INFLUENCE OF NARINGENIN ON PERFORMANCE, MEAT QUALITY AND ANTIOXIDANT STATUS OF BROILERS

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ABSTRACT: The growth promoting antioxidant and meat quality related effects of naringenin in broilers were evaluated in the current study. One hundred and eighty day-older broiler chicks (Hubbard) were randomly selected to four treatment groups containing three replicates of 15 birds each. Feed supplemented with 0, 5, 10 and 20 mg/Kg naringenin was allotted to the experimental treatment groups (T1, T2, T3 and T4) respectively. An increased weight gain and better feed conversion ratio (FCR) ($P<0.05$) was observed by using naringenin at the rate of 10 mg/kg of feed, during the whole experiment period. The results of sensory evaluation showed that the breast meat was better ($P<0.05$) in flavour, mouth feeling and likeness in T2, T3 and T4 as compare to T1. The plasma GSHPx was significantly ($P<0.05$) increased on day 21 and 42 in treated groups as compare to control group. The MDA also showed significant difference between naringenin treated and control groups on day 21 with lower values for the birds treated with higher doses of naringenin. A significantly higher levels of SOD and TAOC were observed in chicken treated with naringenin at 21 or 42 days of age.

Keywords: Broilers, Naringenin, Growth, Immunity, Meat quality.

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INTRODUCTION

Antibiotics are added in feeds for growth promotion, having effective performance improvers in different animals. However, appearance of resistant strains of bacteria is the major problem associated with feed antibiotics (Butaye *et al*., 2003).

Different phytochemicals have been tested as replacers of feed antibiotics. A specific group of phytochemicals viz. flavonoids, has gained increasing interest during recent years as alternative of feed antibiotics. Several *in vitro* studies have shown potential beneficial effects of flavonoids (Halliwell, 2007). However the evidence, *in vivo* contributions of these phytochemicals in improving animal performance is yet not clear. Bioflavonoids, such as naringin are abundant in citrus pulp. It has been extensively used in feeding of animals with a positive effect on preventing problems from its disposal into the environment and the necessity for costly waste management programs. Fibers from citrus fruits have an additional advantage over dietary fibers from other sources due to the presence of associated bioactive compounds (*i.e. flavonoids*) and could be used as functional ingredients, since they can interact physiologically to provide numerous health benefits.

Flavonoids usually contain one or more aromatic hydroxyl groups, which actively remove free radicals and are responsible for the antioxidant activity. They are secondary metabolites synthesized for defense against infection and stress conditions, such as ultraviolet light, pathogens and physical damage. Flavonoids, especially naringin is well known for their antioxidant properties, are health-promoting molecules with multifunctional biological activities; they have been shown to reduce inflammation, stop active oxygen molecules and their intake reduces the risk of cardiovascular disease and several form of cancer.

Poultry industry in Pakistan has been shown to play a major role in the economy of the country, but the continuous use of growth promoters in poultry feed is dangerous for public health. Antimicrobial resistance of different pathogens has been increased to a critical level in the last ten years. This increase in resistance caused a great hindrance in the control of some fatal diseases such as TB, cholera, malaria, dysentery and pneumonia (Augustyniak *et al*., 2017). One of the major causes of antibiotic resistance is the use of antibiotics in poultry feed and there is a need to find some replacements. Although there are some international studies conducted on the use of flavonoids in the same area. The dietary administration of naringin exerted significant effect on broiler breast and thigh meat antioxidative capacity, possibly indicated that these flavonoids were introduced into the cell phospholipids membranes in broiler muscles (Peña *et al.*, 2008), but not much work has been done in this area in Pakistan. This is the first study evaluating naringenin as alternatives to antibiotic growth promoters in Pakistan.

MATERIALS AND METHODS

Birds and treatments: In the present study 180 day old broiler birds (Hubbard) were used. Four groups were made and each group contained three replicates of 15 birds. The test feed was prepared in a home mixer (Table 1) and 0, 5, 10 and 20 mg/Kg naringenin were added to the experimental treatments T1, T2, T3 and T4 respectively. Total duration of experiment was 6 weeks. The birds were feed with a starter-diet for twenty one days and then replaced by a finisher-diet. Body weight, feed consumption and feed conversion ratio (FCR) were recorded at the end of each week for all groups. The mortality and temperature were recorded daily.

