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Research Article

Effect of Multi-enzyme Produced By a Single Fungus on Growth Performance and Some Carcass Parameters of Broiler Chicks Fed on Maize-Soya Based Diets

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Abstract

The present study was conducted to test whether a multi-enzyme produced by a fungus would keep performance of broiler chicks receiving a diet with almost 3% less nutrients (energy, protein, amino acids, calcium and phosphor) under two different housing density. In both experiments, positive control received standard broiler diet, negative control received a diet with 3% less nutrients than standard broiler diet and enzyme group received the negative control diet+supplemental enzyme. In the first trial with 1000, day-old chicks housed 10 birds/m^2, only 1% improvement in feed conversion efficiency and body growth was achieved with enzyme supplementation in contrast to the negative control at the end of 35 days feeding period. In the second trial with 1200, day-old-chicks accommodated 16 birds/m^2. The results showed that enzyme improved broiler performance almost 3.5% in contrast to the performance obtained from the negative control. In conclusion, supplementing broiler diets based on maize/soya with a multi-enzyme produced by a single fungus provides a great potential to gain the gap created by formulating almost 3% nutrients reduction in broilers housed with 16 birds/m^2 density. However, providing high comfort and better rearing condition as the birds at 10 birds/m^2 density, positive effects of the enzyme was limited.

Keywords: *Enzyme, Broiler, Body growth, Feed conversion efficiency, Rearing density*

Bir Mantar Tarafından Üretilen Multi-enzimin Mısır-Soya Bazlı Rasyonla Beslenen Broyler Civcivlerinin Büyüme Performansı ve Karkas Parametrelerine Etkisi

Öz

Mevcut çalışma, 35 günlük bir besi döneminde iki farklı kümes yoğunluğunda, yaklaşık %3 daha az besin (enerji, protein, aminoasit, kalsiyum ve fosfor) içerikli yem tüketen broyler civcivlerde bir mantar tarafından üretilen multi-enzimin performansı koruyup koruyamayacağını test etmek için yapılmıştır. Her iki çalışmada, pozitif kontrol standart broyler yemi, negatif kontrol standart broyler yeminden %3 daha az besin maddesi içeren yem ve enzim grubu negatif kontrol yemi+takviye enzim tüketmiştir. İlk denemede günlük yaşta 1000 civciv 10 civciv/m^2 şeklinde yerleştirilmiş, deneme sonunda enzim takviyesiyle negatif kontrole zıt şekilde yalnızca yemden yararlanma oranı ve vücut gelişiminde %1'lik iyileşme sağlanmıştır. İkinci denemede günlük yaşta 1600 civciv 16 civciv/m^2 şeklinde yerleştirilmiştir. 35 gün sonunda elde edilen sonuçlar enzim takviyesinin negatif kontrolden elde edilen sonuçlara zıt şekilde broyler performansını yaklaşık %3.5 iyileştirdiğini göstermiştir. Sonuç olarak, mısır/soya bazlı broyler rasyonlarına bir mantar tarafından üretilen multi-enzim ilavesi 16 civciv/m^2 yoğunlukta yerleştirilmiş broylerlerde yem formulasyonundan doğan %3 besin maddesi boşluğunu doldurmak üzere büyük potansiyel sağlamıştır. Ancak, yüksek konfor ve 10 civciv/m^2 yoğunluğu gibi daha iyi yetiştirme şartlarında, enzimin olumlu etkisi sınırlandırılmıştır.

Anahtar sözcükler: *Broyler, Büyüme performansı, Yemden yararlanma etkinliği, Enzim, Yerleşim sıklığı*

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INTRODUCTION

The use of exogenous enzymes to enhance nutrient availability of animal feeds has been reported as back as 1925 [1]. The commercial application of feed enzymes as a feed additive however has a history of less than 30 years. Over the last two decades, many studies have been conducted to determine effects of exogenous enzymes and their potential use in poultry nutrition [2-17]. The main focus of those studies with regards to monogastric nutrition was for the use of non-starch polysaccharide (NSP) -degrading enzymes such as β-glucanase and xylanase in barley, rye and wheat, in an attempt to alleviate the problems associated with increased digesta viscosity caused by the use of such viscous grains in animal diets.

