Antioxidant status and growth performance of broiler chickens fed diets containing graded levels of supplementary dihydroquercetin

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Antioxidant status and growth performance of broiler chickens fed diets containing graded levels of supplementary dihydroquercetin

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ABSTRACT

Dihydroquercetin (DHQ), also known as taxifolin, is a natural antioxidant that can be commercially obtained by extraction from Siberian Larch (*Larix sibirica*). Four wheat-soy based diets, formulated to contain 0, 0.5, 1.5 and 4.5 g/kg of supplementary DHQ were prepared. Each diet was fed *ad libitum* to birds in seven pens (three birds in each pen) in a randomised block design from 7 to 21 days of age. The effect of DHQ on weight gain was not significant overall ($P > 0.05$), although there was an indication of a linear increase ($L < 0.05$). The blood glutathione peroxidase responded ($P < 0.001$) in a curvilinear manner ($L < 0.001$ and $Q < 0.05$) to increased dietary DHQ. The results from this study indicate that dietary DHQ supplementation may be beneficial at levels greater than 1.5 g/kg feed, due to improved bird antioxidant status. Further research to define an upper inclusion level and optimal timing for phase feeding programmes is warranted.

Key words: dihydroquercetin, taxifolin, phytochemicals, chickens, antioxidants

Dihydroquercetin (DHQ) (also known as taxifolin) is a flavonoid produced by plants as a polyphenolic secondary metabolite. The effects of DHQ as an antioxidant are well recognised in animals and man (Sunil and Xu, 2019; Zeng et al., 2020), extending to agricultural applications, with inconsistent reports of influencing dietary nutrient availability and growth performance variables in animals (Fomichev et al., 2016; Pirgozliev et al., 2019, 2020). Efforts to optimise the inclusion level for chickens of dietary DHQ indicate a range of 0.1 to 10 g/kg diet may be efficacious on bird growth performance and health (Fomichev et al., 2016; Pirgozliev et al., 2020; Kuzmina and Petrov, 2021). However, for use in commercial poultry production, determining viable inclusion levels and confirming biological effects is essential for optimising inclusion rates. The aim of this study was to refine the range of graded levels of dietary DHQ on growth performance and antioxidant status of broiler chickens. Dietary metabolisable energy and nutrient retention coefficients were also determined.

The experiment was conducted at the National Institute of Poultry Husbandry (NIPH, Newport, Shropshire, UK) and was approved by Harper Adams University Research Ethics Committee. A basal wheat-soya based diet was formulated to meet breeder's recommendations (Aviagen Ltd., Edinburgh, UK; Table 1), with the addition of 5 g/kg of TiO_2 as an inert digestibility marker (total excreta collection was applied at the end of the study to determine available energy and nutrient retention coefficients). The basal diet (mash form) was split into four batches that had (1) no additive (control diet; C), (2) 0.5 g/kg DHQ, (3) 1.5 g/kg DHQ, and (4) 4.5 g/kg DHQ. Dihydroquercetin, as extract of Siberian Larch (*Larix sibirica*) containing over 85% pure DHQ (with the remainder including other flavonoids, saponins and water), was supplied by JSC NPF Flavit (IBI RAS, Pushchino city, Moscow region, Russian Federation 142 290). Each diet was then cold pelleted according to Azhar et al. (2019) using a laboratory

pelleter (KAHL, Amandus Kahl GmbH & Co. KG, Reinbek, Germany). The four experimental diets were fed in one phase from 7 to 21 days of age.

A total of one hundred female Ross 308 broiler chicks were obtained from a commercial hatchery (Cyril Bason Ltd, Craven Arms, UK) and allocated to a single floor pen and offered a proprietary wheat-based broiler starter mash feed (Aviagen Ltd, Edinburgh, UK). At 7 days of age, 84 birds were selected and allocated to 28 raised floor pens (60 x 60 cm), each holding three birds. Each pen had a solid floor, equipped with an individual feeder and drinker. Feed and water were available *ad libitum* throughout the study. Each diet was offered to birds in seven pens following randomisation of dietary treatments to blocks (spatial). Room temperature and lighting regime met commercial recommendations (Aviagen Ltd.). The well-being of the birds was checked twice daily. Between day 17 and 21 of the experiment, the solid floor of each pen was replaced with a wire mesh and all excreta was collected, oven dried at 60 °C and milled through a 0.75 mm screen. Feed intake during this period was also determined to calculate dietary N-corrected apparent metabolisable energy (AMEn), dry matter (DMR), nitrogen (NR) and fat retention (FR) coefficients. Birds and feed were weighed on day 7 and day 21 to determine the average daily feed intake (FI) and the average daily weight gain (WG) and to calculate mortality corrected feed conversion ratio (FCR) on a pen basis. At the end of the study (day 21), one bird per pen was selected at random and was killed by electrical stunning followed by exsanguination. At this time blood was collected into lithium heparin BD Vacutainers®. Blood GSH-Px was determined using a Ransel GSH -Px kit (Randox Laboratories Ltd., Crumlin, UK), as per manufacturer instructions, based on the method of Paglia and Valentine (1967). Proximate analysis, AMEn and retention coefficients of diets were performed as previously explained by Watts et al. (2020).

