Dietary Willow Bark Extract for Broilers Reared Under Heat Stress

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ABSTRACT

A 28-day feeding trial was conducted on 60, Cobb 500 broilers (14 days), assigned to 2 groups (C, E) housed in an experimental hall (32° C, 23 h light regimen). Compared to the conventional diet C, the experimental diet (E) included 1% white willow bark extract (WBE). At 42 days of age, 6 blood samples /group were collected and 6 broilers/ group were slaughtered and caecal content was collected. The dietary WBE didn't influence broiler performance. The serum concentrations of glycaemia, cholesterol and triglycerides were lower ($P < 0.05$) in E group than in C group. E broilers had the lowest count ($P < 0.05$) of Enterobacteriaceae, *E. coli* and staphylococci colony forming units in the caecal content. The inclusion of WBE (1%) in the diet of broilers reared at 32° C had an hypocholesterolemiant and hypoglycaemic effect and reduced the pathogenic bacteria in the caecum.

INTRODUCTION

Heat stress is a major environmental stressor that may have deleterious effects on growth performance, nutrient availability, immunity, intestinal microbiota and welfare of broilers (Lara, and Rostagno, 2013). Gut health problems have increased since the use of antibiotic growth promoters (AGPs) as feed additives was banned in the European Union (Wegener, 2003). Celikbilek *et al.* (2014) showed that poultry are susceptible to the potentially pathogenic microorganism such as *E. coli* and *Salmonella*, even more in heat stress conditions. So, the use of phytogenic compounds in broiler diets can help reducing the harmful microorganisms in the intestine (Celikbilek *et al.* 2014). Nutritional manipulation is one of simplest and most direct methods used in poultry production to alleviate the adverse effects of heat stress (Sahin *et al.* 2009). Many types of plants have been tested, at different levels of inclusion, and received great interest as replacement of

antibiotics in animal production (Akyildiz, and Denli, 2016).

White willow (*Salix alba*) has been used traditionally, as a source of bioactive compounds including salicin, polyphenols, and flavonoids (Vlachojannis *et al.* 2009). Among these active compounds, salicin is known best for its support of the response of the organism to the normal physiological stress (Albrecht *et al.* 1990). For example, *Salix acutifolia* contains up to 12% salicin in its bark (Koptina *et al.* 2010). Also, various in vitro studies have shown antioxidant activity of willow extract (Bonaterra *et al.* 2010; Jukic *et al.* 2012). The willow bark extract has been used for thousands of years but, despite its long history, few studies on animals have been published, which to support these observations (Shara *et al.* 2015).

The aim of the study was to assess the effect of dietary willow bark extract given to broilers (14-42 days) reared at 32° C on performance, serum biochemical parameters and caecal bacterial population.

Table 1. Diet formulation

Ingredient (%)	Grower (14-35 days)		Finisher (35-42 days)	
	C	E	C	E
Corn	62	62	60.45	60.45
Soybean meal	26.58	26.58	25.54	25.54
Gluten	4	4	6	6
Oil	2.5	1.5	3.72	2.72
White willow bark extract	-	1	-	1
Calcium carbonate	1.4	1.4	1.23	1.23
Monocalcium phosphate	1.36	1.36	1.13	1.13
Salt	0.37	0.37	0.33	0.33
Methionine	0.26	0.26	0.25	0.25
Lysine	0.48	0.48	0.20	0.20
Choline	0.05	0.05	0.05	0.05
Vitamin-mineral premix*	1	1	1	1
Total	100	100	100	100
<i>Calculated chemical composition</i>				
Metabolizable energy, kcal/kg	3.250	3.250	3.108	3.108
Dry matter, %	86.48	86.48	86.49	86.49
Crude protein, %	21.5	21.58	20.4	20.5
Ether extractives, %	4.46	4.32	5.66	5.20
Crude fibre, %	3.54	3.54	3.56	3.56
Calcium, %	0.84	0.84	0.78	0.78
Phosphorus, %	0.75	0.75	0.74	0.74

*1kg vitamin-mineral premix contains: = 1.350.000 IU/kg vit. A; 300.000 IU/kg vit. D3; 2700 IU/kg vit. E; 200 mg/kg Vit. K; 200 mg/kg Vit. B1; 480 mg/kg Vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg Vit. B6; 4 mg/kg Vit. B7; 100 mg/kg Vit. B9; 1.8 mg/kg Vit. B12; 2500 mg/kg Vit. C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium; 50 g sodium monensin /kg.