Blood Sampling: The blood sample, from each bird was collected at 21 and 42 days of age. After weighing, two birds from each replicates were sacrificed by slaughtering. Blood samples were collected into plastic vials containing heparin. Each blood sample was centrifuged for 10 minutes at 2800 rpm to separate the plasma. After centrifugation plasma was collected and stored at -20 ºC.

Sensory Quality: The meat from birds was subjected for the evaluation of acceptability of broiler meat by evaluating texture, odour, colour, tenderness, flavour, juiciness, mouth feeling, palatability and likeness/dislikeness.

Laboratory Analyses: Plasma samples were thawed at room temperature for laboratory analysis. Diagnostic kits (Nanjing Jian-cheng Bio engineering Institute, Nanjing,China) were used to determine the antioxidant activities (TAOC, SOD, GSHPx and MDA) in plasma. To minimize variations in the assays, all the samples in the trials were run in one batch.

Statistical Analysis: To evaluate the effect of naringenin on different parameters, SPSS software was used to perform one-way ANOVA. Duncan's multiple range test was also used to determine the difference between means. P-values < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Growth and FCR: Naringenin showed good effect on growth and Feed Conversion Ratio (Table-2 and 3). Naringenin (10 mg/kg) of poultry feed showed an increased weight gain during the whole experimental period. Naringenin level 20 mg/Kg of feed also resulted in increased body weight from 0-42 days of chicken age. No significant difference in feed intake was found between the control and nanrigenin treated groups during the experimental period. Naringenin level 10 mg/ Kg of feed showed a better FCR (P<0.05) from 21-42 days and 0-42 days of chicken age.

The results of isoflavones (ISF) trials on the performance of animals are not consistent. Greiner *et al*., (2001a) and (2001b) reported an increase growth performance in pigs treated with soybean genistein (200mg/Kg) and daidzein (200 or 400mg/Kg). Some other studies also showed promising growth of animals after isoflavones supplementation (Zhengkang *et al*., 2006). In another study Jiang *et al*., (2007) reported significant increase in weight of birds with addition of 10 or 20 mg of ISF/Kg as supplement while no significant change in weight gain was recorded with 40 or 80 mg of ISF/Kg supplementation. Yao (2008) also reported that isoflavones could improve the growth performance of animals due to its positive effects on hormones that are responsible for metabolic activity and immunity.

Meat Quality: The Table 4 shows the effect on the quality of meat with the effect of naringenin. Sensory evaluations showed that the breast meat was better (P<0.05) in flavor, mouth feeling and likeness in T2, T3 and T4 as compare to T1. No significant difference was observed in texture, odour, colour, tenderness, juiciness and palatability among all four treatments.

Similar results of flavonoids and isoflavons supplementation on meat quality of broilers birds were also reported in previous studies (Batista *et al*., 2007; Jiang *et al*., 2007; Zhengkang *et al*. 2006). The naringenin (10 mg/Kg) improved the quality of meat by decreasing the fat contents (2.59 %). It has been reported that poultry meat with less percentage of fat is good for human health (Jaturasitha *et al*. 2017).

Antioxidant response: The results regarding the plasma MDA, SOD and TAOC were depicted in Fig-1 to 4. The plasma GSHPx differed between naringenin treated and control groups on day 21 and day 42 with a significant increase after naringenin supplementation. MDA, an indicator of lipid oxidation, also showed significant difference between naringenin treated and control groups on day 21 with lower values for the birds treated with higher doses (10 mg and 20 mg/Kg) of naringenin. However, no difference was observed at 42 days of chicken age. No significant difference was observed in the blood level of SOD between the control and naringenin treated chickens at 21 days but birds treated with 10 mg of naringenin at 42 days showed higher level. At 42 days of age, a non significant increase was observed after the supplementation of 5 mg/Kg and 10 mg/Kg of naringenin as compared to control group. There were a significant increase in the blood levels of TAOC between the control group and groups supplemented with either 5 or 20 mg /Kg of naringenin at 21 days of chicken age. However, the level of TAOC in the plasma was significantly decreased after 20 mg/kg naringenin supplementation.