73 Statistical analyses were performed using GenStat 19th edition statistical software (IACR
74 Rothamstead, Hertfordshire, England). The data were analysed by one-way ANOVA with
75 blocks. Orthogonal polynomials were used to compare treatment differences for linear and
76 quadratic relationships with increasing DHQ inclusion. In all instances, differences were
77 reported as significant at $P < 0.05$. Means were separated by protected LSD.
78
79 In the reported study, DHQ was added at three different levels at 0.5, 1.5 and 4.5 g per kg feed
80 to determine an appropriate inclusion level for use as an antioxidant in broiler production. On
81 average, birds were consuming approximately 62 g feed per day and their average daily weight
82 gain was approximately 43 g. Thus, the daily consumption of DHQ product varied between
83 0.03 and 0.28 g per bird per day. The obtained results (Table 2) agree with reports on birds at
84 a similar age and previous DHQ studies performed at NIPH (Abdulla et al., 2016; Pirgozliev
85 et al., 2019, 2020). The effect of DHQ on WG was not significant overall ($P > 0.05$) but could
86 be described as a linear increase ($L < 0.05$; Table 2). The response of FCR to DHQ levels
87 tended to be linear ($L = 0.086$), however, there was no overall significant difference ($P > 0.05$)
88 between the individual levels of DHQ or control. The significant response ($P < 0.001$) of blood
89 GSH-Px was best described as curvilinear ($L < 0.001$ and $Q < 0.05$). There was no effect of
90 graded levels of DHQ on AMEn or nutrient retention coefficients ($P > 0.05$).
91
92 The mode of action of flavonoids, e.g. DHQ, is usually associated with their antioxidant
93 properties (Surai 2014). Glutathione peroxidase is a well reported Se-containing enzyme
94 associated with important free radical scavenging ability via oxidative and reductive pathways
95 (Kosower and Kosower 1978). Higher oxidative status is to be expected in animals fed more
96 antioxidants (Woods et al., 2020 b), which was confirmed in the current study whereby birds
97 fed 1.5 g/kg DHQ levels, or above, had greater levels of GSH-Px compared with those fed the

control and the low DHQ supplemented diets. The observed levels were in accordance with other studies involving birds at a similar age (Pirgozliev et al., 2019, 2020; Woods et al., 2020a, c).

This and previous studies have shown that the response of blood GSH-Px to dietary DHQ is not followed by significant growth performance, energy or nutrient availability improvements (Pirgozliev et al., 2019, 2020). However, data published by Fomichev et al. (2016) and Kuzmina and Petrov (2021) indicate a response to DHQ may only be observed in the later stages of growing, suggesting that DHQ dietary incorporation is suited to phase feeding programmes.

The results from this study indicate that dietary DHQ supplementation may be beneficial in broiler production at levels greater than 1.5 g/kg feed, due to improved bird antioxidant status. The determination of an upper inclusion limit and its effect in finisher feeding phases requires further research.

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Table 1.

Diet formulation and determined chemical composition of basal diet

Dietary ingredients (g/kg)	Basal diet
Wheat	545.0
Soybean meal (CP 49 %)	230.0
Full fat soya	50.0
Barley	84.0
Soybean oil	45.0
Monocalcium-phosphate	12.5
Limestone	12.5
Sodium bicarbonate	1.5
Salt	2.5
DL-Methionine	3.5
L-Lysine HCL	3.0
L-Threonine	1.5
Titanium dioxide	5.0
Premix[1]	4.0
Calculated composition	
AME	13.05
Crude protein	202
Crude fat	69
Lysine (available)	11.8
Methionine	6.2
Methionine/Cysteine	9.3
Phosphorus (available)	4.2
Calcium	9.2
Determined values	
DM	923
GE	17.32
Crude protein	196
Crude fat	70
Ca	10.75
P	7.25
Mg	1.43

[1]Provided per kg feed: 2160 µg retinol, 75 µg cholecalciferol; 25 mg α-tocopherol, 1.5 mg menadione, 5 mg riboflavin, 8 mg pantotenic acid, 10 µg cyanocobalamin, 1.5 mg pyridoxine, 1.5 mg thiamine, 0.5 mg folic acid, 30 mg niacin, 60 µg biotin, 0.8 mg I, 10 mg Cu, 80 mg Fe, 0.3 mg Se, 80 mg Mn, 80 mg Zn (Target Feeds Ltd., Whitchurch, UK).

Table 2.
The effect of dietary dihydroquercetin (DHQ) on body weight (BW), daily feed intake (FI), weight gain (WG), feed conversion ratio corrected for mortality (FCR), dietary dry matter (DMR), nitrogen (NR), fat (FR) retention coefficients, N-corrected apparent metabolisable energy (AMEn) and blood glutathione peroxidase as units per red blood cells (GSH-Px), when fed to broiler chickens from 7 to 21 d age.

Treatment	BW 7d (g)	BW 21d (g)	FI (g/b/d)	WG (g/b/d)	FCR (g:g)	DMR	NR	FR	AMEn (MJ/kg DM)	GSH-Px (u/ml RBC)
Control (C)	134	730	60.4	42.6	1.415	0.703	0.645	0.796	13.18	105[a]
C + 0.5 g DHQ	130	710	60.3	40.6	1.488	0.699	0.682	0.757	12.78	97[a]
C + 1.5 g DHQ	133	748	62.7	43.9	1.433	0.708	0.652	0.762	13.18	115[b]
C + 4.5 g DHQ	133	773	62.5	45.7	1.369	0.706	0.638	0.823	13.32	120[b]
SEM	3.4	20.6	1.83	1.40	0.0341	0.0125	0.0137	0.0265	0.213	3.3
P	0.813	0.193	0.677	0.102	0.139	0.960	0.147	0.288	0.347	<0.001
L	0.827	0.059	0.372	0.036	0.086	0.765	0.205	0.192	0.275	<0.001
Q	0.645	0.569	0.708	0.379	0.256	0.904	0.143	0.349	0.345	0.024
CV %	6.7	7.4	7.9	8.5	6.3	4.7	5.5	8.9	4.3	8.0