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Potential use of protexin probiotic and black pepper powder on Cobb 500 broiler chicks

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ABSTRACT

To evaluate the effect of protexin, black pepper powder on performance of broiler chicks 160 one day male broiler chicks were divided into 4 groups of 10 birds each and assigned to 4 treatment diets. Chicks were fed by basal diet as control with or without 0.2 % protexin probiotic or black pepper fine powder respectively. Feed intake (FI), body weight (BW) and feed conversion ratio (FCR) was measured in each period of aviculture. At the end of trial 2 birds from each group were slaughtered and carcass characteristics were evaluated. Blood serum triglyceride, high and low density lipoproteins also were determined. At 42 days old antibody titer against Newcastle Vaccine was estimated. Intestinal microbial populations for Escherichia coli and Lactobacillus were performed. The results showed that body weights and feed conversion ratios were improved significantly ($p<0.05$). As data reveled in this study, using of protexin and black pepper increased carcass yield compared to the control. Data showed those serum blood biochemical were changed significantly by using protexin and black pepper ($p<0.05$). Also E.Coli colonies were decreased and lactobacilli microbial population had significantly ($p<0.05$) increased in chicks were fed by protexin and black pepper powder. It seems that inclusion of protexin and black pepper powder had good effects on performance, some blood biochemical values, and immunity parameters and microbial population in experimental Cobb 500 broiler chicks.

INTRODUCTION

Attempts to use the natural resources such as medicinal plants could be widely accepted as feed additives to improve the efficiency of feed utilization and productivity of performance in poultry (Kirubakaran et al. 2016). Poultry nutritionists are trying to rectify this problem through supplementation of phytogenic additives, which contain antibiotic and antibacterial properties. The majority of medicinal plants do not have the residual effects (Tipuet et al. 2006). Black pepper (*Piper nigrum* L.) is known as spices due to its pungent quality (Zargari 2001). It is a flowering vine in the family *Piperaceae*, genus Piper. It is improves digestibility. Piperine is one of compound of black pepper which has anti-ache effect (Turner and Jack 2004). Additionally, the bioactive molecule, piperine, present in pepper has major pharmacological impact on neuromuscular system exercises and it can help in digestion (Tabidi 2014). Many researchers proved an increase in body weight and best feed conversion ratio when using herbal plants in broiler diets (Dorman et al. 2000; Great head 2003). Platel and Srinivasan (2004) observed an enhanced digestion and a reduction in feed passage time in the digestive tract as a result of piperine supplementation. Abou-Elkhair et al. (2014) showed that black pepper in broiler nutrition had influence on improved health status through increase of serum globulin concentration. Valiollahi et al. (2014) concluded that the use of ginger and black pepper or their mixture already enhanced body performance, cholesterol profile and antibody titer in experimental chicks. Bharambe et al. (2016) was found that the broiler chicks fed of 1% black was more economical as compared to the other supplemented treatment

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levels. Use of probiotics in poultry was pioneered by (Tortuero 1973). He already reported an increase in growth rate in chicks given a Lactobacillus acidophilus culture in drinking water for eleven days from hatching. The same results on the beneficial effects of Lactobacillus cultures on the growth of chickens were also reported by several researchers (Jin et al. 1997; Faghani et al. 2014; Ghaedi et al. 2014). Protexin is one of the probiotics used in poultry feeding. It is a multi-strain probiotic containing live microbes to establish, enhance or re-establish essential micro-flora in the gut (Vali 2009). 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). It can be used to improve the general health of animals or to maximize animal and poultry performance (Abaza 2001). The objective of this study was aimed to determine the effects of protexin and black pepper supplementation on performance in Cobb 500 broiler chickens.

MATERIALS AND METHODS

The birds and diets

This experiment was performed at the Aviculture farm of Chaharmahal and Bakhtiari Province, Vardanjan, Iran. A total of 160 one days male Cobb 500 broiler chicks with an average weight of 40.50 ±50 g were divided into four treatments and were further subdivided into 4 replicates with 10 birds each. The treatments were divided as basal diet with no supplement kept as control, and 0.2 % protexin or black pepper powder respectively. Diets and fresh water were provided adlibitum during aviculture period.

The basal diet was balanced based on corn and soybean meal as recommended by National Research Council (NRC, 1994) as shown in (Table 1). Supplied Per Kilogram of Feed contained 7.500 IU of vitamin A, 2000IU vitamin D3, 30 Mg vitamin E,1.5 µg vitamin B12,2Mg B6,5 Mg Vitamin K,5 Mg vitamin B2,1 Mg vitamin B1,40 Mg nicotinic acide,160µg vitamin Biothine,12 Mg Calcium pantothenate,1MgFolic acid 20 Mg Fe,71 Mg Mn,100µg Se,37Mg Zn,6 Mg Cu,1.14 Mg I,400 µg Cu.

Performance

The feed intake, body weight gain and feed conversion ratio of birds were calculated individually. At the end of experimental period two birds from each replicates were slaughtered, then dressing and internal organs were removed and some edible organs percentage were investigated.