Flavonoids are considered to be powerful antioxidant in *in vitro* (Rice-Evans *et al*., 1996; Frei and Higdon, 2003). The evidence, however, of *in vivo* effects are still unclear. Some studies indicated the ability of tea flavonoids to decrease the susceptibility of LDL oxidation in animal models of atherosclerosis (Vinson, 1998; Frei and Higdon, 2003). On the other, a number of other animal studies reported opposite findings (Tijburg *et al*., 1997; Crawford *et al*., 1998). Some other studies also indicated the prooxidant activities in the gastrointestinal tracts of certain insects consuming high levels of flavonoids and other phenolics (Galati *et al*., 2002; Barbehenn *et al*., 2006).

Table-1: Feed Formulation.

Ingredients	Broiler starter (%)	Broiler finisher (%)
Corn	40	44
Rice broken	12	9
Rice polish	9	10
Wheat bran	3	4
Guar meal	3.6	0
Soybean meal	15	16.7
Canola meal	8	8
Corn gluten 60%	1	1
Corn gluten 30%	3	3
Lime stone	0.6	0.6
Dicalcium phosphate	2.1	2.1
Lysine sul	0.44	0.34
D.L. Methinonine	0.16	0.15
Premix	0.8	0.8
Salt	0.31	0.31
Nahco3	0.05	0.05
Diclazuril	0.02	0.02
Total	100	100

Table-2: Weight gain, feed intake, and feed conversion ratio (FCR) in broilers fed diets supplemented with different concentrations of naringenin at 0-21 days.

Days	Treatment (mg/kg feed)	Weight gain (g)	Feed intake (g)	FCR
0-21	0	390 ^{b}	696	1.79
	5	413 ^{ab}	689	1.67
	10	464 ^{a}	724	1.57
	20	455 ^{ab}	708	1.55

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

Table-3: Weight gain, feed intake, and feed conversion ratio (FCR) in broilers fed diets supplemented with different concentrations of naringenin at 21-42 days and 0-42 days.

Days	Treatment (mg/kg feed)	Weight gain (g)	Feed intake (g)	FCR
21-42	0	814 ^{b}	2047	2.50
	5	844 ^{b}	2005	2.37
	10	994 ^{a}	1798	1.79
	20	903 ^{ab}	2078	2.29
0-42	0	1213 c	2610	2.15 a
	5	257 bc	2695	2.14 a
	10	1458 a	2521	1.72 b
	20	1358 ab	2787	2.04 a

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

Table-4: Effect of naringenin on sensory quality of breast meat of broilers.

Parameters	Naringenin levels (mg)				p value	Trends	
	Control	**5**	**10**	**20**		**Linear**	**Quadratic**
Texture	6.9±0.25	6.9±0.22	6.7±0.21	7.1±0.25	0.634	0.683	0.441
Odour	6.6±0.37	6.7±0.31	6.9±0.36	7.2±0.23	0.635	0.212	0.722
Colour	7.1±0.27	7.1±0.30	7.1±0.29	7.3±0.26	0.963	0.775	0.684
Tenderness	7.3±0.26	6.9±0.24	6.7±0.28	7.0±0.24	0.429	0.372	0.177
Flavour	6.4±0.39 ^{b}	7.0±0.20 ^{ab}	6.7±0.23	7.3±0.24 ^{a}	0.121	0.060	0.952
Juiciness	6.7±0.27	6.9±0.23	6.6±0.22	6.9±0.27	0.791	0.699	0.842
Mouth feeling	6.4±0.29 ^{b}	7.0±0.21 ^{ab}	6.8±0.24	7.4±0.21 ^{a}	0.050	0.016	0.946
Palatability	6.7±0.29	7.1±0.21	6.8±0.08	7.3±0.22	0.352	0.203	0.886
Likeness/ Dislikness	6.2±0.37 ^{b}	7.2±0.19 ^{ab}	6.8±0.24	7.4±0.25 ^{a}	0.015	0.015	0.307

* $^{a\ b\ ab}$ Values with in a row with different superscript differ significantly (P <0.05)

