, where Yij = individual observation, μ = overall mean, Ti = effect of treatment, and eij represents the random error.

Results and discussion

Productive performance

Effects of adding aqueous extract of *T. terrestris* to diet on productive performance of laying hens reared under cold stress condition are presented in Table 1. The dietary treatments used in the current study failed to induce any significant impact on egg weight, body weight, and feed intake at the entire experimental period (6 weeks). In terms of EP, EM, and FCR, significant differences between treatments were detected ($P < 0.001$). Lower FCR and higher EP and EM were found in birds fed the diet included *T. terrestris* compared to those fed the basal diet (Table 1). Cold environment is a stressful factor and has negative effect on economy of production (Yunianto et al. 1997). In the present study, diet supplemented by *T. terrestris* improved productive performance of laying hens reared under cold stress condition. Torki et al. (2014) also found a significant improving effect of dietary supplementation by cinnamon essential oil on productive performance of laying hens reared under cold stress condition (8.8 ± 3 °C). These results show that diet supplementation by medicinal plants could have more beneficial influence on productive performance of birds reared under suboptimal environmental condition. It has been reported that dietary supplemental medicinal herbs (Bampidis et al. 2005; Cross et al. 2002; 2007) or plant extracts (Demir et al. 2003; Lee at al., 2003) have stimulating effect on the productive performance of laying hens. Çek et al. (2007) observed the beneficial effects of *T. terrestris* extract on growth rate of fishes. Sahin and Duru (2010) failed to monitor any significant effect of the diet supplementation by *T. terrestris* extract on broilers' growth performance. Nikolova et al. (2010b) showed that diet supplementation by *T. terrestris* extract increased EP in guinea fowl. Although in the present study, the mechanism of improving effect of diet supplementation by *T. terrestris* extract on productive performance is not understood, but since *T. terrestris* extract is water soluble, it might have increasing effect on nutrient absorption (Pandian and Sheela 1995). As mentioned before, cold stress can disturb gut microflora and shift towards harmful bacteria. Since the antibacterial effect of *T. terrestris* has been already reported (Kiran et al. 2011), it may result in a reducing effect on potentially pathogenic bacteria and shifting the composition of the gut microflora towards more beneficial bacteria which in turn may reduce the competition for nutrients and dietary energy between the host and its microflora. The action mechanism of dietary *T. terrestris* on improving productive performance of laying hens might be due partly to the reducing effect on cholesterol formation needed for cortisol synthesis, because blood cortisol content increases under stress condition and cholesterol is a precursor of cortisol, and in the present study, diet supplementation by *T. terrestris* extract decreased blood content of cholesterol.

Table 1 Effects of supplemental aqueous extract of *T. terrestris* on productive performance, egg quality traits, and blood biochemical parameters of laying hens reared under cold stress condition (6.8 ± 3 °C)

	C	T1	T2	T3	*P*	SEM
EP	81.82^{d}	85.07^{c}	88.01^{b}	90.63^{a}	**	0.47
EW	65.62	66.67	66.06	65.85	–	0.14
EM	53.83^{c}	56.63^{b}	58.13^{ab}	59.61^{a}	**	0.32
FI	118.40	118.30	118.40	118.66	–	0.11
FCR	2.22^{a}	2.10^{b}	2.04^{bc}	1.99^{c}	**	0.01
BW	1411	1402	1401	1409	–	68.9
EI	73.20	73.91	74.83	74.16	–	0.42
YC	6.16	6.58	6.33	6.33	–	6.09
ST	35.18^{b}	36.96^{ab}	38.49^{a}	39.68^{a}	*	0.52
SW	8.35	8.49	9.25	9.13	–	3.32
HU	84.27	82.80	84.09	81.96	–	0.66
Glu	249.17	254.33	255.75	249.50	–	4.97
TG	2172	1922	1871	1998	–	25.3
Chol	198.83^{a}	202.83^{a}	158.83^{b}	144.67^{b}	*	13.4
AL	3.00	3.86	3.49	3.51	–	0.14
UR	5.81	4.71	4.55	4.39	–	0.04

