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EFFECT OF ADDITION OF EXOGENOUS ENZYMES IN HYPOCALORIC DIET IN BROILER CHICKEN ON PERFORMANCE, BIOCHEMICAL PARAMETERS AND MEAT CHARACTERISTICS

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Abstract: In developing countries, broiler farmers often use imbalanced energy diets, hence our study aims to evaluate the combined effect of addition of commercial exogenous enzymes (CEE), in low energy level corn/soybean meal based-diet on performance, serum biochemical parameters, meat characteristics in male and female of broiler chickens. A total of 120 one day old Hubbard F15 broiler chickens were divided on 2 groups (60 animals/group) with 5 replicates/group. The control group received a standard diet, while CEE group received the same diet supplemented with enzymes (250 g/ton). Addition of enzymes reduced significantly feed ($p<0.001$) and water intakes ($p<0.05$); in meantime, feed conversion ratio tended to be lower ($p=0.08$). No changes were observed in pH, protein or moisture contents of meat in both sexes broiler between CEE and control groups. No perturbation was found in all serum biochemical parameters in both sexes between CEE and control groups, except total protein and albumin levels were significantly higher in male birds fed enzymes when compared to male birds of the control group ($p<0.001$; $p<0.01$) respectively. Addition of enzymes allowed a decrease of 950 g/bird in feed intake for the total rearing period, hence save 337 €/1000 birds; thus, use of CEE in hypocaloric diet enhances broilers feed efficiency and procures an economic benefit to farmers.

Keywords: exogenous enzymes, hypocaloric diet, poultry, performance.

Introduction

Poultry production industry, mainly in developing countries, is facing a number of challenges, not the least of which are the pressures to produce more and high quality products to satisfy customer needs in a cost effective manner. As chicken meat is the most affordable in developing country, market demand is increasing rapidly since standard living and population growth continue to rise. While management of the supply chain and its contribution to agricultural production are improving, high production costs, long production cycle and market instability remain challenges of broilers meat producers. High prices of chicken meat at food retailing in developing countries like Algeria are mostly related to the excessive charges of feed, consequence of low feed efficiency and long rearing period. Broilers are usually reared during 42 to 65 days, depending mostly on the quality of feed given to birds. Although nutrients utilization of corn and soybean meal by broilers, which are the major energy and protein contributor to the diets, is generally considered to be high, their energy utilization depends on the amount of indigestible carbohydrates present, specially oligosaccharides and non-starch polysaccharides (NSP). Corn contains 9.7% NSP, whereas soybean meal contains 30.3% NSP (*Bach Knudsen, 2001*). Consequently, the presence of such anti-nutritional factors reduced the nutritional value of corn/soybean meals based diets and thus, mainly soybean is partially digested by poultry (*Pack and Bedford, 1997*). Water soluble NSP fed to broilers is not only indigestible but also interfere with the digestion and absorption of other nutrients by increasing the viscosity of digesta in the gut (*Ward and Marquardt, 1983*.). Thus, NSP are the major cause of growth depression and poor feed conversion in poultry. Exogenous enzymes have been used commercially in poultry diets for over 20 years and their addition is now a routine practice (*Amerah et al., 2011*). Addition of exogenous enzymes to corn/soybean meal based diets can overcome the anti-nutritive effect of water soluble NSP; numerous studies have reported the beneficial impact of exogenous enzymes on chick performance and nutrient digestibility (*Odetallah et al., 2002*; *Silva and Smithard, 2002; Abudabos, 2012*). Although, the value of enzymes for diets based on corn and soybeans is not well established in the scientific literature, few studies reported yet the effect of exogenous enzymes on performances, serum biochemical parameters and meat quality in male and female broiler chickens, as well as on litter moisture. Logic would suggest that if the use of enzymes can improve body weight gain and feed conversion ratio of chickens receiving isocaloric diets, the use of diets containing lower energy levels, might improve the scale and consistency of the response. In developing countries, broiler farmers often use imbalanced energy diets, and as under such us conditions, we attempted therefore in this study to investigate the effect of supplementation of commercial exogenous enzymes (CEE) in hypocaloric corn/soybean meal based-diet on mortality, growth performance, serum biochemical parameters, meat and carcass traits, litter moisture and feed cost in broiler chickens Hubbard F15.