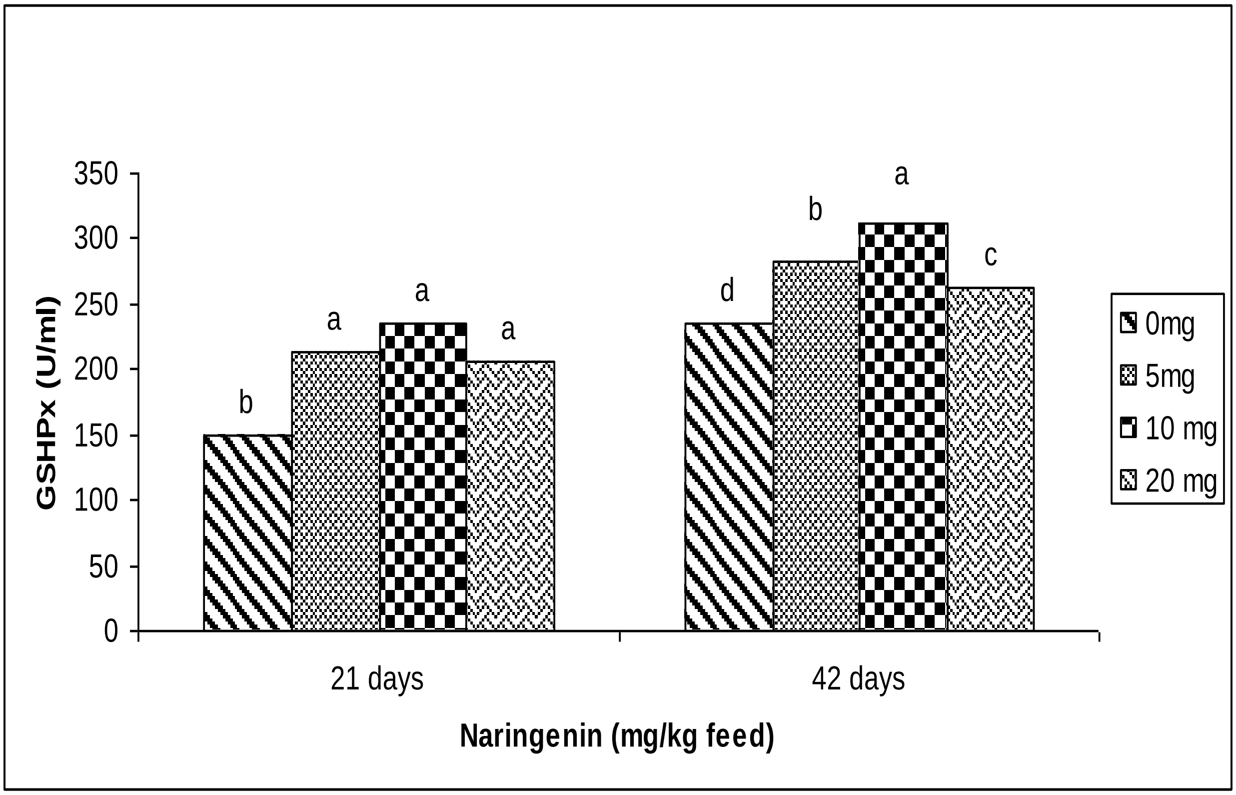

Figure 1: Effects of dietary naringenin supplementation on plasma GSHPx of broilers at 21 and 42 days of age
Values not sharing a superscripts are different at P<0.05.

