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Full Length Research Paper

Intestinal DNA concentration and protein synthesis in response to low quality diets in two strains of Leghorn layers

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Performance, protein synthesis and mucosal DNA in small intestine of Leghorn hens may be affected by low quality feedstuff. An experiment was conducted in completely randomized design (CRD) in 2 × 2 factorial arrangement. Main factors included diets containing 20 and 40 % barley and black and blue strains of leghorn. Layer performance was recorded weekly up to eight weeks. Egg production (%), egg mass and egg number were significantly higher in black strain leghorn on 20% barley diet than blue strain with 40% barley diet (P<0.05). Treatments blue × 40% barley and black × 20% barley showed lowest and highest egg production percentage, egg mass and egg number, respectively (P<0.05). The lowest protein synthesis in small intestine was shown in blue × 40% barley treatment group. Small intestine mucosal DNA quantity in blue strain was significantly higher than that in black strain (P<0.05). In contrast, protein synthesis in blue strain was lower than that in black strain (P<0.05). It was concluded that lower performance in blue × 40% treatment group could be related to lower protein synthesis in small intestine in blue strain, and this may be due to the high barley (40%) contained in diet.

Key words: Performance, protein synthesis, mucosal DNA, small intestine.

INTRODUCTION

Egg production in laying hens is not only related to feed efficiency, but genetic and molecular mechanisms also may play a vital role in protein synthesis from intestine into oviduct during egg production. Barley is one of the low-cost alternative ingredients in poultry nutrition and high in non-starch polysaccharides (NSP) content. The chemical nature of NSP differs from one ingredient to another, but most soluble NSP generally increase digesta viscosity that interferes with nutrient intake and body growth (Evans et al., 1993; Smits and Annison, 1996). Some of the variation in the performance of laying hens on different cereal grains and some other ingredients has been ascribed to the nature and concentration of NSP (Kopinski et al. 1995; Smits and Annison, 1996). Non starch polysaccharides have more undesirable effect on gastrointestinal organs, especially on small intestine. The small intestine is an important organ, contributing 9 to 12% of daily whole-body protein synthesis (Attaix and Arnal, 1987; Simon et al., 1982).

Nutrient absorption is accomplished by two key mechanisms: diffusion and facilitated transport. Nutrient transporters are membrane-bound proteins and operate under varying environmental conditions (McGivan and Pastor-Anglada, 1994). Many amino acid transporters in the brush-border membrane of the intestine are unique to the intestine, unlike the basolateral membrane transporters that are similar to those found on other plasma membranes (Stevens, 1992). Intestinal protein synthesis responds to feeding and to state of protein content in the diet. Deprivation of food for 10 h to 5 days decreases mucosal or whole intestinal fractional protein synthesis rate (Ks) 5 by 20 to 30% relative to the fed state (Burrin

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et al., 1991; McNurlan et al., 1979; Samuels et al., 1996). A unique aspect of intestinal protein synthesis is being sensitive to the route of nutrient supply.

Total parenteral nutrition (vs. enteral feeding) is associated with negative N balance in the intestine, as a result of suppressed fractional and absolute rates of protein synthesis (230 to 240%). When more than one nutrient is co-transported, the efficiency of transport depends on the chemical relationships between the different nutrients and transporter. Nutrients transport relate to one another in a variety of ways, including inhibition and stimulation (Dudley et al., 1998). Although variations in the composition of total parenteral nutrition such as the addition of glutamine have been shown to improve intestinal weight, DNA content and protein synthesis, are not restored to levels seen in enterally fed animals (O'Dwyer et al., 1989; Stein et al., 1994). The impact of grain containing NSP on the intestinal protein synthesis and DNA level has not been widely reported; only in rat, where viscous NSP were shown to increase intestinal weight and mucosal cell proliferation (Johnson and Gee, 1986; Brunsgaard et al., 1995).

The objective of this study was to address the effect of low quality diets (barley), and strains of laying hens (black and blue Leghorn) and their interaction, on performance and molecular aspects reaction in laying hens.

