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Original article

The effects of Ephedra (*Ephedra funereal*) and protexin probiotic on some blood parameters in male Japanese quail (Coturnix Japonica)

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A B S T R A C T

This experiment was conducted to investigate the effects of feeding Ephedra and protexin on performance and some hematological parameters of male Japanese quail. A total of 240 seven days old quail chicks were divided into 8 treatments with 3 replicates as factorial randomized design. The treatments were divided as basal diet with no protexin and Ephedra kept as control, and 0.02 % (E1), 0.04% (E2) and 0.06 % (E3) Ephedra with 0.01 % or without protexin as P0 and P 1 were used respectively. The live body weight gains and feed consumption of birds were measured individually feed conversion efficiency were calculated. At the end of the trial for investigating the effect of using protexin and Ephedra supplementation on performance of quails, 2 birds (male) form each replicates were slaughtered and some blood samples were taken for hematological parameters determination. Data showed that using of protexin and Ephedra increased feed intake (FI) in treatments compared to control. Also body weight (BW) (g/d) and Pre-slaughter weigh (g) were higher in protexin and Ephedra compared to the control. There were no significant differences ($p<0.05$) for feed conversation ratio (FCR) among treatments. As result was relevant from this study there were significant differences ($p\leq0.05$) between blood parameters. Data showed that the glucose level had increased none significantly by using Ephedra and protexin. The triglyceride, cholesterol and LDL were increased significantly ($p\leq0.05$) by using experimental diets. HDL, albumin and globulin levels were decreased by using Ephedra and protexin. In conclusion we demonstrated that protexin and Ephedra may be used as ingredient in quails ration without harming effects on performance and some blood parameters of male Japanese quails.

1. Introduction

Ephedra (*Ephedra funereal*) is a genus of gymnosperm shrubs, the only genus in its family, Ephedraceae, and order, Ephedrales. The various species of Ephedra are widespread in many lands, native to southwestern North America, southern Europe, northern Africa, and southwest and central Asia, northern China, and western South America. Plants of the genus *Ephedra* have traditionally been used by indigenous people for a variety of medicinal purposes, including treatment of asthma, hay fever, and the common cold (Abourashed, 2003). The alkaloids ephedrine and pseudoephedrine are active constituents of Ephedra and other members of the genus. These compounds are sympathomimetics with stimulant and decongestant qualities and are related chemically to the amphetamines. Pollen of Ephedra spp. was found in the Shanidar IV burial site in Iraq, suggesting its use as a medicinal plant dates to over 60,000 years ago(Anon,1996). It has been suggested that *Ephedra* may be the Soma plant of indo Iranian religion (Solecki, 1975). Herbal Ephedra has been used in China to treat respiratory conditions for over 5,000 years; however, the herb is not used for weight loss or physical performance enhancement in eastern medicine. Ephedrine and its isomers were already isolated in 1881 from Ephedra dystachia and characterized by the Japanese organic chemist Nagai Nagayoshi of the 19th century. Its active alkaloid, ephedrine, was first used in western medicine as an asthma treatment in the 1930s. Since then, ephedrine (2-methylamino-1-phenyl-1-propanol) and other sympathomimetic alkaloids have been used in many over the counter decongestants and cold medicines (Cui, 1991). These alkaloids are structurally similar to amphetamines and have direct alpha- and beta agonistic properties and catecholamine releasing actions (Hoffman, 1996). It was not until the 3 early 1990s that herbal *Ephedra* and other products containing ephedrine began to be promoted as weight loss aids in the United States (Abourashed et al., 2003). Probiotics are live microbial feed supplements, which improve the intestinal microbalance (Salminen et al., 1999). The use of probiotics in poultry was pioneered by Tortuero (1973), who reported an increase in growth rate in chicks given a Lactobacillus acidophilus culture in drinking water for 11 days from hatching. Similar results on the beneficial effects of Lactobacillus cultures on the growth of chickens were also reported by several researchers (Kalbane et al., 1992; Jin et al., 1997). One of the probiotics used in poultry feed is Protexin. Protexin is a multi-strain probiotic containing live microbes to establish, enhance or re-establish essential microflora in the gut. Protexin is a highly concentrated pre-mix containing seven strains of bacteria and two yeasts (Lactobacillus plantarum 1.89×1010 cfu/kg (colony forming unit per kilo gram), Lactobacillus delbrueckii subsp. Bulgaricus 3.09×1010 cfu/kg, Lactobacillus acidophilus 3.09×1010 cfu/kg, Lactobacillus rhamnosus 3.09×1010 cfu/kg, Bifidobacterium bifidum 3.00×1010 cfu/kg, Streptococcus salivarius subsp. Thermophilus 6.15×1010 cfu/kg, Enterococcus faecium 8.85×1010 cfu/kg, Aspergillus oryza 7.98×109 cfu/kg, Candida pintolopesii 7.98 × 109 cfu/kg). All the microorganisms in the protexin are naturally occurring and have been isolated from a wide range of feed, plant, animal, bird and human sources (Ayasan et al., 2006). Protexin can be used in a wide range of circumstances, either to improve the general health of animals, address specific problems or to maximize animal's performance. Under general conditions Protexin has been promoted to: improve health naturally, stimulate appetite, aid in establishment of gut flora in immature animals like day old chicks, calves, lambs, kids, kittens, re-establish gut microflora after antibiotic treatment, optimize digestion of feed and reduce stress (Rajmane, 1998; Cyberhorse, 1999; Panda et al., 2000, Vali.,2009). Many studies have been conducted to test the efficacy of protexin on animal growth and performance. Balevi et al, (2000) indicated that supplementation of diets with protexin at 500 gr/tonne quality was shown to cause some improvement in feed intake. Ayasan and Okan (2001) investigated the effect of four different levels of protexin on fattening performance and carcass characteristics of Japanese quails. Because of the importance of birds as an economic and nutritious form of animal protein and the fast growing characteristics of this animal, research workers have devoted studies to the use of probiotics and some medical in poultry and quails. The objective of this study was conducted to evaluate the effects of protexin and *Ephedra* on performance, carcass characteristics and intestinal morphology in male Japanese quail (Coturnix japonica).