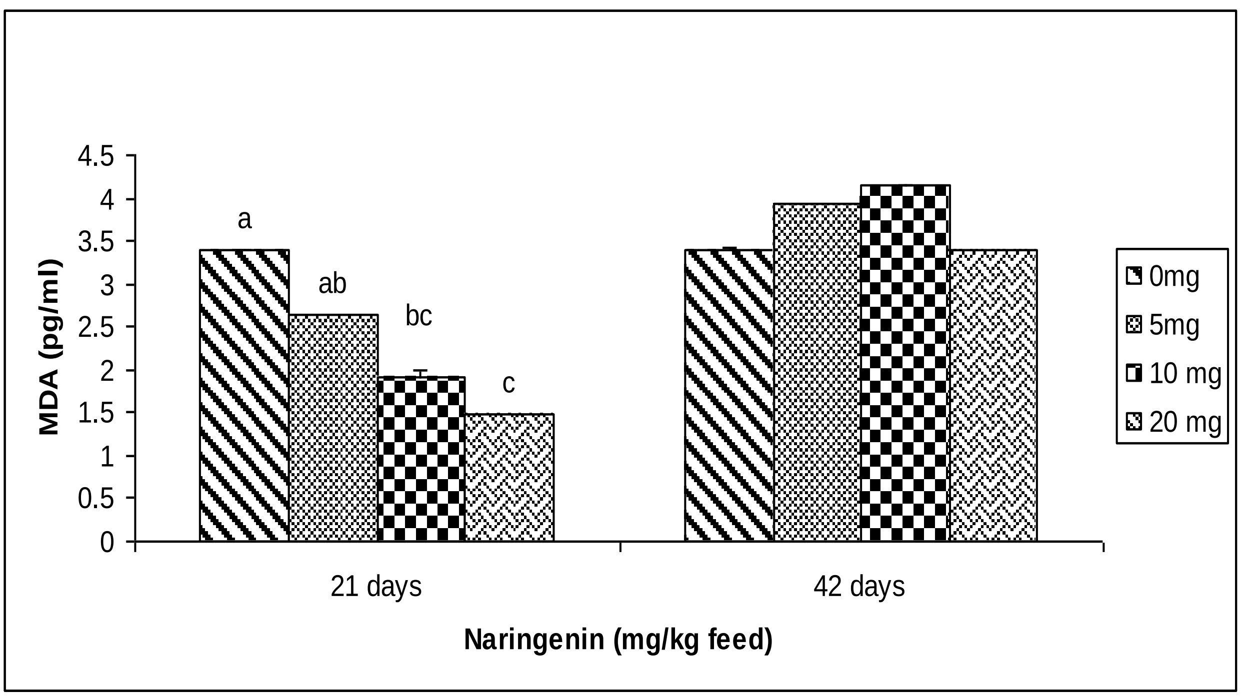

Figure-2: Effects of dietary naringenin supplementation on plasma MDA of broilers at 21 and 42 days of age
Values not sharing a superscripts are different at P<0.05