Materials and Methods

Experimental design

The experiment was conducted at ORAVI (Res ELMA, Setif), a public commercial poultry farm. A total of 120 one day old Hubbard F15 broiler chickens were divided randomly on 2 groups (60 animals/ group) that were replicated five times with twelve animals per replicate. The first group is a control group received a standard corn/soybean meal based-diet, while the second one is CEE group received the same diet supplemented with commercial enzyme at a concentration of 250 g/ton of feed as indicated in its commercial card. CEE is a multi-enzyme complex consisting of endo-ß-1,4-xylanase derived from *Trichoderma longibrachiatum*, endo-ß-1,4-glucanase, amylase and protease and the side activities cellulase and galactomannase..

A standard corn/soybean meal based-diet was formulated by the National Board of Livestock Feed (ONAB of El Eulma, Algeria), adapted to each phase of rearing broilers, was used in our experiment. The basal diet given in our experimental was hypocaloric, detailed formulation is shown in Table 1. Birds were fed starter diet from 1 to 9 days, grower diet from 10 to 41 days and finisher diet from 42 to 60 days. Feed and water were served *ad libitum* throughout the whole experimental period. All animals were exposed to a similar rearing management. The chicks were kept in ventilated broiler house in ten pens (1.2 m x 1.25 m) on wood dust litter within floor pens with stainless-steel shaving. Pens were gradually widened during grower and finisher periods as well as bird densities were respectively 8, 7 and 4 chicks per meter square at the end of starter, grower and finisher periods.

There were one hanging feeder and one drinker in the middle of each pen. A thermometer was put in the middle of the rearing area of each group to register daily at 12:00 pm the ambient temperature. The average mean temperature was initially 31 ± 0.75°C, then 30 ± 1.09°C during starter period, 26 ± 3.04°C during grower period and 22 ± 2.62°C during finisher period. The lighting program was as follows: 24h light/d until 3 day, 23h light/d from day 4 to 7, 18h light/d for the 2nd week, 14h light/d for the 3rd week; then light period was increased by 2 hours per week until 6th week when light was maintained at 22h/d. Chicks were vaccinated via drinking water against Gumboro and New Castle Disease (Ceva vaccines) at day 12 and 15 respectively.

Table 1. Formulation and calculated analysis of broiler chicken diet

Ingredients (g/kg)	Starter	Grower	Finisher
Corn	610.00	648.00	670.00
Soybean meal (48.6% CP)	297.00	270.00	180.00
Milling issues	50.00	50.00	120.00
Limestone	6.00	12.00	10.00
Di calcium phosphate	16.7	10.00	10.00
DL Methionine	0.30	-	-
Vitamin premix anti-stress	10.0	-	-
Mixture premix[1]	10.0	10.00	
Mixture premix [2]	-	-	10.00
Calculated analysis			
ME (Kcal/kg)	2781	2816	2826
Crude protein (%)	20.45	18.68	16.46
DL Methionine (%)	0.49	0.44	0.41
Calcium (%)	0.85	0.90	0.79
Phosphorus (%)	0.69	0.56	0.55

All premix mixtures were supplied by kg of diet.

Vitamin anti-stress premix: Retinol, 0.15 mg; Tocopherol, 1500 mg; Menadione, 250 mg ; Thiamine, 50 mg; Riboflavin, 400 mg; Pyridoxine, 400 mg; Cobalamin, 1 mg; Ascorbic acid, 15000 mg; Niacin, 2000 mg; Folic acid, 100 mg; Biotin, 10 mg.

