Evaluation of two commercial dietary enzyme products in broiler chicken fed reduced energy diets based on corn and soybean meal

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SUMMARY

This study evaluated the effects of two commercial enzyme products incorporated into reduced energy diets on growth performance, mortality and litter quality of broiler chicken. A total of 1620 7-d-old male Arbor Acres broiler chicks were randomly divided into 3 groups of 540 chicks each (60 chicks x 9 replications) and assigned to one of the following treatments: (1) corn-soybean meal based diet as control (C), (2) C with a 60 Kcal/kg AME reduction supplemented with NSP-degrading enzymes (Rovabio Excel) at 0.05 g/kg (D1), and (3) C with a 120 Kcal/kg AME reduction supplemented with multi-enzyme preparation (Natuzyme) at 0.1 g/kg (D2). There were 4 dietary phases: starter (d1-d7), grower (d8-d21), finisher 1 (d22-d28), and finisher 2 (d29-d37). The experimental period was d7-d37. Live body weight (LBW), daily weight gain (DWG), daily feed intake (FI), feed conversion ratio (FCR) and mortality rate were measured by production phase and for the whole rearing period (d1-d37). Production index (PI) and litter quality were also measured. No difference was seen between diets C and D1 during any stage or overall rearing period, showing that Rovabio Excel supplementation with reduced energy diet formulation completely compensated for the reduced energy amount. Natuzyme partially restored broiler performance equal to standard diet formulation, except for LBW which was lower ($p<0.05$) during all phases, DWG which was lower ($p<0.05$) on days 29-37, and FCR which was higher ($p<0.05$) on days 22-28 in Natuzyme supplemented group. Natuzyme supplemented diet produced lighter broilers with higher FCR at d 37 ($p<0.05$). Mortality and litter quality were not affected by enzyme supplementation. Rovabio Excel supplementation reduced the cost per kilogramme of live body weight. In conclusion, a 60 Kcal/kg AME reduced energy diet supplemented with Rovabio Excel had equivalent performance to a standard diet and provided the best economic result. This approach can be used to reduce the amount of primary ingredients needed to formulate poultry diets namely corn which is exclusively imported and consequentlty to decrease production costs.

KEY WORDS

Broilers performance; energy level; litter quality; Natuzyme; Rovabio Excel.

INTRODUCTION

In Tunisia, broiler chicken feed is based primarily on corn and soybean meal, which supplies the majority of energy and protein in the diet. Utilization of the nutrients contained in both feed ingredients by broilers is generally considered to be high. Nevertheless, it has been shown that about 400-450 Kcal of energy per kg of diet is not digested when birds are fed a typical corn-soya diet[1]. These feed ingredients are exclusively imported into Tunisia. In addition, the high demand for corn and soybean for human consumption and biofuel production has led to a surge in their prices world wide, consequently increasing feed cost which represents between 60 and 80% of the production cost of broiler chicken. Thus, the application of nutritional approaches that optimize feed utilization is needed to increase broiler efficiency and reduce production costs.

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Over the last two decades, several exogenous enzymes have become readily available and commonly used in poultry diets to improve feed utilization and performance. These exogenous enzymes are used either to supplement a lack of specific endogenous enzymes for degrading certain nutrients or to hydrolyse anti-nutritional compounds in feed ingredients. For example, dietary non-starch polysaccharides (NSP)-degrading enzymes and phytase have been used to reduce the negative effects of NSP[2;3;4] and enhance dietary phytate utilization[5;6], respectively. More recently, multiple-enzyme preparations with broad spectrum activity (e.g. NSPase activity, amylase activity, protease activity and phytase activity) are also being developed and used commercially. Their application has been shown to result in additive or synergistic effects on nutrient utilization and animal performance[7;8]. In some cases, these enzyme cocktails have been shown to improve nutrient utilization in poultry diets better than single enzyme products[9] but bird responses to enzyme supplementation are variable. Several factors contribute to these inconsistencies, principally enzyme type and concentration, diet, and bird factors such as genetics and the composition of the gastrointestinal microbiome. Among dietary factors, the nutrient density of the diet is particularly important. Exogenous enzymes can be beneficial in diets containing low-digestible feedstuffs and a marginal nutrient density[9] or with diets containing high-digestible feedstuffs. Responses to exogenous enzymes are predicted to be minimal if there is a surplus of nutrients (energy, amino acids, phosphorus) in the diet. Accordingly, the use of some dietary enzymes has been suggested as a tool that can improve nutrient utilization in diets formulated with reduced available metabolic energy, crude protein or amino acids, available phosphorus or calcium[1;9;10;12]. This approach could be used to reduce the amount of primary ingredients such as corn and soybean meal needed to formulate poultry diets and consequentlty to decrease production costs. The purpose of this study was therefore to compare standard diet formulation to reduced energy diet supplemented with one of two commercial enzyme products, with respect to performance parameters, mortality and litter quality.