2. Materials and methods

This experiment was carried out at the Aviculture farm of Islamic Azad University, Shahrekord, Iran. A total of 240 seven days old quail chicks with an average weight of 18.50±50 g were divided into 4×2 treatments and were further subdivided into 3 replicates with 10 birds on each. Ephedra was purchased from local market Shahrekord, Iran. Corn, soybean meal and were analyzed in the lab for determine amount of dry matter, crude protein, calcium, phosphorus and its crude fiber with Association of official analytical chemists (AOAC, 2000). The basal diet was balanced on the basis of corn and soybean meal as recommended by National Research council (NRC, 1994). The treatments were divided as basal diet with no protexin and Ephedra kept as control, and 0.02 % (E1), 0.04% (E2) and 0.06 % (E3) Ephedra with (0.01%) or without protexin as P0and P1 were used respectively. Diets and fresh water were provided adlibitum during this experiment. The live body weight gains and feed consumption of quails were measured individually, feed conversion efficiency were calculated weekly. At the end of experimental period, 2 birds (male) form each replicates (totally 48 birds) were slaughtered for determination of other parameters.

2.1. Evaluation of some blood parameters

After 12 h of fasting, blood samples were taken from the brachial vein from four birds per replicate and stored at refrigerator at 4°C. Individual serum samples were analyzed for, Glucose, Cholesterol, Triglyceride, Albumin, Globulin, HDL and LDL by an automatic biochemical analyzer following the instructions of the corresponding reagent kit (Pars Azmoon Co., Teheran, Iran).

2.2. Statically model and data analysis

The statically model was: Yijk = μ+ ά i+ ßj+ (ά +ß)ij+ eijk, Yijk = average effect observed, μ = total average, άi= effect of Ephedra, ßj = effect of protexin, (ά +ß) ij = interactions (Ephedra × protexin), eijk = effect of errors.

The GLM procedure of SAS software (SAS, 2001) was used for data analysis of variance as completely randomized design. The significant difference among the mean were calculated by Duncan's multiple range tests (1995).