[1]**Mixture premix:** Retinol, 306.69 mg; Cholecalciferol, 5.11 mg; Tocopherol, 1532 mg; Menadione, 152.9 mg; Thiamine, 202.92 mg; Riboflavin, 420 mg; Pyridoxine, 97.6 mg ; Cobalamin, 2 mg; Folic acid, 53.2 mg; Niacin, 2530.8 mg; Ca Panthotenat, 872 mg, Cholin Chloride, 106000 mg; Anticoccidial (Salinomycin), 6000 mg; Cu, 228 mg; Co, 22.8 mg; Fe, 1520 mg; Mg, 1216 mg; Mn, 5700 mg; Zn, 1900 mg; I, 114 mg; Se, 6.08 mg; DL Methionin, 80000 mg; Antioxidant BHT, 13000 mg.

[2]**Mixture premix:** Retinol, 306.69 mg; Cholecalciferol, 5.11 mg; Tocopherol, 1072 mg; Menadione , 121.6 mg; Riboflavin, 420 mg; Pyridoxine, 97.6 mg ; Cobalamin, 2 mg; Niacin, 1529 mg; Ca Panthotenat, 872 mg, Cholin Chlorure, 106000 mg; Anticoccidial (salinomycin), 6000 mg; Cu, 228 mg; Co, 22.8 mg; Fe, 1520 mg; Mg, 1216 mg; Mn, 5700 mg; Zn, 1900 mg; I, 114 mg; Se, 6.08 mg; DL Methionin, 80000 mg; Antioxidant BHT, 13000 mg.

Data collection and sampling

Mortality was recorded daily and expressed as frequency; birds were weighed at 21, 35, 49 and 60 days of age. However, the weight of birds was not recorded at the end of grower period (41 days). Feed and water consumptions were measured every 2 to 3 days at the beginning of experiment and daily at the end of experiment. Then, daily feed and water intakes, and ratio of water/feed were calculated obviously for alive birds and for each rearing period. Feed conversion ratio during the entire experiment was also calculated. At the end of the experimental period, 1 to 3 chickens per pen, with identical weights were selected to obtain 10 males and 10 females per group (control and CEE) to measure serum biochemical parameters, carcass weights and their different part yields, and estimate meat characteristics per treatment and sex.

Serum biochemical analysis

Blood samples were collected from wing vein after fasting animals 4 hours. Blood glucose was immediately measured using Ultra One Touch strips. However, serum was separated after centrifugation at 2500 g for 15 min and frozen at -20°C until analysis. Serum concentrations of urea, total cholesterol (TC), triglyceride (TG), total protein (TP) and albumin were determined automatically with Technicon RA-XT Biochemistry Analyzer, while serum calcium was measured with Beckman coulter automate, using enzymatic colorimetric Cypress Reagent Kits (Belgium).

Animal sacrifice and dissection

At 60 days of age, all chickens were sacrificed by slitting their throat after 6 hours of fasting; feather, heads and shanks were removed. All carcasses were weighed, and then carcasses of 10 males and 10 females were dissected by the same butcher to different parts as: thighs (remained linked to tail), breast, back, wings, neck, liver, gizzard, spleen, heart, abdominal and neck fats. Parts were weighed and their yield expressed as percentage of carcass weight.

Meat characteristics

Meat characteristics were estimated in the thigh muscles by determination of pH, moisture and protein contents. After slaughter, the right thigh muscles were labelled and conserved about 12 hours at 4°C before evaluating pH and moisture content. All muscle thighs were stored individually at -20°C until analysis of crude proteins. Moisture content was determined after drying a sample of 5 g at 105°C for 24 hours (*AOAC, 1990*), while pH was determined using digital pH meter after homogenizing a sample of 5 g in 10 ml distilled water (*Patsias et al., 2008*). Crud proteins were measured following the Kjeldahl method of nitrogen analysis.

Litter moisture

At the end of the experiment, moisture content of the litter was measured in all pens. Litter was collected using an empty 200 ml of beaker, consisted of 12 cm core sample of litter (*Eichner, 2007*). Samples were taken from 3 different areas located far from the drinker of each pen; two litter samples were taken from the area where chickens slept while the third sample was taken from the middle of the left side of the pen. Litter samples were mixed in a plastic bag, and then a quantity of 100 g of each mixed sample was oven-dried at 105°C for 30 hours. The dry litter was then weighed and litter moisture determined.