SEM standard error of means, *EP* egg production (%), *EW* egg weight (g), *EM* egg mass (g/hen/day), *FI* feed intake (g), *FCR* feed conversion ratio (g), *BW* body weight (g), *EI* egg index, *YI* yolk color (Roch unit), *ST* shell thickness (0.01 mm), *SW* shell weight (%), *HU* Haugh unit, *Glu* glucose(mg/dl), *TG* triglycerides(mg/dl), *Chol* cholesterol (mg/dl), *AL* albumin (mg/dl), *UR* uric acid (mg/dl)

– Nonsignificant; *$P < 0.05$; **$P < 0.001$

$^{a–c}$ Means within the same row with no supercript lowercase letters significantly differ

Egg quality characteristics

Egg quality characteristics are shown in Table 1. Egg quality traits were not significantly influenced by dietary treatments,

except for eggshell thickness which increased in laying hens fed the T2 or T3 diets ($P < 0.05$). Nikolova et al. (2010b) found no difference in yolk color of laying hens fed the diets included *T. terrestris* extract, whereas Nickolova and Dimo (2010a) observed significant effect of diet supplementation by *T. terrestris* extract on yolk weight and albumin of egg in Japanese quails. Similar to the result of the present study, Grigorova (2008) and Grigorova and Kashamov (2007) also showed no significant effect of diet supplementation by *T. terrestris* extract on egg index, Haugh unit, and yolk color. The lack of concurrence among the results of the motioned studies may be partially explained by *T. terrestris* variety. In addition, there are other variables such as differences in background of the targeted populations, birds' type and age, overall farm hygiene, and stress that may influence the efficacy of dietary supplementation by *T. terrestris* and thus it was difficult to directly assess different studies using *T. terrestris*. As it is indicated in Table 1, egg shell thickness of birds received T2 and T3 diets was significantly higher than control group ($P < 0.05$). It has been suggested that alkaline phosphatase secretion decrease under stress condition and this enzyme prevent calcium storage of bone (Brandae-Neto et al. 1995). Dry extract of *T. terrestris* significantly increased calcium in the blood serum of broilers' parent (Grigorova et al. 2008a). Preventing negative effects of environmental stress on shell thickness of eggs by dietary *T. terrestris* extract could be partly justified by the report by Coetzee (2002) who showed the effect of calcium levels in drinking water on shell integrity in South African laying hens. In this study demonstrated that the birds supplied an additional 200 mg of calcium per liter of drinking water laid eggs with increased shell strength.

Blood biochemical parameters

Effects of dietary supplementation by aqueous extract of *T. terrestris* on blood parameters of laying hens are shown in Table 1. There was no significant effect of dietary supplemental *T. terrestris* extract on serum albumin, uric acid, triglycerides, and glucose, whereas hens fed on the diet supplemented by *T. terrestris* extract (T2 and T3) significantly showed lower level of blood cholesterol ($P < 0.05$). In contrast to these finding, Amin et al. (2006) and Kohli et al. (2004) reported differences in the serum concentration of glucose of hens given10 mg/kg body weight/day the *T. terrestris* extract for a period of 12 weeks. Christev et al. (2011) indicated that dietary supplemental *T. terrestris* extract decreased the serum concentration of cholesterol and triglycerides in guinea fowls compared to the birds in the control group. The mode of action of *T. terrestris* might be explained by the enhancement of testosterone level which has reducing effect on cholesterol (Malkin et al. 2004) and steroid hormones as well. Lee et al. (2004) showed a decrease in HMG-COA reductase activity in broiler chicks fed dietary supplemental medicinal plants. HMG-COA reductase is a key regulatory enzyme in cholesterol synthesis and consequently has the hypocholesterolemic effect.

Conclusion

In conclusion, based on the results of the present study, dietary supplemental *T. terrestris* extract had an improving effect on performance parameters (increased EP, EM and decreased FCR) of laying hens reared under cold stress condition. Eggshell thickness was significantly improved in hens by dietary supplemental *T. terrestris* extract. The results also suggest that the *T. terrestris* extract can reduce the serum concentration of cholesterol. The serum content of albumin, glucose, triglycerides, and uric acid was not affected by dietary supplemental *T. terrestris* extract in laying hens. Comparison of extract effects between thermoneutral condition and stress condition could be subject for further research in this area.