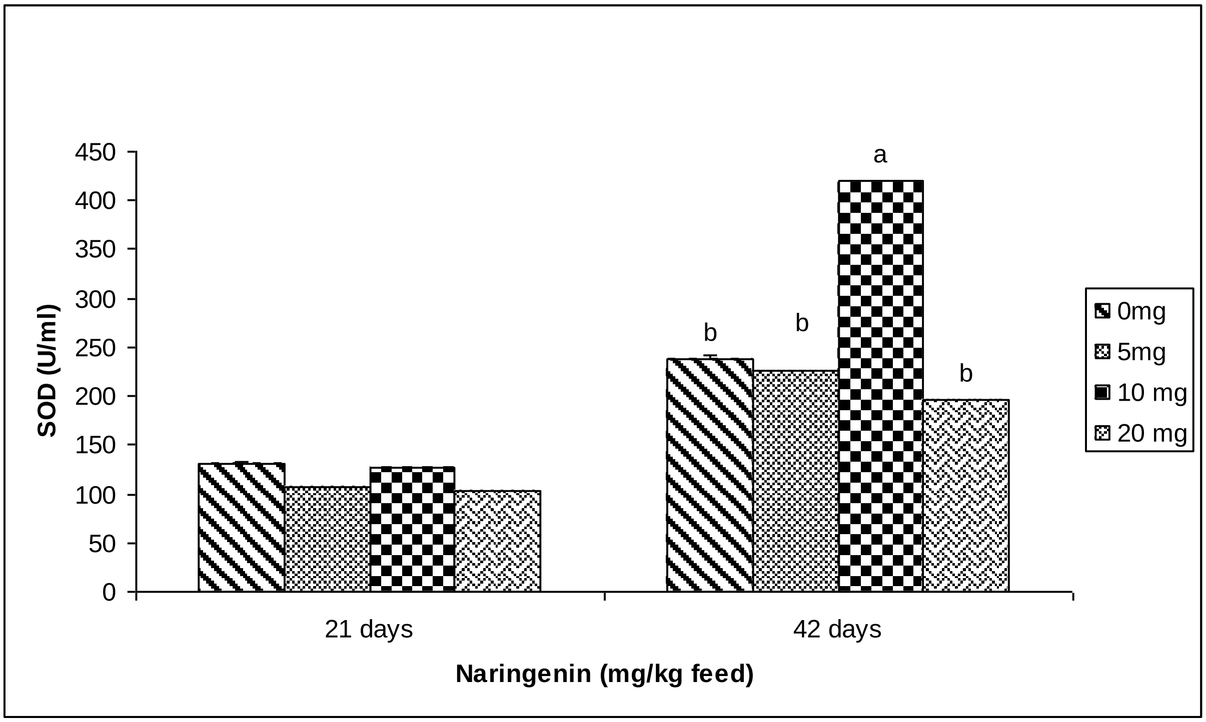

Figure-3: Effects of dietary naringenin supplementation on plasma SOD of broilers at 21 and 42 days of age
Values not sharing a superscripts are different at P<0.05

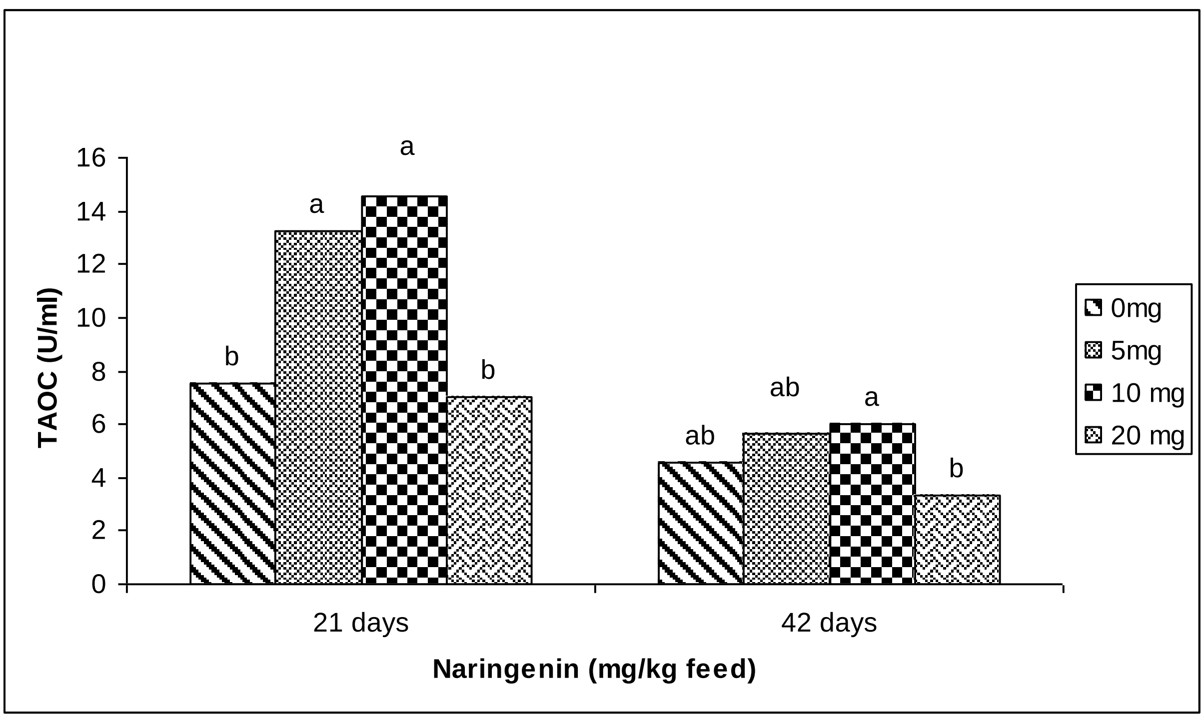

Figure-4: Effects of dietary naringenin supplementation on plasma TAOC of broilers at 21 and 42 days of age
Values not sharing a superscripts are different at P<0.05

Conclusion: The current pilot study demonstrated the enhancement of SOD and TAOC activity in the plasma of broilers in the dose dependent manner of naringenin supplementation (broiler or chicken). However further dose dependent study would be essential to ascertain the beneficial antioxidative role of naringenin in the broiler's diet.

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