

Full Length Research Paper

Digestibility of nutrients and metabolisability of energy in broiler diets with different ME level and supplemented with exogenous enzyme

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This experiment was carried out to assess the effects of exogenous enzyme (ROXAZYME®, G2G) supplementation on digestibility of nutrients and metabolisability (ME/GE) in 28-31 days old male Ross 308 broilers. A total of 96 Ross 308 hybrids were allocated randomly to 6 dietary treatments with 4 replicates per treatments, and 4 birds per replicates. In this 3x2 factorial design trial with three different levels of metabolizable energy (13.4, 13.00, 12.6 MJ/kg), with and without exogenous enzyme, the nutrition density was reduced by introducing sunflower meal in different percentages to diet (0, 8 and 16%). The methods used to determine digestibility of nutrients and energy were total and partial collection method and ileal digesta (one percent of Celite was added to the diet for this purpose). Metabolizable energy level had a significant effect ($p < 0.05$) on digestibility of DM, hemicelluloses and ME/GE. By lowering metabolizable energy level, the digestibility decreased. Enzyme supplementation significantly ($p < 0.05$) improved the digestibility of NDF, hemicelluloses and ash determined by partial and total collection method, while statistically significant improvement in digestibility of crude protein was observed only by total collection method. Digestibility determined by ileal digesta showed only significant improvement ($p < 0.05$) in digestibility of hemicelluloses, while the other nutrients had a certain improvement that was not significant. The results suggested that exogenous enzyme, used in this trial, increased digestibility of crude proteins, NDF, hemicelluloses and ash ($p < 0.05$). However, the effects were most evident in diets with reduced level of metabolizable energy level and were determined by total and partial collection method. It can be concluded that the ME level affected digestibility of nutrients and energy, and that enzymes supplementation had the most profound effect on digestibility of nutrients and energy in diets with reduced ME level.

Key words: Exogenous enzymes, digestibility, broilers.

INTRODUCTION

Exogenous enzymes are widely used in intensive animal production. The first report about positive effects of enzyme supplementation dates from the end of 1940s (Hastings, 1946). Since then, the concept of using enzymes has changed significantly, from a single enzyme to multiple enzyme products which combine several types of enzymes (Cmiljanic et al., 2007).

Soluble non-starch polysaccharides (NSP content

varying between plant species) are considered as anti-nutritive factors which cause depression in digestibility and metabolisability of energy (ME/GE), and the supplementation by enzymes can reduce, to some extent, these effects (Choct et al., 1995; Nian et al., 2011). Exogenous enzymes, which act on NSP, improved digestibility partially by reducing intestinal viscosity. The other way of action of this enzymes are by modification of gut microflora (Choct et al., 1992; Bedford, 1996; Apajalahti and Bedford, 1999; Choct et al., 1999; Bedford, 2000; Bedford and Apajalahti, 2001).

Sunflower meal, usually found on markets in Serbia,

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Table 1. Composition and nutrient analyses of experimental diets.

Ingredient	Diet 1	Diet 2	Diet 3
Corn	62.21	59.86	57.54
Soybean meal	13.64	7.96	2.25
Full-fat soybeans	20.00	20.00	20.00
Sunflower meal	0.00	8.00	16.00
L-lysine HCL	0.00	0.07	0.16
DL-Methionine	0.18	0.16	0.15
Monocalcium phosphate	1.36	1.38	1.39
Salt	0.43	0.42	0.40
Limestone	1.17	1.15	1.12
Premix	1.00	1.0	1.00
Calculated analyses			
Crude protein (%)	19.00	19.00	19.00
Crude fat	6.12	6.08	6.05
Crude fiber	3.57	4.82	6.07
ME (MJ/kg)	13.40	13.00	12.60
Lysine (%)	1.01	1.00	1.00
Methionine (%)	0.49	0.49	0.49

contains 33% of crude protein and varies in the level of crude fibre [21% is the legal limit (Sl. list SRJ br. 20/2000 i 38/2001)]. The effect of exogenous enzyme in some studies is more visible when metabolizable energy is reduced (Zhou et al., 2009). It is widely accepted that enzyme supplementation of poorly digested diets enhances digestibility of nutrients and AME (Scott et al., 1998; Cantor et al., 2009).

The goal of this experiment was to investigate the effects of added exogenous enzyme, Roxazyme G2G [cellulase (endo-1,4- β -glucanase), β -glucanase (endo-1,3(4)- β -glucanase) and xylanase (endo-1,4- β xylanase)] on digestibility of nutrients and on energy utilization of (28-31 days old male Ross 308) broilers whose diet was different in metabolizable energy level. Nutrition density was reduced by introducing sunflower meal with 33% CP in diet in different percent (0, 8 and 16%).

