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EFFECT OF DIET ON AMINO ACID PROFILE OF EGG YOLK

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Key words: amino acid, laying hen, egg yolk, enzymes

The aim of the study was to investigate the effect of diet on amino acid profile of egg yolk. Laying hens (Hy-line Isa brown) were fed two diets based on wheat, barley and rye with or without supplementation of enzymes. Enzymatic preparation consisted of endo-1,4-β-xylanase and endo-1, 4-β-glucanase. Egg yolks were tested on the amounts of amino acids in 22nd, 28th and 41th week of hen's age. The amino acid content was estimated using an automatic amino acid analyzer (AAA 400).

The results of experiment showed that supplementation of enzymes to diet improved the amino acid content of egg yolk.

The results obtained in this study cannot only be applied on more accurate evaluation of the nutritional value of egg yolk, but also on the formation of feed mixtures to control the conversion process of feed.

INTRODUCTION

For a long time man has benefited from the high nutritional value of the egg yolk. Today the egg yolk is still an important source of nutrients. Cereal–based diets contain a class of poorly digestible substances called non-starch polysaccharides (NSP), which are also associated with the endosperm cell wall of the grain. In wheat, arabinoxylans are the major NSP [Choct *et al*., 1995; Choct & Annison, 1992], while in barley, β-glucans are the major NSP [Rotter *et al.,* 1990; White *et al*., 1983]. The NSP, as an anti-nutritional factor, can not be hydrolyzed by enzymes that are produced endogenously by the hens. The anti-nutritional effect can be explained in such a way that these NSP may prevent access of endogenous enzymes to the nutrients contained within grain cells. The other prevailing explanation is that some of the cell wall NSP of these cereals dissolve in the digestive tract and form high molecular weight aggregates while increasing viscosity [White *et al.,* 1981]. Enzymes have been developed to reduce the negative effects of NSP and improve the feeding value of cereal-based diets. Xylanase and β-glucanase are enzymes the most effective for supplementing cereal-based diets. Studies have shown that application of xylanases and β-glucanases in cereal-based diets improve the performance and increase nutrient digestibility [Petterson *et al.*, 1990; Bedford & Classen, 1992; Friesen *et al*., 1992; Marquardt *et al*., 1994].

The purpose of the experiment was to establish the effect of supplementation of enzymes to diets on the amino acid profile of egg yolk.

MATERIAL AND METHODS

The experiment was conducted using 22-week-old Hyline- Isa brown hens (n=108) housed five birds per cage (50 cm x 40 cm) in a three-level cage system. All birds were supplied with bulk feed and water *ad libitum* in an environmentally controlled house where day and night temperatures were maintained at approximately 16-27°C, respectively.

The laying hens were fed on conventional layer diets with or without enzymes (Table 1). Diets were supplemented with vitamins, mineral salts and carotenoid premix. The composition of both diets is presented in Table 2.

The experiment was designed to determine the influence of adding enzyme preparation to the hen's diets on amino acid profile of egg yolk. The experiment consisted of three treatments.

Treatment 1 subsumed the egg yolk of 22 week old hens. Treatment 2 took into consideration in the yolks of 28 week old hens and treatment 3 comprised the yolks of 41 week old laying hens. A total of 108 egg yolks were analyed for each of three treatments in six replicates.

Chemical analyses of feeds and egg yolks were performed following the procedures of AOAC [1990]. The total protein was determined by the Kjeldahl method. Egg yolks were frozen at -40°C before determination of amino acids. The levels of amino acids in egg yolks were related to 100% of dry matter.

After acid hydrolysis in 6 N hydrochloric acid at 110°C for 24 h the contents of amino acids were determined on the basis of a colour reaction between the amino acid and ninhidrin using an automatic amino acid analyzer (AAA 400 manufactured by INGOS Praha).The following amino acids

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TABLE 1. Feeding of basal diet with /without enzymes supplementation.

Period/ weeks	Control group	Trial group
22- 28	D1	D1 with ES
over 28	D2	D2 with ES

D1 – based diet 1, D2 – diet with lower content of crud protein than diet 1(Table 1), ES – enzymes preparation

TABLE 2. Composition of experimental diets.

Ingredient (g/kg of diet)	D1[1]	D2[6]
Wheat	266	271
Rye	150	150
Barley	200	250
Soybean meal (47% CP)	220	180
Soybean oil	25	5
Fat	20	45
Monocalcium phosphate	17	12
Limestone	90	75
Salt (38% Na)	3	3
Sodium bicarbonate (28% Na)	1	1
DL – methionine (50% M)	3	3
Vitamin premix[2]	4	4
Mineral premix[3]	1	1
Choline chloride (50%)	2	2
Carotenoid premix[4]	1	1
Enzymatic preparation[5]	0.08	0.08
Calculated nutrient composition	D1	D2
Crude protein (g/kg)	177	165
Energy(MJ/kg)	11.5	11.7
Lysine (g/kg)	8.81	7.90
Methionine (g/kg)	4.17	4.03
Methionine+ cysteine (g/kg)	7.41	7.15
Threonine(g/kg)	6.27	5.80
Linoleic acid (g/kg)	19	10.8
Ca (g/kg)	39.1	32.4
Available P (g/kg)	3.8	3.0
Na (g/kg)	1.5	1.5