Feed cost

To estimate feed cost through the entire experiment period, quantity of CEE supplemented was subtracted from all amount of the diet consumed during each period to obtain the quantity of diet consumed per bird knowing that CEE was added to diet at 0.025%, then the cost of feed intake was calculated. Prices of different diets considered in our study were: starter diet: 41. €/quintal; grower and finisher diet: 39.19 /quintal; CEE: 23.52 €/kg.

Statistical analysis

All data are expressed as mean ± SE. For each group, means data of five replicates were calculated. Data means between both groups were then compared using Student test when variances were equal, or Mann–Whitney U test when variances were unequal. Khi-square test was used to compare mortality frequencies between both groups. $p<0.05$ was considered as statistically significant and trends were discussed when $p<0.10$.

All statistical analyses were performed using SPSS package program, version 17.0.

Results

Mortality, feed and water consumption, water/feed ratio and feed conversion

No difference was observed in the frequency of mortality in CEE group compared to control group (8% vs 13%). Table 2 shows that feed intake was similar between both groups during starter period, then decreased during grower and finisher periods in CEE group compared to control group ($p<0.01$; $p<0.001$) respectively. Water intake was lower during starter and grower periods in CEE group when compared to control group ($p<0.05$), while this difference was not observed during the finisher period. Water/feed ratio was lower during the starter period in CEE group compared to control group ($p<0.05$), then increased during the grower period ($p<0.05$) and tended to be higher during the finisher period ($p=0.07$) (Table 2). Concerning the entire experimental period, animals fed CEE consumed less feed ($p<0.001$) and water ($p<0.05$) when compared to control animals. Total feed conversion ratio tended also to be significantly reduced in CEE group compared to control ($p=0.08$). However, water/feed ratio was significantly higher in CEE group ($p<0.05$) when compared to control group (Table 2).

Table 2. Feed and water intakes, water/feed ratio and total feed conversion ratio during experimental period

Periods	Groups	Mean ± S.E.	Signification
Starter (1-9 days)			
- Feed intake (g/bird)	Control	170.67 ± 3.04	NS
	CEE	173.13 ± 4.17	
- Water intake (ml/bird)	Control	293.15 ± 2.16	p<0.05
	CEE	264.17 ± 8.54	
- Water/feed ratio	Control	1.72 ± 0.02	p<0.05
	CEE	1.53 ± 0.06	
Grower (10-41days)			
- Feed intake (g/bird)	Control	3360.34 ± 52.33	p<0.01
	CEE	2890.52 ± 76.33	
- Water intake (ml/bird)	Control	4232.93 ± 71.82	p<0.05
	CEE	3945.58 ± 65.46	
- Water/feed ratio	Control	1.26 ± 0.03	p<0.05
	CEE	1.37 ± 0.03	
Finisher (42-60 days)			
- Feed intake (g/bird)	Control	3398.80 ± 65.64	p<0.001
	CEE	2917.48 ± 53.80	
- Water intake (ml/bird)	Control	5325.51 ± 149.76	NS
	CEE	5088.90 ± 125.46	
- Water/feed ratio	Control	1.57 ± 0.04	p =0.07
	CEE	1.75 ± 0.08	
All experiment period			
-Total feed intake (g/bird)	Control	6929.80 ± 97.96	p<0.001
	CEE	5981.13 ± 87.95	
-Total water intake (ml/bird)	Control	9851.60 ± 126.60	p<0.05
	CEE	9298.75 ± 185.93	
- Total feed conversion Ratio (g:g)	Control	3.07 ± 0.11	p=0.08
	CEE	2.67 ± 0.15	
- Total water/feed ratio	Control	1.42 ± 0.03	p<0.05
	CEE	1.56 ± 0.04	

NS: not significant. CEE: commercial exogenous enzymes

Live weight and carcass characteristics

There were no significant differences in carcass or live weights recorded for any rearing period between CEE and control groups (Table 3).