In fact, formulating poultry diets based on their indigestible components has been a common concept [18]. Maize is considered a homogenous commodity that is highly digestible for broilers [19], although some reports have found considerable variation between samples [20] and others that maize starch digestibility may be as low as 85% at the terminal ileum [21]. This may be due to the fact that some forms of starch cannot be degraded by endogenous carbohydrases [19] because of their different chemical structure and physical properties [22]. Such starch is called as resistant starch and presents the opportunity for the use of exogenous feed enzymes in poultry diets [23].

As the second main ingredient of poultry feeds, soya is considered a good source of protein and amino acids and is probably the most popular vegetarian protein source used in broiler feeds. There is, however considerable variability for digestibility between soybean meal samples. Therefore, corn-soya based poultry diets are suggested to be supplemented with exogenous feed enzymes not as a pro-nutrient, but rather as a means of improving homogeneity and digestibility.

In practice, exogenous enzymes in poultry diets are used in three ways. Firstly, it could be used as a feed additive with a potential nutritional (protein, amino acids, energy etc.) value contributing the diet on top, secondly, reducing the nutrient (protein, amino acids, energy etc.) content of diet up to a potential amount which would be gained by supplemental enzyme, and thirdly, on top without calculating any nutritional contribution. It has practically been claimed that most of NSP-enzymes used in broiler diets provide an almost 2-3% improvement of feed digestibility on average, meaning a significant feed cost reduction without impairing bird performance.

The present study was conducted to evaluate the effects of dietary supplemental multi-enzyme produced by a single fungus *(Talaromyces versatilis)* in maize-soya diets with reduced nutrient contents (energy, protein, amino acids, calcium and phosphor) according to its potential enhancement (almost 3%) on the growth performance, mortality, carcass weight, yield and abdominal fat weight of broiler chicks fed on maize-soya based diets under our experimental condition in two consecutive trials differing in animal density during the rearing period from day 0 to 35.

MATERIAL and METHODS

The present study comprising two trials differing in animal density were carried out in the Broiler Unit of Experimental Farm of the Department of Animal Science, Faculty of Agriculture, University of Çukurova-Turkey. All the protocols used in this experiment are approved by the Animal Experiments Local Ethics Committee of Çukurova University, Adana-Turkey.

Day old broiler chicks (ROSS 308) were used in both trials. The first experiment was conducted using one thousand chicks (500 male and 500 female) accommodated in 20 pens with a density of 10 birds/m^2 for 35 days. While the second experiment was run with a density of 16 birds/m^2 using 1200 (600 males and 600 females) chicks up to 35 days of age. A conventional feeding programme was applied during the both experiments, in which standard starter (days 0 to 10), grower (days 11-21) and finisher (days 22 to 35) diets were used. All diets were based on corn and soya. Ingredient and nutritional composition of the diets used in Experiments 1 and 2 are given in *Table 1* and *Table 2,* respectively.

As the possible effect(s) of supplemental enzyme (50 g/ton feed) on enhancing nutrient value of the diet is known to be varied according ingredient content of the diet, potential reduction in nutrients contents of the experimental diet was determined using Predictor of Rovabio-Advance [25], in which the ingredient composition and consumption level of each diet of each trial were loaded then the Predictor calculated what the reduction level as same as the levels gained by supplemental enzyme should be for energy, protein, digestible amino acids, calcium and available phosphorus for each diet used in Trials 1 and 2 *(Table 3)*.

At the beginning of the trials all the chicks were weighed, sexed and grouped with a similar mean weigh and same number of each sex, then accommodated in 20 pens in a completely randomized design with 3 treatments with 6 or 7 replicates *(Table 4)* each. Each pen (replicate) was equipped with a tube feeder and water drinkers on litter; wood shaving litter 7-8 cm height. Chicks were fed 35 days *ad libitum* under a commercial rearing condition (23:1 light: dark photoperiod, thermoneutral temperature). In the first trial total 50 chicks (25 females+25 males) were accommodated in each pen sized 5 m^2 (2x2.5m; having 10 birds/m^2), while in the second trial total 60 chicks (30 females+30 males) were housed in each pen sized 3.75 m^2 (1.875 x 2.0m; having 16 birds/m^2 as applied by the industry).

