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Formononetin Influences Growth and Immune Responses in Broilers

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Abstract.- Many *in vitro* studies indicate that flavonoids could exhibit a variety of potential beneficial effects as non-antibiotic feed additives. However, there is a dire need to ensure the *in vivo* contribution of flavonoids in animals. Present study was aimed at determining the growth promoting and immune modulating effects of formononetin in broilers. A total of 135 one day old Hubbard broiler chicks, randomly divided into 3 treatment groups, received a common basal diet with formononetin inclusion at 0 (control), 10 and 20 mg/kg of diet. The results indicated that 10 mg formononetin/kg of feed resulted in better FCR ($P<0.05$) from 21-42 and 0-42 days and significant increase in weight gain from 0-42 days of age. The dressing percentage was higher ($P<0.05$) in birds fed the diet supplemented with either 10 or 20 mg/kg of formononetin as compared to control group at 42 days of age. The dose of 10 mg/kg significantly enhanced the meat quality. Analysis of immunity related indices showed significant difference in the blood levels of PGE_2, IL-10, IFN-γ and LTB_4 between the birds supplemented with either 10 or 20 mg formononetin/kg feed and control group depending upon the age of the birds. Overall, the results suggested that formononetin could positively affect the immune response and improve growth in broiler depending on their age and its dose.

Keywords: Broiler, formononetin, flavonoids, immune modulating effects, feed conversion rate.

INTRODUCTION

Antibiotics are added at low concentrations to chicken feeds as growth stimulants to modify bacterial, protozoal or fungal populations. There are many reports regarding the beneficial role of antibiotics which have proven to be effective in improving the performance of poultry especially broilers (Harms *et al*., 1986; Rosen, 1996; Taylor, 1997; Engberg *et al*., 2000). However, veterinary feed antibiotics have resulted in the appearance of resistant strains of bacteria. Resistant bacteria which are also human pathogens may cause diseases that are difficult to treat. Even if the resistant bacteria are not human pathogens, they may still be dangerous because they can transfer their antibiotic resistant genes to other pathogenic bacteria (Barton, 1998; Khachatourians, 1998).

The quest for non-antibiotic feed additives has included the testing of a number of phytochemicals. Flavonoids, a group of polyphenolic compounds, found mainly in fruits and vegetables, have gained increased attention especially for use in poultry as can be derived from a significant increase in the number of scientific publications since 2000. This appears to be strongly driven by the ban on most of the antibiotic feed additives within the European Union in 1999, a complete ban enforced in 2006, and on-going discussions to restrict their use outside the European Union (Windisch *et al*., 2008). The results of several *in vitro* studies indicate that flavonoids could exhibit a variety of potential beneficial effects, including antioxidant, antiviral, anti-allergic and anti-inflammatory activities (Manthey *et al*., 2001; Nijveldt *et al*., 2001;, Khan *et al*., 2010). The *in vivo* evidence, however, is conflicting and the real contributions of such compounds to animal performance (including health challenges) are still unclear (Skibola *et al*., 2000; Ferreira *et al*., 2002; Halliwell, 2007).

Present study was, therefore, conducted to evaluate the effects of formononetin, an isoflavonoid, as non-antibiotic feed additive in broiler diet.

MATERIALS AND METHODS

Animals and dietary treatments

A total of 135 one day old Hubbard broiler

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chicks were included in the study. The birds were randomly assigned, according to their initial body weights, to 3 treatment groups having 3 replicates of 15 birds each. The antibiotic-free feed containing 0, 10 and 20 mg/kg formononetin were prepared in a home mixer according to Hubbard requirements (Table I) and fed to 3 treatment groups of broilers. The energy and protein were adjusted to local environmental conditions. The total duration of the experiment was 6 week. Feed consumption was recorded daily, whereas body weight was recorded weekly.

Table I.- Formulation of broiler starter and broiler finisher feed*.

Ingredients	Broiler starter (%)	Broiler finisher (%)
Corn	40	44
Rice broken	12	9
Rice polish	9	10
Wheat bran	3	4
Guar meal	3.60	0
Soybean meal	15	16.70
Canola meal	8	8
Corn gluten 60%	1	1
Corn gluten 30%	3	3
Limestone	0.60	0.6
Dicalcium phosphate	2.10	2.10
L-Lysine	0.36	0.27
DL-Methionine	0.16	0.15
Vitamin-mineral premix	0.80	0.80
Salt	0.31	0.31
$NaHCO_3$	0.05	0.05
Diclazuril	0.02	0.02

*Taken from NRC (1994)

Carcass composition and quality

At 21st and 42nd day of age, six birds from each group (two birds from each replicates) were sacrificed and dressing percentage, weight of breast meat and weight of legs (thigh and shank) were recorded for growth evaluation. At the end of the trial, the composition of breast meat and meat quality via sensory evaluation was determined by following the standardized protocol and procedures (AOAC, 2000; Brenda and Lyon, 2001).