MATERIALS AND METHODS

This experiment was conducted at the in Pacific Agri-food Research Center (PARC) Agassiz in British Columbia Canada. This project was approved by the Animal Care Committee (ACC) prior to the commencement under No: 0512. One hundred ninety two. Leghorn laying hens in their mid-way through egg production were used in this study. A total of 96 hens from each of two genetic lines were used. A total of 192 laying hens were randomly assigned into four treatments with eight replicates (48 hens per treatment and six per pen), based on a completely randomized design (CRD). Formulation rations in this experiment and nutrients calculation are given in Table 1.

Sample collection

At the end of the experimental period, one bird form each replication (eight birds per treatment) were chosen and killed by cervical dislocation and their intestines removed for DNA extraction and protein synthesis. After that, weights of full and empty small intestine were recorded. The duodenum and ileum samples were held by 10 and 20 ml phosphate-buffered saline (PBS) in pH 7.4 and then frozen. These tissues were subsequently used for protein synthesis and DNA quantity. The assays of protein synthesis were made based on the methods of Bicinchoninic Acid kit (BCA) (SIGMA-product code BCA-1 AND B 9643-Saint Louis, Missouri 63103 USA). Assertion of the DNA quantity of diploid cells based on fastDNA® kit (application manual revision 6540-400-4H01) and fluorometric method using ethidium bromide was made.

Protein measurement in intestinal tissue

The protein content in the mucosal was measured according to the method of BCA Kit as follow: sodium phosphate buffer (10 mM) was added to 200 mg of intestinal homogenate tissue. 1.5 ml mannitol buffer was then added to homogenizing samples and centrifuged at 14000 g for 5 min. Supernatant was removed and 600 ml phosphate buffer (PB) at pH 7 was added to samples and removed to spin tube. Pellets (samples) were transferred to filter tubes and filtered for 2 min using Keptin microtubes for protein work. Samples were diluted 50, 40, 30 and 20 times in PB pH 7. BCA was made by mixing bicinchoninic acid (as reagent A) and copper sulfate penthahydrate (as reagent B) in 50:1 part, respectively Bovine serum albumin (BSA) was used as standard with different concentrations (0 as blank, 200, 400, 600, 800 and 1000 mg/ml with PB). Finally, 0.1 ml of the mixed reagent A and B in blank, standard and sample separately were kept at room temperature for 2 h and then read at 562 nm in a spectrophotometer. In order to increase accuracy, for each treatment, a standard curve was provided with equation for each treatment and this was used to determine protein concentration in each treatment.

Used fish sperm 2.5, 5, 10 and 20 mg/ml (Fig. 1) and without fish meal as blank and transformed to ethidium bromide plastic sheet.

DNA measurement in intestinal tissue

DNA was extracted from crude mucosal homogenates of the jejunum and quantified by fluorescent microscope using the method described by Lemmens (1995). All chemicals were obtained from Q-BIO gene (FastDNA® kit) Pty Ltd. In order to prepare standards, fish sperm (2.5, 5, 10 and 20 mg/ml) (Figure 1) and without fish meal (blank) were used and transformed to ethidium bromide plastic sheet. Fluorescent microscope was used to get an image after scanning preparation by Image Quant software and the images were changed to data. In order to increase accuracy, one standard curve was provided for each treatment. The equation given for standard from each treatment was used to determine the DNA content in each treatment.

Experimental design and data analysis

The experimental design was a 2 × 2 factorial in completely randomized form. Main factors include strains (black and blue) and diets (20 and 40% barley). Four treatments of eight replications and six hens were used in each replication. Performance parameters and body weight change were analyzed weekly. All data of variables were analyzed on the basis of number of birds selected from each cage within a treatment. In feed intake (FI) parameter remote data were deleted and then analyzed. All collected data were analyzed by the analysis of variance technique, using SAS 9.1 (2004). Comparisons of means were done by Duncan's multiple range tests, assuming error level of 0.05.

