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Effect of Chicory Plant (*Cichorium intybus* L.) Extract on Performance and Blood Parameters in Broilers Exposed to Heat Stress with Emphasis on Antibacterial Properties

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Abstract
The antimicrobial effectiveness of different extracts of Chicory (*Cichorium intybus* L.) plant including methanolic, ethylic acetate, chloroformic, and aqueous extracts was evaluated by Disk Diffusion method. The ethylic acetate extract showed higher antibacterial activity against *E. coli* compared with others. Then, effects of different levels of ethylic acetate extract on growth performance and blood parameters of broilers subjected to high ambient temperature was investigated. The treatments were; a control diet, 3 levels of the Chicory ethylic acetate extract (150, 250, and 350 mg/kg feed) and one level of probiotic with 4 replicates of 20 broiler chicks in each. The temperature was increased to 35°C with 50% relative humidity for 5 h daily, starting from 11 d until 42 d of the experimental period. Results indicated that inclusion Chicory extract at 350 mg/kg and probiotic increased body weight gain and improved feed conversion ratio during 11-24 d and 0-42 d ($P < 0.05$). There were no significant differences in feed intake of broilers treated with Chicory extract and probiotic compared with control. The serum concentrations of triglyceride and very low-density lipoprotein significantly decreased in birds received Chicory extract at the levels of 250 and 350 mg/kg feed compared with the other treatments ($P < 0.05$). No significant difference was observed between treated groups and control for serum high-density lipoprotein and low-density lipoprotein concentrations. It seems that dietary supplementation of Chicory ethylic acetate extract at levels higher than 250 mg/kg feed has growth promoting effect which can result in improving growth performance and decreasing blood lipids of broilers exposed to heat stress condition.

Introduction

The harmful effects of heat stress on performance, health and physiology of broilers can be a cause of concern, especially in tropical regions. The microbes of gastrointestinal tract play a key role in production efficiency, enhancing immunity and the health of the bird.

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The imbalance in the microbes of the gastrointestinal tract, by weakening of beneficial microorganisms, causes more colonies of pathogens to be formed (Lin *et al*., 2011). Antibiotic growth promoters improve the public performance of birds with improving bowel and strengthen beneficial microorganisms. Furthermore, they prevent the creation of some pathogenic bacteria, but their harmful effects on human health due to antibiotic resistance and residues remaining in poultry products, results in an effort to find materials that can improve animal growth without adverse effects on consumer's health (Eckert *et al*., 2010).

Today, many materials have been introduced as an alternative to antibiotics such as probiotics, prebiotics and medicinal plants. Probiotics increase production efficiency by reducing the nutrients available for harmful bacteria and also reduce the production of toxic bacterial metabolites (Patterson & Burkholder, 2003). Chicory plant due to its special characteristics and ingredients is used as an herbal medicine. All parts of this plant especially roots have medicinally important compounds such as alkaloids, fructooligosaccharides, inulin (about 98%), flavonoids and variety of terpenoids (Saxena *et al*., 2014). Active ingredients in the herb Chicory have the property of increasing beneficial microbes (lactobacilli, bifidobacteria and butyrate-producing bacteria) and reducing pathogenic bacteria (*Escherichia coli* and *Salmonella*) in the intestines of chickens (Chow, 2002).

There are not many reports regarding the use of Chicory plant extract in broilers during heat stress. Therefore, in this study antibacterial properties of different extracts of Chicory (*Cichorium intybus* L.) plant was investigated and then the effects of the ethylic acetate extract as a growth-promoting additive on performance and blood parameters of broiler chickens was examined under heat stress condition.

Material and Methods

The protocol and all procedures were approved by Gonbad Kavous University, Gonbad-e-Kavous, Iran.