Immunity parameters

The blood samples were collected at 21st and 42nd day of age. After slaughtering, blood samples were harvested into heparinized plastic tubes. Individual blood samples were challenged with Calcium ionophore (A23187) diluted in Dimethyl sulfoxide (DMSO) @ 10 µg/ml of blood within 15 minutes of collection. After incubation at 37ºC for 1 hour and centrifugation for 10 min @ 2800 rpm, the plasma was collected and preserved at -20ºC until further analysis.

Plasma samples were thawed at room temperature to determine immunity parameters. The concentration of PGE_2, LTB_4, IFN-γ, and IL-10 were determined using the ELISA kits (Adlitteram Diagnostic Laboratories) according to the manufacturer instructions.

Statistical analysis

The effect of formononetin treatment on different parameters was evaluated by one-way ANOVA. Differences between means were determined by Duncan's multiple range (DMR) test and $P<0.05$ were considered statistically significant.

RESULTS

Growth and feed conversion ratio (FCR)

The effects of formononetin on growth and meat yield are shown in Table II. Formononetin level @10mg/kg of feed resulted in better FCR ($P<0.05$) from 21-42 and 0-42 days and significant increase in weight gain from 0-42 days of age.

Meat yield

The effects of formononetin on meat yield are summarized in Table III. The dressing percentage was higher ($P<0.05$) in broilers fed the diet supplemented with either 10 or 20 mg/kg of formononetin compared to those fed the control diet from 21 to 42 days of age. There were no significant differences in other parameters (breast weight, weight of leg and shank) between control and formononetin supplemented groups during the entire experiment period.

Composition of breast meat

The protein and moisture contents of breast meat showed non-significant difference ($P>0.05$) among the treatments (Table IV). However, fat contents were tended to be low (2.6%) in birds supplemented with formononetin at 10 mg/kg diet

as compared to control and dose of 20 mg/kg.

Table II.- Effect of different concentrations of formononetin on growth, feed intake and FCR in broilers

Days	Parameters	Formononetin levels (mg/kg)		
		0	10	20
0-21	Weight gain (g)	412±24	464±59	455±19
	Feed intake (g)	689±52	723±28	708±55
	FCR	1.66±0.04	1.58±0.20	1.55±0.10
21-42	Weight gain (g)	845±27	995±120	903±57
	Feed intake (g)	2005±119	1798±280	2078±281
	FCR	2.37±0.2^{a}	1.80±0.06^{b}	2.29±0.2^{a}
0-42	Weight Gain (g)	1257±50^{b}	1458±99^{a}	1359±50^{ab}
	Feed intake (g)	2522±96	2695±262	2787±277
	FCR	2.14±0.12^{a}	1.72±0.08^{b}	2.04±0.15^{a}

Values within a row with different superscript differ significantly (P<0.05)

Table III.- Effect of different concentrations of formononetin on meat yield related parameters in broilers

Days	Parameters	Formononetin levels (mg/kg)		
		0	10	20
0-21	Dressing percentage	56±0.65	52±0.57	54±4.30
	Breast weight (%)	26±2.48	25±1.32	27±2.62
	Weight of leg & shank (%)	34±1.86	32±1.23	28±4.02
0-42	Dressing percentage	52±1.93^{b}	59±2.67^{a}	58±3.68^{a}
	Breast weight (%)	32±2.74	35±3.43	32±1.58
	Weight of leg & shank (%)	32±1.13	32±1.63	31±0.93

Values within a row with different superscript differ significantly (P<0.05)

Table IV.- Effect of formononetin on chemical composition of breast meat of broiler at 42nd day of age

Parameters	Formononetin levels (mg/kg)		
	0	10	20
Dry Matter (DM) %	26.8±0.34	26.8±0.39	27.0±0.85
Moisture contents %	73.1±0.34	73.2±0.25	72.8±0.83
Protein % in DM	75.5±0.58	74.9±2.91	75.8±3.91
Fat % in DM	13.3±1.00	10.7±0.29	13.3±1.00

Meat quality

The results of sensory evaluation (Fig. 1) showed that the breast meat was significantly (P<0.05) harder in birds fed the control diet as compared to those fed the diets supplemented with either 10 or 20 mg/kg of formononetin. No significant difference was observed in texture, odour, colour, tenderness, flavour, juiciness, mouth feeling and palatability among all the three treatments. However, overall liking/disliking differed significantly (P<0.05) among the treatments. Breast meat of the birds fed the diet supplemented with 10 mg/kg of formononetin was liked more than other treatments.