Compliance with ethical standards All experimental protocols were adhered and approved by the guidelines of the Animal Ethics Committee of Razi University (Kermanshah, Iran).

References

Amin A, Lotfi M, Shafiullah M, Adeghate E (2006) The protective effect of *Tribulus terrestris* in diabetes. Ann Acad Sci 4:391–401

Bampidis VA, Christodoulou V, Florou-Paneri P, Christaki E, Chatzopoulou PS, Tsiligianni T, Spais AB (2005) Effect of dietary dried oregano leaves on growth performance, carcass characteristics and serum cholesterol of female early maturing turkeys. Br Poult Sci 46:595–601

Blahova J, Dobsikova R, Strakova E, Suchy P (2007) Effect of low environmental temperature on performance and blood system in broiler chickens (*Gallus domesticus*). Acta Vet Brno 76:S17–S23. doi:10.2754/avb200776S8S017

Bölükbaşi SC, Erhan MK (2007) Effect of dietary thyme (*Thymus vulgaris*) on laying hens performance and *Escherichia coli* (*E. coli*) concentration in feces. Int J Natural Engine Sci 1(2):55–58

Brandae-Neto J, Stefan V, Mendonca BB, Bloise W, Castro AWB (1995) The essential role of zinc in growth. Nutr Res 15:335–358

Christev C, Nickolova M, Penkov D, Ivanova R, Abadjieva D, Grigorova S (2011) Investigation of the effect of *Tribulus terrestris* extract on the main biochemical and hematological indices of the blood in guinea fowls (Numida meleagris). J Center Europ Agric 12(1):16–26. doi:10.5513/JCEA01/12.1.875

Coetzee C (2002) The effect of elevated calcium levels in drinking water on shell integrity. Specified News summer

Çek S, Turan F, Atik E (2007) Masculinization of convict cichlid (*Cichlasoma nigrofasciatum*) by immersion in *Tribulus terrestris* extract. Aqua Int1 5(2):109–119

Cross DE, Acamovic T, Deans SG, McDevitt RM (2002) The effect of dietary inclusion of herbs and their volatile oils on the performance of growing chickens. Br Poult Sci 43:33–35

Cross DE, McDevitt RM, Hillman K, Acamovic T (2007) The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Br Poult Sci 48:496–506

Demir E, Sarica S, Ozcan MA, Suicmez M (2003) The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. Br Poult Sci 44:44–45

Eisen EJ, Bohren BB, McKean HE (1962) The haugh unit asa measure of egg albumen quality. Poult Sci 41:1461–1468

Grigorova S (2008) Investigation the effect of *Tribulus terrestris* on laying productivity and eggs morphological characteristics of hens from the population White Plymouth Rock-mini. Processing scientific conference "Food science Engineering and Technologies" Plovdiv, UHT, v. LV1:487–492.

Grigorova S, Kashamov B (2007) Effect of *Tribulus terrestris* extract on morphological and sensor properties of table eggs. Processing scientific conference "Food science Engineering and Technologies" Plovdiv. UHT, v 2:241–246

Grigorova S, Kashamov B, Sredkova V, Surdjiiska S, Kurtenkov A (2007) Effect of *Tribulus terrestris* extract on semen quality and serum cholesterol content in White Plymouth Rock-mini cocks. Sovremennii Nauchnii Vestnik 13:11–18

Grigorova S, Kashamov B, Vasileva D, Sredkova D, Surdjiiska S (2008a) Investigation the effect of *Tribulus terrestris* extract on the egg yolk lipids and some biochemical parameters of the blood serum in broilers parents. Science conference with international participation "Ecology and Health Plovdiv Proceedings 93–98.

Grigorova S, Vasilkeva D, Kashamov B, Sredkova V, Surdjiiska S (2008b) Investigation of *Tribulus terrestris* extract on biochemical parameters of eggs and blood serum in laying hens. Arch Zootechnica 11(1):39–45

Hernandez F, Madrid J, Garcia V, Orengo J, Megias MD (2004) Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poult Sci 83:169–174

Jing F, Liu C, Zhang Z, Xing M, Shi-wen X (2013) Influence of inflammatory pathway markers on oxidative stress induced by cold stress in intestine of quails. Res Vet Res 95:495–501

Kashamov B (2008) Investigation the effect of supplementation of *Tribulus terrestris L*.on poultry, Dissertation.

