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Short Communication

Effect of dietary energy density and folic acid supplementation on white striping occurrence and growth performance of broiler chickens

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Abstract

Broilers were allocated to eight treatments consisting of two energy levels: Normal (NE) and High (HE), and four folic acid (FA) levels (2.2, 5, 10 and 15 ppm). On d 42, two male and two female chickens were euthanized per pen and white striping (WS) evaluation was performed on the *Pectoralis major*. Birds fed HE diets had reduced ($P<0.05$) FI and FCR than those on NE diets. With increasing FA levels, there was reduced ($P<0.05$) WS score, increased ($P<0.05$) normal breast fillet percentage in female but not in the male chickens. WS scores were higher ($P<0.01$) in male chickens than in the females.

Keywords: energy density, folic acid, growth performance, white striping.

Introduction

White striping (WS) is an emerging disease in poultry. Lately, worldwide attention has been drawn to the incidence of this myopathy, which negatively affects the visual appearance and nutritional value of broiler breast meat, and thus has an adverse effect on consumer perception, leading to

economic losses (Zanetti et al. 2018). Even though the exact etiology for WS is still unknown, there are reports that the myopathy is associated with oxidative stress and inflammation (Kuttappan et al. 2016).

Folic acid (FA) is a water-soluble vitamin belonging to the B-complex group, and exogenous intake is required for optimal health, growth, and development (Kennedy 2016). As a cofactor or co-enzyme, FA plays vital biological roles in a variety of physiological processes, including the synthesis of nucleic acids and methionine regeneration (Kennedy 2016). It has been reported that FA has a homocysteine-lowering effect, and one of the potential mechanisms for this effect include anti-oxidation (Brattstrom 1996). In addition, FA has been found to exert anti-inflammatory effects in vivo, including reducing the circulating level of several inflammatory mediators in healthy overweight subjects (Kennedy 2016). The FA requirements of broiler chickens are estimated in the 90's to be between 1.3 and 3 mg/kg (Whitehead et al. 1995). However, the current requirements are likely to be underestimated in broiler chickens, especially in fast-growing and high breast yield chickens. It has been demonstrated that WS was more prevalent in broiler chickens fed high energy density diet compared to those fed low energy density diets (Kuttapan et al., 2012). The objective of the proposed research was to investigate the effect of increasing levels of FA supplementation on the occurrence of WS in broiler chickens.

MATERIALS AND METHODS

Day-old male and female broiler chickens (Ross 308) were obtained from Atlantic Poultry Incorporated, Port Williams, Nova Scotia. Upon arrival at the Atlantic Poultry Research Center, Dalhousie University Faculty of Agriculture, Truro, NS, chicks were weighed in groups of 25 birds and assigned to floor pens (0.93 m x 2.14 m), at a stocking density of 0.076 m²/ bird. Room temperature was monitored daily and was gradually reduced from 30 to 22.6°C from d 0 to 42.

The lighting program was set to produce 18 hours of light and 6 hours of darkness throughout the experimental period, and illumination was gradually reduced from 20 lx on d 0 to 5 lx. Broilers were placed in environmentally controlled rooms with the same conditions set for temperature, humidity, light scheduling, and bird density. The experimental methods for this research were approved by Dalhousie University Animal Use and Care Committee and all chickens were handled and cared for according to the recommendations of the Canadian Council of Animal Care (CCAC, 2009). Broilers were raised on a phase-feeding program, which consisted of starter (0-14 days), grower (15-24 days), and finisher (25-42 days) and received feed and water ad libitum. Diets were fed as crumbled pellets during the starter phase and as pellets during the grower and finisher phases. Birds were randomly sorted into eight dietary treatments, consisting of eight replicate pens per treatment. Each pen consisted of 25 chicks. The experiment was designed as a 2 x 4 factorial design consisting of two levels of energy: 1) Normal (NE) and 2) High energy density (HE) and four levels of supplemental folic acid (2.2, 5, 10 and 15 ppm). The HE diets were formulated to contain an additional 100 Kcal/kg ME above the NE diets. The composition of NE diets were similar to the basal diets used in our previous study (Adewole et al., 2020). The NE and HE diets were formulated to have a similar ratio of metabolizable energy (ME) to crude protein and digestible amino acids.

Feed intake, body weight, and mortality were recorded weekly, and BWG and FCR were calculated. On d 42 of age, four chickens (two males and two females) per pen (32 chickens per treatment) were randomly selected and euthanized by electrical stunning and exsanguination. Birds were bled for 3 – 5 minutes after carotid and jugular veins were cut. All carcasses were manually de-feathered and eviscerated, and breast fillets were manually deboned from the carcasses. White striping evaluation was immediately performed on the deboned, skinless breast fillets of the

pectoralis major muscles according to the procedures of Kuttappan et al. (2016), in which the degree (normal, moderate, severe, and extreme) of WS was determined. Data were subjected to analysis of variance using the GLM procedure of SAS with FA level, energy level, sex, and energy level x FA interaction as factors and the following parameters as variables: feed intake, body weight, body weight gain, and feed conversion ratio. White striping scores were subjected to a non-parametric Kruskal-Wallis test.