Preparation of extract

Chicory plant used in this experiment was collected in September 2013 from the mountains near Bojnoord, North Khorasan province, Iran. The collected plant was dried under 25ºC shadow. The dried samples were grounded into 3 to 5 mm particles using a laboratory mill. Solvents (methanol, ethyl acetate, chloroform and water) were added to the powdered plant and kept for 4 to 5 days at room temperature and were filtrated through Whatman No. 1. The solvent was then removed using rotary evaporator. The extracts became viscous, dried on a water bath and then stored at 4ºC.

Antibacterial activity of different solvent extracts

The sensitivity of *E. Coli* to different *Cichorium intybus* plant extracts was determined accordance with the Disc Diffusion method by Mueller Hinton agar medium plate (Merck Co., Germany). Antimicrobial discs of Gentamicin used as positive control for comparing with different Chicory extracts. The antimicrobial activity was evaluated by measuring the inhibition zone at 50 and 100 µg/mL.

Experimental design

An experiment was conducted as a completely randomized design with 5 dietary treatments and 4 replicates of twenty broiler chicks (mixed sex) in each pen. For preparing dietary treatments, a basal diet was formulated to meet or exceed the nutrient recommendations for broiler chickens according to Ross 308 requirements (Avigen, 2009; Table 1). Then, five dietary treatments were prepared by addition of 4 levels (0, 150, 250, and 350 mg/kg feed) of the ethylic acetate Chicory plant extract and 1 level of probiotic Primalac to the basal diet. The ethylic acetate extract and probiotic were first mixed very well with corn and then gradually was added to the basal diet. Probiotic Primalac (StarLabs Inc., Clarksdale, MO, USA) contained a total of 2×10^8 colony forming unit of *L. acidophilus, L. casei, E. faecium and B. bifidium*.

Birds had free access to feed and water throughout the experiment. Body weight gain, feed intake, and feed conversion ratio were determined during each period of the experiment. Birds were reared on litter floor pen and a continuous lighting program with 23 h light and 1 h darkness was used. The temperature at 1-10 days was kept according to the Ross 308 manual recommendations. Birds were exposed daily to heat stress for 5 hrs at 35°C and 50% relative humidity during 11 days of age up to the end experiment.

Blood sampling

At 35 d of the age, after 6 h of fasting, two male chickens from each replicate were selected. Blood samples were collected from the brachial vein and Serum was obtained by centrifugation of the coagulated blood at 3000 × g for 10 min. The sera samples kept in the deep freezer until analysis for determining total protein, albumin and globulin (Doumas *et al.*, 1981), cholesterol, High-density lipoprotein (HDL) (Wybenga *et al.*, 1970) and triglyceride (Neri & Frings, 1973). Very low-density lipoprotein (VLDL) was determined by dividing triglyceride concentration to five. Low-density lipoprotein (LDL) calculated by subtracting HDL and VLDL from cholesterol concentration (Warnick *et al.*, 1990).

Statistical analysis

Analysis of variance was performed using the GLM procedure with SAS software (SAS, 2003) based on a completely randomized design. Significant differences among treatment means were determined by Duncan's multiple range test at a 5% probability level.

Table 1. Ingredients and nutrient composition of the basal diet

Ingredient (%)	(1-10 d)	(11-24 d)	(25-42 d)
Corn	55.88	58.96	62.67
Soybean meal	37.16	34.06	29.73
Soybean oil	2.22	2.95	3.75
Limestone	1.31	1.07	1.06
Dicalcium phosphate	1.79	1.55	1.49
Salt	0.50	0.50	0.43
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL-Methionine	0.35	0.27	0.24
L-Lysin	0.29	0.14	0.13
Nutrient composition			
ME (Kcal/kg)	2900	3000	3100
CP (g/kg)	21.1	20	18.41
Ca (%)	1.00	0.86	0.82
Available P (%)	0.48	0.43	0.41
Lysine (%)	1.37	1.18	1.05
Methionine	0.68	0.57	0.52
Methionine + Cystine (%)	1.02	0.90	0.83
Sodium (%)	0.21	0.21	0.19

[1]Each Kg of vitamin premix contained: vitamin A, 3,600,000 IU; vitamin D_3, 800,000 IU; vitamin E, 7.2 g; vitamin K_3, 0.8 g; thiamine, 0.72 g; riboflavin, 3.3 g; pantothenic acid, 4 g; vitamin B_6, 1.2 g; vitamin B_{12}, 6 mg; niacin, 12 g; biotin, 40 mg; folic acid, 0.4 g; choline chloride, 100 g; antioxidant, 40 g.