3. Results and discussion

Data showed that use of protexin and Ephedra had increased feed intake (FI) significantly ($p<0.05$) in comparison to control (Table 1). Result showed that body weight BW (kg) was higher significantly when the birds fed by protexin and Ephedra compared to control. Although feed conversion ratio (FCR) were lesser in protexin and Ephedra group but there were no significant differences compared to the control.

The effects of Ephedra and protexin of some blood parameters on quails are shown on table 2. As result was relevant from table 2 there were significant differences ($p\leq0.05$) between blood parameters. Data showed that the glucose level had increased none significantly by using Ephedra and protexin. The triglyceride, cholesterol and LDL were increased significantly ($p\leq0.05$) by using experimental diets. HDL, albumin and globulin levels were decreased by using Ephedra and protexin.

In the present study, protexin and Ephedra supplementation had significant effects on the measured values in growing Japanese quails. The usage of protexin and Ephedra was significant influences on FI, BW, FCR and carcass yield. Balevi et al, (2001) showed that diet supplementation with probiotic could improve FI and FCR. Parreira (1998) has showed that dietary supplementation of protexin increased growth performance and decreased mortality in broilers. Shabani et al, (2012) showed that the chicken broilers feed with protexin have the lowest feed conversion ratio and was the most favorable. Zamiri and Karimi (2005) showed that weights of internal organs were not affected by ephedrine in their experiment on ram lambs and also they showed that ephedrine was effective in increasing the leg meat and leg meat as a percentage of live weight. In disagreement with our result, Toubro et al, (1993) concluded that the ephedrine and caffeine combination is safe and effective in long term treatment in improving and maintaining weight loss.

Many scientists showed that beneficial effects of herbal or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and antihelminthic actions (Janssen, 1989; Manzanilla et al., 2001; Jamroz et al., 2003).

Oskbjerk and Sorensen (1996) reported that ephedrine was effective in reducing fat and increasing protein deposition in finishing pigs, Michael E. Powers (2001) mentioned that ephedrine is a sympathomimetic and a central nervous system stimulant and it is commonly used as an energy enhancer. Supplementation with ephedrine increases plasma insulin, glucose, and c-peptide in a dose dependent manner, possibly due to the state of transient insulin resistant classical stimulants induce (Vansal, 1999) Ephedrine is able to directly agonize all three subsets of β-adrenergic receptors of brown adipose tissue, leading to increased thermo genesis without significantly activating the α-adrenergic receptor class and possibly antagonizing activation from agonists of these receptors. The β-class of adrenergic receptors is seen as the classes that stimulate lipolysis, where the alpha class is inhibitory (Buemann B, et al (1994).

Sarica et al, (2009) showed that use of essential oils in combination with the enzyme complex, a probiotic and a mannan oligosaccharide with or without the enzyme complex in the wheat based diet significantly reduced the intestinal viscosity compared to the control diet, these treatments negatively decreased plasma total cholesterol and triglyceride on quails. In (Moon-Koo Song et al., 2012) study using Ephedra improved insulin responsiveness. Ephedra reduced both fasting and postprandial glucose levels, decreased triglyceride, increased HDL compared to the control group. Buemann B, et al (1994) showed that ephedrine with using caffeine has been noted to reduce the rate of HDL-C decreases seen with hypocaloric dieting without significantly affecting overall total cholesterol.

Table 1

The effects of *Ephedra* and protexin on growth performance of Japanese quails.

