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## Performance and Some Intestinal Functions of Broilers Fed Diets with Different Inclusion Levels of Sunflower Meal and Supplemented or Not with Enzymes

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### ■ Keywords

Carcass characteristics, enzyme activity, performance, viscosity.

### ABSTRACT

Enzyme supplementation of diets enhances broiler performance by improving some of the basic production parameters such as average feed intake, feed conversion ratio, or average weight gain. The enzyme NSPase is commonly used in broiler diets containing high levels of viscous cereals such as barley, oat, wheat, or sorghum. The use of NSPase in diets with different levels of sunflower meal has been not been extensively explored. The experiment was carried out to evaluate the effects of the inclusion of sunflower meal levels in grower and finisher broiler diets supplemented or not with enzymes (cellulase,  $\beta$ -glucanase, and xylanase) on broiler performance, intestinal function, and carcass traits. A completely randomized experimental design, with 3\*2 factorial arrangement with five replicates, was applied (1200 Ross 308 broilers). Sunflower meal inclusion and enzyme supplementation started in grower phase. Broiler performance significantly improved in grower phase (weight gain and feed conversion ratio) by enzyme supplementation, while the effect of sunflower meal was evident in finisher phase, when it significantly reduced weight gain. Sunflower meal increased ileal viscosity, and the interaction between diet and enzyme supplementation was statistically significant. Maltase activity was reduced with sunflower meal dietary inclusion, while enzyme supplementation had no effect either on maltase or sucrase activity. There were no any effects of sunflower meal inclusion with or without enzyme supplementation on carcass characteristics. It can be concluded that high inclusion of sunflower meal in broiler diets may impair broiler performance, but this may be overcome by enzyme supplementation. The effect of enzyme supplementation more evident in the grower phase than in the finisher phase.

### INTRODUCTION

Conventional broiler production is based on corn as a source of energy and soybean meal as a source of protein. Sunflower meal is byproduct of the oil industry and, in the Serbian market, it usually contains 33% crude protein. It may be a good protein source for broiler diets, although its use may be limited by its low lysine and high fiber contents (Sredanovic *et al.*, 2005). The use of sunflower meal made from dehulled seeds significantly improves the quality of the meal and reduces its percentage of fiber (Levic *et al.*, 1998). However, this process makes production more expensive and diminishes the competitiveness of sunflower meal as an alternative protein source.

Previous studies investigating the effects of the use of sunflower meal as a replacement for soybean meal show inconsistent results. The inclusion of sunflower meal resulted in worse broiler performance in some studies (Abdelrahman *et al.*, 2007; Peric *et al.*, 2010), whereas



in others, the inclusion of sunflower meal up to 20% (El-Sherif *et al.*, 1997; Tavernari *et al.*, 2008), or at even higher levels (Rama Rao *et al.*, 2006; Mushtaq *et al.*, 2009) did not have any effects on average body weight or weight gain.

One of the first studies on the use of exogenous enzymes as a supplement of animal diets with the purpose of improving dietary nutrition value was performed in the 1940s (Hastings, 1946). Research on this subject achieved its full extension in the 1980s and 1990s in a broad area of biotechnology (Bhat, 2000).

Enzymes that act on non-starch polysaccharides are mainly used in diets based on wheat, barley, or in some cases, corn for its proven performance improvement of monogastric animals (Bedford, 2000).

The first studies with broiler feeds containing high NSP levels, such as barley, wheat or sorghum, reported poor broiler performance and high gut viscosity. There are limited data on the influence of the dietary inclusion of sunflower meal on broiler gut viscosity. Brazilian research studies showed that 15% inclusion of sunflower meal in the diet caused a statistically significant increase in viscosity (Araújo *et al.*, 2011). However, the inclusion of sunflower meal up to 35% in broiler diets did not increase viscosity either in the jejunum or in the ileum. The addition of an exogenous enzyme also had no effects (Kocher *et al.*, 2000).