Table 1. *Ingredient and nutritional compositions of the diets used in Experiment 1*

Ingredients (%)	Standard (for control diets)			Reduced Nutrient Contents (for negative control diets)		
	Starter (0-10d)	Grower (11-21d)	Finisher (22-35d)	Starter (0-10d)	Grower (11-21d)	Finisher (22-35d)
Yellow corn	48.49	51.33	46.30	52.06	53.26	49.76
Fullfat soya	23.41	23.00	26.00	14.66	22.36	26.00
Soybean meal (46% CP)	16.29	10.00	-	21.80	9.84	-
Soybean meal (44% CP)	-	-	5.83	-	-	4.20
Rice (feed grade)	-	-	5.00	-	-	5.00
Maize gluten meal (60% CP)	2.85	3.22	-	2.63	2.60	-
Meat bone meal (35% CP)	2.00	2.00	2.50	2.00	2.00	2.50
Poultry offal meal	2.00	2.00	2.50	2.00	2.00	2.50
Sunflower meal (34% CP)	2.00	3.00	4.00	2.00	3.00	4.00
DDGS (Corn)	-	2.00	3.00	-	2.00	3.00
Soya Oil	-	0.99	3.07	-	0.50	1.26
Marble powder	0.80	0.76	0.59	0.70	0.75	0.59
DCP (18% P)	0.60	0.30	-	0.62	0.31	-
DL-Methionine	0.33	0.27	0.27	0.33	0.27	0.25
Sodium bicarbonate	0.33	0.27	0.18	0.33	0.27	0.18
L-Lysine	0.31	0.32	0.22	0.30	0.30	0.23
L-Threonine	0.12	0.09	0.06	0.11	0.08	0.05
Common salt	0.17	0.15	0.18	0.16	0.16	0.18
Trace Mineral Premix*	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin Premix**	0.10	0.10	0.10	0.10	0.10	0.10
Choline-60	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat (Sacox)	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrients (%)						
ME-Poultry (MJ/kg)***	12.55	12.97	13.39	12.17	12.59	13.01
Dry matter	88.37	88.35	88.05	88.06	88.20	87.98
Crude protein	24.24	22.46	20.37	23.68	21.90	19.85
Crude fibre	3.71	3.74	4.05	3.63	3.75	4.05
Crude fat	7.83	8.42	10.42	5.60	7.85	8.69
Crude ash	5.79	5.25	4.88	5.69	5.22	4.84
Ca	1.00	0.90	0.85	1.00	0.90	0.85
Available P	0.49	0.44	0.43	0.49	0.44	0.42
Na	0.18	0.17	0.16	0.18	0.17	0.16

** Each kg of vitamin premix contains 13.500.000 IU Vit. A, 4.000.000 IU Vit. D_3, 100.000 mg Vit. E, 5.000 mg Vit. K_3, 3.000 mg Vit. B_1, 8.000 mg Vit. B_2, 60.000 mg Niacin, 18.000 mg Ca-D-Pantotenate, 5.000 mg Vit. B_6, 30 mg Vit. B_{12}, 2.000 mg Folic Acid, 200 mg D-Biotin and 100.000 mg Vit. C*
*** Each kg of trace mineral premix contains 100.000 mg Manganese, 80.000 mg Iron, 80.000 mg Zinc, 8.000 mg Copper, 200 mg Cobalt, 1000 mg Iodine, 150 mg selenium (sodium selenite), 500.000 choline chloride*
**** ME value of the diet is calculated by individual ME value of each feedstuff [24] multiple by its ratio in the diet*

During the experiment, body weight, feed intake, feed conversion efficiency and mortality were recorded on days 7, 14, 21, 28, 35 on subgroup (replicate) bases. At the end of the trial on day 35 all the birds were weighed, 10 birds were sampled for each subgroup, then total 200 birds were transferred to a commercial slaughter house to slaughter for carcass analyses. Live weight, slaughter weight, hot and cold carcass weight, carcass yield (100 x [cold carcass weight/slaughter weigh]) abdominal fat (consists of a mass of adipose tissue located in the abdominal cavity adjacent to the pelvic bones; [26]) weight were measured. Feeds used in the trial were analysed for dry matter, ether extract, crude ash, crude protein and crude fibre according to AOAC [27] procedure.

