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Feeding dihydroquercetin to broiler chickens

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Abstract 1. A total of 80 male Ross 308 broilers were used in a study to investigate the effect of dietary dihydroquercetin (DHQ) on growth performance variables, gastrointestinal tract (GIT) and immune organ development, glutathione peroxidase (GPx) and haemoglobin in blood, hepatic vitamin E content, dietary N-corrected metabolisable energy (AMEn), and nutrient retention coefficients when fed to broiler chickens from 7 to 35 days of age.

2. Two treatments were used in this study: control (C) and C + 0.5 g/kg extract of Siberian Larch (*Larix sibirica*) per kg feed, containing 85 % DHQ. The diets were fed over two feeding phases, a grower phase from 7 to 28 d age, and a finisher phase from 28 to 35 d age. The birds were reared under breeder's recommended conditions.

3. In general, there were no effects of DHQ on growth performance of broiler chickens. However, the results of this experiment have shown that there can be changes in redness colour of the breast meat when DQH is fed. No negative effects of feeding DHQ at 0.5 g/kg diet were observed in this study.

4. Supplementation of poultry diets with DHQ under standard industry rearing conditions, did not improve production performance or any of the studied health variables, except an increase of redness index of the breast fillets. Feeding DHQ at different doses and/or under more challenging conditions, e.g. heat stress, may however, bring positive responses.

Key words: broilers, dihydroquercetin (DHQ), phenols, growth performance, antioxidants

INTRODUCTION

The popularity of natural antioxidants to protect human and animal health and to increase the shelf life of products from animal origin has increased during the past decade (Weidmann 2012; Iskender et al. 2017). Flavonoids being a major sub-group representing plant polyphenols, are considered antioxidants from natural sources and as such, have been attracting attention for use in animal nutrition (Surai 2014). Dihydroquercetin (DHQ), also known as taxifolin, a flavonoid extracted from various conifers including Siberian Larch (*Larix sibirica*), longleaf Indian Pine (*Pinus roxburghii*), Himalayan Cedar (*Cedrus deodara*) and Chinese Yew (*Taxus chinensis var. mairei*), has been widely applied as an antioxidant for the surface treatment of fresh meat and fish (Semenova et al. 2008; Ivanov et al. 2009; Balev et al. 2011; Dragoev et al. 2014). Dihydroquercetin has also been incorporated in animal diets in order to enhance production performance. Fomichev et al. (2016) extensively reviewed the effect of DHQ as dietary supplement in animal production and reported enhancement in growth performance and blood variables of poultry and pigs. Research by Nikanova (2017) with piglets further supported the observations of Fomichev et al. (2016). However, Balev et al. (2015) did not find significant difference in growth performance of broilers fed DHQ from day old to 49 days when compared to the control fed birds. Torshkov (2011) reported that the breast meat from 42 d age broilers fed DHQ supplemented diet had higher dry matter, lower fat, lower tryptophan and the same protein content when compared to birds fed control diet only. In addition, Torshkov et al. (2014) found that feeding DHQ to broilers increases the number of red blood cells and haemoglobin concentration compared to control. However, there was no information on growth performance variables in both reports. Omarov et al. (2016) reported increased protein concentration in the organs and tissues of broiler chickens when fed DHQ, however, the experiment was not designed to study the effect of DHQ on growth performance variables. The

majority of experiments involving DHQ emphasise more on its impact on the composition of various tissues (muscle, blood), while the information on performance is rarely presented. It is likely that improvements seen in productive performance in some papers may be due to heat stress (Fomichev et al. 2016). Rearing animals at temperatures exceeding their thermal comfort zone (e.g. during summer) may be a reason for depleting levels of tissue antioxidants, thus the antioxidant status of animal may be associated with the mode of action of DHQ. Since DHQ is a natural flavonoid with recognised antioxidant properties, understanding its mode of action may be important for enhancing health and productivity of intensely reared animals. In addition, there is no information on the impact of DHQ on the development of the gastrointestinal tract (GIT), immune organs, dietary available energy and nutrient retention coefficients.