Where: C- conventional diet; E- conventional diet + 1% white willow bark extract

MATERIALS AND METHODS

The feeding trial was conducted in the experimental halls of the National Research-Development Institute for Animal Biology (IBNA-Balotesti, Romania), according to an experimental protocol approved by the Ethics Commission of the Institute. A total of 60, day-old Cobb 500

broiler chicks were purchased and received a conventional starter (1-14 days) diet (3000 kcal/kg metabolisable energy, 22% crude protein). At the age of 14 days, the broilers were weighted and assigned to 2 groups, control group (C) and experimental group (E). The chicks were housed in an experimental hall with 32°C constant

Table 2. Effect of dietary willow bark extract on broiler performance (14-42 days)

Item	Days	C	E	SEM	p-value
Body weight (g/broiler)	14	379.26 ^a	379.26 ^a	4.686	0.9993
	42	1923.18 ^a	1861.82 ^a	29.677	0.2524
Average daily feed intake (g/broiler/day)	14-42	92.105 ^a	91.59 ^a	1.788	0.6385
Average daily weight gain (g/broiler/day)	14-42	55.14 ^a	52.95 ^a	1.400	0.2696
Feed conversion ratio (g feed/g gain)	14-42	1.67 ^a	1.73 ^a	0.035	0.9644

Where: ^a - Not significant difference ($P > 0.05$); SEM: standard error of the mean.
C- conventional diet; E- conventional diet + 1% white willow bark extract

temperature, humidity 36% and 23 h light regimen. The broilers had free access to the feed and water. The conventional diet formulation (group C) had corn and soybean meal as basic ingredients and with sodium monensin (50 g/kg premix), as COXIDIN (monensin concentration, 20%) supplied by HUVEPHARMA (Sofia, Bulgaria) in the premix (Table 1). Compared to the diet formulation for the control group (C), the formulations for the experimental group (E) included 1% white willow bark extract (WBE) from Plant Extract, Radaia, Cluj County, Romania.

The following parameters were monitored between days 14-42: body weight (g); average daily feed intake (g feed/broiler/day); average daily weight gain (g/broiler/day); feed conversion ratio (g feed/g gain). Mortality was recorded throughout the experimental period.

At 42 days of the feeding trial, blood samples were aseptically collected into 9-mL Vacutainer containing 14.3 U/mL of lithium heparin (Vacutest®, Arzergrande, Italy) for serum biochemical assessment on an automatic BS-130 Chemistry analyser (Bio- Medical Electronics Co., LTD, China). After blood sampling, 6 broilers/group were slaughtered by cervical dislocation and immediately bled. Carcasses were eviscerated manually and the gastrointestinal tract was excised. Caecal contents (2 caeca per bird) were collected aseptically in sterilized plastic tubes and preserved at -20° C until the bacteriological analyses (*Enterobacteriaceae*, *E. coli*, staphylococci, lactobacilli, *Salmonella spp.*).

A classical medium of isolation, G.E.A.M. or Levine, was used to determine the *Enterobacteriaceae* and the *E. coli* in the samples of caecal content. The samples were first immersed into a medium with lauryl sulphate (enrichment medium), properly homogenized, and left for 20-30 minutes at room temperature (23-24° C). Decimal solutions up to 10^{-5} in medium with lauryl sulphate were prepared. Dilutions 10^{-2} – 10^{-5} were used to seed 2 Petri dishes/dilution, on Levine medium. The Petri dishes were incubated for 48h at 37° C, and the colonies which developed in the dishes were thereafter counted. *E. coli* developed characteristic colonies (dark violet with metallic shining). The other *Enterobacteriaceae* formed either intense red, opaque colonies (lactose-positive species), or pale pink or colourless, semi-transparent colonies (lactose-negative species). The results were expressed as $\log_{10}$ colony-forming units (CFU) per gram of caecal contents. The colony forming units from *Enterobacteriaceae*, *E. coli*, staphylococci and lactobacilli was determined by a colony counter (Scan 300, INTERSCIENCE France).

The effects of treatments were tested by analysis of variance using the GLM procedure of the Minitab software (version 17, Minitab® Statistical Software), with treatment as fixed effect, according to the model $Y_i = T_i + e_i$, where Y_i was the dependent variable, T_i is the treatment and e_i is the error. When overall F-test was significant, differences between means were declared significant at $p < 0.05$ using the test of Tukey.