Table 3. Live and carcass weights of broiler chickens

Weights	Groups	Mean ± S.E.	Signification
Live weights (g)			
21 days	Control	408.84 ± 8.65	NS
	CEE	388.92 ± 7.67	
35 days	Control	972.54 ± 35.42	NS
	CEE	965.66 ± 18.71	
49 days	Control	1547.80 ± 63.17	NS
	CEE	1599.56 ± 71.58	
Final live weights (g)	Control	2269.00 ± 103.47	NS
(60 days)	CEE	2252.80 ± 109.82	
Carcass weights (g)	Control	1847.71 ± 52.87	NS
	CEE	1741.95 ± 46.50	

NS: not significant. CEE: commercial exogenous enzymes

Likewise, supplementation of diet with exogenous enzymes did not influence the carcass weights and parts yields of male or female broiler chickens (Table 4).

Table 4. Carcass weights and relative weight of different parts of carcass in male and female broiler

		Male Birds			Female Birds		
		n	Mean ± SE	P	N	Mean ± SE	P
Carcass (g)	Control	10	1926.56 ± 80.13	NS	10	1818.30 ± 59.40	NS
	CEE	10	1807.10 ± 59.67		10	1704.00 ± 71.24	
Thighs (%)	Control	10	43.55 ± 0.40	NS	10	41.30 ± 0.44	NS
	CEE	10	44.20 ± 0.46		10	41.44 ± 0.36	
Breast (%)	Control	10	29.69 ± 0.49	NS	10	31.68 ± 0.56	NS
	CEE	10	29.29 ± 0.45		10	31.72 ± 0.60	
Back (%)	Control	10	8.60 ± 0.34	NS	10	8.93 ± 0.31	NS
	CEE	10	8.67 ± 0.27		10	8.85 ± 0.36	
Wings (%)	Control	10	11.84 ± 0.23	NS	10	12.30 ± 0.31	NS
	CEE	10	11.61 ± 0.54		10	11.94 ± 0.31	
Neck (%)	Control	10	3.35 ± 0.15	NS	10	3.00 ± 0.13	NS
	CEE	10	3.35 ± 0.24		10	3.00 ± 0.12	
Liver (%)	Control	10	2.75 ± 0.09	NS	9	2.74 ± 0.12	NS
	CEE	10	2.69 ± 0.11		10	2.91 ± 0.11	
Gizzard (%)	Control	9	1.89 ± 0.11	NS	9	1.70 ± 0.09	NS
	CEE	10	1.79 ± 0.10		10	1.70 ± 0.05	
Spleen (%)	Control	8	0.16 ± 0.01	NS	6	0.20 ± 0.01	NS
	CEE	9	0.17 ± 0.01		7	0.22 ± 0.02	
Heart (%)	Control	10	0.54 ± 0.02	NS	10	0.57 ± 0.03	NS
	CEE	10	0.53 ± 0.02		10	0.53 ± 0.02	
Abdominal fat (%)	Control	10	0.79 ± 0.10	NS	10	1.50 ± 0.22	NS
	CEE	8	1.11 ± 0.17		9	0.98 ± 0.19	
Neck fat (%)	Control	10	2.87 ± 0.20	NS	9	3.11 ± 0.22	NS
	CEE	10	2.88 ± 0.12		10	3.05 ± 0.18	

NS: not significant. n: number of birds. CEE: commercial exogenous enzymes

Serum biochemical parameters levels and meat characteristics in male or female broilers

Levels of serum TP and albumin were higher in male birds of CEE group compared to male birds of the control group ($p<0.01$; $p<0.001$) respectively, while all other serum parameters were not influenced neither in male nor in female birds by CEE addition to diet (Table 5). There is no modification in pH, protein and moisture contents in thigh muscles of CEE group compared to control group (Table 6).