[2]Each Kg of mineral premix contained: manganese, 40 g; zinc, 40 g; iron, 20 g; copper, 4 g; iodine, 0.4 g; selenium, 80 mg.

Results

The results of the antibacterial effect of Chicory extracts are shown in Table 2. All extracts showed good antibacterial activity against *E. coli* but the greatest inhibition was seen on the ethylic acetate extract. The inhibition zone in ethylic acetate extract was significantly higher than the other extracts at both concentrations of 50 (7.4 mm) and 100 μg/mL (15.2 mm) ($P < 0.05$).

The effects of different levels of Chicory ethylic acetate extract and probiotic on the body weight gain, feed intake and feed conversion ratio are shown in Table 3. There was no significant difference between Body weight gain, feed intake and feed conversion ratio in birds received different dietary treatments at 1-10 d of age.

Table 2. Antibacterial activity of plant extracts of Chicory

Solvent	Zone of inhibition against *E.Coli* (mm)	
	50 μg/mL concentration	100 μg/mL concentration
Chloroform	5.4[c]	9.9[d]
Ethyl acetate	7.4[b]	15.2[b]
Methanol	6.2[bc]	12.6[c]
Water	3.7[d]	6.7[e]
Gentamicin[1]	10.5[a]	22[a]
SEM[2]	0.37	0.53

[1]The positive control was Gentamicin; [2]Standard error of means.

[a-e]Means within the same column without common letters differ significantly ($P < 0.05$).

Table 3. The effect of feeding Chicory extract on performance parameters of broilers

Item	Control	Chicory extract (mg/kg)			Probiotic	*SEM*[1]
		150	250	350		
0-10						
Body weight gain (g)	117	115	119	129	118	4.16
Feed intake (g)	198	195	190	185	187	4.49
Feed conversion ratio	1.70	1.82	1.59	1.44	1.58	0.16
11-24						
Body weight gain (g)	821[b]	820[b]	835[ab]	902[a]	898[a]	26.01
Feed intake (g)	1469	1476	1494	1542	1526	37.72
Feed conversion ratio	1.79[ab]	1.80[a]	1.79[ab]	1.71[b]	1.70[b]	0.04
25-42						
Body weight gain (g)	1272[b]	1353[b]	1474[ab]	1416[ab]	1518[a]	28.70
Feed intake (g)	2505[b]	2665[b]	2874[a]	2719[ab]	2899[a]	34.76
Feed conversion ratio	1.97[a]	1.97[a]	1.95[ab]	1.92[b]	1.91[b]	0.03
0-42						
Body weight gain (g)	2210[b]	2292[b]	2430[ab]	2449[ab]	2536[a]	41.2
Feed intake (g)	4172	4336	4558	4446	4612	77.7
Feed conversion ratio	1.89[a]	1.89[a]	1.87[ab]	1.81[b]	1.82[b]	0.03

[1]Standard error of means. [a,b]Means within the same row without common letters differ significantly ($P < 0.05$).

During 11 to 24 d of age, birds fed diets containing 350 mg/kg Chicory extract or probiotic had greater body weight gain ($P < 0.05$) than birds fed other diets. However, no significant difference was found between control diet with 150 mg/kg and 250 mg/kg Chicory extract. There was no significant difference between dietary treatments for feed intake (g) at 11-24 d. Birds fed diets containing 350 mg/kg Chicory extract or probiotic had significantly better feed conversion ratio than those birds fed 150 mg/kg Chicory extract ($P < 0.05$).