MATERIALS AND METHODS

Enzymes products used

The two commercial enzymes products tested in this study are as follows: (1) **Rovabio Excel** (ADISSEO, Alpharetta GA, USA) which is a combination of non-starch polysaccharides (NSP) degrading enzymes produced by the non-genetically modified fungus *Penicillum funiculosum*. The main enzymes are xylanase (Endo - 1,4 - β - xylanase, 30,000 unit/kg) and β-glucanase (Endo - 1,3 (4) - β - glucanase, 25,000 unit/kg). This product hydrolyzes pentosans and β glucans in plant raw materials; and (2) **Natuzyme** (Bioproton Pty Ltd., Sunnybank, Australia) is composed of xylanase (10,000,000 unit/kg), cellulase (5,000,000 unit/kg), β-glucanase (1,000,000 unit/kg), pectinase (140,000 unit/kg) from *Trichoderma reesei* and *Trichoderma longibrachiatum*. It also contains protease (6,000,000 unit/kg) and phytase (500,000 unit/kg) from *Aspergillus niger*, and α-amylase (1,800,000 unit/kg) from *Bacillus subtilis*. Each of these two enzyme products are claimed to enable better nutrient utilisation from feed, resulting in better growth performance and lower total costs.

Experimental feed preparation

A conventional feed (control diet; C) for broiler chicken based on corn and soybean-meal and two reduced energy diets supplemented with enzyme preparations (D1 and D2) were individually prepared for each production phase: grower phase (d8-d21), finisher 1 phase (d22-d28), and finisher 2 phase (d29-d37). D1 corresponds to C with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg. D2 correspond to C with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1 g/kg. Each enzyme preparation was incorporated into the assigned diet at the concentration recommended by the manufacturer. Enzymes were heat stable and able to with stand pelletization temperature up to 90°C for 90 s. For each enzyme product, the amount of reduced energy corresponds to the amount of improvement provided by its inclusion as compared to a standard corn-soybean meal based diet (without energy reduction) as claimed by the producer. To verify the producers' affirmation, the two reduced energy diets supplemented with enzyme preparations were individually compared to the standard diet. Since we were not interested to assess the amount of improvement provided by the dietary enzyme supplementation as compared to low energy diet without enzyme, no negative control diet was included in this study.

The composition and nutrient calculated content of the four diets for each production phase are given in Table 1.

Experimental design

One thousand six hundred and twenty (1,620) 1-day-old male Arbor Acres broiler chicks from a local commercial hatchery (Couvoir SAVINORD, Jendouba, Tunisia) were used to evaluate the effects of the dietary incorporation of enzyme preparations on the growth performance and litter quality. Upon their arrival, chicks were individually weighed and randomly distributed into 27 floor pens (2 m × 2 m i.e. 15 chicks per m^2) in a completely randomized design (3 treatments × 9 replications, each replication included 60 chicks). The 3 experimental groups were designed as follows: (1) a control group was fed a standard diet unsupplemented with enzymes (C), and (2) two groups were each fed one of two energy deficient diets supplemented with enzymes (D1or D2) described above.

