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EFFECTS OF DIETARY ADDITION OF CHLOROGENIC SUBSTANCES ON GROWTH PERFORMANCE, INTESTINAL MICROFLORA POPULATION AND SERUM BIOCHEMISTRY IN BROILERS

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Abstract

The aim of this study was to evaluate dietary effects of chlorogenic substances (Igusafe) on growth performance, intestinal microflora population and serum biochemistry in broilers. A total of 144 Ross 308 one-day-old male broiler chicks were randomly allocated to 3 treatment groups consisting of 8 replications of 6 chicks per pen for 42 days. There treatments were used: chicks were fed by basal diet as control group, basal diet plus 80 mg Igusafe/kg diet, and basal diet and 160 mg Igusafe/kg diet. Results showed that the addition of 80 mg Igusafe to the diet resulted in significantly higher body weight gains and lower aspartate amino transferase (AST) and serum total protein (TP) concentrations compared with control group (P<0.05). Intestinal tract weight was also significantly higher in the group fed 160 mg Igusafe/kg diet (P<0.05). Supplementing both levels of Igusafe significantly reduced Escherichia coli count (P<0.05). There were no treatments effects on carcass yield, liver weight and Lactic acid bacteria population (P>0.05). In conclusion, our results indicated that supplementation of Igusafe may be useful as enhancer of growth performance by reducing Escherichia coli count with a positive trend to decrease the broilers mortality.

Key words*: chlorogenic substances, growth performance, serum biochemistry, microbial population, broiler.*

INTRODUCTION

Many kinds of antibiotics have been used extensively as growth promoters in animal feeds for a large number of years, especially in the fields of poultry production. Antibiotic feed additive bans have forced in investigators to research novel approach for alternative feed additives in poultry production.

Various herbs, plant extracts, spices, essential oils, prebiotics, and probiotics have received great attention being used as feed supplements to improve growth performance. These alternatives are of great interest to the poultry industry. In recent years, several studies have been done to identify the potential beneficial effects of plants or plant extracts and spices or their active compounds in animal nutrition. Various kinds of plant extracts were being used as feed additive to improve growth performance of animals (William and Losa, 2001).

Previous studies suggested that several herb and plants compounds indicated antimicrobial, anti-inflammatory and antioxidant activities (Hernandez et al., 2004; Lawrence, 2005; Khalaji et al., 2011). Beneficial actions of herbal extracts or their active compounds in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion (Rahimi et al., 2011). In addition Allen et al. (1998) reported beneficial effects in the control of coccidiosis in poultry. However, there is limited published data concerning with using plant extracts as liver protector in poultry. Igusafe has developed with choleretic, cholagogue and antioxidant properties which protect the liver cells reinforcing the liver functions and enhancing performance in the animal.

The aim of the present study was to investigate the effect of dietary supplementation with chlorogenic substances (Igusafe) on growth performance, intestinal

microflora population and serum biochemistry in broilers.

MATERIALS AND METHODS

This trial was performed at the Dicle University, Animal Research Center according to the guidelines for animal experimentation of Dicle University and approved by the Ethical Committee.

A total of 144 Ross 308 one-day-old male broiler chicks were randomly allocated to 3 treatment groups consisting of 8 replications of 6 chicks per pen for 42 days. There treatments were used: chicks were fed by basal diet as control group, basal diet plus 80 mg Igusafe/kg diet, and basal diet and 160 mg Igusafe/kg diet. Two diets were formulated according to the NRC (1994) recommendations to meet the nutrient requirements of broilers from d 1 to 21 (grower diet) and from d 22 to 42 (finishing diet). The composition of the basal diets is presented in Table 1. Feed and water were provided *ad libitum* throughout the experiment.