The highest body weight gain belonged to probiotic treatment that had a significant difference with 150 mg/kg Chicory extract and control treatments at 25-42 d ($P < 0.05$). However, there was no significant difference between different levels of Chicory treatments for body weight gain during this period. Birds fed diets containing probiotic or 250 mg/kg Chicory extract had significantly higher feed intake than those birds fed control and 150 mg/kg Chicory extract ($P < 0.05$). No significant difference was found between treatments for feed conversion ratio during this period.

For the overall experimental period (0-42 d), there was no significant difference between different levels of Chicory extract for body weight gain; Although it was improved by increasing amounts of Chicory extract. The highest body weight gain was related to birds fed probiotic. Neither Chicory extract nor probiotic had a significant effect on feed intake during this period. Birds fed 350 mg/kg Chicory extract or probiotic had significantly better feed conversion ratio than those birds fed control or 150 mg/kg Chicory extract ($P < 0.05$).

Table 4. The effect of feeding Chicory extract on blood serum components in broilers at 35 d of age

Serum components	Control	Chicory extract (mg/kg)			Probiotic	*SEM*[1]
		150	250	350		
Total protein (g/dL)	3.12	3.24	3.16	3.25	3.23	0.17
Albumin (g/dL)	1.29	1.47	1.45	1.51	1.50	0.15
Globulin (g/dL)	1.83	1.77	1.71	1.74	1.73	0.09
Triglyceride (mg/dL)	95.90[a]	86.37[ab]	79.22[b]	69.30[b]	89.0[ab]	8.01
Cholesterol (mg/dL)	121.70[ab]	128.85[a]	118.62[ab]	115.21[b]	129.4[a]	4.17
HDL (mg/dL)	37.60	49.57	37.78	38.35	50.60	4.31
LDL (mg/dL)	65.00	62.00	65.00	63.00	61.00	5.60
VLDL(mg/dL)	19.10[a]	17.28[ab]	15.84[b]	13.86[b]	17.8[ab]	1.48

[1]Standard error of means. [a,b]Means within the same row without common letters differ significantly ($P < 0.05$).

The effect of feeding Chicory plant extract and probiotic on some serum components in broilers at 35 d of age are shown in Table 4. Broilers received 250 and 350 mg/kg Chicory extract had significantly lower serum triglyceride, cholesterol and VLDL concentrations than other treatments. These amounts were decreased by elevating the levels of Chicory plant extract. There was no significant difference between broilers fed probiotic and control treatment. No significant effect was observed in serum total protein, albumin, globulin, HDL and LDL concentration of treated groups and control ($P < 0.05$).

Discussion

There are some components in the extracts of Chicory such as flavonoids, phenolic compounds and alkaloids that have antimicrobial properties (Saxena *et al.,* 2014). The antimicrobial and antioxidant effects of phenolic compounds have been reported by Akbarian *et al.* (2011) and Viveros *et al.* (2011). It has been reported that ethylic acetate fraction showed more strong activity against *E. coli* than chloroformic and methanolic extracts (Petrovic *et al.,* 2004; Mehmood *et al.,* 2012). The hexane and ethyl acetate root extracts of Chicory showed a greater zone of inhibition than chloroformic, petroleum ether and water extracts on *E. coli* (Nandagopal *et al.,* 2007). Flamini *et al.* (2001) and Dendougui *et al.* (2006) showed that polar compounds in the Chicory plant can be dissolved in polar solvents. The higher antimicrobial property of ethylic acetate extract may be related to this fact that flavonoids and phenols which are polar and semi-polar compounds can be dissolved in organic solvents such as ethyl acetate and chloroform (Ceksteryte *et al.*, 2007). Plant extracts in poultry diets can play an important role in improving animal performance by two mechanisms including endogenous enzyme stimulation and regulation of intestinal microflora balance (Deans and Waterman, 1993). Shapiro and Guggenheim, (1995) and Stiles *et al.* (1995) reported that terpenes and phenols propane have lipophilic properties and are able to penetrate the bacterial cell membrane and reach the inner membrane and are capable of disrupting the enzymatic activity of bacteria.