Treatments[*]	**FI** $_{(Kg)}$ [**]	**BW** $_{(Kg)}$	**FCR** $_{(kg/kg)}$
(*Ephedra*)			
Control	20.45^{c}	6.55^{ab}	3.71
E (1)	20.63^{c}	6.62^{ab}	3.41
E (2)	21.01^{b}	6.28^{ab}	3.24
E (3)	21.48^{a}	7.42^{a}	3.09
P Value	0.687	0.310	0.002
(Protexin)			
P (0)	20.32^{b}	6.17^{a}	3.54
P (1)	21.06^{a}	6.38^{a}	3.20
P Value	0.378	0.006	0.003
(*Ephedra* × Protexin)			
Control× P (0)	20.41^{b}	7.36^{bc}	3.76
E (1) × P (0)	21.07^{ab}	7.79^{bc}	3.3.
E (2) × P (0)	21.27^{ab}	7.81^{ab}	3.24
E (3) × P (0)	21.54^{ab}	8.25^{b}	3.11
Control× P (1)	20.59^{b}	7.33^{bc}	3.45
E (1) × P (1)	21.67^{b}	8.51^{b}	3.02
E (2) × P (1)	22.42^{a}	8.67^{b}	2.93
E (3) × P (1)	22.87^{a}	8.89^{a}	2.80
P Value	0.63	0.641	0.921
SEM	0.918	0.310	0.055

*No protexin and Ephedra kept as control, and for others 0.02% (T1), 0.04% (T2) and 0.06% (T3) Chicoridin without (P0) or with (P1) (0-0.01% g/kg) protexin. **Feed intake (FI), body weight (BW), feed coefficient (FCR). ***Means within row with no common on letter are significantly different (p<0.05).

Table 2
The effects of *Ephedra* and protexin of some blood parameters on quails.

Treatments*	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)
(*Ephedra*)							
Control	169.22	211.83[a]	211.21[a]	140.54[d]	94.14[d]	1.44	2.61[a]
E (1)	170.45	206.53[b]	207.46[b]	134.23[c]	102.22[c]	1.38	2.20[b]
E (2)	172.34	202.23[c]	202.24[b]	122.54[b]	113.45[b]	1.25	2.08[c]
E (3)	174.45	197.65[d]	191.17[c]	109.31[a]	123.78[a]	1.17	1.96[d]
P Value	0.0101	0.421	0.0911	0.0240	0.045	0.011	0.031
(Protexin)							
P (0)	170.17	200[b]	195.38[b]	125.34[a]	116.11	1.48	2.44[a]
P (1)	172.36	204.2[a]	204.64[a]	116.28[b]	117.31	1.31	2.16[b]
P Value	0.0031	0.0121	0.0101	0.631	0.021	0.001	0.021
(*Ephedra* × Protexin)							
Control×p(0)	170.10	206.16[b]	208.43[a]	134.93[a]	98.88[c]	1.32	2.14[a]
E (1) × P (0)	171.25	203.45[b]	207.21[a]	132.16[a]	102.16[cb]	1.27	2.10[b]
E (2) × P (0)	169.45	200.16[dc]	206.52[a]	127.51[ab]	107.47[bc]	1.21	2.02[b]
E (3) × P (0)	168.54	197.36[d]	202.74[ab]	116.23[b]	110.33[b]	1.11	1.94[c]
Control× P (1)	170.00	207.6[a]	208.34[a]	127.07[ab]	123.14[ab]	1.16	2.04[c]
E (1) × P (1)	169.31	202.25[ab]	205.65[a]	118.29[b]	126.65[ab]	1.24	2.23[a]
E (2) × P (1)	170.25	200.39[ab]	205.33[a]	113.34[b]	129.76[ba]	1.28	2.41[a]
E (3) × P (1)	171.63	198.65[d]	206.55[a]	108.77[c]	131.66[a]	1.30	2.64[a]
P Value	0.0106	0.121	0.086	0.930	23.12	0.0002	0.106
SEM	3.240	8.210	5.161	23.61	0.415	0.062	1.24

* No protexin and Ephedra kept as control, and for others 0.02% (T1), 0.04% (T2) and 0.06% (T3) Chicoridin without (P0) or with (P1) (0-0.01% g/kg) protexin. **Feed intake (FI), body weight (BW), feed coefficient (FCR). ***Means within row with no common on letter are significantly different ($p<0.05$).

4. Conclusion

We could be explained by the facts that Ephedra and protexin could benefit acts on performance for broilers chicks. This improvement may be due to the biological functions of Ephedra and protexin to improve growth or that may be due to its role as stimulant, carminative, enhanced digestibility antimicrobial properties. However further studies are needed for more explanations.

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