Table 3. Serum biochemical parameters (average values/group)

Item	C	E	SEM	p-value
Energy profile				
Glycaemia, mg/dL	273.482 ^a	228.756 ^b	8.281	0.0004
Cholesterol, mg/dL	133.178 ^a	118.200 ^b	3.741	0.0350
Triglycerides, mg/dL	109.208 ^a	85.01 ^b	4.378	0.0002
Protein profile				
Total protein (g/dL)	2.514 ^a	2.502 ^a	0.9228	0.057
Albumin (mg/dL)	1.596 ^a	3.89 ^b	0.398	<0.0001
Other biochemical parameters profile				
Total bilirubin (mg/dL)	0.536 ^a	0.670 ^a	0.3134	0.063
Creatinine (mg/dL)	0.426 ^a	0.340 ^a	0.3967	0.039
Urea (mg/dL)	3.324 ^a	5.966 ^b	0.0013	0.511
Mineral profile				
Calcium (mg/dL)	8.702 ^a	10.202 ^b	0.0398	0.382
Phosphorus (mg/dL)	4.496 ^a	5.922 ^b	0.0062	0.300
Magnesium (mg/dL)	1.232 ^a	1.500 ^b	0.0009	0.051
Iron (µg/dL)	90.870 ^a	106.284 ^a	0.1903	5.690
Enzyme profile				
ALT (TGP), U/L	44.186 ^a	34.988 ^b	0.0223	2.169
AST (TGO), U/L	59.832 ^a	49.036 ^b	0.0069	2.287
Alkaline phosphatase, U/L	63.100 ^a	47.246 ^b	0.0033	3.200
Gama GT, U/L	20.906 ^a	19.926 ^a	0.6991	1.164
LDH, U/L	948.840 ^a	782.272 ^b	0.0002	30.293
Creatine kinase, U/L	1022.718 ^a	947.860 ^a	0.1327	24.498

Where: C- conventional diet; E- conventional diet + 1% white willow bark extract.

ALT- Alanine aminotransferase; AST- aspartate aminotransferase; LDH- Lactate Dehydrogenase.

^{a-c} Mean values within a row having different superscripts are significantly different by least significant difference test (P<0.05). SEM: standard error of the mean.

RESULTS AND DISCUSSIONS

Table 2 data show that although at the start of the feeding trial all the chicks had similar body weight (379.26 g), at 42, the body weight of E group was lower than that of C group, but the difference was not statistically significant (P>0.05).

Compared to the Management guide of Cobb 500 hybrid, (reared under thermoneutral conditions), the broilers reared under heat stress had 32.68% (C) and 34.83% (E) lower final body weight. There were no significant differences in the average daily feed intake, average daily weight gain and feed conversion ratio (14-42 days) between

Table 4. Effect of dietary willow bark extract on the caecal microbiota of broilers (14-42 days) ($\log_{10}$ CFU*/g caecal content)

Specification	C	E	SEM	p-value
<i>Enterobacteriaceae</i> , $\log_{10}$	10.780 ^a	10.094 ^b	0.066	<0.0001
<i>Escherichia coli</i> , $\log_{10}$	8.982 ^a	8.800 ^b	0.018	<0.0001
Staphylococci, $\log_{10}$	8.826 ^a	8.100 ^b	0.071	<0.0001
Lactobacilli, $\log_{10}$	10.210 ^a	10.450 ^a	0.051	0.310
Salmonella spp.	Absent	Absent	-	-

Where: C- conventional diet; E- conventional diet + 1% white willow bark extract

*colony-forming units; SEM: standard error of the mean; means in the same row no common superscript significantly different ($P < 0.05$).

the two groups. No mortalities were recorded in the two groups throughout the experimental period. Even though not statistically significant, feed conversion ratio was higher in group E than in group C. Abidin and Khatoon, (2013) revealed that the impaired growth performance of broilers subjected to high ambient temperatures may be associated with decreased feed intake, impaired digestion and metabolism. Also under heat stress condition (32° C), Criste *et al.* (2017) showed that the average daily weight gain and the feed conversion ratio of Cobb 500 broilers (14-35 days), were not different between the groups fed diet with rosehip or oregano powder and the C group. In contrast with this study, but under normal conditions of temperature, Harfinda *et al.* (2016) have shown that the use of 1000 mg willow bark extract/l in the drinking water for the broilers improved the feed conversion ratio.