Table 2. *Ingredient and nutritional compositions of the diets used in Experiment 2*

Ingredients (%)	Standard (for control diets)			Reduced Nutrient Contents (for negative control diets)		
	Starter (0-10d)	Grower (11-21d)	Finisher (22-37)	Starter (0-10d)	Grower (11-21d)	Finisher (22-37d)
Yellow corn	41.32	46.70	43.64	45.88	50.50	48.30
Fullfat soya	24.00	18.00	25.00	16.18	16.01	25.00
Soybean meal-44	15.23	15.04	6.40	20.22	16.09	4.98
Wheat shorts	0.00	0.00	6.00	0.00	0.00	6.00
Corn gluten meal-60	6.82	3.93	0.00	3.52	2.41	0.00
Corn flour	3.00	3.00	3.00	3.00	3.00	3.00
Meat & bone meal-35	3.00	2.50	2.73	3.00	2.50	2.58
DDGS (Golden)	2.00	3.00	3.00	2.50	3.00	3.00
Sunflower meal-34	2.00	3.00	5.00	3.00	3.00	4.00
Marble powder	0.70	0.69	0.60	0.69	0.69	0.67
Soybean oil	0.00	2.34	3.24	0.00	1.00	1.08
DCP-18	0.37	0.28	0.00	0.41	0.30	0.00
L-Lysine	0.32	0.31	0.24	0.32	0.29	0.24
DL-Methionine	0.29	0.29	0.29	0.32	0.30	0.29
Sodium bicarbonate	0.26	0.22	0.16	0.26	0.22	0.17
Common Salt	0.17	0.18	0.20	0.17	0.18	0.20
L_Threonine	0.11	0.12	0.10	0.13	0.11	0.09
Trace Mineral Premix*	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin Premix**	0.10	0.10	0.10	0.10	0.10	0.10
Toxin Bindere (Unike Plus)	0.10	0.10	0.10	0.10	0.10	0.10
Choline-60	0.06	0.05	0.05	0.05	0.05	0.05
Coccidiostat (Sacox)	0.05	0.05	0.05	0.05	0.05	0.05
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Nutrient (%)						
ME-Poultry (MJ/kg)***	12.55	12.97	13.39	12.17	12.59	13.01
Dry matter	88.56	88.23	88.53	88.02	87.88	88.12
Crude protein	25.71	22.51	19.96	24.00	21.60	19.22
Crude fiber	3.87	3.88	4.01	4.07	3.93	3.85
Crude fat	7.05	8.31	10.53	5.69	6.70	8.49
Crude ash	6.04	5.52	5.16	6.04	5.50	5.07
Ca	1.00	0.90	0.83	1.00	0.90	0.83
Available P	0.49	0.44	0.41	0.49	0.44	0.40
Na	0.18	0.17	0.16	0.18	0.17	0.16

** Each kg of vitamin premix contains 13.500.000 IU Vit. A, 4.000.000 IU Vit. D_3, 100.000 mg Vit. E, 5.000 mg Vit. K_3, 3.000 mg Vit. B_1, 8.000 mg Vit. B_2, 60.000 mg Niacin, 18.000 mg Ca-D-Pantotenate, 5.000 mg Vit. B_6, 30 mg Vit. B_{12}, 2.000 mg Folic Acid, 200 mg D-Biotin and 100.000 mg Vit. C, Phytase (500 U/kg)*
*** Each kg of trace mineral premix contains 100.000 mg Manganese, 80.000 mg Iron, 80.000 mg Zinc, 8.000 mg Copper, 200 mg Cobalt, 1000 mg Iodine, 150 mg selenium (sodium selenite), 500.000 choline chloride*
**** ME value of the diet is calculated by individual ME value of each feedstuff [24] multiple by its ratio in the diet*

The data obtained in the study were analysed using GLM procedure of SAS; the Statistical Analysis System [28] and Duncan's New Multiple Range Test in SAS were used to identify significant differences among treatments means.

RESULTS

The results obtained in the experiments 1 and 2 are presented according to the statistical analyses, given in the relevant tables and discussed in the frame of present literature.