(1) diet 1, 2; (2), (3) provided per kilogram diet: vitamin A 12.000 IU; vitamin D_3 2.400 IU; vitamin E 20 mg; vitamin K_3 1 mg; vitamin B_1 2 mg; vitamin B_2 6 mg; vitamin B_6 4 mg; vitamin B_{12} 20 μg; biotin 40 μg; folic acid 0.8 mg; nicotin acid 30 mg; panthotenic acid 8 mg; choline chloride 1000 mg; Fe 88mg; Zn 44 mg; Cu 6 mg; Mn 44 mg; I 0.44 mg; Co 0.1 mg; Se 0.13 mg; (4) provided per kilogram diet: consisted of Lucanthin Yellow 10% 30 mg and Lucanthin Red 10% 40 mg; (5) consisted of endo-1, 4-β-xylanase (7.820 TXU/g) and endo-1,4-β-glucanase (2.940 TGU/g), it used for trial group; (6) diet 2– after 28 weeks.

were monitored: Aspartic acid (Asp), Treonine (Thr), Serine (Ser), Glutamic acid (Glu), Proline (Pro), Glycine (Gly), Alanine (Ala), Cysteine (Cys), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Tyrosine (Tyr), Phenylalanine (Phe), Histidine (His), Lysine (Lys) and Arginine (Arg). Each analysis was performed in duplicate.

The experimental data were subjected to a statistical evaluation by analysis of variance for the amino acid contents in egg yolks (ANOVA) using the software package STATGRAPHICS. Means were separated using the LSD Multiple Range Test.

The experiment was carried out at the Department of Animal Nutrition at the Slovak University of Agriculture in Nitra, Slovak republic.

RESULTS AND DISCUSSION

The highest average value of crude protein (CP) in egg yolk was observed in the trial group (Treatment 2). The least value of CP of in yolk was observed in the control group (Treatment 1). In Treatment 3, the crude protein (CP) of egg yolk was lower in the trial group than in the control group. The highest difference in dry matter (DM) was registered under Treatment 2 (control group compared to trial group). The highest value of DM was observed in the control group under Treatment 1 (Table 3).

In an early work, it was noted that diet and greed had no effect on amino acid composition of hen's eggs [Lunven *et al.,* 1973]. Presently, the researchers investigated only effects of enzyme supplementation to viscous cereal diets on the relative size of organs and digestive tract of broiler chickens [Brenes *et al.*, 1993; Yu *et al.*, 1998]. The effects of diet types and enzyme supplementation on hen's performance, egg quality, organ weight, intestinal viscosity and digestive system characteristics for laying hens [Çiftci *et al.*, 2003] were studied, but not the effect of enzymatic preparation on the composition of amino acids in egg yolks.

The effect of enzymatic preparation on the composition of amino acids in egg yolks is presented in Table 2.

The results of the experiment showed that the amino acid content of yolk under Treatment 2 and Treatment 3 was higher than the amino acid content of yolk under Treatment 1 (in both groups). The enzyme supplementation significantly improved the contents of cysteine, methionine, serine in egg yolk ($p<0.05$, Treatment 1) and the amounts of amino acids without Val, Ile, Leu, Tyr ($p< 0.05$, Treatment 2). The amino acid profile was increased except Asp, Pro, Gly, Met, Ile, significantly at $p<0.05$ under Treatment 3 (the control group compared to the trial group). The contents of amino acids significantly increased in the control group ($p<0.05$) but not Thr, Pro, Gly Ala, Ile, Phe, Lys and Arg (period 22-41 weeks). The amounts of Thr, Phe, Val, Arg significantly increased in the trial group compared with the same amino acids of the control group (period 22-41 weeks).

CONCLUSIONS

The results of the experiment showed that the amino acid content of egg yolk (Treatment 3) was higher in comparison to amino acid content of egg yolk (Treatment 1).

Experimental results demonstrated that the enzyme supplementation mainly improved the contents of treonine, valine, phenylalanine and arginine.

The results obtained in this study cannot only be applied on more accurate evaluation of the nutritional value of egg yolk but also for the formation of feed mixture to control the conversion process of feed.

TABLE 3. Effects of enzyme supplementation on amino acid content of egg yolk.