MATERIALS AND METHODS

A metabolism trial was used to estimate the nutrient digestibilities (dry matter, crude protein, crude ash, NDF, ADF, hemicelluloses) and energy metabolisability (ME/GE). Chicks were reared in a broiler house from 1 to 24 days of age and then transferred to metabolic cages. A total of 96, 24 days old Ross 308 hybrids were allocated randomly to 6 dietary treatments with 4 replicates per treatments, and 4 birds per replicates (three levels of metabolizable energy: 13.40; 13.00; 12.60 MJ/kg; with or without an enzyme complex). The composition of the diets is presented in Table 1. Total experimental period was seven days, with four days for bird adaptation to the experimental diets and cages, and the remaining three days for excreta collection which was carried out once a day. The methods used to determine digestibility of nutrients and energy

metabolisability (ME/GE) were total and partial collection method (one percent of Celite was added to the diet for this purpose) and ileal digesta. Collected excreta samples were placed in plastic bags, weighed and stored in a freezer on -20°C. At the end of the experimental period, feed intake and total amount of produced excreta were determined. Samples were thawed at environment temperature. Excreta was homogenized and dried in a forced-ventilation oven at 55°C for 72 h. Dried excreta samples were grounded and submitted to the Animal Nutrition Lab at Department of Animal Science, Faculty of Agriculture, Novi Sad. AIA (Celite) was analyzed according to the procedure described by Vogtmann et al. (1975). At the end of the trial broilers were killed by cervical dislocation in order to obtain the ileal digesta (the content of digesta was taken between Mackel's diverticulum and ileocecal junction). Procedures related to the preservation, storage and analysis of the ileal digesta were identical to the procedure for excreta.

Crude protein, crude fibre, crude fat were analyzed by using Wendee method, while NDF, ADF and hemicelluloses (NDF-ADF) were analyzed by using the procedure of Van Soest (1983).

Data were analyzed by GLM using StatSoft software (STATISTICA 8, 2009) to determine the effect of enzymes addition, diet type and interaction between these two factors. The results are considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

The results of this study are presented in Table 2. Digestibility was determined by three methods, total and partial collection method of faeces and ileal digesta.

Total and partial collection method was able to measure digestibility differences between diets with and without enzyme supplementation, while the analysis of ileal digesta showed differences which were not statistically significant. Statistical significance ($p < 0.05$) occurred only in digestibility of hemicelluloses as the effect of enzymes supplementation determined by ileal digesta. Digestibility of dry matter, hemicelluloses and energy (ME/GE) was affected by metabolizable energy level, while the effect of enzymes supplementation was significant on digestibility of crude protein, NDF, hemicelluloses and ash determined by total collection method. A significant ($p < 0.01$) interaction between ME level and enzymes supplementation on digestibility of ash and ADF was also observed by this method.

The differences in digestibility of nutrients and energy determined by partial and total collection method were in digestibility of energy (ME/GE), where, except the effect of ME level, the effect of enzyme supplementation was also evident. ME level had a significant effect on digestibility of crude protein determined by total collection method and there was a significant effect of enzymes supplementation on digestibility of crude protein determined by partial collection method.

The digestibility of DM, CP and AME was linearly decreased as reducing level of metabolizable energy in diets in the study of Zhou et al. (2009). Effects of enzymes supplementation were much more obvious in diets with reduced ME in the same study. Some studies showed that improved digestibility and value of AME were greater in lower energy diets than that of higher energy diets

Table 2. Digestibility of nutrient and metabolisability determined by three methods.

Collection method	Diet type	ME	DM	CP	NDF	ADF	Hemicelluloses	Ash
Total collection	13.4	0.837	0.811	0.744	0.355	0.298	0.433	0.480
	13.4+e	0.815	0.790	0.724	0.271	0.089	0.454	0.464
	13.0	0.775	0.739	0.653	0.160	-0.032	0.378	0.259
	13.0+e	0.825	0.807	0.745	0.374	0.223	0.543	0.512
	12.6	0.758	0.719	0.648	0.143	0.138	0.152	0.312
	12.6+e	0.796	0.769	0.716	0.325	0.233	0.454	0.414
Probability	me	0.040	0.042	0.135	0.389	0.331	0.043	0.059
	enzymes	0.142	0.067	0.034	0.037	0.429	0.005	0.006
	me *e	0.116	0.095	0.089	0.310	0.014	0.109	0.025
Partial collection	13.4	0.781	0.774	0.671	0.132	0.058	0.233	0.298
	13.4+e	0.783	0.752	0.660	0.140	-0.074	0.354	0.363
	13.0	0.747	0.707	0.608	0.061	-0.130	0.308	0.167
	13.0+e	0.801	0.723	0.637	0.106	-0.100	0.335	0.305
	12.6	0.715	0.670	0.604	-0.004	-0.012	0.012	0.195
	12.6+e	0.750	0.716	0.651	0.160	0.034	0.337	0.268
Probability	me	0.007	0.035	0.038	0.191	0.106	0.045	0.018
	enzymes	0.020	0.162	0.078	0.017	0.709	0.003	0.005
	me *e	0.220	0.590	0.773	0.079	0.288	0.051	0.528
Ileal digesta	13.4	0.692	0.643	0.680	0.062	-0.031	0.274	0.327
	13.4+e	0.647	0.611	0.639	-0.140	-0.466	0.354	0.373
	13.0	0.610	0.575	0.616	0.023	-0.267	0.345	0.289
	13.0+e	0.632	0.594	0.633	-0.040	-0.562	0.594	0.276
	12.6	0.650	0.617	0.672	-0.075	-0.075	0.089	0.312
	12.6+e	0.668	0.629	0.674	0.140	-0.067	0.213	0.340
Probability	me	0.419	0.448	0.742	0.653	0.099	0.138	0.329
	enzymes	0.952	0.991	0.689	0.719	0.051	0.019	0.572
	me *e	0.602	0.744	0.851	0.300	0.226	0.364	0.793