Blood bichemical analaysis

Blood samples were obtained from brachial vein after 12 hours of fasting in the 42th day of experiment then centrifuged in order to getting serum. Blood serums have been analyzed for cholesterol, low density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride by ELISA set.

Microlplate heamagglutination inhinition (HI) test

Micro-plate HI test was performed (Anon 1971) to determine the antibody level of the sera samples collected from the chicks of different groups. The test was conducted using constant 4HA unit New Castle diseases virus and diluted serum (β

Table 1. The composition of the experimental diets to experimental chicks

Ingredients%	0-02(weeks)	02-04(weeks)	04-06(weeks)
Corn	51.64	56.61	60.37
Soybean meal	37.74	32.30	27.81
Wheat	5	5	5
Soybean oil	1.40	2.03	2.84
Di Calcium Phosphate	1.56	1.47	1.39
Oyster shells	1.17	1.13	1.08
Methionine D-L	0.30	0.29	0.27
Lysine-L	0.13	0.13	0.30
Nacl	0.26	0.24	0.14
Vitamin Premix*	0.3	0.3	0.3
Mineral Premix*	0.3	0.3	0.3
Protexin / Black Pepper powder	0.2	0.2	0.2
Calculated nutrient content			
ME (kcal.kgr)	2.850	2.950	3.050
CP (%)	22	20	18.5
Ca(%)	0.90	0.85	0.80
Available Phosphorus (%)	0.45	0.42	0.40
Lysine (%)	1.35	1.20	1.16
Na (%)	0.16	0.15	0.15
Methionine + Cystine (%)	0.97	0.87	0.85

method).

Microbial count determination

To determine the microbial count, about 7 cm from the length of the ileum was sampled to determine the microbial population. Also 1 g of ileum content was used to make 10 fold dilutions using buffered peptone water and then 0.1 mL of the appropriate ileum dilution was spread on Lactobacillus MRS Agar-Hi Media Laboratories to detect lactic acid bacteria and violet red bile Agar todetect E-coli form. The plates were incubated at 37.5 ^{o}C for 48 h. After counting the number of colonies in each plate, the number was multiplied by inverse of the dilution and the result was stated as the number of colony forming unit (cfu) in 1 g of the sample.

Statically analysis

The GLM procedure of SAS software (SAS 2001) was used for data analysis of variance as completely randomized design. The significant difference among the means was calculated by (Duncan 1995) multiple range tests.The statically model was: $Yij = \mu + ti + eij$.

Whereas: Y ij = Average effect observed, μ = Total average, t i = Effect of treatments, e ij = Effect of errors.

RESULTS AND DISCUSSION

The results of broilers performance are shown in table 2. As result showed, there were significant differences for FI in experimental groups ($p<0.05$). The highest BW was for protexin and black pepper and FCR was at the lowest in these groups. Data from this study showed that highest pre-slaughter weigh was for chicks that fed by protexin.

These changes on broilers performance may be due to the digestibility property of black pepper included in the broiler diet, which was similar to the findings of Ghazalah et al. (2007). Additionally, Mansob (2011) showed that black pepper increases digestion through arousing digestive liquids of stomach and eradication infectious bacteria. Black pepper affects the absorption power, decrease material transit velocity and increase digestive enzymes acts and increased chicks dietary and weighs gain. Al-Kassie et al. (2011) showed that the high activity of Piperazine citrate included in the broilers diet which may have affected the flow of digestive juices across the stomach. Hernandez et al. (2004) reported the improved digestibility of the feeds for broilers supplemented with phytogenic growth promoters like black pepper powder. Other researchers found that this may be due to its stimulant, carminative, digestion and anti-microbial properties (Moorthy et al. 2009). Also (Parreira 1998) noted 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. It has been observed that bile acid secretion not increased in laboratory animals fed by piperine (Platel and Srinivasan 2004). This result is agreed with (Kavyani et al. 2012) who indicated that carcass yield increased in broilers fed diets containing probiotic. The findings of our research are in agreement with the other scientific findings (Al-Kassie et al. 2001; Khalaf et al. 2008).

Data from this study showed that liver percentage was significantly increased ($p<0.05$) when broilers fed with protexin and black pepper compared to the control. Additionally, there were significant differences in drumstick and breast meat percentage in all treatments. Data showed that using of protexin and black pepper has reduced abdominal fat percentage statistically ($p<0.05$). Also gizzard percentage was higher for protexin than others. Borsal .F decreased by using protexin and black pepper. As result revealed from this study carcass yield percentage was higher in protexin and black pepper than control ($p<0.05$). The results are in contrary with finding of Mansoub (2011), Ghaedi et al. (2013), Shverdi et al. (2013), who recoded significant increase in carcass yield.

