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Feeding dry stevia leaf (*Stevia rebaudiana*) or xylanase improve the hepatic antioxidative status of broiler chickens

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Abstract Four diets, formulated with and without stevia and with and without exogenous xylanase, following 2 x 2 factorial design, were prepared. Each diet was fed ad libitum to birds in eight pens (three birds in each pen) in a randomised block design. It was found that birds fed xylanase grew faster, used the feed more efficiently and had an increased concentration of hepatic α-tocopherol and vitamin E concentrations ($P < 0.05$). Feeding stevia did not affect growth performance ($P > 0.05$), but increased hepatic CoQ_{10} ($P = 0.05$), lutein, zeaxanthin and total carotenoids ($P < 0.001$) concentrations. There were no dietary stevia by xylanase interactions ($P > 0.05$) for any of the studied variables. The results showed that alone, dietary stevia and dietary xylanase can improve the antioxidative status of birds through enhancing dietary antioxidants availability.

Key words: stevia, xylanase, chickens, hepatic antioxidants.

The antioxidant activities of stevia were recognised with mammals and in vitro studies, but the results with chickens are not consistent (Geuns et al., 2003a, 2003b). Stevioside induced nephrotoxicity in chickens (Jahanbakhsh et al., 2015), did not bring differences in antioxidant capacity in liver of chicken embryo (Sadighara et al., 2017), and alleviate intestinal mucosal damage through anti-inflammatory and antioxidant activity in broiler chickens (Jiang et al., 2019). In rats, Awney (2011) observed high doses of stevioside induce lipid oxidation while low doses have an antioxidant effect. Xylanase is widely used in poultry production in the world and improves not only productive performance of but also hepatic antioxidative status of birds (Pirgozliev et al., 2015). However, information on the interaction between stevia and xylanase is lacking. The aim of the study was to study the impact of dietary stevia with or without exogenous xylanase on antioxidavtive status of broiler chickens. Bird growth performance variables were also measured.

A wheat-soy-based basal grower diet that meet breeder's recommendations (Aviagen Ltd., Edinburgh, UK) was prepared (Table 1). Another diet was mixed as the only difference with the basal diet was an inclusion of 20 g/kg of milled dry stevia leaf (*Stevia rebaudiana*) from cultivar Stela on the expense of wheat. Both diets were then split into two batches as one of them was supplemented with *Aspergillus oryzae* commercial preparation of endo-1,4-beta-xylanase at 100 g/kg (100 FXU/kg, Ronozyme WX, DSM, Switzerland).

The experiment has been conducted at the National Institute of Poultry Husbandry and approved by Harper Adams University Research Ethics Committee.

All birds were fed a common commercial starter ration until 7 d of age at which time 96 male broiler chickens (Ross 308), excluding ill and malformed, were allocated at random to the 4 experimental diets. Each diet was fed to 8 pens (3 birds per pen) following randomisation. Each of the pens had a solid floor and were equipped with an individual feeder and drinker. Feed and water were offered *ad libitum* to birds throughout the experiment. The ambient temperature and the lighting programme were maintained following breeder's recommendation. The well-being of the birds was checked regularly every day. Birds and feed were weighed on days 7 and 21 in order to determine average daily feed intake (FI), average daily weight gain (WG) and to calculate the feed conversion efficiency (FCE) on a

pen basis. At the end of the study, at 21d of age, one bird per pen, selected at random, was electrically stunned and killed by cervical dislocation. The livers of the birds were immediately collected, freeze dried and stored at minus 80°C for further analysis.

The proximate analysis of diets were performed as previously explained (Pirgozliev et al., 2009). Stevioside and rebaudioside in stevia were determined following reports of Geuns et al. (2003a, 2003b). Antioxidants in feed and liver were determined as described by Karadas et al. (2014).

Data was analysed using Genstat (18^{th} edition) statistical software package (IACR Rothamstead, Hertfordshire, UK). Comparisons among studied variables were performed by 2 X 2 ANOVA using a factorial design (dietary stevia X xylanase). In all instances, differences were reported as significant at $P < 0.05$.