The activity of two important disaccharidases (maltase and sucrase) is influenced by nutrition (Shakouri *et al.*, 2008; Yang *et al.*, 2008; Zdunczyk *et al.*, 2009). In some studies, the addition of exogenous enzymes that enhance nutrient digestibility by reducing viscosity or increasing nutrient availability in the gastrointestinal tract increased activity of maltase or sucrase or both (Fan *et al.*, 2008; He *et al.*, 2010), while in other studies, the effect of addition of exogenous enzyme did not cause any changes in the activities of maltase or sucrase (Shakouri *et al.*, 2008; Yang *et al.*, 2008).

## **MATERIAL AND METHODS**

### **Birds and experimental treatments**

The study was conducted in floor pens in an environmentally controlled poultry house, on the experimental farm of the School of Agriculture in Novi Sad, Republic of Serbia. A total of 1200 day-old Ross 308® broilers were distributed in six groups with five replicates each, with 40 (as hatched) birds per pen. Birds were fed *ad libitum*.

The control diet did not include sunflower meal (SFM). In the first treatment, birds were fed diets with

sunflower meal inclusion at 6% in the grower and 10% in the finisher phases, respectively, and the second treatment, diets contained sunflower meal (SFM) at 8% in the grower and 16% in the finisher periods, respectively. Both the treatment and the control diets were supplemented or not with an enzyme blend (cellulase,  $\beta$ -glucanase, and xylanase) at 0.01% in both stages.

Bird body weight and feed intake were weekly monitored.

This experiment was conducted in accordance with the guide of EU directive for the protection of animals used for scientific purposes.

This study was the part of the technology project N° 31033, which was funded and approved by the appropriate committees of the Republic of Serbia, and registered under n. 401-00-9/2011-01 in January 25, 2011.

### **Viscosity**

At the end of the experiment (day 42), four birds per replicate were randomly selected and sacrificed. Euthanasia was performed according to the standard procedure used in processing plants. Birds were immediately dissected to obtain intestinal digesta and gut (jejunum and ileum) samples. The collected digesta samples (approximately 2g) were centrifuged at 6000 rpm for 10 min. The obtained supernatant (0.5 ml) was used to determine viscosity. A HAAKE Mars rheometer (Thermo Scientific, Karlsruhe, Germany) was used to perform rheological measurements using C35/2 cone and plate measuring geometry (diameter: 35 mm, cone angle: 2°). Samples were allowed to rest for 5 min after loading. The flow curves were obtained by recording shear stress at shear rates from 0 to 500 s<sup>-1</sup> in 180 s, at 25 ± 0.1 °C. All the examined samples exhibited Newtonian flow behavior and thus were characterized by a single coefficient of viscosity.

### **Enzyme assay**

The samples of the jejunum and the ileum were obtained immediately after evisceration, weighed, labeled, and stored in liquid nitrogen at -80 °C until analyses. After thawing and homogenization of the samples, disaccharidase activity was determined according to the method of Dahlqvist (1984). Shortly, the amount of the released glucose was measured spectrophotometrically (UV PG Instruments T80) at 505 nm, and enzyme activity was expressed as unit per minute per gram of protein. One unit of disaccharidase activity hydrolyzed 1  $\mu$ mol of disaccharide/min at 39 °C. Protein content was determined by the method of biuret.