RESULTS

Performance

The results of this experiment showed that feed intake, percentage of egg production and egg mass (g) were significantly increased by black strain and diet containing 20% barley rather than blue strain by 40% barley diet, respectively ($P<0.05$). No significant responses were observed in black × 40 and blue × 20 treatments in these cases ($P>0.05$) (Table 2). Also, no significant differences

Table 1. Composition nutritional levels (g/kg and %) in 20 and 40% barley diets.

Feedstuff	20% Barley	40% Barley
Corn	46.43	25.57
Barley	20	40
Soy bean meal	12.88	13.57
Canola meal	8	6.11
Limestone	7.8	7.87
Canola oil	2.92	5
DCP	0.71	0.63
Silt	0.5	0.5
Salt	0.34	0.33
Mineral premix[1]	0.11	0.11
Vitamin premix[1]	0.11	0.11
Lysine HCl	0. 10	0.08
Choline	0.06	0.06
DL-Methionine	0.05	0.06
Calculated[2]		
Energy	2800	2800
Protein	15	15
Calcium	3.25	3.25
Ava. Phophorous[3]	0.25	0.25
Lys	0.76	0.76
Met	0.3	0.3
Met + Cys	0.58	0.58

[1]Premix provided (%): NaCl 20; Na $(HCO_3)_2$ 10; choline chloride 10; Rovimix 428 2.3; Cimbria L513 10; Carophyl rot 10 0.6; Avizant Gelb 20S 2; lime 28.4; ground wheat 16.7. Rovimix 428 provided (per kg) retinol, 50 Mio IU; cholecalciferol, 10 Mio IU; α-tocopheryl acetate, 75 g; thiamine nitrate, 10 g; niacin, 30 g; Ca-D-panthothenate, 60 g; nicotinic acid, 20 g; pyridoxine hydrochloride, 20 g; folic acid, 5 g; cobalamine, 0.1 g; menadione, 10.2 g. Cimbria L513 contained (per kg) FeSO4 7H2O, 45 mg; CuSO4 5H2O, 6 mg; MnSO4H2O, 90 mg; ZnCO3, 450 mg; I 50 mg; Se, 150 mg; $CoCl_2$ $6H_2O$, 15 mg. [2]Calculated by Weender analysis (Naumann et al., '93); [3] available phosphorous