There was no effect of diets on FI ($P>0.05$), but birds fed xylanase had an improved WG and FCE ($P<0.05$) (Table 2). Dietary stevia increased hepatic CoQ_{10} ($P=0.05$), lutein, zeaxanthin and total carotenoids ($P<0.001$) concentrations, and tended ($P<0.01$) to increase hepatic α-tocopherol and total vitamin E concentrations. Feeding xylanase increased ($P<0.05$) hepatic α-tocopherol and total vitamin E concentrations, but did not change ($P>0.05$) the rest of the studied antioxidants.

Phytogenic feed additives, including stevia, and exogenous xylanases were widely studied recently as a result of their health and growth-promoting properties (Atteh et al., 2008; Pirgozliev et al., 2015). However, while plant extracts are involved in the regulation of many physiological processes in animals and humans, their mode of action in poultry physiology and nutrition remains unclear (Geuns et al. 2003a, 2003b; Karadas et al., 2014). Dietary xylanases can improve growth performance, nutrient availability and bird health via reduced digesta viscosity, hydrolysing dietary fibres and generation of prebiotics. To our knowledge, this is one of the first report on the expression of effects of dietary stevia on the total carotenoids and coenzyme Q_{10} in liver of broiler chickens. The concentration of total vitamin E and carotenoids in the liver of chickens was comparable with previously reported values for broiler chickens (Karadas et al., 2014; Pirgozliev et al., 2015).

78 While the diet is the major determinant of the carotenoid composition in liver tissue, some results
79 suggest that feed supplements other than carotenoids, e.g. phytase, xylanase and plant extracts, may be
80 a reason for differences in efficiency of carotenoid assimilation from the diet and their accumulation in
81 the liver (Pirgozliev et al., 2015; Karadash et al. 2014). The improved carotenoid concentration in the
82 liver of stevia fed birds suggests that the supplement either increases carotenoid absorption, or for some
83 reason reduces oxidative stresses, thereby preventing carotenoid reserves from depletion, or perhaps a
84 combination of the two. Overall feed intake did not differ between treatments, suggesting that efficiency
85 of absorption and/or deposition was higher or reduced metabolism occurred.

86 Carotenoids possess antioxidant properties and can inhibit tumour growth and the induction of apoptosis
87 (Milani et al., 2017). Epidemiological studies have shown a correlation between a high carotenoid
88 intake in the diet with a reduced risk of various disease, suggesting that higher concentrations of
89 carotenoids in body tissues may decrease the challenge provoked by various diseases. This suggests
90 that when birds are exposed to more stress, e.g. reared in big commercial facilities with high stocking
91 density, reduced environmental control, lees hygienic conditions etc., feeding stevia may potentially
92 improve their antioxidant status and resistance to diseases. Anyway, it is not clear if carotenoids express
93 their health-promoting properties directly or as a result of interactions with other antioxidants. Surai
94 (2002) stated that carotenoids can potentially recycle vitamin E or other antioxidants. However, in
95 agreement with previous research (Karadas et al., 2014), there was no evidence of interactions with
96 vitamin E in this study, there being no effect of carotenoids on vitamin E concentration in the liver of
97 the birds. On the other hand, again in accord with Karadas et al. (2014), the increase in the
98 concentrations of total carotenoids was associated with increased concentrations of another antioxidant,
99 coenzyme Q_{10}; thus the carotenoid participation in antioxidant interactions within the liver of growing
100 chickens cannot be excluded. The improvements seen may indicate that the carotenoids and coenzyme
101 Q_{10} may be effective at reducing production and effects of free radicals (Surai, 2002). Coenzyme Q_{10}
102 can be obtained from the diet but, more importantly, it is synthesised in the body. Therefore, an
103 increased concentration of coenzyme Q_{10} in the liver of the growing chickens as a result of dietary stevia
104 supplementation could be considered beneficial. Indeed, Jiang et al. (2019) concluded that dietary

stevioside supplementation could alleviate lipopolysaccharide-induced intestinal mucosal damage by ameliorating inflammation and improving the antioxidant status of intestinal mucosae.