**Table 1** – Composition of the experimental diets.

Ingredients	Treatment					
	Control		I		II	
	grower	finisher	grower	finisher	grower	finisher
Corn	56.09	62.40	52.80	56.89	51.91	53.47
Soybean oil	0	0	0	2	0	3.24
Soybean meal	21.56	17.07	13.09	10.61	10.22	6.77
Full-fat soybeans	17.62	16.00	23.35	16.01	25.00	16.00
Sunflower meal	0	0	6	10	8	16
L-lysine HCL	0.12	0.12	0.18	0.22	0.30	0.28
DL-Methionine	0.26	0.22	0.24	0.20	0.24	0.19
Limestone	1.54	1.38	1.53	1.25	1.52	1.23
Monocalcium phosphate	1.38	1.45	1.38	1.48	1.39	1.49
Salt	0.43	0.36	0.43	0.34	0.42	0.33
Premix	1.00	1.00	1.00	1.00	1.00	1.00
Calculated composition						
Crude protein,%	21.00	19.00	21.00	19.00	21.00	19.00
ME, MJ/kg	12.60	12.80	12.60	12.80	12.60	12.80
Lysine,%	1.24	1.09	1.24	1.09	1.24	1.09
Methionine and cystine,%	0.95	0.86	0.95	0.86	0.95	0.86
Ca,%	0.95	0.95	0.95	0.85	0.95	0.85
P (available),%	0.45	0.42	0.45	0.42	0.45	0.42

### Carcass traits

At the end of feeding trial, two birds (with the average body weight of the pen) per pen, totaling twelve birds per replicate, were sacrificed and eviscerated. Carcass, breast, thigh and drumstick, and abdominal fat weights were recorded and expressed as a percentage of live weight.

### Data analysis

Data were analyzed by two-way analysis of variance using StatSoft software (STATISTICA 8, 2009) to determine the effects of enzyme addition, diet type, and the interaction between these two factors. The results were considered significant when  $p < 0.05$ . Enzyme (maltase, sucrase) activity data were logarithmically transformed prior to the analysis of variance.

## RESULTS

### Growth performance

Performance response of the broilers fed diets with different sunflower meal levels and with or without enzyme supplementation during the grower and finisher phases are presented in Table 2. The effects of enzyme supplementation on weight gain were evident in the grower phase. The inclusion of sunflower meal at 6 and 8% in grower diet had no effect on growth performance, but at 10% and 16% in finisher diet, weight gain was significantly affected. Weight gain statistically improved with dietary enzyme

supplementation during the entire experimental period. Neither sunflower meal nor enzyme addition had any effect on feed intake (g/day).

### Enzyme activity and gut viscosity

The results of digestive enzyme activities and gut viscosity are summarized in Table 3. Diet statistically reduced maltase activity ( $p = 0.0162$ ), but not sucrose activity. The effect of region was statistically significant for maltase and sucrose ( $p < 0.001$ ;  $p = 0.0005$ ).

The high level of sunflower meal inclusion (16%) increased gut viscosity, particularly in the ileum. The effects of diet and of the interaction between diet and enzyme on gut viscosity was significant ( $p = 0.001$ ).

The effects of sunflower meal inclusion and enzyme supplementation on carcass traits (dressing percentage, and breast, thigh and drumstick and abdominal fat yields) are shown in Table 4. There was no significant effect of sunflower meal or enzyme supplementation on the evaluated parameters.

## DISCUSSION

Dietary sunflower meal inclusion reduced weight gain in the finisher phase, but not in the grower period. This can be explained by the relatively small amount of sunflower meal in diet (6% and 8%) in grower phase. However, some authors have reported the influence of the inclusion of sunflower meal at 5% and 8% in grower diets on broiler average body weight (Peric et al., 2010).



**Table 2** – Effects of different dietary inclusion levels of sunflower meal and of enzyme supplementation on feed intake, body weight gain, and feed conversion ratio of broilers.