Table 3. *Calculation of potential reduction in nutrient contents of the experimental diets containing 50 grams enzyme over its define matrix value per kg using Predictor for Experiments 1 and 2*

Nutrients	Experiment 1		Experiment 2	
	Nutritional Up-Lift Potential of 50 g Enzyme	Matrix Value of Each kg Enzyme	Nutritional Up-Lift Potential of 50 g Enzyme	Matrix Value of Each kg Dietary Enzyme
ME-Poultry (kcal/kg)	92.8	1.855.499	93.3	1.866.555
Crude Protein	0.530	10.567	0.550	11.045
Dig. Lysine	0.029	579	0.032	641
Dig. Methionine	0.005	94	0.007	136
Dig. Cystine	0.012	241	0.013	266
Dig. Meth. + Cyst.	0.017	335	0.020	401
Ca	0.025	588	0.024	478
Available P	0.029	500	0.028	563

Table 4. *Treatment groups and replicates number used in the Experiments 1 and 2*

Treatment Groups	No of Replicates (subgroups)	Treatment Groups	Enzyme (50 g/ton)
1	6	Positive Control-standard diet	NO
2	7	Negative control (reduced nutrient contents) diet	NO
3	7	Negative control + Enzyme supplemented diet*	YES

Table 5. *Effect of multi-enzyme produced by a single fungus on feed intake (g/bird) of broilers in Experiment 1*

Age (day)	Treatment Groups			SED	P=
	Positive Control	Negative Control	Negative Control + Enzyme		
7	143.6^{b}	147.3^{ab}	148.9^{a}	0.862	0.072
14	544.5^{b}	558.6^{ab}	566.3^{a}	3.086	0.034
21	1269	1275	1302	6.993	0.147
28	2357	2328	2365	11.14	0.362
35	3544	3560	3560	15.22	0.878

SED: *Standard error of difference between means;* ^{a,b} *means in the same row with different letters are significantly different (P<0.05)*

The results obtained in the experiment 1 with respect to feed intake, body weight gain, feed conversion efficiency, slaughter weight and carcass parameters and mortality are presented in *Tables 5, 6, 7, 8* and *9* respectively.

During the first two weeks of the experiment 1, the birds of the groups receiving reduced nutrient contents (negative control and/or negative control + enzyme) consumed feed higher amount than the birds of positive control *(Table 5)*. However, from the week 3 and later, all birds attained similar feed intake without any significant difference (P>0.05). The results with respect to feed intake during the first two weeks suggest that the birds receiving diets with reduced nutrient contents tried to compensate nutrient intake by increasing feed consumption for the period in which broilers grow fast and almost increased their body weight 12 folds.

At the beginning of the experiment 1, all the groups had similar body weight. Throughout the experiment, all the groups had similar growth performance. At the end of the experiment 1, the group receiving positive control diet achieved the best performance. The groups receiving supplemental enzyme with negative control diet had a performance, which was 1% better than the group receiving negative control diet but 0.5% lower than the group receiving positive control diet *(Table 6)*.

Similar to body weight gain, feed conversion efficiency was also not affected by enzyme significantly during the first four weeks of the experiment 1 (P>0.05). However, the cumulative data obtained at the end of the experiment showed that the group receiving positive control diet attained the best performance. Their feed conversion rate was found to be numerically better than that of the group receiving negative control diet with enzyme, but significantly better than that of the group receiving only negative control diet. 3% reduction in nutrient content of

Table 6. *Effect of multi-enzyme produced by a single fungus on body weight gain (g/bird) of broilers in Experiment 1*

Age (day)	Treatment Groups			SED	P=
	Positive Control	Negative Control	Negative Control + Enzyme		
Initial Body Weight (g/bird)	44.4	44.4	44.8	0.099	0.189
7	128	130	129	0.927	0.630
14	459	470	473	3.118	0.210
21	1014	1004	1008	4.045	0.609
28	1753	1702	1727	9.433	0.165
35	2479	2442	2467	10.69	0.371

SED: *Standard error of difference between means*

Table 7. *Effect of multi-enzyme produced by a single fungus on feed conversion efficiency (g intake : g gain) of broilers in Experiment 1*