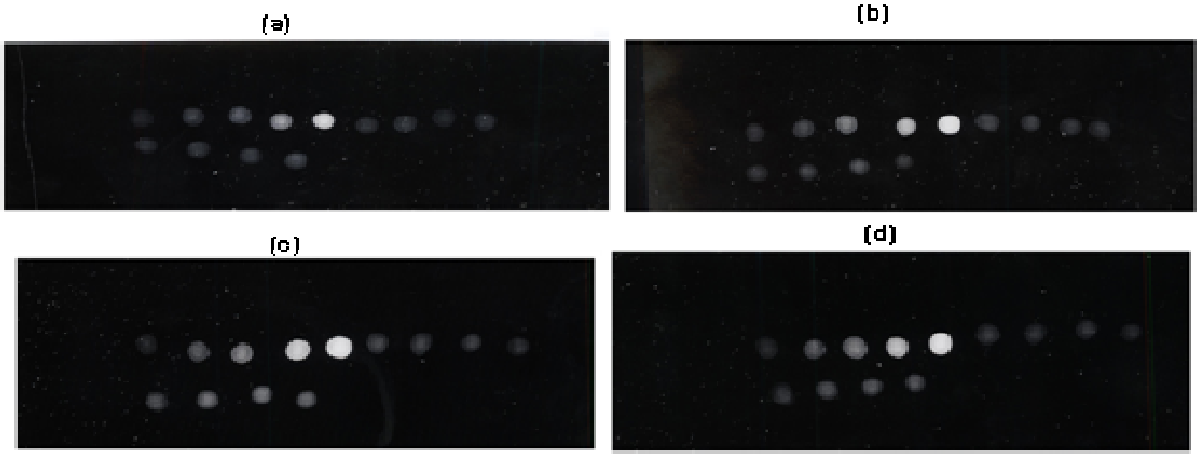

Figure 1. The image of standard and sample DNA on ethidium bromide in different treatments. (a) Treatment 1, Black × 20% ; (b) Treatment 2, Black × 40%; (c) Treatment 3 , Blue × 20% and (d) Treatment 4 , Blue × 40%. In each figures, 1 to 5 dots refer to standards, while 6 to 13 refer to samples.

Table 2. Effects of strain and diet on performance.

Parameter		FI[1]	EP [2] (%)	EW[3]	EM[4]	BW[5]	FCR[6]
Strain	Black	100.82[a] ± 10.46	79.12[a] ± 13.41	57.94[a] ± 2.65	32.88[a] ± 5.96	1786.50[a] ± 248.27	1.74[a] ± 0.174
	Blue	97.22[b] ± 12.94	74.01[b] ± 15.29	57.68[a] ± 2.78	30.70[b] ± 6.48	1627.19[a] ± 192.93	1.98[a] ± 0.619
Diets	20%	101.57[a] ± 9.69	79.13[a] ± 12.77	58.02[a] ± 2.69	32.89[a] ± 5.36	1686.93[a] ± 257.45	1.75[a] ± 0.165
	40%	96.47[b] ± 13.28	73.98[b] ± 15.84	57.59[a] ± 2.73	30.70[b] ± 6.98	1726.75[a] ± 212.78	1.97[a] ± 4.60
Black × 20%		103.51[a] ±8.79	82.46[a] ± 11.59	57.96[a] ± 2.62	34.11[a] ± 4.94	1749.75[a] ± 312.29	1.79[a] ± 0.152
Black × 40%		98.14[b] ± 11.31	75.96[b] ± 14.39	57.92[a] ± 2.69	31.66a[b] ± 6.62	1823.25[a] ± 177.41	1.69[a] ± 0.183
Blue × 20%		99.64[b] ± 10.20	75.99[b] ± 13.15	58.08[a] ± 2.77	31.67[ab] ± 5.51	1624.12[a] ± 188.37	1.72[a] ± 0.171
Blue × 40%		94.80[c] ± 14.85	72.03[c] ± 16.98	57.27[a] ± 2.75	29.73[b] ± 7.22	1630.25[a] ± 210.36	2.24[a] ± 6.54
P-Value[7]		0.0004	<0.0001	0.1487	<.0001	0.0548	0.4354
P-Value [8]		0.0001	<0.0001	0.3173	0.0325	0.6208	0.4836
P-Value [9]		0.7911	0.3334	0.4015	0.7798	0.542	0.3089
P-Value[10]		0.0001	<0.0001	0.5365	0.0252	0.2533	0.5465
MSE[11]		132.44	62.126	22.036	122.78	50699	10.494

maens in a column bearing different superscript are significantly different (p<0.05). 1: Feed Intake; 2: Egg production; 3: Egg Weight; 4: Egg mass; 5: Body weight; 6: Feed conversion ratio; 7: Strain; 8: Diets; 9: Interaction; 10: Model; 11: Means Square of Error

were found in egg weight, body weight and feed conversion ratio in this experiment. (P>0.05).

Egg characteristics

The results of the effect of strains, diets and treatments on egg characteristics are shown in Table 3. Egg numbering in diet including 20% barley was significantly higher than diet which contained 40% barley (P<0.05). Treatment black × 20% barley showed significantly higher egg number than blue × 40% barley (P<0.05). On the other hand, no significant differences were found in crack, soft and double yolk eggs in this case (P>0.05).

Small intestinal region

Small intestinal length and weight was not affected by diets, strains and treatments (P>0.05) (Table 4).