ME (metabolisability, ME/GE), DM (digestibility of dry matter), CP (digestibility of crude protein), NDF (digestibility of NDF), ADF (digestibility of AD F), hemicelluloses (digestibility of hemicelluloses), ash (digestibility of ash).

(Zhoe et al., 2009; Kocher et al., 2003; Cowan et al., 1996). Enzymes caused the increase in digestibility of poorly digested diets to much bigger extent than well digested diets (Bedford, 2000). This is also the case in our study. Enzyme addition significantly improved ME/GE (determined by partial collection method but not with total collection method) of diets in which we reduced the energy by introducing sunflower meal at various inclusion levels (0, 8, 16%). Cell wall of sunflower seed contained considerable amount of β -glucans, xylana, arabans, pectins, and various other oligosaccharides.

These polysaccharides caused the depression in the productivity of poultry (Senkoylu and Dale, 1999). Enzymes treatments significantly increased digestibility of CP determined by total collection method. Reports regarding effects of enzyme supplementation on digestibility of crude proteins are not very consistent. In some reports, such as Marsman et al. (1997), there was

the effect of enzymes supplementation on digestibility of protein. However, effect was absent in the growth performance. In the work of Zhou et al. (2009), ME level had the effect on protein digestibility. The effect of enzymes supplementation also existed, which is similar to our results. A significant effect of enzyme supplementation on digestibility of protein was absent in diets with a high level of sunflower meal and reduced energy level (Mushtaq et al., 2009). This is not consistent with our data. Results from this study can be partially explained by the fact that chickens benefited more from enzyme supplementation at a younger age and that the contribution of enzymes on digestibility of nutrients and energy decreased with the age of birds (Olukosi et al., 2007). In addition, the effects of enzymes are more obvious under harsh production conditions rather than good animal laboratory conditions.

The important part of digested fiber, which cannot be

digested by endogenous enzymes of digestive tract of broilers, is digested through microflora of cecum. There's not much information on the digestibility of NDF, ADF and hemicelluloses in broilers diets with different ME level. NDF is the estimation of the cell wall carbohydrates which are hemicelluloses, cellulose and lignin and ADF is estimation of cellulose and lignin (hemicelluloses=NDF-ADF). NDF and ADF were not affected by different ME level, but hemicelluloses was. The effect of enzyme supplementation was evident on digestibility of hemicelluloses which is consistent with data from the study of Nian et al. (2011). Vranjes et al. (1994) in digestibility trial with 20% of barley found that there was no effect of enzyme supplementation on digestibility of NDF, ADF or hemicelluloses in broilers diets. By another authors (Cantor et al., 2009) the digestibility of NDF was enhanced by enzyme supplementation in low-energy basal diet. Negative value digestibility of NDF and ADF fraction in ileal digest can be partially explained by the appearance in the chyme of the small amount of these fractions from endogenous secretion, desquamated mucosa cell and microbes or as the effect of separation between the indigestible marker and digesta.

The digestibility of ash is not usually common approach. It most often keeps track of digestibility of calcium or phosphorous. However, some authors still express digestibility through ash and in the works of these authors the usage of multicarbohydase is accompanied by the increase in digestibility (Saleh et al., 2005).

The decrease of ME level decreased digestibility of DM, hemicelluloses, ash and metabolisability (ME/GE) of energy while the digestibility of crude protein and NDF and ADF was reduced but not significantly. The enzyme supplementation increased significantly the digestibility of crude protein, NDF, hemicelluloses and ash, but the effect was most obvious in diets with reduced energy density.

It can be concluded that the ME level affected digestibility of nutrients and energy, and that enzymes supplementation had most profound effect on digestibility of nutrients and energy in diets with reduced ME level.

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