

EFFECTS OF ENZYMES SUPPLEMENTATION ON DIGESTIBILITY AND ENERGY UTILIZATIONS OF BROILERS DIETS WITH DIFFERENT METABOLIZABLE ENERGY LEVEL

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Invited paper

Abstract: 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 enzymes (nutrition density was reduced by introducing sunflower meal in different percentage to diet (0, 8 и 16%)), the goal was to investigate the effects of exogenous enzymes 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 and energy utilization. Effects of enzymes were most visible in diet with 13.00 MJ/kg and 12.6 MJ/kg while this effect was unnoticed in the group with 13.4 MJ/kg. Reducing of ME caused the decrease in digestibility and metabolizability (ME/GE). The results suggest that exogenous enzymes, used in this trial, increase digestibility of crude proteins, NDF, hemicelluloses and ash ($p < 0.05$), but the effects were most evident in diets with the reduced level of metabolizable energy.

Key words: broilers, exogenous enzymes, digestibility,

Introduction

Early beginnings of introducing exogenous enzymes in nutrition of monogastrics animals are from the end of 1940. The first researcher who reported about a positive effect of exogenous enzymes in nutrition with high level of fiber was Hastings 1946, until then scientists believed that introducing exogenous enzymes in nutrition of non ruminant animal wouldn't have any effect after passing upper part of digestive tract. Since then, the concept regarding the usage of exogenous enzymes has been changed significantly, from the usage of only single type of enzyme to the usage of multiple enzymes products which act on divers group of nutrients.

Sunflower meal which can most often be found on our market contains 33% crude protein, and high level of cellulose (21% is the legal limit (*Sl. list SRJ*“, br. 20/2000 i 38/2001)). Cell wall of sunflower seed contains considerable amount of β -glucans, xilana, arabans, pectins, and various other oligosaccharides, this polysaccharides cause the increase of intestinal viscosity and consequently the depression in productivity of poultry (*Senkoylu, 1999*).

The reduction of metabolizable energy level linearly decreases digestibility of DM and AME (*Zhou, 2009*). The effect of the usage of exogenous enzyme in some studies is more visible when metabolizable energy level is reduced (*Zhou, 2008*).

Enzymes have clearly been demonstrated to increase the digestibility of poorly digested diets to a much greater extent than well digested diets (*Classen et al., 1995; Scott et al., 1998*). A researcher concluded that chicks benefited more from enzymes at a younger age (*Olukosi et al., 2007*), but the effect of age was not observed in another trail (*Fuente, 1995*). The effect of added enzymes is mediated through gut flora, whereas in young animals which have poorly developed digestive system, and not fully develop gut flora the effect is probably more direct (*Bedford, 2001; Bedford, 2000*).

The goal of this trail was to investigate effects of added exogenous enzymes 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 energy utilization of broilers diet variably in metabolic energy, of 28-31 days old male Ross 308 broilers. Nutrition density was reduce by introducing sunflower meal with 33% CP in diet in different percent (0,8,16%).

Materials and Methods

A metabolism trail was used to estimate the nutrients digestibility (organic mater, crude protein, fat, crude ash, NDF, ADF, hemicelluloses, crud fiber) and metabolizability (ME/GE). Chicks were reared in the masonry broiler house from 1 to 24 days of age, and than transferred to metabolic cages. A total 96, 24 day old Ross 308 hybrids were allocated randomly to 6 dietary treatments with 4 replicates per treatments, and 4 birds per replicates (three level of metabolic energy: 13.40; 13.00; 12.60 MJ/kg; with or without of an enzyme complex). The 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 metabolic energy were total collection method and partial collection method (one percent of Celit was added to the diet for this purpose). Collected excreta samples were placed in plastic bags, duly identified, 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 were homogenized and dried in a forced-ventilation oven at 55°C for 72 hours. Dried excreta samples were ground 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)*. Crude protein, crude fiber, crude fat were analyzed using Wende method, while NDF, ADF and hemicelluloses (NDF-ADF) were analyzed using the procedure of Van Soest (*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 this two factors. Linear contrast of enzymes effect (i.e. with vs. without enzymes addition) within each diet was made. The results are considered significant when $P < 0.05$.

Table 1. Composition and nutrient analyses of experimental diets

Ingridients	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

Results and Discussion

Metabolizability (ME/GE) and digestibility of nutrients determined by two methods (total and partial excreta collection method) are shown in tables 2 and 3. Enzyme complex had a significant effect ($P < 0.05$) on digestibility of NDF, hemicelluloses, and ash determined by both methods. The metabolizability (ME/GE) decreases with the reduction of energy density of diet. Enzyme

supplementation enhanced metabolizability but the effect of enzyme supplementation wasn't statistically significant ($P>0.05$).

The present study demonstrated that the addition of the multicarbohydase supplement of cell wall degrading enzymes did not improve digestibility of nutrients and energy in diet with not reduced metabolizable energy level. These results are consistent with some other trials conducted so far on the effect of exogenous enzymes in diet with normal nutrition density based on corn and soybean (Dourado et al., 2009; Saleh, 2005). Moreover some trial conducted on the diet in which sunflower meal was concluded up to 20%, with normal nutrition density, also didn't give any improvements in digestibility of nutrients or AME (Tavernari, 20008). The effect of multicarbohydase supplementation is closely connected with ME level by some researchers (Zhou, 2008). The most advanced effect of enzymes in this trial was on diets with reduced nutrition density (13.00 and 12.6 MJ/kg), but most often statistically significant differences between diets with and without enzymes complex were in 13 MJ/kg, determined by the total collection method.