Biochemical indices of intestinal growth and metabolic activity

Intestinal mucosal protein (µg/ml) in black strain and diet containing 20% barley was significantly higher than blue strain and diet containing 40% barley (Table 5). Also, protein content in small intestine was significantly decreased in treatment including blue strain and 40% barley (blue×40%barley treatment) than the other treatments (P<0.05). Small intestine mucosal DNA content (µg/ml) in blue strain was significantly higher than black strain. More also, small intestine mucosal DNA content significantly increased (P<0.05) in diet containing 20% barley than 40% barley. Treatment blue × 20% barley significantly increased DNA of small intestine mucosal contents (P<0.05) than the other treatments. The lowest small intestine mucosal DNA content was indicated by black × 40% barley treatment.

DISCUSSION

The role of the gastrointestinal tract (GIT) in

Table 3. Effects of strain and diet on egg characteristics.

Parameter		EN[1]	CE[2]	SE[3]	DYE[4]
Strain	Black	11.43[a] ± 2.10	0.039[a] ± 0.194	0.004[a] ± 0.063	0.004[a] ± 0.063
	Blue	11.10[a] ± 2.29	0.051[a] ± 0.212	0.027[a] ± 0.186	0.00[a] ± 0.00
Diets	20%	11.69[a] ± 1.96	0.0429[a] ± 0.203	0.016[a] ± 0.153	0.004[a] ± 0.063
	40%	10.85[b] ± 2.35	0.047[a] ± 0.212	0.016[a] ± 0.124	0.00[a] ± 0.00
Black × 20%		11.97[a] ± 1.91	0.039[a] ± 0.195	0.00[a] ± 0.00	0.008[a] ± 0.088
Black × 40%		10.89[ab] ± 2.15	0.039[a] ± 0.194	0.008[a] ± 0.088	0.00[a] ± 0.00
Blue × 20%		11.39[ab] ± 1.97	0.047[a] ± 0.212	0.031[a] ± 0.215	0.00[a] ± 0.00
Blue × 40%		10.80[b] ± 2.55	0.054[a] ± 0.228	0.023[a] ± 0.152	0.00[a] ± 0.00
P-Value[5]		0.0557	0.5038	0.0417	0.3179
P-Value[6]		0.0285	0.8568	1	0.3213
P-Value[7]		0.5132	0.8568	0.6531	0.3213
P-Value[8]		0.1115	0.9478	0.5676	0.3992
MSE[9]		17.917	0.0595	0.03459	0.001953

Means in a column bearing different superscripts are significantly different (P<0.05). [1]Egg number; [2]crack eggs; [3]soft eggs; [4]double yolk eggs; [5]strains; [6]diets; [7]interaction; [8]model; [9]means square of error.

Table 4. Effects of strain and diet on small intestine length and weight.

Parameter		Length (cm)	weight (g)
Strain	Black	50.19[a] ± 4.96	12.69[a] ± 1.79
	Blue	52.03[a] ± 6.45	11.93[a] ± 2.01
Diets	20%	50.31[a] ± 4.93	12.64[a] ± 2.12
	40%	51.91[a] ± 6.51	11.98[a] ± 1.67
Black × 20%		51.00[a] ± 4.12	13.09[a] ± 1.78
Black × 40%		49.39[a] ± 5.86	12.29[a] ± 1.83
Blue × 20%		49.63[a] ± 5.83	12.19[a] ± 2.46
Blue × 40%		54.44[a] ± 6.48	11.66[a] ± 1.55
P-Value[1]		0.377	0.2639
P-Value[2]		0.441	0.3318
P-Value[3]		0.2744	0.5343
MSE[4]		33.56	3.611

Means in a column bearing different superscripts are significantly different (P<0.05). [1]Strains; [2]diets; [3]model; [4]means square of error.

nutrient processing and acquisition is evident from the pattern of its performance on laying hens observed in this study. Diets containing 40% barley decreased egg production percent, egg mass, feed intake and egg number. Intestinal physiology, morphology and ecosystem of gastrointestinal tract may be changed by high level of NSP content of barley and this may lead to unfavorable effects on nutrient solubility (Almiral et al., 1995; Leeson and Caston, 2000). High viscosity is the principle factor that increases anti-nutritive activity of these nutrients, which may have negative effect on poultry performance (Combell et al., 1993). In fact, soluble NSP reaction by membrane glycocalyx may cause an increase in mucosal layer of small intestine and decrease in protein synthesis, thus resulting to reduction in nutrient absorption as well as performance (Marquardt et al., 1983).