Kiran B, Lalitha V, Raveesha KA (2011) In vitro evaluation of aqueous and solvent extract of *Tribulus terrestris L*. leaf against human bacteria. Int J Pharm Techno Res 3: 1897–1903.

Kohli RK, Giri S, Kolhapure A (2004) Evaluation of clinical efficacy and safety of Ciabecon in NiDD. Antiseptic 11:487–494

Lee KW, Everts H, Kappert HJ, Frehner M, Losa R, Beynen AC (2003) Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br Poult Sci 44:450–457

Lee KW, Everts H, Kappert HJ, Wouterse H, Frehner M, Beynen AC (2004) Cinnamaldehyde, but not thymol, counteracts the carboxymethyl cellulose-induced growth depression in female broiler chickens. Int J Poult Sci 3:608–612

Lohmann LSL-Classic international (2011) Variety LSL-Lite commercial management guide. Lohmann international, Tierzucht. www. Itz.de

Malkin S, Pugh P, Jones R, Kapoor D, Channer K, Jones T (2004) The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypo gonadal men. J Clin Endocrine Metabolism 89:3313–3318

Neu HC (1992) The crisis in antibiotic resistance. Sci 257:1064–1073

Nickolova M, Dimo P (2010a) Influence of *Tribulus terrestris* extract supplementation on laying productivity and eggs quality in Japanese quails. Center Europe Agric 11:373–380

Nikolova M, Grigorova S, Abadjieva D, Penkov D (2010b) Investigation of the effect of *Tribulus terrestris* extract on some characteristics of the reproductive capacity of guinea fowl. Biotechnol Anim Husbandry 26(3–4):259–266

Pandian TJ, Sheela SG (1995) Hormonal induction of sex-reversal in fish. Aquaculture 138(1):1–22

Petkova M, Grigorova S, Abadjieva D (2009) Blood biochemical changes and sperm quality in bulls fed diet supplemented with dry extract from *Tribulus terrestris*. Book of abstracts of the 60th Annual meeting of the EAAP, Barcelona, Spain, Session 36, Poster51

Sahin A, Duru M (2010) Effects of *Tribulus terrestris* (Puncture Vine) supplementation on performance and digestive system of broiler chicks. J Agric Sci 16:271–277

Şahin K, Onderci M, Şahin M, Aydin S (2002) Effects of dietary chromium picolinate andascorbic acid supplementation on egg production, egg quality and some serum metabolites of laying hens reared under a low ambient temperature. Arch Anim Nutr 56:41–49. doi: 10.1080/00039420214174

SAS software (2001) SAS User's Guide: Statistics, Version 9.2, SAS Institute, North Carolina, USA.

Torki M, Akbari M, Kaviani K (2014) Single and combined effects of zinc and cinnamon essential oil in diet on productive performance, egg quality traits, and blood parameters of laying hens reared under cold stress condition. Int J Biometeorol. doi:10.1007/s00484-014-0928-z

Valchev G, Ivanov A, Grigorova S, Zlateva N (2008) A study on the effect of *Tribulus terrestris L*. extract as a growth promoter for rabbits. J Anim Sci 45(3):96–101

Williams P, Losa R (2001) The use of essential oils and their compounds in poultry nutrition. World Poult Sci J17:14–15

Xue WL, Li XS, Zhang J, Liu YH, Wang ZL, Zhang RJ (2007) Effect of *Trigonella foenum-graecum* (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats. Asia Pac J Clin Nutr 16:422–426

Yang XJ, Li WL, Feng Y, Yao JH (2011) Effects of immune stress on growth performance, immunity, and cecal microflora in chickens. Poult Sci 90:2740–2746

Yunianto BVD, Hayashi K, Kaneda S, Ohtuska A, Tomita Y (1997) Effect of environmental temperature on muscle protein turnover and heat production in tube-fed broiler chickens. Br J Nutr 77: 897–909

Zhao F, Zhang Z, Yao H, Wang L, Liu T, Yu X, Li S, Xu S (2013) Effects of cold stress on mRNA expression of immunoglobulin and cytokine in the small intestine of broilers. Res Vet Sci 95:146–155