Table 5. Concentration of serum biochemical parameters in male and female broiler chickens

	Groups	Male Birds			Female Birds		
		n	Mean ± SE	P	n	Mean ± SE	P
Urea (g/l)	Control	10	0.06 ± 0.01	NS	7	0.05 ± 0.01	NS
	CEE	9	0.06 ± 0.01		9	0.06 ± 0.02	
TP (g/l)	Control	10	32.90 ± 1.07	0.01	8	42.75 ± 2.38	NS
	CEE	9	43.00 ± 2.89		9	37.89 ± 2.29	
Albumin (g/l)	Control	10	10.70 ± 0.21	0.001	8	15.25 ± 0.62	NS
	CEE	8	14.25 ± 0.65		9	14.67 ± 0.67	
TC (g/l)	Control	10	0.96 ± 0.04	NS	8	1.16 ± 0.07	NS
	CEE	9	0.99 ± 0.05		9	1.05 ± 0.08	
TG (g/l)	Control	10	0.61 ± 0.05	NS	8	0.62 ± 0.07	NS
	CEE	9	0.66 ± 0.07		9	0.58 ± 0.04	
Calcium (g/l)	Control	9	67.89 ± 3.14	NS	8	68.50 ± 3.96	NS
	CEE	9	71.67 ± 1.93		9	69.22 ± 3.22	
Glycemia (g/l)	Control	10	2.37 ± 0.08	NS	8	2.35 ± 0.06	NS
	CEE	9	2.44 ± 0.15		9	2.18 ± 0.09	

Cholesterol (TC), Triglyceride (TG), Total Protein (TP). NS: not significant. n: number of birds. CEE: commercial exogenous enzymes

Table 6. Meat characteristics in male and female broiler chickens

	Groups	Male Birds			Female Birds		
		n	Mean ± SE	P	n	Mean ± SE	P
PH	Control	10	6.02 ± 0.05	NS	10	6.00± 0.05	NS
	CEE	10	6.00 ± 0.11		10	6.06 ±0.10	
Protein (%)	Control	10	21.46 ± 0.51	NS	10	19.82 ± 0.36	NS
	CEE	10	21.79 ± 1.02		10	20.72 ± 0.97	
Moisture (%)	Control	10	78.80 ± 0.44	NS	10	79.20 ± 0.44	NS
	CEE	10	78.00 ± 0.52		10	79.40 ± 0.31	

NS: not significant. n: number of birds; CEE: commercial exogenous enzymes

Litter moisture

Litter moisture of birds fed diet supplemented with CEE was increased ($p<0.01$) when compared to control group (46.20% ± 1.39 vs. 33.40% ± 3.66).

Feed cost and benefit

Addition of exogenous enzymes did not influence the cost of feed intake during the starter period, but they reduced feed cost during grower and finisher periods ($p<0.01$; $p<0.001$) respectively, as well as during the entire experimental period ($p<0.001$) (Table 7). For the total rearing period, feed intake was lower after CEE addition, corresponding to 950 g/bird less than the control group; hence, use of CEE save 337.2 €/1000 reared birds (Table 7).

Table 7. Cost of total feed intake with or without addition of exogenous enzymes through all period of experiment

Periods	Net diet intake (g/bird)	Net CEE intake (10^{-2} g/bird)	Cost of net diet intake (10^{-2} €)	Cost of net CEE intake (10^{-2} €)	Cost of total diet intake (10^{-2} €)
Starter					
Control	170.67± 3.04	/	7.02±0.13	/	7.02±0.13
CEE	173.09 ± 4.17	4.32±0.12	7.12±0.17	0.10±0.00	7.22±0.17
Signification	NS		NS		NS
Grower					
Control	3360.34±52.33	/	131.70±2.05	/	131.70±2.05
CEE	2889.79±76.32	72.28±1.91	113.26±2.99	1.70±0.04	114.96±3.04
Signification	0.01		0.01		0.01
Finisher					
Control	3398.80±65.64	/	133.21±2.57	/	133.21±2.57
CEE	2916.75±53.78	72.96±1.34	114.31±2.11	1.72±0.03	116.03±2.14
Signification	0.001		0.001		0.001
All period					
Control	6929.80±97.96	/	271.59±3.84	/	271.59±3.84
CEE	5979.63±87.93	149.54±2.20	234.35±3.45	3.52±0.05	237.87±3.50
Signification	0.001		0.001		0.001
Benefit feed (g/bird)	950.17				
Benefit (€/bird)	0.3372				
Benefit (€)/ 1000 birds	337.2				