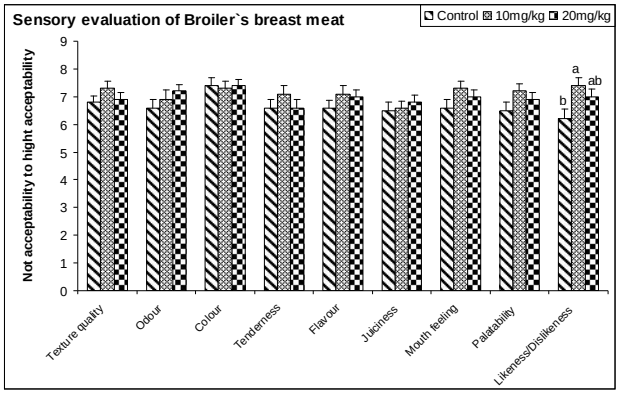

Fig. 1. Effect of formononetin on various sensory attributes of breast meat of broiler at 42nd day of age

* $^{a\ b\ ab}$ Means within the same scale with different letters are significantly different (P<0.05). Errors bars indicate standard deviations.

**Scale: 1-2, Extremely Not-Acceptable; 3-4, Moderately Not-Acceptable; 5, Slightly Not-Acceptable; 6, Slightly Acceptable; 7-8, Moderately Acceptable; 9-10, Extremely Acceptable

Immune responses

The impact of formononetin on different immunity parameters is depicted in Figure 2 and Figure 3. There was a significant difference (P<0.05) in the blood levels of PGE_2, IL-10, IFN-γ and LTB_4 between the control group and the groups supplemented with either 10 or 20 mg/kg formononetin depending upon the age of the birds. Formononetin suppressed the level of PGE_2 in birds both at 21 and 42 day of age except that the suppression was not significant with 10 mg/kg of formononetin inclusion at 21 day. Significantly elevated levels of LTB_4 were observed with 20 and 10 mg/kg of formononetin supplementation at 21 and 42 days respectively. The level of IL-10 showed non-significant difference at 21 day of age while

significant increase at 42 days was noted after formononetin supplementation. High level of IFN-γ in the blood plasma was found in birds receiving 20 mg/kg of formononetin at 21 day, whereas 10 mg/kg dose resulted in increased level of IFN-γ at 42 days of age.

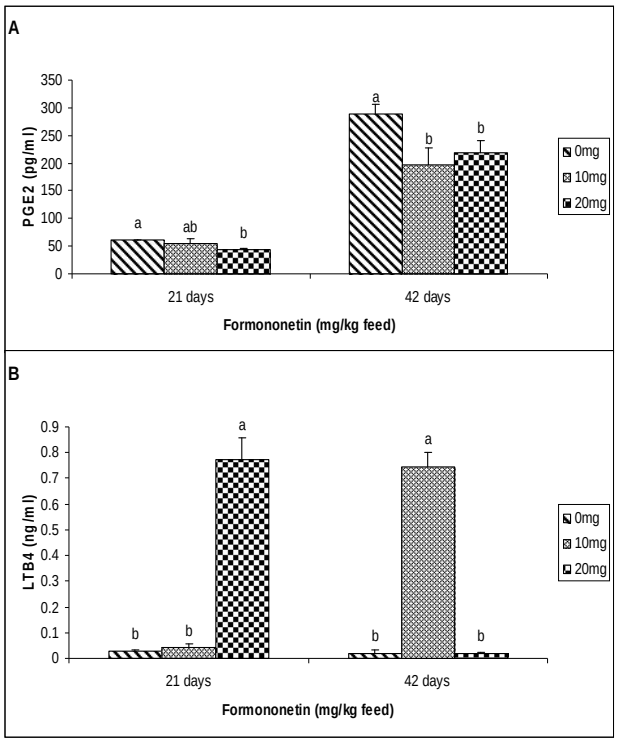

Fig. 2: Effect of formononetin on PGE_2 and LTB_4 production in plasma of broilers. Means within the same time point with different letters are significantly different (P<0.05). Errors bars indicate standard deviations.