The aim of the study was to assess the impact of DHQ on growth performance variables, GIT and immune system organ development, glutathione peroxidase (GPx) and haemoglobin concentration in blood, dietary N-corrected metabolisable energy (AMEn), dry matter (DMR), nitrogen (NR) and fat retention (FR) coefficients when fed to broiler chickens from 7 to 35 days of age.

MATERIALS AND METHODS

Experimental diets

Two wheat-soy-based diets were offered to the birds during the experiment. The diets were fed over two feeding phases, a grower phase from 7 to 28 d age, and a finisher phase from 28 to 35 d age. The grower and finisher basal diets were formulated to meet breeder's recommendations (Aviagen Ltd., Edinburgh, UK) (Table 1). All diets included 5 g/kg of TiO_2 as a marker. The basal diets were then split into two batches that had 1.) no additive (control diet; C) and 2.) 0.5 g/kg extract of Siberian Larch (*Larix sibirica*) (JSC NPF Flavit, IBI RAS,

74 Pushchino city, Moscow region, Russian Federation 142290). According to the company
75 producer, this extract contains over 85 % pure DHQ.

76 **Husbandry and sample collection**

77 The experiment was conducted at the National Institute of Poultry Husbandry and approved by
78 the Research Ethics Committee of Harper Adams University. A total of 85 male Ross 308
79 broilers were obtained from a commercial hatchery (Cyril Bason Ltd, Craven Arms, UK),
80 allocated to a single floor pen and offered a standard wheat-based broiler starter feed
81 formulated to meet Ross 308 nutrient requirements (Aviagen Ltd., Edinburgh, UK). At 7 d age,
82 80 of the birds were allocated to 16 raised floor pens (60 x 60 cm) each holding 5 birds. Each
83 of the pens had a solid floor and were equipped with an individual feeder and drinker. Feed
84 and water were fed *ad libitum* to birds throughout the experiment. Each diet was offered to
85 birds in 8 pens following complete randomisation. The birds were fed the experimental diets
86 from 7 to 35 d age, when the experiment ended. Room temperature and lighting regime met
87 commercial recommendations (Aviagen Ltd, Edinburgh, UK). The well-being of the birds was
88 checked regularly every day.

89 Birds and feed were weighed on days 7, 28 and 35 in order to determine average daily feed
90 intake (FI), average daily weight gain (WG) and to calculate the gain:feed ratio (G:F) on a pen
91 basis. For the last 3 days of the study, the solid floor of each pen was replaced with a wire
92 mesh. Excreta were collected each day for the last three days of the experiment, stored at 4 °C,
93 and a representative subsample was dried at 60 °C and then milled through 0.75 mm screen.
94 At the end of the study, one bird per pen, selected at random, was electrically stunned and
95 blood was obtained in heparin coated tubes from the jugular vein. The organs from the GIT,
96 including proventriculus and gizzard (PG), duodenum, pancreas, jejunum, ileum, caeca and
97 liver, the heart, the spleen and the bursa of Fabricius were weighed. The colour on the surface

of the left breast fillet was determined, and the left breast muscles were used to determine the chilling yield.

Laboratory Analysis

Dry matter (DM) in feed and excreta samples was determined by drying of samples in a forced draft oven at 105 °C to a constant weight (AOAC 2000; method 934.01). Crude protein (6.25 × N) in samples was determined by the combustion method (AOAC 2000; method 990.03) using a LECO FP-528 N (Leco Corp., St. Joseph, MI). Oil (as ether extract) was extracted with diethyl ether by the ether extraction method (AOAC 2000; method 945.16) using a Soxtec system (Foss Ltd., Warrington, UK). The gross energy (GE) value of feed and excreta samples was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL) with benzoic acid used as the standard. Titanium in feed and excreta was determined as explained by Short et al. (1996).