Data showed that using of protexin and black pepper decreased blood serum triglyceride, cholesterol and LDL but increased HDL content significantly ($p<0.05$). Al-Kassie et al. (2011) showed that the reduction of the blood pocket cell may be due to the activity of black pepper which may act on oestrogen hormone. Shahverdi et al. (2013) showed that the triglyceride, glucose, cholesterol blood levels were changed, when chicks used black and red pepper respectively. Deconjugation of gallbladder acids in small intestine may control of serum cholesterol, while deconjugated acids are not capable to solve and

Table 2. The effect of added protexin and black pepper on broilers performance

Treatments	$FI_{(g.d)}$	$BW_{(g.d)}$	FCR	$TFI_{(g)}$	Pre slaughter weigh $_{(g)}$
Control	87.20^{c}	40.54^{b}	2.06^{a}	3692.0^{c}	1765.5^{b}
Protexin	89.20^{a}	42.24^{a}	1.99^{b}	3714.1^{b}	1816.7^{a}
Black pepper	88.16^{b}	43.17^{a}	1.96^{b}	3723.2^{a}	1812.5^{a}
P value	**	**	**	**	**

*FI: Feed Intake ,BW:Body Wieght,FCR: Feed Conversion Ratio,TFI:Total Feed Intake.

** Means within row with no common on letter are significantly different ($p\leq 0.05$)

Table 3. The effect of added protexin and black pepper on percentage of some visceral organs.

Treatments	Liver (%)	Abdominal.F (%)	Drumstick (%)	Breast Meat (%)	Gizzard (%)	Borsal.F (%)	Carcass yield (%)
Control	2.96[b]	4.85[a]	21.65[b]	24.40[a]	3.54[b]	0.228[a]	66.70[b]
Protexin	3.00[b]	4.00[b]	22.14[a]	25.46[a]	3.62[a]	0.200[a]	69.26[a]
Black pepper	3.16[a]	3.60[c]	22.07[a]	24.98[b]	3.30[b]	0.165[a]	70.14[a]
P value	**	**	**	**	**	**	**

Different letters in each column indicate significant difference at p≤0.05.

absorb fatty acids as conjugated acids. Ghaedi et al. (2013) reported that some plants or specific combinations of herbs in formulations may act as antioxidants by exerting superoxide scavenging activity or by increasing superoxide dismutase activity in various tissue sites. These results mentioned that black pepper feed additives though being less effective performed like antibiotic to certain extent and have a great potential to be utilized as an alternative. Protexin was associated with increased abundance of many of the targets in the proximal gastrointestinal tract, with fewer targets affected in the distal regions. By the fact active compounds in black pepper having receptors on adrenal gland and may affect the nervous system so they lead to decrease Adrenocorticotropic hormone (ACTH) secretion which may cause to increase blood glucose concentration (Akbarian et al. 2012).

Data obtained from table 5 showed that antibody titer against New Castle vaccine increased by using protexin and black pepper. Additionally, microbial count of E-Coli were decreased and Lactobacillus colonies were increased significantly ($p<0.05$).It may be due to its stimulant, carminative, digestion and anti-microbial properties. Black pepper has been found to have antioxidant properties and anti-carcinogenic effect (Nalini et al. 2006). Piperine is an excellent bactericidal activity against all the gram positive and gram negative bacteria tested. In manystudies the alkaloids like piperine, piperidine, volatile oil and resins might be responsible for the antibacterial activity. According to (Hosseini 2011) study the effect of using different levels of black pepper on sheep red blood cell (SRBC) count were significantly higher than control. Ghaedi et al. (2014) showed that antibody titers were significantly higher when broilers were fed by black pepper and virginiamycine antibiotic ($p<0.05$). Shahverdi et al. (2013) demonstrated that pathogenic microbial flora in the small intestine compete with host for nutrients and at the same time and they able to inhibit the binding of the bile acids to the pertinent substances, hence they may decrease the digestion of fats and fat-soluble vitamins. The results of this study were in line with finding of (Al-Kassie et al. 2012) who reported that, black pepper increases digestion through arousing digestive liquids of stomach and eradication infectious bacteria.

Table 4. The effect of added protexin and black pepper on some blood parameters

Treatments	TG (Mg.dl)	Chol (Mg.dl)	HDL (Mg.dl)	LDL (Mg. dl)
Control	70.68[a*]	132.25[a]	58.47[c]	71.20[a]
Protexin	68.22[b]	128.88[b]	61.28[b]	70.00[b]
Black pepper	66.14[c]	126.25[c]	62.87[a]	66.14[c]
P value	**	**	**	**

TG: Triglyceride, Chol: Cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein.
Different letters in each column indicate significant difference at p≤0.05.

Table 5. The effect of protexin and black pepper on HI titer and intestinal microbial populations

Treatments	HI (42 days) (Log2)	E.Coli (cfu.gr)	Lacto.B (cfu.gr)
Control	3.68[c*]	7.01[a]	4.50[b]
Protexin	4.11[b]	6.24[b]	5.11[a]
Black pepper	4.29[a]	5.99[c]	5.02[a]
P value	**	**	**

HI: heamagglutination inhinition test: E.Coli: Eshcherchiacoli , Lacto.B: Lactobacillus
Different letters in each column indicate significant difference at p≤0.05.

CONCLUSION

In present experiment, we already attempted to study the effect of supplementation of black pepper in diets on performance of broiler. In conclusion, we could demonstrate that black pepper and protexin probiotic may have beneficial actions on performance of experimental broilers chicks. This improvement may be due to the biological functions of that to improve growth or its role as stimulant, carminative, enhanced digestibility antimicrobial properties.

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