Serum biochemical parameters directly express the status of metabolism, nutrition and health of the animals (Zhu *et al.* 2014). The same authors showed that these indices can be used to assess the effect of willow bark extract on growth conditions, metabolism, immunity and mechanisms of broilers.

The biochemical parameters determined in the serum (Table 3) revealed several benefits of feeding the broilers with willow bark extract. Thus, all the parameters of the plasma energy profile (glycemia, cholesterol and triglycerides) were significantly ($P < 0.05$) lower compared to the group C. In contrast with present work, Tang *et al.* (2013) show that the acute heat stress

decreased insulin concentration in broilers. The reduced content of total cholesterol may reflect the hypocholesterolemic properties attributed to the dietary willow bark extract (1%) which has a major influence on the health status of the broilers. In agreement with these results, Karimi *et al.* (2015) showed in a study on normolipidemic rabbits that aromatic water of *Salix aegyptiaca* decreased level of total cholesterol and this effect was comparable to that of simvastatin. On the other hand, Gursu *et al.* (2004) found increased concentrations of triglyceride, cholesterol, and HDL cholesterol in heat-stressed broilers.

Excepting the albumin and urea levels, which were significantly lower in E group than in C, the level of total protein, bilirubin and creatinine in broiler serum showed no significant differences ($P > 0.05$) between groups. Except for the iron, the calcium, phosphorus and magnesium levels in the serum of E group were significantly ($P < 0.05$) higher than those in C group. The same trend was noticed in the enzymatic profile, where LDH concentration was significantly ($P < 0.05$) lower, by 17.55%, in E group than in C group (Table 3). Although the broilers from both groups were reared under heat stress, the ALT and AST levels in the serum of broilers fed experimental diet was significantly ($P < 0.05$) lower compared to conventional diet (Table 3). In contrast with the present results, Hosseini-Vashan *et al.* (2015) showed that the serum activity of AST was increased significantly by the heat stress (34±1° C, 55% RH, 5 h/day) even if the broilers received diets with tomato pomace. Serum ALT and AST are

very low under normal conditions, but when liver damages or an increase in the permeability of liver cells are present, ALT and AST are released into the blood to increase their activity (Zhang, 2011). Also, Zhang, (2011) revealed that their serum levels are sensitive indicators of liver cell damage.

The number of colony forming units of *Enterobacteriaceae*, *E. coli* and *staphylococci* was significantly ($P < 0.05$) lower in the caecal content of E group compared to C group (Table 4). The *Enterobacteriaceae* populations in the caecum of E group were 6.36% lower, the *E. coli* were 2.03% lower, while the *staphylococci* were 8.22% lower. Many studies support the idea that, under heat stress, different dietary phytoadditives can reduce the pathogenic bacteria in the intestinal and caecal content of broilers (Criste *et al.* 2017; Vlaicu *et al.* 2017). A significantly decrease in the caecal populations of *Escherichia coli* was noticed by Khalaji *et al.* (2011) too, when *Artemisia sieberi* leaves was added to broiler diets, however, under normal conditions of temperature. Sulaiman *et al.* (2013) revealed that *S. alba* extract possesses antimicrobial activities, positively correlated with their antioxidant potential.

The lactobacilli populations from the caecal content of E group were higher than in the C group, but no statistically significant ($P > 0.05$). Suzuki *et al.* (1983) noticed that the heat stress (35° C) increased the counts of *E. coli* and streptococci in the small and large intestine of broilers (7-28 days), but without any change in the lactobacilli count. *Salmonella spp.* were absent in all samples of caecal content.

CONCLUSIONS

Broilers treated with 1% willow bark extract did not show significant differences of the growth performance. Heat (32° C) decreased the body weight compared to the reference values in the Cobb 500 Management guide for broilers (42 days) reared under normal environmental temperatures. The serum levels of glycemia, cholesterol and triglycerides were lower ($P < 0.05$) in E group than in C group, thus, showing the hypocholesterolemic and hypoglycaemic effect of willow bark extract. The dietary inclusion of willow bark extract (1%) had a positive effect ($P < 0.05$) compared to group C in reducing, under heat stress conditions, the proliferation of

pathogenic bacteria (*Enterobacteriaceae*, *E. coli*, *staphylococci*) in the broiler caecum.

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