The colour score on the surface of the left breast meat within 5 minutes after slaughter was carried out using a Chroma Meter CR-400 from Konica Minolta (Sunderland, UK) to determine luminance and chromaticity scores using CIELAB scoring (where L* refers to lightness, a* refers to redness, and b* refers to yellowness). Areas were selected that were free of any obvious blood-related defects, such as bruises, haemorrhages, or full blood vessels (Fletcher et al. 2000). Two readings of CIE L*, a*, and b* were obtained for the breast fillet for each bird (2 readings/left side). The chilling yield determined on the left breast of each slaughtered chicken was also determined (Jeong et al. 2011).

The glutathione peroxidase in blood was determined using a Ransel GPx kit (Randox Laboratories Ltd., UK) that employs the method based on that of Paglia and Valentine (1967).

The concentration of hepatic vitamin E was determined using an HPLC system as previously described (Karadas et al. 2010).

Calculations

Dietary nutrient retention coefficients were calculated using the following equation:

$$Nutrient\ retention\ coefficients = 1 - \frac{exnut/exti}{dietnut/dietti}$$

where *exnut* is the concentration of the respective nutrient in the excreta, *exti* is the concentration of titanium dioxide in the excreta, *dietnut* is the concentration of the respective nutrient in the diet and *dietti* is the concentration of titanium in the diet.

The AMEn value of the experimental diets was determined following the method of Hill and Anderson (1958).

$$AMEn = GE\ diet - \frac{(GE\ ex\ X\ dietti)}{exti} - 34.39\ X\ N\ retained$$

where AMEn (MJ/kg) = N-corrected apparent metabolizable energy content of the diet; GE diet and GE ex (MJ/kg) = GE of the diet and excreta, respectively; *dietti* and *exti* (%) = titanium in the diet and excreta, respectively; 34.39 (MJ/kg) = energy value of uric acid; and *N retained* (g/kg) is the N retained by the birds per kilogram of diet consumed. The retained N was calculated as

$$N\ Retained = N\ diet - \frac{N\ ex\ X\ dietti}{exti}$$

where N Diet and N *ex* (%) = N contents of the diet and excreta, respectively.

The relative development of organs was determined as percent by dividing the organ weight to body weight by the respective bird and multiplying by 100 (data not included in tables).

Chilling yield of breast meat was determined from 8 carcasses per diet as follows:

$$\%\ Chilling\ yield = \frac{Post\ chill\ breast\ weight}{Pre\ chill\ breast\ weight} X\ 100\ \%$$

where Post chill breast weight is the weight of the left breast after 24h in a fridge at 4° C and Pre chill breast weight is the weight of the left breast immediately after dissection, respectively.

Statistical Analysis

Statistical analysis was performed using GenStat 19th edition statistical software (IACR Rothamstead, Hertfordshire, England). A completely randomised one-way analysis of variance was performed to investigate the effect of dietary DHQ on the studied variables. Differences were reported as significant at $P < 0.05$.

RESULTS AND DISCUSSION

All birds were healthy throughout the study period and there was no mortality. There was no effect of treatment on any of the studied growth performance variables (Table 2).

The results on AMEn and nutrient retention coefficients are in accordance with previous research (Pirgozliev et al. 2006, 2015; Whiting et al. 2016) and there were no differences ($P>0.05$) between treatments (Table 3).

There were no differences ($P>0.05$) in the relative weights of the studied organs measured as percentage of body weight (data not in tables) and the results were in agreement with previous research (Dror et al.1977; Abdulla et al. 2016; 2017).

The results on chilling yield and the colour score were similar to these reported by Jeong et al. (2011) (Table 4). The breast fillets of the birds fed DHQ had a higher red colour index (a*) compared to the control fed birds ($P<0.05$).