Table 1. Composition of experimental diets (%)

Ingredients	Starter (1-22 day)	Finisher (23-42 day)
Maize	57.4	59.5
Soybean meal (46 % CP)	28.3	28.9
Full fat soybean	6.8	4.0
Fish meal (60 % CP)	4.7	-
Sunflower oil	-	4.7
Dicalciumphosphate[a]	1.7	2.1
NaCl	0.35	0.40
Vitamin premix[b]	0.10	0.10
Mineral premix[c]	0.15	0.15
Lysine	0.26	0.18
Calculated composition		
Crude Protein	23.0	20.0
ME (kcal/kg)	3010	3240
Calcium	1.0	0.91
Available phosphorus	0.5	0.47
L-lysine	1.3	1.02
Methionine+cystine	1.1	0.62

[a] Contains 240 g Ca and 17.5 g P/kg;
[b]Provided (per kg of diet): vitamin A, 8,000 IU; vitamin D3, 1,200 IU; vitamin E, 10 IU; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 5 mg; pyroxidine, 0.2 mg; vitamin B12, 0.03 mg; pantothenic acid, 10 mg; niacin, 50 mg; biotin, 0.1 mg; folic acid, 0.5 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg
[c]Provided (per kg of diet): Iron, 80 mg; zinc 40 mg; manganese 60 mg; iodine 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg.

The experiment lasted 42 days, including 21 days on the grower diet and from day 22 to 42 on the finishing diet. Igusafeis is a commercial product which has developed by Igusol Advance SA, Spain. It is a combination of a standardized mixture of plant extracts with choleretic, cholagogue and antioxidant properties which protect the liver cells reinforcing the liver functions and enhancing performance in the animal.

The body weight gains of chickens were measured individually and feed intake, and feed conversion rate per cage were recorded weekly from day 7 to 42 (n = 6). Mortality was recorded daily throughout the experimental period.

At the end of the experiment, blood samples (2 mL per bird) were collected from 10 chickens per treatment for serum biochemical determination. Within 1 h, the serum was obtained by centrifugation (2.500 × *g* for 15 min) and stored at −80°C until further analysis. Serum biochemical parameters were measured by using Architech System Reagents and an automatic clinical chemistry analyzer. The concentration of total protein (TP) was measured by following the Biuret method; uric acid (UA) by following the uricase method; cholesterol by following the cholesterol esterase-peroxidase method; respectively; triglyceride by following the glycerol phosphate oxidase method; and the enzymatic activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) by using the recommended International Federation of Clinical Chemistry and Laboratory Medicine reference methods. After taking blood samples, chickens were euthanized with an intravenous injection of sodium pentobarbital and immediately intestinal tract, liver and spleen were removed and weighed (data expressed as relative organ weight; grams of organ per 100 g of BW). Small intestine was immediately removed and digesta contents (from final part of small intestine) from 40 chickens (10 chickens per treatment) were collected separately, cooled at once used for microbial assays *(Escherichia coli and Lactobacillus).* The data were analyzed by using the ANOVA procedure of SPSS 16.0 (2011). Tukey's multiplerange test was used to detect the differences ($P<0.05$)

RESULTS AND DISCUSSIONS

The body weight gain, feed intake, feed conversion rate and mortality are given in

Table 2 Data from this study showed that supplementation of broiler's diet with Igusafe at the level of 80 mg/kg feed significantly increased body weight compared to the control (P<0.05). Also, the mortality rate of chickens fed diet supplemented Igusafe was lower than control. However, there were no differences (P > 0.05) in feed intake and feed conversion rate between treatment groups. (P>0.05).

Table 2.Effects of dietary inclusion of Igusafe on growth performance of broilers

Treatments	Body Weight Gain (BWG, g)	Feed Intake (FI, g)	Feed conversion rate (FI/BWG)
Control	2395.6[b]	4072.4	1.69
Igusafe (80 mg/kg)	2526.2[a]	4089.4	1.66
Igusafe (160 mg/kg)	2452.0[ab]	3913.1	1.65
SEM	20.66	41.95	0.013
P-Value	0.030	0.170	0.486

Pooled SEM: pooled standard error of the mean
[a,b,] Means within column with different superscripts differ significantly P < 0.05