Age (day)	Treatment Groups			SED	P=
	Positive Control	Negative Control	Negative Control + Enzyme		
7	1.12	1.13	1.15	0.007	0.352
14	1.19	1.18	1.19	0.007	0.772
21	1.25	1.26	1.29	0.007	0.127
28	1.34	1.36	1.36	0.005	0.239
35	1.43^{b}	1.45^{a}	1.44^{ab}	0.004	0.081

SED: *Standard error of difference between means;* ^{a,b} *means in the same row with different letters are significantly different (P<0.05)*

Table 8. *Effect of multi-enzyme produced by a single fungus on slaughter weight and carcass parameters (g/bird) of broilers in Experiment 1*

Parameters	Treatment Groups			SED	P=
	Positive Control	Negative Control	Negative Control + Enzyme		
Slaughter weight (at 35 d)	2504	2471	2498	29.75	0.893
Hot carcass weight	1912	1882	1910	21.70	0.814
Cold carcass weight	1814	1790	1814	21.56	0.864
Carcass yield (%)	72.47	72.42	72.65	0.055	0.183
Abdominal fat weight	28.40^{b}	34.47^{a}	33.04^{a}	0.475	**0.001**

SED: *Standard error of difference between means;* ^{a,b} *means in the same row with different letters are significantly different (P<0.05)*

the diet deteriorated feed conversion efficiency at about 1.4%, but enzyme supplementation could compensate the half (0.7%) of this deterioration *(Table 7)*.

The results with respect to carcass analyses showed that there were no differences obtained for slaughter weight, hot carcass weight, cold carcass weight and carcass yield of the treatment groups (P>0.05). However, the groups receiving negative control diet with or without enzyme attained significantly heavier abdominal fat than the group receiving positive control diet *(Table 8)*.

The results obtained from the first experiment suggest that almost 3% reduction in nutrient contents of the diet reduce broiler performance numerically (P>0.05) but increased abdominal fat statistically (P<0.05) under our condition of Experiment 1. The increase in abdominal fat may be attributed to increased feed/energy intake of the birds to meet their protein requirement. The increase in abdominal fat should also be associated with the deterioration in feed conversion efficiency. The results with respect feed intake, body weight gain and feed conversion efficiency suggest that positive effects of supplemental enzyme to the diet with reduced nutrients contents were not significant and also lower than the expected.

The results obtained in the experiment 2 with respect to feed intake, body weight gain, feed conversion efficiency, slaughter weight and carcass parameters are presented in *Tables 9, 10, 11* and *12*, respectively.

The results with respect to feed intake showed that nutrient reduction almost 3% affected feed intake significantly (P<0.001). During the experiment 2, the birds receiving reduced nutrient contents consumed feed higher (2-3%) amount than the birds receiving positive control or

Table 9. *Effect of Effect of multi-enzyme produced by a single fungus on feed intake (g/bird) of broilers in Experiment 2*

Age (day)	Treatment Groups			SED	P=
	Positive Control	Negative Control	Negative Control + Enzyme		
7	140[b]	144[a]	137[c]	0.648	0.0007
14	585[b]	630[a]	594[b]	2.715	<.0001
21	1248[a]	1244[a]	1163[b]	7.068	0.0001
28	2218[ab]	2249[a]	2158[b]	11.90	0.0164
35	3494[ab]	3552[a]	3462[b]	12.24	0.0217

SED: *Standard error of difference between means;* [a,b] *means in the same row with different letters are significantly different (P<0.05)*

Table 10. *Effect of multi-enzyme produced by a single fungus on body weight gain (g/bird) of broilers in Experiment 2*

Age (day)	Treatment Groups			SED	P=
	Positive Control	Negative Control	Negative Control + Enzyme		
Initial Body Weight (g/bird)	44.4	45.7	44.6	0.473	0.518
7	110	110	106	0.825	0.139
14	400	399	398	1.876	0.914
21	885[a]	863[ab]	854[b]	5.192	0.082
28	1568	1568	1542	8.573	0.382
35	2371[a]	2330[b]	2359[ab]	7.340	0.088

SED: *Standard error of difference between means;* [a,b] *means in the same row with different letters are significantly different (P<0.05)*