The values for haemoglobin concentration and glutathione peroxidase were in agreement with published reports (Tanaka and Rosenberg 1954; Elagib and Ahmed 2011; Popović et al. 2016)

and there were no differences (P>0.05) between diets (Table 5). However, the results did not support the finding of Torshkov et al. (2014) for increased haemoglobin concentration in birds fed DHQ.

The values of hepatic vitamin E were in the expected range (Karadas et al. 2010, 2014; Whiting et al. 2018). However, no differences between dietary treatments were observed (P>0.05).

In the literature, dietary DHQ concentrations varied between studies and species. In poultry diets the concentration of supplemented DHQ varied from 1 mg per kilogram live weight (Torshkov et al. 2014) to 40 mg per kg live weight (Balev et al. 2015); in calves and cows, from 20 to 200 mg per head daily (Fomichev et al. 2016); in weaning piglets from 10 mg per kg feed (Fomichev et al. 2016), to 50 mg per animal per day (Nikanova 2017). Research by Dunnick and Halley (1992) did not find any toxic effect of quercetin when fed to rats for 6 months at concentrations of up to 40000 ppm, and the estimated dose delivered was approximately 40–1900 mg/kg/day. Similarly, DHQ, which is closely related to quercetin in chemical structure, has been shown to be nontoxic when fed to albino rats and humans at much higher levels than in the reported study (Booth and DeEds 1958). There were no treatment-related effects on survival and no treatment-related clinical signs of toxicity for this period.

In the reported study, DHQ was added at 0.5 g per kg feed or 500 ppm. On average, birds were consuming approximately, 100 g feed per day, and their average daily weight gain was approximately 60 g. Thus, the average daily consumption of DHQ was 0.05 g per bird, or 0.83 g per kilogram daily growth. The lack of adverse effects on birds health further confirms that DHQ is generally safe to use in broiler production. Further exploration of graded levels of dietary DHQ should be considered to optimise the dose required for enhanced production performance.

188 In the reports by Fomichev et al. (2016) and Nikanova (2017), feeding DHQ generally
189 improved the growth performance variables of animals reared in challenging conditions, i.e.
190 high temperature. Fomichev et al. (2016) also reported that the response at later stage of
191 growing, i.e. 42 d old, was more pronounced compared to the early stage of growth (28 d age).
192 However, Balev et al. (2015) reared birds under industry-recognised conditions and did not
193 observe difference in growth performance of broilers fed DHQ for the entire period of 49 days.
194 Heat stress stimulates the release of corticosterone and catecholamines, increase the level of
195 free radicals and initiates lipid peroxidation in cell membranes (Freeman and Crapo 1982).
196 Prochazkova et al. (2011) suggested that flavonoids could prevent injury caused by free
197 radicals by the following mechanisms: direct scavenging of reactive oxygen species (ROS),
198 activation of antioxidant enzymes, metal chelating activity, reduction of a-tocopheryl radicals,
199 inhibition of oxidases, mitigation of oxidative stress caused by nitric oxide, increase in uric
200 acid levels, and increase in antioxidant properties of low molecular antioxidants. The
201 mechanism of flavonoids health-promoting abilities is usually associated with their antioxidant
202 properties (Andriantsitohaina et al. 2012) although recent findings suggest that flavonoids do
203 not behave the same way *in vitro* and *in vivo* (Veskoukis et al. 2012). However, in the present
204 study, birds were reared under standard industry recommended conditions, and no challenges
205 were applied, possibly limiting the detection of the benefits of DHQ as an antioxidant.

206 In conclusion, supplementation of poultry diets with 0.5 g DHQ per kg feed, under standard
207 industry rearing conditions, did not improve production performance or any of the studied
208 health variables. However, the redness index of breast fillet was increased. Feeding DHQ at
209 different doses and/or under more challenging conditions, e.g. heat stress, may bring positive
210 responses.

211

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DISCLOSURE STATEMENT

The authors reported no potential conflict of interest.