RESULTS AND DISCUSSION

The main effects of FA and energy level on growth performance parameters are presented in Table 1. There was no interaction between FA and energy level on growth performance parameters, except for FCR (d 0-42), where there was a significant interaction ($P = 0.048$) between FA level and energy density. During the grower phase (d 15-24), birds fed 10 ppm FA had significantly lower ($P < 0.05$) FI and BWG, and higher FCR, compared to the other treatments. Birds fed HE diets had significantly lower ($P < 0.05$) FI and FCR compared to those fed NE diets. There was no effect energy level on BW and BWG throughout the study. The effects of FA, energy, and sex on slaughter weight, breast weight, and WS scores for male and female broiler chickens are presented in Table 2. For the male chickens, there was no effect of FA or energy level on WS score and the percentages of normal, moderate, severe, and extreme WS breast fillets. However, birds fed 10 ppm FA had lower ($P < 0.05$) slaughter weight compared to those fed basal diet (2.2 ppm FA), while those fed 5 and 15 ppm FA were intermediate. Birds fed 5, 10, and 15 ppm FA had lower WS score compared to those fed the basal diet. Birds fed 15 ppm FA had the highest percentage ($P < 0.01$) of normal breast fillets compared to those fed the basal diet, while other treatments were intermediate. Generally, FA is considered a nontoxic vitamin. Leeson and Summer (2001) documented that birds are very tolerant of high levels of FA and that up to 5,000 times regular

intake would be needed to induce toxicity. Though there are limited studies on feeding high doses of FA to broiler chickens, recent studies with laying hens have demonstrated non-toxicity of high doses of FA (Tactacan et al. 2012). A possible explanation for the reduced growth performance in chickens fed 10 ppm FA in the current study is the metabolic relationship between FA and methionine. Hyperhomocysteinemia may have been present in the birds fed 10ppm FA diet. It is speculated that under this condition, FA would not have been enough to neutralize homocysteine to methionine and consequently less amounts of this limiting amino acid were available for protein biosynthesis. Dietary energy level is a vital element that influences feed efficiency and growth performance in chickens. The reduced feed intake and better FCR observed among birds fed HE diets compared to those fed NE diets demonstrates that the HE diets helped birds to meet their requirement with a lower amount of feed. This finding is consistent with several reports in the literature (Kuttapan et al. 2012). Irrespective of energy level, a reduction in WS score and an increase in the percentage of normal breast fillets, with increasing FA levels, was observed for female broiler chickens in the current study while there was no effect on the male chickens. This is associated with the significant differences in slaughter weight, breast weight and WS occurrence between male and female broiler chickens. Male and female broiler chickens potentially have different rates of growth and breast muscle development. This might have elicited differences in breast muscle capillary development and energy reserve. The potential ameliorative effect of FA on WS occurrence in female and not in male chickens could be an indication that female chickens require additional dietary FA compared to the male chickens. The beneficial effects of FA on WS occurrence may also be related to FA effects on lipid metabolism. A proteomics and metabolomics study by Liu et al. (2018) reported that lipid metabolism was disturbed by FA addition and that FA substantially disrupted *de novo* fatty acid synthesis and promoted triglyceride hydrolysis. It

has been previously reported that white striped breast muscle fillets are characterized by increased fat deposition (Kuttappan et al., 2012). Therefore, FA may be considered as a precautionary strategy for fat deposition in broiler chickens but this requires further investigation.

In conclusion, dietary supplementation with 5 and 15 ppm FA reduced WS occurrence in female broiler chickens, without a negative effect on growth performance. Dietary energy level did not affect WS occurrence, but growth performance, was enhanced when chickens were fed with high energy compared to normal energy diets. Male broiler chickens showed a higher degree of WS occurrence than female broiler chickens. Irrespective of energy level, dietary inclusion of 5 to 15 ppm FA did not have effect on WS occurrence in male broiler chickens. It is suggested that female broiler chickens may have a higher dietary requirement for FA than their male counterparts, but further studies are required in this area.