During the first week of age (d1-d7), chicks in the control group received a standard starter diet, while those in D1 and D2 group received starter diets with reduced levels of energy corresponding to the same amount of reduced energy to be applied during the following production phases but without enzyme supplementation since exogenous enzyme supplementation during the starter period could have detrimental impact on chicks' health. Thus, the distribution of enzyme-supplemented diets started from the 8th day of age.

In all groups, feed and water were offered *ad libitum*. The lighting schedule was 23h light/1h darkness. Ambient temperature was equal to 33°C during the first week and it was subsequently reduced by 4°C each week. Wood shavings were used as litter material and spread in each pen to a thickness of 6 cm. The experimental protocol was approved by the Official Animal Care and Use Committee of the College of Agriculture of Mateur - University of Carthage before the initiation of research and followed the Tunisian guidelines approved by the committee on care, handling, and sampling of the animals.

Performance monitoring

During the experimental period (d8-d37), feed intake per pen and individual body weight were recorded for each production phase to calculate daily body weight gain (g/bird/day) and feed conversion rate (**FCR**). Mortality was daily monitored. Production index (**PI**) was also calculated as follows: [liveability (%) × final live body weight (kg) / growing period (37 days) × FCR] × 100.

Litter quality

Litter quality was assessed on d21, d28 and d37. Each pen was divided into 2 halves and litter quality was scored for each half of the pen and averaged. Litter quality scores were taken visually, and ranged from 1 to 4, with 1 being extremely dry and no caked litter and 4 being total pen coverage of caked litter. Three independent observers were involved in the litter scoring process to obtain the average value.

Table 1 - Composition and nutrient calculated content of experimental diets.

Item	Grower (8-21d)			Finisher 1 (22-28d)			Finisher 2 (29-37d)		
	C[1]	D1[2]	D2[3]	C	D1	D2	C	D1	D2
INGREDIENT (%)									
Corn	44.27	42.12	40.82	40.93	39.07	38.21	37.39	35.99	34.58
Soybean meal	23.21	22.47	23.75	19.53	19.29	19.29	17.42	19.10	18.93
Wheat	20.11	23.22	23.22	26.91	29.24	30.00	32.85	32.75	34.50
Vegetable oil	07.50	07.35	07.36	08.00	07.80	07.96	08.10	07.95	07.80
Premix[4]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	0.74	0.68	0.68	0.52	0.50	0.42	0.20	0.16	0.14
Limestone	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
NaCl	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
DL-Met	0.30	0.30	0.30	0.26	0.26	0.26	0.23	0.23	0.23
L-Lys HCl	0.26	0.26	0.26	0.26	0.24	0.26	0.23	0.23	0.23
L-threonine	0.13	0.11	0.12	0.11	0.11	0.11	0.10	0.10	0.10
Rovabio Excel	–	0.005	–	–	0.005	–	–	0.005	–
Natuzyme	–	–	0.01	–	–	0.01	–	–	0.01
NUTRIENT									
AME (Kcal/kg)	3050	2990	2930	3180	3120	3060	3240	3180	3120
Crude protein (%)	20.00	20.00	20.00	18.20	18.20	18.20	18.00	18.00	18.00
Ca (%)	0.95	0.95	0.95	0.90	0.90	0.90	0.90	0.90	0.90
Digestible P (%)	0.36	0.36	0.36	0.32	0.32	0.32	0.28	0.28	0.28
Na (%)	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Lys (%)	1.11	1.11	1.11	1.02	1.02	1.02	0.98	0.98	0.98
Met + Cys (%)	0.85	0.85	0.85	0.78	0.78	0.78	0.74	0.74	0.74

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg.
[4] Premix Leg 2% (Provimi b.v., Rotterdam, The Netherlands), provides (per kg of diet): vitamin A (retinyl acetate), 12 188 IU; cholecalciferol, 2438 IU; vitamin E (DL-α-tocopheryl acetate), 18.3 IU; pantothenic acid, 42.6 mg; vitamin B_1, 1.2 mg; vitamin B_2, 7.3 mg; vitamin B_3, 9.7 mg; vitamin B_6, 1.2 mg; vitamin B_{12}, 0.024 mg; vitamin K_2, 1.2 mg; folic acid, 0.62 mg; choline chloride, 622.2 mg; calcium, 8784 mg; phosphorus, 3660 mg; sodium, 366 mg; magnesium 36.6 mg; iodine, 0.59 mg; cobalt, 0.59 mg; copper, 2.42 mg; iron, 45.75 mg; manganese, 97.36 mg; zinc, 85.39 mg; selenium, 0.11 mg; methionine, 1647 mg.