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363

364

365 Table 1. Ingredient composition of the control experimental diets (as fed).

Ingredients (g/kg)	Grower	Finisher
Barley	79	67
Wheat	550	600
Soybean meal	230	190
Full-fat soybeans	50	50
L Lysine HCL	3	3
DL Methionine	3.5	3
L Threonine	1.5	1.5
Soya oil	45	47.5
Limestone	12.5	12.5
Monocalcium phosphate	12.5	12.5
Salt	2.5	2.5
Sodium bicarbonate	1.5	1.5
Premix[1]	4	4
Titanium Dioxide	5	5
Calculated values (as fed)		
Crude protein (N x 6.25, g/kg)	201	187
Crude oil (g/kg)	68	71
ME (MJ/kg)	12.99	13.17
Calcium (g/kg)	9.3	9.2
Av Phosphorus (g/kg)	4.2	4.2
Av Lysine (g/kg)	11.8	10.8
Lysine (g/kg)	12.7	11.6
Methionine + Cysteine (g/kg)	9.4	8.4
Tryptophan (g/kg)	8.5	7.8
Determined values		
Dry matter (g/kg)	894	893
Gross energy (MJ/kg)	17.43	17.39
Crude protein (N x 6.25, g/kg)	194	181
Crude oil (g/kg)	69	66

366

367 [1]The Vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by
368 NRC (1994). All the experimental diets were designed to be low in P. The premix provided (units/kg diet): retinol
369 3600 µg, cholecalciferol 125 µg, α-tocopherol 34 mg, menadione 3 mg, thiamine 2 mg, riboflavin 7 mg, pyridoxine
370 5 mg, cobalamin 15 µg, nicotinic acid 50 mg, pantotenic acid 15 mg, folic acid 1 mg, biotin 200 µg, iron 80 mg,
371 copper 10 mg, manganese 100 mg, cobalt 0.5 mg, zinc 80 mg, iodine 1 mg, selenium 0.2 mg and molybdenium
372 0.5 mg.

373

374 Table 2. Production performance of broiler chickens fed control or dihydroquercetin (DHQ)
375 supplemented diets.

Item	Control	DHQ	SEM (DF=14)	P-value
Feed Intake 7-35 d (g/b)	2737	2788	29.5	0.268
Weight Gain 7-35 d (g/b)	1609	1666	36.0	0.300
Feed Conversion Efficiency 7-35 d (g/g)	0.588	0.599	0.0104	0.497
Body Weight 35 d age (g)	1735	1790	29.9	0.232

376

377 Table 3. Dietary apparent N-corrected metabolisable energy (AMEn) and nutrient retention
378 coefficients

Item	Control	DHQ	SEM (DF=14)	P-value
AMEn (MJ/kg DM)	13.60	13.52	0.0710	0.470
Dry Matter Retention	0.811	0.808	0.0062	0.574
Nitrogen Retention	0.738	0.737	0.0131	0.963
Fat Retention	0.844	0.835	0.0065	0.244

379

380 Dietary AMEn and nutrient retention coefficients were determined between 32 and 35 d of age.

381

382 Table 4. Chilling yield and surface colour of broiler breast fillets (within 5 minutes after
383 slaughter) fed control or dihydroquercetin (DHQ) supplemented diets.

Item	Control	DHQ	SEM (DF=14)	P-value
Chilling yield (%)	96.26	95.78	0.219	0.138
L*	49.1	52.1	2.83	0.484
a*	2.24	3.42	0.170	0.002
b*	1.02	0.73	0.191	0.322

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386 Table 5. Haemoglobin and glutathione peroxidase (GPx) in blood, and hepatic vitamin E of
387 broiler chickens fed control or dihydroquercetin (DHQ) supplemented diets.

Item	Control	DHQ	SEM (DF=14)	P-value
Haemoglobin (g/l)	132.1	133.6	3.07	0.735
GPx (u/ml RBC)	67.6	64.2	2.85	0.410
Hepatic Vitamin E (µg/g)	19.03	17.03	0.780	0.120

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