Although some strain have indicated a high capacity for

Table 5. Effects of strains and diets on small intestine mucosal DNA contents and protein concentration.

Parameter		DNA concentration (µg/ml)	Protein concentration (µg/ml)
Strain	Black	$0.875^{b} \pm 0.65$	$579.71^{a} \pm 168.07$
	Blue	$1.405^{a} \pm 0.50$	$443.96^{b} \pm 142.92$
Diets	20%	$1.448^{a} \pm 0.64$	$538.56^{a} \pm 191.14$
	40%	$0.832^{b} \pm 0.459$	$485.12^{b} \pm 222.96$
Black × 20%		$1.164^{b} \pm 0.73$	$499.81^{a} \pm 164.46$
Black × 40%		$0.585^{c} \pm 0.420$	$659.62^{a} \pm 137.43$
Blue × 20%		$1.732^{a} \pm 0.399$	$577.31^{a} \pm 218.65$
Blue × 40%		$1.079^{bc} \pm 0.367$	$310.62^{b} \pm 134.31$
P-Value[1]		0.0051	0.0433
P-Value[2]		0.0015	0.0433
P-Value[3]		0.8364	0.0012
P-Value[4]		0.0012	0.002
MSE[5]		0.253987	39527.9

Means in a column bearing different superscripts are significantly different ($P<0.05$). [1]Strains; [2]Diets; [3] interaction; [4]model; [5] means square of error.

consumption of NSPs in the diets in comparison to others, still, high production hens need to meet a good and high level of diet sources. On the other hand, proliferation of intestinal cells could be changed by some fiber diets, thereby promoting cell by increasing DNA quantity and protein synthesis (Sonoyama et al., 1994; Iji et al., 2001; Olasunkanmi et al., 2003; Nishibori et al., 2003). Reduction in rate of digestion and absorption could occur due to decrease in protein synthesis in small intestine (Sell et al., 1991). Apart from the direct physical effects of barley in producing viscosity in the gastrointestinal tract, there are a number of indirect negative effects on the nutritive value of poultry diets, including high level of barley (40%) in diets. This could reduce DNA concentration, as well as protein synthesis in small intestine mucosal (Table 5). Dudley et al. (1998) confirmed that decrease in protein synthesis in small intestine could be produced by diet including high level of fiber (NSP).

Differentiations between two strains in small intestinal mucosal DNA are referred to the strain characteristics. Similar to this study, Uni et al. (1995) observed an increase in DNA content of the duodenal mucosa in the two different strains. The highest protein synthesis by blue × 20% barley treatment rather than black × 40% barley could be due to strains and diets effects (Table 5), although black strain compared to blue had a better egg production, egg mass and protein synthesis in the small intestine. In addition, treatment black × 20% barley had higher egg production percent and egg mass than blue × 40% barley treatment ($p<0.05$; Table2). In contrast however, the blue strain had higher small intestine mucosal DNA contents than the black one ($p<0.05$; Table5).

Conclusion

The results of this study show that treatment including 40% barley and blue strain, reduced performance of laying hens. However, DNA alone could not show the performance of the strain. Hence, protein concentration in small intestine mucosal is a better index to performance, rather than DNA concentration. Based on these conflicting results, further investigation are needed to modulate bacterial population and strain in small intestine reactions by low quality diets. Also, more considerations are necessary to make clear the low quality diet on types of birds (layers, broilers and breeder) and their molecular reactions.

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