Table 11. *Effect of multi-enzyme produced by a single fungus on feed conversion efficiency (g intake : g gain) of broilers in Experiment 2*

Age (day)	Treatment Groups			SED	P=
	Positive Control	Negative Control	Negative Control + Enzyme		
7	1.28	1.32	1.29	0.009	0.2377
14	1.46[c]	1.58[a]	1.49[b]	0.014	**<.0001**
21	1.41[b]	1.44[a]	1.36[c]	0.003	**<.0001**
28	1.41[b]	1.44[a]	1.40[b]	0.003	**0.0005**
35	1.47[b]	1.52[a]	1.47[b]	0.002	**<.0001**

SED: *Standard error of difference between means;* [a,b,c] *means in the same row with different letters are significantly different (P<0.05)*

negative control + enzyme diets *(Table 9)*. The difference between feed intake of the negative control and negative control+enzyme groups was significant (P<0.05). This result suggest that birds receiving negative control diets tried to sustain nutrient intake by increasing feed intake throughout the study. Supplemental enzyme seems to be worked as expected by liberating some extra nutrients, which helps birds to sustain nutrient flow through intestines.

At the beginning of the experiment 2, all the groups had similar body weight. Throughout the experiment, all the groups had similar growth performance but at the end of the experiment, the group receiving positive control diet achieved the best performance. The group receiving supplemental enzyme had a performance, which was 1% better than the group receiving negative control diet but 0.5% lower than the group receiving positive control diet *(Table 10)*.

From the second week of the experiment 2, feed conversion efficiency was also affected by enzyme supplementation significantly similar to body weight gain (P<0.001). The overall data obtained at the second, third, fourth and last weeks of the experiment 2 showed that the best feed efficiency was obtained by the group receiving positive control or negative control+enzyme diets. Their feed conversion rate was found to be significantly (P<0.05) better than that of the group receiving negative control diet. Enzyme supplementation was improved feed conversion efficiency by about 3.5% *(Table 11)*.

The results with respect to carcass analyses showed that there were no significant differences obtained for slaughter weight, hot carcass weight, cold carcass weight and carcass yield of the treatment groups. However, the groups receiving negative control diet with or without enzyme attained significantly heavier abdominal fat

Table 12. *Effect of multi-enzyme produced by a single fungus on slaughter weight and carcass parameters (g/bird) of broilers in Experiment 2*

Parameters	Treatment Groups			SED	P=
	Positive Control	Negative Control	Negative Control + Enzyme		
Slaughter weight (at 37d)	2622	2582	2619	25.01	0.769
Hot carcass weight	1954	1910	1946	21.05	0.670
Cold carcass weight	192	1882	1916	19.68	0.680
Carcass yield (%)	73.25	72.92	73.17	0.241	0.840
Abdominal fat weight	30.73[a]	32.89[ab]	35.93[a]	0.784	**0.036**

SED: *Standard error of difference between means; [a,b] means in the same row with different letters are significantly different (P<0.05)*

than the group receiving positive control diet *(Table 12)*.

The results of the second experiment suggest that almost 3% reduction in nutrient contents of the diet reduce broiler performance (P<0.05) under the condition of Experiment 2. The results also suggest that positive effects of supplemental enzyme to the diet with reduced nutrients contents were significant for feed intake and feed conversion efficiency as expected.

DISCUSSION

The results of the present study showed that exogenous multi-enzyme produced by a single fungus used in maize-soya based diets improve nutritional value of the diets up to 3%. The improvement found to be varies according to animal density. In the first experiment during which the animals reared at low density (10 birds/m^2), the improvement with the supplemental enzyme was about only 1%, but when the birds were accommodated at high density (16 birds/m^2 industrial level) the improvement with the supplemental enzyme in performance was over 3%. As the source of main ingredients, maize and soya, of the diets and the other experimental conditions used in the both trials were the same, the difference between the improvement obtained from the both experiments could be attributed to the stocking density.