period	Treatment				enzyme		SEM	Probability		
	days	control	I	II	-	+		Diet	enzyme	D*E
Feed intake (g/bird)										
grower	14-21	578	592	571	584	577	8.37	0.608	0.725	0.952
	22-28	911	897	910	897	910	10.22	0.660	0.554	0.882
finisher	29-35	1200	1180	1216	1182	1214	12.24	0.510	0.231	0.700
	36-42	1279	1274	1283	1269	1289	13.88	0.986	0.507	0.377
	14-42	3970	3939	3980	3934	3992	29.89	0.835	0.326	0.691
Weight gain (g/bird)										
grower	14-21	377	411	384	380	402	12.26	0.539	0.412	0.979
	22-28	604	568	592	573	603	8.63	0.225	0.049	0.943
finisher	29-35	585	576	571	584	571	9.82	0.759	0.456	0.005
	36-42	583	505	552	535	558	5.50	0.012	0.227	0.667
	14-42	2149	2060	2099	2071	2134	16.87	0.062	0.039	0.290
Feed conversion ratio										
grower	14-21	1.492	1.433	1.511	1.517	1.441	0.03	0.551	0.226	0.929
	22-28	1.512	1.544	1.587	1.582	1.514	0.02	0.267	0.049	0.650
finisher	29-35	2.047	2.036	2.061	2.006	2.090	0.03	0.933	0.159	0.026
	36-42	2.309	2.561	2.376	2.443	2.386	0.04	0.027	0.437	0.781
	14-42	1.850	1.913	1.896	1.901	1.871	0.02	0.271	0.362	0.562

The effect of the use of exogenous enzymes on feed conversion rate and growth rate in this trial was evident in the grower phase, but not in the finisher phase. Results on the use of exogenous enzyme in broiler diets are contradictory. Some researchers undoubtedly point out that the increase in body weight gain is due to the effect of the use of carbohydratases (Mathlouthi *et al.*, 2002; Francesch & Geraert, 2009; Hajati, 2010). Other authors reported an increase in diet digestibility, but not in broiler performance (Marsman *et al.*, 1997; Kocher *et al.*, 2000; Preston *et al.*, 2000), whereas some did not observe any effect (Aftab, 2009).

Sunflower meal had no effect on feed conversion ratio. Literature studies reported that high levels of sunflower meal inclusion in grower and finisher broiler diets (up to 20%) had no effect on feed conversion ratio (El-Sherif *et al.*, 1997; Furlan *et al.*, 2001; Tavernari *et al.*, 2008; Aftab, 2009; Peric *et al.*, 2010). Moreover, some researchers found that the highest level sunflower meal inclusion (20%) in the diet improved feed conversion ratio which was explained by the fact that the oil inclusion level was increased in order to supply birds' energy needs (Tavernari *et al.*, 2008).

In this study feed conversion ratio improved with the supplementation of exogenous enzymes to the grower

diets, indicating that digestibility was enhanced. Some studies reported stronger effects of enzyme addition on nutrition digestibility and feed conversion ratio in younger broilers (up to three weeks of age) (Shakouri *et al.*, 2008). The digestive tract of young broilers has not reached its maximum capacity, which may explain those effects.

Sunflower meal significantly increased digesta viscosity in the ileum, while enzyme supplementation decreased digesta viscosity only in the treatments with sunflower meal inclusion. Viscosity increased from the proximal to the distal digestive tract. This is probably due to the effect of the concentration of compounds that produce viscosity during the process of digestion or perhaps due to the increased hydration of those compounds (Boros *et al.*, 1998). The main effect of enzyme supplementation on viscosity was not observed. However, the interaction between enzyme and diet was statistically significant, which indicates that the enzymes reduced digesta viscosity only when the diet with the high sunflower meal inclusion was fed, and therefore, when the diet contained higher NSP levels. The supplementation of the enzyme glucanase in barley-based diets fed to chickens of two different ages (21-day-old broilers and 1-year-old roosters)





**Table 3** – Effects of different dietary inclusion levels of sunflower meal (SFM), of intestinal segment (jejunum or ileum), and of enzyme supplementation on sucrose and maltase activities (U/g proteins) and viscosity (mPa\*s).