The results from this experiment showed that dietary xylanase not only improved bird growth but also increased the concentration of hepatic vitamin E. These results are in line with previous observations that feeding a low viscosity, in comparison to a high viscosity, diet improves growth and the hepatic antioxidant content of broilers (Pirgozliev et al., 2014). There are a number of factors that could explain this effect: first, there could have been improved bioavailability of these fat-soluble compounds. Although dietary fat digestibility was not examined in the present study, high viscous diets thicken the unstirred water layer of the mucosa which has been suggested (Palliyeguru and Rose, 2014) to thicken the intestinal mucous layer and reduce nutrient absorption. Second, a decreased digesta viscosity may have reduced dysbacteriosis in the small intestine. Dysbacteriosis is a disease condition caused by imbalance of the normal microbial flora in the distal part of the small intestine and the pathogenesis can be initiated by a mixture of opportunistic pathogens (Palliyeguru and Rose, 2014). In general, an increased dysbacteriosis may result in more toxins produced in the gut of the birds, which will be absorbed across the intestinal mucosa and stimulate the immune system and increase the demand for vitamin E.

The results from this study showed that chickens can benefit from supplementing diets with xylanase. The experimental data also suggest that dietary stevia and xylanase may improve the anti-oxidative status of birds through enhancing dietary antioxidants availability.

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Table 1.
Diet formulation and determined chemical composition of basal diet and stevia leafs

Dietary ingredients (g/kg)	Basal diet	Diet with stevia leafs
Stevia leafs	-	20.00
Wheat	670.00	650.00
Soybean meal (48 CP)	219.70	219.70
Soybean meal (full fat)	50.00	50.00
Vegetable oil	20.00	20.00
Dicalcium phosphate	14.50	14.50
Limestone	12.50	12.50
NaCl	2.70	2.70
Lysine	2.07	2.07
Methionine	3.90	3.90
Vitamin mineral premix[1]	4.00	4.00
	1000	1000
Calculated analysis (as fed)		
Crude Protein g/kg	206	204
ME MJ/kg	12.67	12.42
Crude Fat g/kg	44.4	43.9
Ca g/kg	9.7	9.5
Available P g/kg	4.6	4.5
Lysine g/kg	12.4	12.2
Met + Cysteine g/kg	9.9	9.7
Determined values	Basal diet	Stevia leaf
DM (g/kg)	878	881
CP (g/kg)	212.9	119.2
CF (g/kg)	40.2	39.8
Stevioside (mg/100g)	nd	7.61
Rebaudioside (mg/100g)	nd	4.81
Lutein	0.9	243.1
Zeaxanthin	0.1	32.8
β-cryptoxanthin	0.01	3.0
β-carotene	0.1	68.0
total carotenoids	1.0	346.8
α-tocopherol	19	35.2
γ-tocopherol	10.4	4.9
δ-tocopherol	5	4.7
Coenzyme Q10	0.6	3.1
Sum of peaks total carotenoids	1.4	469.4

[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).

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Table 2.
The effect of dietary stevia and xylanase on daily feed intake (FI), weigh gain (WG), feed conversion efficiency (FCE), liver weight and concentration of hepatic coenzyme Q_{10}, α -, γ - tocopherol, vitamin E, lutein, zeaxantin, β – cryptoxantin, β – carotene and total carotenoids.

Treatment	FI (g/b/d)	WG (g/b/d)	FCE (g;g)	Liver (g)	DM liver (kg/kg)	Q_{10}	α-tocopherol	γ-tocopherol	Vit E	Lutein	Zeaxanthin	β-cryptoxanthin	β-carotene	Total carotenoids
ST														
No	54.5	33.2	0.610	10.1	0.261	219	56.6	2.8	59.5	1.9	0.30	0.02	0.57	2.8
Yes	54.0	32.3	0.600	17.4	0.259	252	66.3	3.4	69.7	5.2	0.76	0.03	0.49	6.4
XYL														
No	53.5	31.8	0.594	17.7	0.263	234	52.8	2.8	55.6	3.6	0.53	0.03	0.56	4.7
Yes	55.0	33.8	0.615	18.8	0.257	238	70.1	3.4	73.5	3.5	0.53	0.02	0.50	4.5
SEM	0.75	0.60	0.0060	0.64	0.0028	11.0	5.16	0.28	5.42	0.38	0.027	0.005	0.046	0.38
Probabilities														
ST	0.627	0.306	0.269	0.088	0.547	0.050	0.077	0.154	0.074	<0.001	<0.001	0.823	0.249	<0.001
XYL	0.186	0.029	0.023	0.219	0.111	0.806	0.003	0.111	0.003	0.892	0.963	0.194	0.392	0.802
ST x XYL	0.120	0.116	0.477	0.711	0.746	0.431	0.366	0.947	0.392	0.483	0.862	0.786	0.245	0.393