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Table 1. Effect of increasing levels of folic acid fed at different energy levels on growth performance of broiler chickens

	Folic acid level (ppm)					Orthogonal Polynomial P-values			Energy level			Energy*FA ³
	2.2	5	10	15	SEM ¹	Linear	Quadratic	Cubic	Normal	High	SEM ²	
Feed intake, g/bird												
d0-14	263	259	256	262	2.09	0.6351	0.3876	0.0314	264 ^a	256 ^b	1.48	0.9488
d15-24	1097 ^a	1101 ^a	1056 ^b	1070 ^{ab}	8.50	0.5177	0.0002	0.3332	1108 ^a	1054 ^b	6.17	0.1714
d25-42	1517	1448	1451	1489	22.47	0.1089	0.5906	0.0773	1501 ^a	1450 ^b	16.35	0.6163
d0-42	2877	2818	2765	2821	28.99	0.3639	0.0637	0.0788	2873 ^a	2767 ^b	20.51	0.6050
Body weight, g												
d14	342	340	329	336	4.97	0.9275	0.0864	0.3326	338	335	3.51	0.3238
d24	1297	1300	1235	1277	9.30	0.2247	<0.0001	0.0041	1283	1272	6.58	0.1408
d42	3240 ^a	3242 ^a	3118 ^b	3199 ^a	12.6	0.2022	<0.0001	0.0054	3204	3195	11.46	0.7119
Body weight gain, g/bird												
d0-14	230	228	220	223	4.71	0.9423	0.1130	0.6439	226	225	3.33	0.5130
d15-24	794 ^a	800 ^a	751 ^b	788 ^{ab}	10.29	0.2482	0.0113	0.0266	787	780	7.28	0.3930
d25-42	971	971	965	960	10.98	0.9082	0.4657	0.7727	973	962	7.76	0.1485
d0-42	2006 ^a	1999 ^{ab}	1942 ^b	1972 ^{ab}	16.19	0.8941	0.0068	0.1851	1992	1967	11.67	0.0965
Feed conversion ratio												
d0-14	1.18	1.20	1.21	1.18	0.013	0.5893	0.8859	0.1215	1.21	1.18	0.01	0.1651
d15-24	1.37 ^{ab}	1.34 ^b	1.41 ^a	1.36 ^{ab}	0.016	0.0812	0.1035	0.0829	1.40 ^a	1.35 ^b	0.01	0.4442
d25-42	1.56	1.54	1.56	1.55	0.009	0.1687	0.3940	0.8374	1.58 ^a	1.52 ^b	0.01	0.6050
d0-42	1.43	1.41	1.43	1.43	0.012	0.2059	0.5707	0.4084	1.44 ^a	1.41 ^b	0.01	0.0484

¹Standard error of the mean for the main effect of folic acid. ²Standard error of the mean for the main effect of energy. ³Energy x Folic acid interaction. In a row, means assigned different lowercase letters are significantly different, P < 0.05 (Tukey's procedure). n = 8.

Table 2. Effect of folic acid and energy levels on slaughter weight, breast weight and white striping occurrence in broiler chickens

			Slaughter weight, g	Breast weight, g/kgBW	WS ¹ score	WS ² , %			
						Normal	Moderate	Severe	Extreme
Folic acid (ppm)	Male	2.2	3805	780	1.38	18.8	34.4	37.5	9.40
		5	3762	795	1.21	18.1	43.8	31.3	6.31
		10	3668	764	1.31	12.5	46.9	37.5	3.12
		15	3760	781	1.41	6.50	50.0	40.6	3.10
		SEM ³	42.7	14.2	0.14	5.60	8.88	8.08	3.87
	Female	2.2	3150 ^a	688	1.41 ^a	3.12 ^b	53.1	37.5	6.31
		5	3097 ^{ab}	709	0.97 ^b	28.1 ^{ab}	59.4	9.4	3.13
		10	3004 ^b	677	0.98 ^b	18.8 ^{ab}	59.4	18.8	3.10
		15	3089 ^{ab}	703	0.94 ^b	37.5 ^a	34.4	25.0	3.10
		SEM	37.0	12.6	0.14	6.94	9.15	8.54	3.93
Energy density	Normal	3395	734	1.18	18.8	46.1	31.25	3.91	
	High	3440	740	1.22	17.2	49.2	28.1	5.47	
	SEM	19.97	6.72	0.07	3.15	4.51	4.16	1.82	
	FA x energy ⁴	0.061	0.130	-	-	-	-	-	
Sex	Male	3749 ^a	780 ^a	1.34 ^a	14.1	43.8	36.7 ^a	5.47	
	Female	3085 ^b	694 ^b	1.07 ^b	21.9	51.6	22.7 ^b	3.91	
	SEM	19.97	6.72	0.07	3.15	4.51	4.16	1.82	
	FA x sex ⁵	0.998	0.964	-	-	-	-	-	
	energy x sex ⁶	0.229	0.457	-	-	-	-	-	

¹White striping. ²Percentages of normal, moderate severe and extreme white striped breast fillets. ³Standard error of the mean. ⁴Energy x folic acid interaction p-value. ⁵Folic acid x sex interaction p-value. ⁶Energy x sex interaction p-value. Interactions could not be determined with the non-parametric Kruska-Wallis test for WS scores. In a column, means assigned different lowercase letters are significantly different, $P < 0.05$.