	Sucrase activity, U/g	Maltase activity, U/g	Viscosity, mPa*s
Jejunum			
control	21.66	138.10	2.395
Treatment I	16.84	137.36	3.059
Treatment II	15.86	106.06	2.518
enzyme			
-	19.03	139.62	2.92
+	17.46	117.79	2.461
Ileum			
control	15.22	78.71	2.336
Treatment I	9.95	67.41	2.513
Treatment II	7.26	49.28	7.214
Enzyme			
-	6.28	68.51	4.89
+	15.35	67.76	3.407
SEM	1.49	7.04	0.072
Probability			
Diet	0.151	0.0162	0.001
Enzyme	0.227	0.292	0.135
Region	0.0005	0.0000	0.018
Diet*enzyme	0.954	0.404	0.010
Diet*region	0.781	0.796	0.000
Region*enzyme	0.141	0.589	0.592
D*E*R	0.535	0.252	0.341

Enzyme activity was expressed as unite per minute per gram of protein.

reduced digesta viscosity in both periods. However, diet digestibility significantly increased in younger birds, suggesting that the effect of enzyme supplementation was not only due to viscosity reduction (Almirall *et al.*, 1995).

Although enzyme supplementation reduced digesta viscosity in the finisher phase in the present study, it did not improve broiler performance, especially weight gain and feed conversion ratio. The improvement was evident, but not statistically significant.

The dietary supplementation of exogenous enzyme enhanced diet digestibility possibly because it promoted an increase in the activity of digestive enzymes by increasing the availability of substrates. When adding exogenous amylase and protease to broiler diets, researchers reported higher activity of pancreatic and intestinal enzymes measured in 14- and 42-d-old broilers, especially in the younger birds (14 days) (Pinheiro *et al.*, 2004). Diets based on barley, maize, sorghum, and wheat significantly affected the activity of intestinal enzymes, while enzyme supplementation did not (Shakouri *et al.*, 2008). According to another study (Zdunczyk *et al.*, 2009), maltase and sucrase activity was reduced when turkeys were fed diets with high fiber levels. The lower intestinal enzyme activity due to the effect of diet observed in the present study may be explained by the higher fiber level the diets with high sunflower meal content of the finisher diets.

Enzyme activity was reduced from the proximal to distal part of intestine. These results are in agreement with the previous studies (Cavides-Vidal *et al.*, 2000; Uni *et al.*, 1999).

Carcass and parts traits determine the purchase decision of chicken meat consumers. Neither sunflower meal nor exogenous enzymes had any effect on carcass traits in this trial. These results are in agreement with reports from other authors, who also did not find any effects of sunflower meal or exogenous enzymes on carcass traits (Tavernari *et al.*, 2008; Mushtaq *et al.*, 2009). In the work of Seleh *et al.* (2005), the addition of cellulase to a broiler diet based on corn and soybean meal significantly reduced abdominal fat. The authors concluded that cellulase affected fat metabolism in an unknown way. However, some studies suggest that enzyme supplementation may improve carcass yield (Omojola & Adesehinwa, 2007). The use of NSPase in diets with a high sunflower meal inclusion levels

**Table 4** – Effects of different dietary inclusion levels of sunflower meal and of enzyme supplementation on carcass traits of broilers.

Treatment	enzyme					Probability		
control	I	II	-	+	SEM	Diet	Enzyme	D*E
Dressing percentage (%)								
83.64	82.54	82.66	82.70	83.19	0.3	0.275	0.418	0.577
Breast (%)								
27.60	27.11	26.40	26.74	27.32	0.3	0.187	0.275	0.674
Thigh and drumstick (%)								
21.41	21.40	22.20	21.51	21.83	0.2	0.119	0.358	0.911
Abdominal fat (%)								
1.61	1.60	1.57	1.64	1.55	0.07	0.984	0.612	0.998

Carcass, breast, thigh and drumstick, and abdominal fat weights were recorded and expressed as a percentage of live weight.



resulted in better carcass yield (Khan *et al.*, 2006), while in the study of Hajati (Hajati *et al.*, 2010), there were evident effects on some carcass traits. However, in a broader context, carcass quality was not significantly affected by the use of enzymes.

It can be concluded that high inclusion levels of sunflower meal in broiler diets may impair broiler performance, but this effect can be overcome by enzyme supplementation. The effects of enzyme supplementation are stronger during the grower phase, and are much less obvious in the finisher phase.

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