Statistical analysis

Data obtained throughout the experiment were analysed by one-way analysis of variance as completely randomized block design with diet as fixed effect and block as random effect using the GLM procedure of SAS[13]. Means were separated by Dunnett's multiple comparison test[14] at $p<0.05$.

RESULTS

Mortality

Mortality rate in the enzymes-supplemented chicken groups (D1 and D2) was statistically similar to that of the control group (C) for the growth phase (d7-d21), the two finisher phases (d22-d28) and (d29-d37), and the entire rearing period (d1-d37; Table 2). This observation is an indication that the dietary incorporation of the enzyme preparations tested here had no deleterious effect on broiler mortality.

Growth performance

At the beginning of the experiment (d7), live body weights were similar for all the groups (Table 3). On d21, the live body weight of broilers fed the reduced energy diet supplemented with Rovabio Excel (D1) was statistically identical (P = 0.053) to that of the control birds. Birds on the diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme (D2) had lower (-2.17%) body weight than those

Table 2 - Effect of enzyme dietary supplementation on the broilers mortality.

Diets	Mortality (%)			
	d8-d21	d22-d28	d29-d37	d1-d37
C[1]	1.91[a]	1.29[a]	2.42[a]	8.13[a]
D1[2]	3.39[a]	1.31[a]	1.78[a]	7.94[a]
D2[3]	2.33[a]	0.87[a]	1.34[a]	6.01[a]
SEM[4]	0.93	0.58	0.65	1.70
P-value	0.688	0.626	0.574	0.647

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg.
[4] SEM: standard error of the mean
[a] means within a column with no common superscript differ significantly (P<0.05)

Table 3 - Effect of enzyme dietary supplementation on the performance of broilers fed reduced energy diets.

Diets	Live body weight (g)				Daily weight gain (g)			Daily feed intake (g)			FCR		
	d7	d21	d28	d37	d7-d21	d22-d28	d29-d37	d7-d21	d22-d28	d29-d37	d7-d21	d22-d28	d29-d37
C[1]	193.84^{a}	1022.7^{a}	1657.98^{a}	2494.57^{a}	59.20^{a}	90.75^{ab}	92.95^{a}	87.34^{a}	147.40^{a}	184.15^{a}	1.47^{a}	1.62^{b}	1.98^{a}
D1[2]	195.10^{a}	1022.14^{a}	1666.57^{a}	2497.32^{a}	59.07^{a}	92.06^{a}	92.3^{ab}	86.82^{a}	152.63^{a}	185.72^{a}	1.47^{a}	1.66^{ab}	2.01^{a}
D2[3]	194.51^{a}	1000.53^{b}	1618.32^{b}	2419.62^{b}	57.57^{a}	88.256^{b}	89.03^{b}	86.06^{a}	148.65^{a}	180.15^{a}	1.49^{a}	1.68^{a}	2.03^{a}
SEM	9.34	11.46	14.90	2.07	0.62	1.03	1.25	0.97	1.91	3.26	0.03	0.02	0.04
P-value	0.69	0.053	0.048	0.033	0.098	0.047	0.048	0.842	1.169	0.843	0.781	0.028	0.783

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg
[4] SEM: standard error of the mean
[a-b] means within a column with no common superscript differ significantly (P<0.05)

Table 4 - Effect of enzyme dietary supplementation on performance parameters of broilers fed reduced energy diets overall rearing period (1-37 days).