NS: not significant. CEE: commercial exogenous enzymes

Discussion

Obviously the live weight of birds in our research, fed a low energy level corn/soybean meal based-diet, was too below to the target weight performed under commercial conditions by Hubbard F15 birds. Interestingly, addition of CEE enzymes improve feed efficiency by reducing feed intake, 950 g per bird, so birds receiving enzymes consumed 13.68 % less of feed compared to other birds who did not feed exogenous enzymes; in the meantime, feed conversion tended to be lower in CEE group compared to the control group. It was demonstrated that feed intake reduced as the energy content of feed decreased in diet having low energy and high protein diet content (*Dairo et al., 2010*). Indeed, reduced feed intake could be fully compensated by the effect of enzyme supplementation on feed efficiency so birds fulfil their nutrient requirement by taking fewer amounts of feed. The improvement of feed conversion efficiency of diets supplemented with enzymes was the result of increased metabolizable energy, consequence of higher digestibility of crude protein and NSP of diet. Our results agree with findings obtained by previous studies (*Zhou, et al., 2009; Aok, 2012*), when addition of exogenous enzymes improved energy digestibility in diets with lower levels of metabolizable energy, it was effective in overcoming anti-nutritive effects of NSP on broiler performance only at low energy levels. However, improved feed efficiency and body weight with no change in feed consumption were observed when CEE was added to isocaloric corn/soybean meal diets (*Abudabo, 2010*). Contrary, *Zakaria et al.*, (*2008*) reported no effect of CEE on body weight, feed consumption or feed conversion ratio. The heterogeneity in the findings on the effect of CEE on performance of broiler chickens could be explained by the different diets quality and chicken breed used in each experiment. Hence, use of CEE improves the nutritional value of a hypocaloric corn/soybean meal based-diet in birds. Until now, no study reported the effect addition of enzymes in this kind of diet on broiler chicken performance.

Our data suggest that enzyme supplemented diet did not interact with sex to influence organs weight or meat yield. Also, it is important to underline that the weight of fats (abdominal or neck) were not increased to the detriment on weight of different meat parts which is beneficial in animal production. Our findings is in agreement with work of *Alam, et al.*, (*2003*) who reported that dietary enzyme did not interact with sex to influence any meat yield characteristic. On other hand, *Abudabo, A.* (*2010*) reported, an increase in breast yield after addition of CEE but not in other meat part yields in Cobb chickens. In the current study, no perturbation of serum biochemical parameters concentrations was observed in both sexes of broilers between CEE and control groups, except for serum albumin and TP levels, which were higher in male birds fed CEE-supplemented diet. Our results are consistent with those reported by a recent study (*Abudabo, 2010*); it was shown that animals that have low feed intake require higher dietary with protein content (*Larbier and Leclercq, 1992*). These suggest that addition of enzymes increases protein digestibility, consequently decreased feed intake as discussed previously. Thus, energy and amino acid values of maize based-diets for broilers can be enhanced by supplementation with an enzyme cocktail of xylanase, amylase and protease (*Zanella et al., 1999; Cowieson and Ravindran, 2008*), which are components present in our CEE. Nevertheless, increase in serum albumin, and therefore total serum protein were found when the dietary protein level was increased beyond the requirement for growth; this reflects the ability of the chicks to store "reserve" protein even after the animal has reached its maximum capacity for depositing tissue or less "labile" protein (*Leveille, and Sauberlich, 1961*). Previous studies showed an increase in protein digestibility in bird fed corn/soy bean meal diet supplemented with CEE or with other commercial enzyme as Avizyme® 1500 containing some identical enzymes present in our CEE such as xylanase, protease and amylase (*Zanella et al., 1999; Abudabo, 2010*).

Our results revealed that frequencies of mortality are similar between birds fed or no CEE. Also, addition of exogenous enzymes did not influence pH, moisture and protein contents of thigh meat neither in male nor in female broiler chickens.

Our findings indicate that meat pH and protein content were close to those found in thigh muscle of male Hubbard F15 chickens aged 65 days and receiving a isocaloric diet (*Mikulski et al., 2011*). No study until now reported effect of CEE on meat quality in both sexes broiler chickens. Thus, we can suggest that meat of broiler chickens fed diet supplemented with CEE conserved its characteristics although feed intake reduction.