In agreement with our results, da Silva *et al.* (2011), Safamehr *et al.* (2013) and Saeed *et al.* (2015) reported better body weight gain in broilers consumed Chicory extract. The improvement in body weight gain may be attributed to better mucosal growth, villus height and width, crypt depth and ratio of villus height to crypt depth, which might have resulted in increased absorption of nutrients (Zyl *et al.*, 2010; Awad *et al.*, 2011). It is expected that Chicory extract is more effective in improving broilers performance expose to heat stress when physiological and biochemical metabolites change. Reducing feed intake and lack of nutrient efficiency occurred in heat stress conditions (Cooper & Washburn, 1998). Birds consume energy to cool down body during heat stress. This energy has a significant impact on their performance and reduces the production indexes (Tuzcu *et al.,* 2008). No significant difference was observed for feed intake between the birds received Chicory extract during the whole period of the experiment. This is in accordance with findings of others (Liu *et al.*, 2011; Elrayeh and Yildiz, 2012; Saeed *et al.*, 2015) who reported that Chicory extract did not affect feed intake. On the contrary, Behboud *et al.* (2011) and Safamehr *et al.* (2013) reported a significant increase in feed intake in birds that consumed Chicory extract.

Better feed conversion ratio in birds given Chicory ethylic acetate extract may probably be due to its complex carbohydrates such as oligofructose and oligosaccharides which might have resulted in efficient meat production (Park & Park, 2012), an improvement in digestibility of nutrients and reduction in pathogenic microbes (Anderson *et al.,* 2000). These findings are in agreement with the results of da Silva *et al.* (2011) and Saeed *et al.* (2015), however, the findings of Liu *et al.* (2011) and Elrayeh and Yildiz (2012) were different, in which they showed that feed conversion ratio of broilers treated with Chicory was not improved.

Plant extracts can stimulate secretions in the small intestinal, liver and pancreas, and increase the rate of digestion (Khajuria *et al.,* 2002). Brenes and Roura (2010) reported that plant extracts may stimulate crypt cell proliferation. The Compounds derived from medicinal plant of Chicory such as inulin can remove pathogenic microbes and replace them with beneficial bacteria and improve the performance of poultry (Biggs *et al.,* 2007). The final product of fermentation of inulin and oligofructose are short-chain fatty acids (SCFA) such as acetate, propionate and butyrate (Blottiere *et al.*, 1999). Butyrate production increase microvilli height and absorption of nutrients in the digestive system and improve the growth performance of broiler chickens (Van Leeuwen *et al.*, 2005). Many studies have confirmed the beneficial effects of probiotics on performance and the microbial population of the gastrointestinal tract and serum parameters in broiler chickens (Lin *et al.*, 2011).

Our results on cholesterol are consistent with Velasco *et al.* (2010) and Saeed *et al.* (2015). The decrease in blood cholesterol level might be due to the properties of Chicory extract to stimulate lactic acid producing bacteria and secreting the hydrolase enzyme (Hinton *et al.*, 2000) that converts bile salts into deconjugated bile acids and reduced serum cholesterol level (Safamehr *et al.*, 2013). Chicory inulin reduces serum triglycerides and lipoprotein levels by reducing fatty acid synthesis (Williams, 1999).

Conclusion

Ethylic acetate Chicory extract had more antibacterial activity and more effective properties against *E.coli* compared to other Chicory extracts. Supplementation of Chicory ethylic acetate extract at the level of 350 mg/kg has growth promoting effect and improves growth performance and decreases blood lipids in broilers exposed to heat stress.

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