DISCUSSION

Neither the weight gain nor the feed intake or FCR was affected during the starter phase suggesting that formononetin supplementation (up to 20 mg/kg of feed) did not have any role in growth performance during the initial stages of development. However, the greater FCR in the finisher phase indicated the significant dose dependent effect of formononetin on growth performance during this phase. The effects of isoflavones (ISF) on growth related traits are somewhat variable. Greiner *et al.* (2001a,b) found that soybean genistein (200 mg/kg) and daidzein (200 or 400 mg/kg) could improve growth in virally challenged pigs. Some other studies also indicated that isoflavonic phytoestrogens encouraged growth of animals (Zhengkang *et al.*, 2006). Jiang *et al.* (2007) showed that dietary supplementations with 10 or 20 mg of ISF/kg significantly increased weight gain of birds while 40 or 80 mg of ISF/kg did not show any significant change in the said parameter. Yao (2008) reported that isoflavones could promote the growth of male animals with reference of their effect on metabolic hormone and immunity.

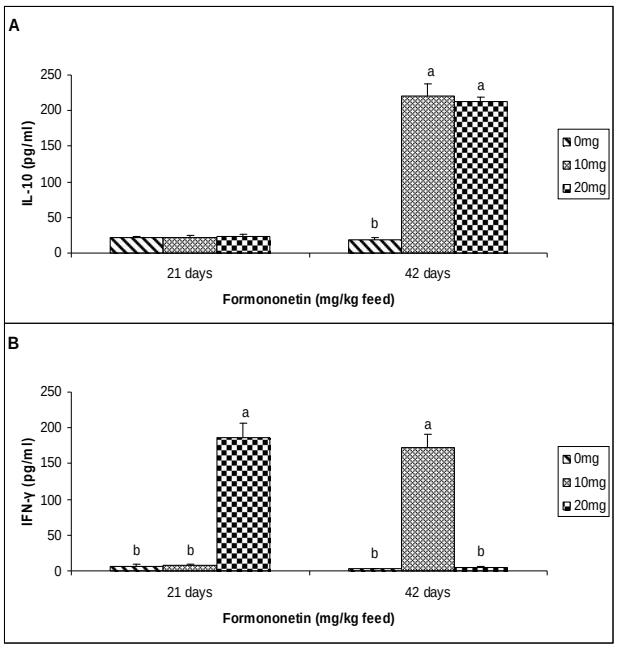

Fig. 3: Effect of formononetin on IL-10 and IFN-γ production in plasma of broilers. Means within the same time point with different letters are significantly different (P<0.05). Errors bars indicate standard deviations.

The sensory evaluation indicated that the meat quality was enhanced by supplementary diet of formononetin as compared to the control group. The findings of present study are in agreement with previous studies which showed that flavonoids and isoflavonoids improved meat quality of broilers (Batista *et al.*, 2007; Jiang *et al.*, 2007; Wei *et al.*, 2011). The formononetin (10 mg/kg) also resulted a 2.6% decrease in fat percentage which indicated the

tendency of formononetin for improving the quality of meat as low fat poultry meat is considered good for health (Jaturasitha *et al.*, 2002).

Although there is scarcity of published data regarding the effects of formononetin on the immune status in broiler, however, the decrease in PGE2 concentration in the present study is partially in line with the effects of other isoflavones. Takano-Ishikawa *et al.* (2006) demonstrated that genistein and daidzein inhibited PGE_2 production in the LPS-stimulated macrophage, but the data indicated that genistein was markedly more active than daidzein. The age and dose dependent effects of formononetin on LTB_4 was in contrast to the inhibition of LTB_4 by soy protein in rat peritoneal exudates cells as reported by Yamada *et al.* (1999). De Paula *et al.* (2008) reported that elevated IL-10 levels in the brain of the genistein-treated mice which is in agreement to the effects of formononetin at 42 days of chick life in the present study. The increase in IFN-γ by supplementation of formononetin (10 mg/kg) is, however, not parallel to the results of De Paula *et al.* (2008) who found an impressive suppression of IFN-γ in the brain of the mice treated with genistein. Similarly, Curran *et al.* (2004) reported that dietary genistein or soy could inhibit the amount of IFN-γ normally produced in response to a bacterial infection in mice.

Overall, our data indicate the usefulness of formononetin as non-antibiotic feed additive. Supplementing broiler diet with 10 mg formononetin/kg could induce positive influences on growth and immune responses of broiler chicken besides enhancing meat quality.

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