Table 2. Digestibility (%) of organic matter (OM), metabolizability ME/GE (ME), crude protein (CP), NDF, ADF, hemicelluloses (HEM), crude fiber (CF) and crude ash (ASH) determined by total collection method

diet	OM	ME	CP	NDF	ADF	HEM	CF	ASH
13.4MJ/kg								
-	0.835	0.838	0.744	0.355	0.298	0.433	0.878	0.480
+	0.813	0.815	0.724	0.271	0.089	0.454	0.759	0.464
p	0.280	0.318	0.385	0.262	0.066	0.745	0.072	0.698
13.0MJ/kg								
-	0.775	0.775	0.653	0.160	-0.032	0.378	0.824	0.259
+	0.828	0.826	0.745	0.374	0.223	0.543	0.835	0.512
p	0.005	0.005	0.005	0.022	0.048	0.024	0.646	0.003
12.6MJ/kg								
-	0.747	0.759	0.648	0.143	0.138	0.152	0.840	0.312
+	0.793	0.797	0.716	0.325	0.233	0.454	0.849	0.414
p	0.300	0.333	0.249	0.113	0.401	0.062	0.871	0.288
Probability								
diet	0.036	0.040	0.135	0.389	0.331	0.043	0.716	0.059
enzymes	0.115	0.142	0.034	0.037	0.429	0.005	0.219	0.006
d*e	0.119	0.116	0.089	0.310	0.014	0.109	0.094	0.025

Table 3. Digestibility (%) of organic matter (OM), metabolizability ME/GE (ME), crude protein (CP), NDF, ADF, hemicelluloses (HEM), crude fiber (CF) and crude ash (ASH) determined by partial collection method

diet	OM	AME	CP	NDF	ADF	HEM	CF	ASH
13.4MJ/kg								
-	0.777	0.743	0.660	0.132	0.058	0.233	0.837	0.298
+	0.779	0.744	0.671	0.140	-0.074	0.354	0.722	0.363
p	0.893	0.956	0.641	0.859	0.034	0.152	0.055	0.181
13.0MJ/kg								
-	0.750	0.711	0.608	0.061	-0.130	0.308	0.799	0.167
+	0.754	0.721	0.637	0.106	-0.100	0.335	0.826	0.305
p	0.751	0.536	0.230	0.241	0.738	0.688	0.424	0.017
12.6MJ/kg								
-	0.701	0.683	0.565	-0.004	-0.012	0.012	0.814	0.195
+	0.750	0.712	0.651	0.160	0.034	0.337	0.823	0.268
p	0.300	0.275	0.195	0.035	0.688	0.018	0.841	0.273
Probability								
diet	0.033	0.010	0.120	0.191	0.106	0.045	0.370	0.018
enzymes	0.266	0.231	0.075	0.017	0.709	0.003	0.280	0.005
d*e	0.479	0.562	0.372	0.079	0.288	0.051	0.049	0.528

Exogenous enzymes had no effect on digestibility of crude fat in diets with normal energy density as well as in diet with reduced ME. This finding is consistent with the results of other authors (*Saleh et al., 2005; Marsman et al., 1997*).

The digestibility of NDF, hemicelluloses and crude ash was significantly improved by the supplementation with exogenous enzymes determined by both methods, while digestibility of crude protein was significantly improved by enzymes determined by total collection method. The improvement in digestibility of crude protein is well known (*Zanella et al., 1999; Mushtaq et al., 2008; Cantor et al., 2009*). Enzymes need to cleave only a few places in polysaccharide chain to grate the reduction of viscosity and enhance digestibility by softening “cage effect” made by NSP in intestines.

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

NDF and hemicelluloses, fraction which are under direct impact of enzymes supplementation, had a significant improvement in digestibility after enzymes supplementation in all treatments, this is consistent with the data from the study by *Vranjes (1994), Cantor et al. (2009)* and *Nian et al. (2011)*.

Conclusion

Reducing of metabolizable energy level caused decrease in digestibility of nutrients and metabolizability (ME/GE). Results suggest that exogenous enzymes, used in this trial, increase digestibility of crude proteins, NDF, hemicelluloses and ash ($p < 0.05$), but the usefulness were most evident in diets with the reduce level of metabolic energy.

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Efekat egzogenih enzima u ishrani brojlera na svarljivost i metaboličnost u smešama sa različitim nivoom metaboličke energije

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Rezime

U ovom 3x2 faktorijalnom ogledu, dizajniranom sa tri nivoa metaboličke energije (13,4, 13,00, 12,6 MJ/kg), sa i bez dodataka egzogenih enzima (metabolička energija smeša smanjivana je dodavanjem suncokretove sačme u različitom procentu (0, 8 i 16%) cilj nam je bio da ispitamo efekat egzogenih enzima Roxazyme G2G (celulaze (endo-1,4- β -glukanaza), β -glukanaze (endo-1,3(4)- β -glukanaze) i ksilanaze (endo-1,4- β -ksilanaze)) na svarljivost hranljivih materija i metaboličnost. Efekat enzima je bio najuočljiviji u smeši sa 13,00 MJ/kg i 12,6 MJ/kg, dok je kod smeše sa 13,4 MJ/kg efekat izostao. Smanjenje metaboličke energije uzrokovalo je smanjenje svarljivosti i metaboličnosti. Rezultati ukazuju da enzimi, korišćeni u ovom ogledu, povećavaju svarljivost sirovih proteina, NDF, hemiceluloze i pepela ($p < 0,05$), ali efekat je bio najizraženiji u smešama sa smanjenom metaboličkom energijom.

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