Mortalities were 3 and 1 for control, and Igusafe (80 mg/kg) respectively. No mortality was seen in the Igusafe 160 mg/kg feed group. Many researchers obtained that various plant extracts as mixed preparations may play role in improving growth performance and health status of broilers (Manzanilla et al., 2001; Alcicek et al., 2004; Rahimi et al., 2011). Our BWG findings are in agreement with those of Brzoska et al. (2010) who investigated the effects of herbs and herbal products in broiler diets and those of Shafey et al. (2013) who used olive leaves extract in broiler diets.Table 3 shows the effects of treatments on serum concentration of cholesterol, creatinine, total proteins, triglyceride and uric acid and activities of ALP and AST. No significant differences were observed among treatments in serum concentration of cholesterol, triglyceride, creatinine uric acid (P<0.05). These results are in agreement with the results of Amouzmehr et al. (2012) that showed plant extracts had no significant effects on these serum parameters in broilers. Liver enzymes activities have been reported to be sensitive indicators of hepatocellular damage (Shi et al., 2006). Our results showed that chickens fed diet with Igusafe had significantly lower AST and higher total protein compared with the control group (P<0.05). It is possible that the antioxidant properties of Igusafe protected hepatocellular.

Table 3. Effects of dietary inclusion of Igusafe on serum biochemistry of broilers

Treatments	ALP (U/L)	AST (U/L)	CHOL (mg/dL)	CRE (mg/dL)	TP (g/dL)	TRG (mg/dL)	UA (mg/dL)
Control	1649.6	320.6[a]	109.3	0.297	3.43[ab]	55.0	6.70
Igusafe (80 mg/kg)	1401.2	275.6[b]	112.2	0.314	3.33[b]	46.1	7.12
Igusafe (160 mg/kg)	1443.6	258.9[b]	119.2	0.306	3.78[a]	49.7	7.05
SEM	57.41	9.22	2.44	0.004	0.07	2.20	0.24
P-Value	0.170	0.013	0.242	0.380	0.038	0.260	0.760

Pooled SEM: pooled standard error of the mean
[a,b,] Means within column with different superscripts differ significantly P < 0.05
ALP = alkaline phosphatase; AST = aspartate amino transferase; TP = total protein; UA = uric acid; CRE =creatinine
CHOL = cholesterol; TRG = triglyceride.

The measures for carcass yield, liver weight and intestinal tract weight are presented in Table 4.

No significant differences were detected for carcass yield and liver weight, whereas, intestinal tract weight was significantly higher in chickens fed with Igusafe at the level of 160 mg/kg feed (P < 0.05).

Table 4. Effects of dietary inclusion of Igusafe on internal organs weight and carcass yield in broilers

Treatments	Carcass Yield, (%)	Liver weight, (g/BW, %)	Intestinal tract weight (g/BW, %)
Control	75.9	2.40	4.81[b]
Igusafe (80 mg/kg)	76.2	2.35	4.79[b]
Igusafe (160 mg/kg)	75.6	2.39	5.55[a]
SEM	0.44	0.04	0.12
P-Value	0.877	0.871	0.010

Pooled SEM: pooled standard error of the mean
[a,b,] Means within column with different superscripts differ significantly P < 0.05

Plant extracts are feed additives which beneficially affect the host by improving its intestinal microbial microflora to animal health and nutrition. Beneficially affect can be more evident when animals are challenged by pathogens or chemicals (Yirga, 2015). In earlier studies, significant reductions of *E. Coli* number have been obtained after application of various plant extract (Jamroz et al. 2005; Tiihonen et al. 2010). In the present study, reduction of *E. coli* and increase of *Lactobacillus* spp. were observed in the end of the 42 days old chickens from the Igusafe supplemented groups (Table 5).

Table 5. Effects of dietary inclusion of Igusafe on *E.coli* and *Lactobacillus* numbers in ileal contents of broilers.

Treatments	***E. coli* (log CFU g-1)**	***Lactic acid bacteria* (log CFU g-1)**
Control	5.55[a]	7.78
Igusafe (80 mg/kg)	4.06[b]	7.11
Igusafe(160 mg/kg)	4.11[b]	7.98
SEM	0.152	0.162
P-Value	0.0001	0.064

Pooled SEM: pooled standard error of the mean

[a,b] Means within column with different superscripts differ significantly $P < 0.05$

CONCLUSIONS

Results of this study showed that the addition of Igusafe to broilers diets may be useful as enhancer of growth performance by reducing *Escherichia coli* count with a positive trend to decrease the broilers mortality.

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