In fact, studies on stocking densities in broiler production have produced variable conclusions. Some studies show large benefits in reducing stocking density, while others show little or no differences. BILGILI and HESS [29] conducted a study examining densities of 0.8, 0.9 or 1.0 square foot per bird. Body weight, feed conversion, mortality, carcass scratches and breast meat yield were improved significantly when birds were given more space. A limited improvement with enzyme addition under better welfare condition should be expected, as modern broilers perform better when given more space. However, the ultimate goal in broiler production is to maximize pounds of chicken produced per square meter while preventing production losses due to overcrowding. Under this condition, enzyme supplementation seems to offer easy and effective way to maximize the product by promoting feed conversion efficiency. The improvements in feed conversion efficiency could be attributed to better digestion and higher absorption of nutrients liberated by the enzyme. It is well known that the most abundant carbohydrates in maize and soya are cellulose, arabinoxylans, pectins, oligosaccharides and starch [30]. Some of these compounds are relatively resistant to digestion such as cellulose, arabinoxylans and pectins. Many different primary enzymes are required to degrade these carbohydrates such as cellulase and cellobiohydrolyse to degrade cellulose, xylanase and arabinofuranosidase to degrade arabinoxylans, α-galactosidase to degrade the oligosaccharides and amylase to degrade starch.

The enzyme, produced by *Talaromyces versatilis*, used in the present study, has been introduced by ADISSEO [25]. It does have many different types of xylanases (Endo -1.4 β-xylanase β-xylosidase), β -glucanases (Endo - 1.3 1.4 β-glucanase Laminarinase), pectinases (polygalacturonase, Pectin esterase, Endo-1.5 α-arabinanase, α-galactosidase, rhamnogalacturonase), proteases (aspartic protease, metallo protease) and celluloses (Endo-1.4 β-glucanase, cellobiohydrolase, β-glucosidase) are associated with arabinofuranosidases, essential debranching enzymes (α-arabinofuranosidase, α-glucuronidase, ferulic acid esterase) besides some others (Endo-1.4 β-mannanase, β-manosidase).

It is well known that arabinoxylan is one of the main non-starch polisaccarides components in cell wall of most cereals and can partially be solubilised by processing [31]. It is composed of a xylose backbone chain carrying arabinose branches [32]. Arabinoxylans are primarily hydrolyzed by endo-1,4-b-xylanase activity, cleaving the (1,4)-linkages of the xylan backbone [20]. Due to their narrow specificity towards a given bond, distinct enzymes are required for the degradation of arabinoxylans. While xylanases hydrolyze the xylose backbone, their activity is frequently hampered by the presence of arabinose branches which prevent access to the central xylose chain. FOURIE [23] reported remarkable improvements with multi-enzym (xylanase, glucanase, cellulase, xyloglucanase, endo-xylanase, galactomannanase, mannanase and pectinase) in energy and protein digestibility of broiler received maize-soya based diets. He suggest that enzyme addition to balanced vegetarian maize-soya diets may result in significant financial gains. Similar results have also been

reported in more recent studies; using a combination of amylase, xylanase and protease was reported to be effective in improving the growth profiles of broilers fed maize-soybean-rapeseed-cotton mixed diets [33]. The multy-enzyme used in the present study does also have arabinofuranosidases, which is known as debranching enzymes, are able to cleave arabinose from the backbone and enhance the efficacy of xylanases. Removing the branches greatly helps the xylanases gain access to the xylose backbone [34].

The results obtained in the present study suggest that exogenous multi-enzyme containing many different types of xylanases, ß-glucanases, pectinases, celluloses and arabinofuranosidases is able to improve nutritional value of maize-soya up to 3%, as it can compensate the reduction made 3% nutrients (energy, protein, amino acids, calcium and phosphor) by better feed conversion efficiency.

As a result, in the first trial, providing no feeding competition with high comfort and better rearing condition as the birds were accommodated with low density (10 birds/m^2), positive effects of the enzyme was shadowed. Supplemental multi-enzyme seems be work partly and it gains almost 1% nutrients through better feed conversion efficiency and growth performance. In the second trial, providing feeding competition with low comfort and moderate rearing condition similar to the industry, as the birds were accommodated with high density (16 birds/m^2), positive effects of the enzyme was obvious. Supplemental multi-enzyme seems to work markedly and it gains weight almost 3.5% nutrients by better feed conversion efficiency and growth performance.

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