In a well-managed broiler house, litter moisture normally averages between 25 to 35 % (*Butcher and Miles, 1996*). Our study revealed that litter moisture was higher in pens of birds fed enzymes supplemented diet compared to control birds and exceeded the recommended normal range; also higher water/feed ratio observed in chickens fed enzymes supplemented diet can be attributed to the increase in protein digestibility as discussed previously. It was demonstrated that addition of enzymes makes more protein available so diet allows high protein concentration, increases water consumption and water/feed ratio (*Larbier and Leclercq, 1992; Francesch and Brufau, 2004*). Therefore, Dietary protein in excess of requirements causes an increased heat increment and water intake which results in elevated litter moisture content (*Alleman and Leclercq, 1997*). There are not yet published studies demonstrating effect of CEE on litter moisture; however, no effect of CEE supplemented diet on clean excreta moisture was reported (*Abudabo, 2010*).

Poultry industry is the important sector that can provide animal protein in a cost effective manner, mainly for poor people. However, feed is the major component of the total cost of broiler production (*Khan, 2004*). In our study, addition of CEE in corn/soybean diet procures lower diet charge by reducing feed intake, so use of CEE save 337 €/1000 birds. However, *Abudabo, A.* (*2010*), reported that addition of CEE offers potential to reduce diet cost commensurate with enhanced production. Exogenous enzymes show up as tools on poultry diets formulation flexibilization, allowing the utilization of non-conventional ingredients without impairment to birds' performance, with consequent reduction on production costs (*Costa et al., 2008*).

Conclusions

Addition of CEE in hypocaloric corn/soybean meal based-diet reduced feed and water consumptions. Feed intake decreased with no change in carcass and life weights, meat part yields, organs, fats, meat characteristics or serum biochemical levels neither in male nor in female birds, except a high serum albumin and protein levels was observed in male birds fed CEE probably by improving nutrient digestibility, mainly protein; Albeit high litter moisture found, addition of enzymes in low diet energy allows producers to raise birds with an economic feed benefit cost and contribute to enhance broiler chicken production.

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Efekat dodavanja egzogenih enzima u hipokaloričnoj ishrani brojlerskih pilića na performanse, biohemijske parametare i osobine mesa

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Rezime

U zemljama u razvoju, u ishrani brojlera na farmama se često koriste obroci koji nisu izbalansirani sa aspekta energetske vrednosti, pa naša studija ima za cilj da ispita kombinovani efekat dodavanja komercijalnih egzogenih enzima (CEE), u ishrani koja se zasniva na obrocima od kukuruza/sojine sačme, niskog nivoa energije, na performanse, serum biohemijske parametre, osobine mesa muških i ženskih pilića. Ukupno 120 jednodnevnih Hubbard F15 brojlera je podeljeno u 2 grupe (60 životinja/grupa) sa 5 ponavljanja/grupi. Kontrolna grupa je dobila standardi obrok, dok je CEE grupa dobila isti obrok sa dodatkom enzima (250 g/tona). Dodavanje enzima značajno smanjuje unos hrane ($p<0,001$) i vode ($p<0,05$); istovremeno, konverzija hrane ima tendenciju smanjenja ($p=0,08$). Nema promena u pH, sadržaju proteina ili vlage mesa oba pola brojlera između CEE i kontrolne grupe. Nisu utvrđene bilo kakve smetnje u biohemijskim parametrima seruma oba pola izmedju CEE i kontrolne grupe, osim ukupnih proteina i albumin nivoa koji su bili značajno veće kod muških pilića hranjenih enzimima kada se uporedi sa muškim pilićima iz kontrolne grupe ($p<0,001$; $p<0,01$) respektivno. Dodavanje enzima omogućilo je smanjenje unosa hrane od 950 g/brojleru za ukupni period odgoja, čime se ostvaruje ušteda od 337 €/1000 brojlera; dakle, upotreba CEE u hipokaloričnoj ishrani povećava efikasnost korišćenja hrane kod brojlera i osigurava se ekonomska korist za proizvođače.

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