		Treatment 1						Treatment 2						Treatment 3					
		control group			trial group			control group			trial group			control group			trial group		
		$\overline{x}$	s	v	$\overline{x}$	s	v	$\overline{x}$	s	v	$\overline{x}$	s	v	$\overline{x}$	s	v	$\overline{x}$	s	v
DM (g/kg)		499.97	2.639	0.528	497.33	6.308	1.268	490.18	3.621	0.739	483.20	15.471	3.201	493.05	3.935	0.798	494.42	6.032	1.220
CP (g/kg DM)		307.18	3.083	1.00	313.43	2.993	0.955	314.62	3.493	1.110	318.43	11.074	3.478	316.80	5.463	1.724	312.23	3.855	1.234
		$\overline{x}$	s	v	$\overline{x}$	s	v	$\overline{x}$	s	v	$\overline{x}$	s	v	$\overline{x}$	s	v	$\overline{x}$	s	v
Amino acid (g/100g DM)	Asp	2.10^{a}	0.144	6.892	2.28^{a}	0.366	15.862	2.19^{a}	0.015	0.689	2.33^{b}	0.053	2.280	2.38^{bc}	0.027	1.142	2.49^{c}	0.001	1.189
	Thr	1.21^{a}	0.081	6.730	1.26^{ab}	0.078	6.185	1.23^{a}	0.007	0.537	1.31^{bc}	0.030	2.269	1.26^{ab}	0.019	1.503	1.35^{c}	0.026	1.932
	Ser	1.82^{a}	0.108	5.934	1.92^{bc}	0.101	5.242	1.87^{ab}	0.007	0.375	1.99^{c}	0.073	3.677	2.00^{c}	0.059	2.970	2.17^{d}	0.013	0.608
	Glu	2.53^{a}	0.132	5.232	2.62^{ab}	0.143	5.469	2.53^{a}	0.026	1.045	2.71^{b}	0.069	2.534	2.86^{c}	0.026	0.884	3.02^{d}	0.014	0.464
	Pro	1.17^{ab}	0.078	6.678	1.20^{b}	0.073	6.231	1.17^{ab}	0.039	3.331	1.30^{c}	0.095	7.311	1.12^{a}	0.043	3.845	1.16^{ab}	0.043	3.677
	Gly	0.76^{a}	0.048	6.304	0.77^{a}	0.043	5.507	0.75^{a}	0.006	0.781	0.82^{b}	0.021	2.604	0.75^{a}	0.017	2.278	0.77^{a}	0.008	1.082
	Ala	1.22^{a}	0.067	5.511	1.27^{bc}	0.070	5.508	1.24^{ab}	0.017	1.401	1.34^{de}	0.056	4.215	1.30^{cd}	0.025	1.916	1.37^{e}	0.011	0.813
	Val	1.45^{a}	0.090	6.171	1.49^{a}	0.097	6.469	1.51^{ab}	0.046	3.010	1.57^{b}	0.029	1.848	1.50^{a}	0.052	3.472	1.57^{b}	0.012	0.739
	Met	0.81^{ab}	0.055	6.720	0.67^{c}	0.049	7.389	0.77^{a}	0.012	1.560	0.82^{b}	0.022	2.621	0.88^{d}	0.041	4.756	0.91^{d}	0.043	4.657
	Ile	1.32^{a}	0.078	5.820	1.34^{ab}	0.077	5.705	1.34^{ab}	0.038	2.870	1.38^{b}	0.029	2.112	1.29^{a}	0.042	5.692	1.35^{ab}	0.012	0.871
	Leu	2.02^{a}	0.101	4.980	2.08^{abc}	0.119	5.714	2.02^{ab}	0.027	1.329	2.11^{bc}	0.089	4.190	2.12^{c}	0.046	2.173	2.24^{d}	0.010	0.453
	Tyr	1.24^{a}	0.067	5.361	1.28^{ab}	0.063	4.954	1.27^{ab}	0.015	1.192	1.32^{b}	0.057	4.295	1.11^{c}	0.023	2.069	1.17^{d}	0.007	0.589
	Phe	1.09^{a}	0.061	5.644	1.12^{abc}	0.058	5.127	1.14^{bc}	0.017	1.477	1.19^{d}	0.029	1.996	1.10^{a}	0.029	2.630	1.16^{cd}	0.011	0.978
	His	0.71^{ac}	0.041	5.810	0.74^{acd}	0.036	4.917	0.71^{ac}	0.018	2.468	0.75^{d}	0.020	2.726	0.74^{b}	0.036	2.450	0.68^{ab}	0.013	1.856
	Lys	1.89^{a}	0.097	5.148	1.98^{abd}	0.105	5.338	1.93^{ab}	0.024	1.236	2.02^{d}	0.090	4.479	1.94^{ab}	0.032	1.655	2.02^{d}	0.012	0.604
	Arg	2.08^{a}	0.108	5.212	2.17^{bd}	0.136	6.273	2.16^{ab}	0.024	1.095	2.26^{de}	0.085	3.748	2.14^{ab}	0.045	2.085	2.29^{e}	0.008	0.340
	Cys	0.55^{a}	0.035	6.330	0.46^{b}	0.018	3.864	0.59^{c}	0.016	2.681	0.65^{d}	0.010	1.449	0.81^{e}	0.011	1.344	0.81^{e}	0.233	2.866

(a-e) Means within rows with no common letters are significantly different at $p<0.05$

Asp – Aspartic acid, Thr – Treonine, Ser – Serine, Glu – glutamic acid, Pro – Proline, Gly – Glycine, Ala – Alanine, Val – Valine, Met – Methionine, Ile – Isoleucine, Leu – Leucine, Tyr – Tyrosine, Phe – Phenylalanine, His – Histidine, Lys – Lysine, Arg – Arginine, Cys – Cysteine.

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