Diets	Initial live body weight (g)	Final live body weight (g)	Daily weight gain (g)	Daily feed intake (g)	Feed conversion ratio	Production index
C[1]	41.78^{a}	2494.57^{a}	66.29^{a}	112.17^{a}	1.69^{a}	366.5^{a}
D1[2]	41.66^{a}	2497.3^{a}	66.37^{a}	113.75^{a}	1.71^{a}	363.37^{a}
D2[3]	41.87^{a}	2419.62^{b}	64.26^{b}	111.19^{a}	1.73^{a}	355.29^{b}
SEM	0.33	2.07	0.4	1.39	0.02	1.08
P-value	1.12	0.033	0.003	0.974	1.41	0.041

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg
[a-b] means within a column with no common superscript differ significantly (P<0.05)

in the control group. A similar trend was observed in live body weights recorded on d 28. At the end of the experiment (d37), the diet D1 resulted in the same final body weight as the control diet, whereas D2 produced broilers with significantly ($p<0.05$) lower body weight almost 75 g lighter than those fed the control diet (Table 3).

During the grower phase (d7-d21) and the finisher phase 1 (d22-d28), the incorporation of Rovabio Excel (D1) or Natuzyme (D2) into reduced energy diets resulted in weight gain statistically comparable to that obtained with the control diet (Table 3). Throughout the finisher phase 2 (d29-d37), broilers fed D diet grew at the same rate as those given the control diet while broilers fed D2 diet grew at a significantly ($p<0.05$) slower rate (-4%) than control broilers. A similar trend was observed for body weight gain during the overall growing period (d1-d37) wherein body weight gain of broilers on diet D2 decreased by 3% (Table 4).

Regardless of the growing period, no significant difference was found when comparing feed intake between the control diet and the 2 reduced energy diets supplemented with enzymes (D1 and D2, Tables 3 and 4).

FCR was found to be non-significantly affected among all dietary treatments through the period days 7-21, 29-37 (Table 3) and overall rearing period (1-37 days; Table 4). During days 22-28 of age, the FCR of birds fed the reduced energy diet and supplemented with Rovabio Excel (D1) was equal to that of birds fed the control diet while those of birds fed reduced energy diet but supplemented with Natuzyme (D2) was significantly ($p<0.05$) higher (+4%; less efficient) than that of control birds (Table 3).

Control chickens had the highest realized value of PI (366,51) followed by those fed the reduced energy diet and supplemented with Rovabio Excel (363,37; Table 4). Both PI values were statistically similar. However, Natuzyme (D2) supplementation decreased significantly ($p<0.05$) the PI by 3% compared to that achieved with the control diet (Table 4).

Litter quality

No significant difference among dietary treatments was observed, either during the grower phase (d8-d21) or the finisher phases (d22-d28) and (d29-d37; Table 5). Litter quality

Table 5 - Effect of enzyme dietary supplementation on the broilers litter quality.

Diet	Scoring day		
	d21	d28	d37
C[1]	1.81^{a}	2.750^{a}	3.625^{a}
D1[2]	1.88^{a}	2.625^{a}	3.625^{a}
D2[3]	2.00^{a}	2.750^{a}	3.700^{a}
SEM	0.14	0.17	0.16
P-value	0.811	0.79	0.669

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg.
[a] means within a column with no common superscript differ significantly (P<0.05)

Table 6 - Economic analysis of broiler chicken fed reduced energy diets supplemented with enzyme.

Item	Diets		
	C[1]	D1[2]	D2[3]
Feed intake (d1-d7) (kg/bird)	0,209	0,209	0,208
Cost of feed (d1-d7) (TND/kg)	0,7665	0,7541	0,7510
Cost of consummed feed (d1-d7) (TND/bird)	**0,160**	**0,158**	**0,157**
Feed intake (d8-d21) (kg/bird)	1,223	1,215	1,205
Cost of feed (d8-d21) (TND/kg)	0,722	0,7105	0,7049
Cost of consummed feed (d8-d21) (TND/bird)	**0,882**	**0,864**	**0,849**
Feed intake (d22-d28) (kg/bird)	1,032	1,068	1,041
Cost of feed (d22-d28) (TND/kg)	0,7428	0,7412	0,7428
Cost of consummed feed (d22-d28) (TND/bird)	**0,766**	**0,792**	**0,773**
Feed intake (d29-d37) (kg/bird)	1,657	1,671	1,621
Cost of feed (d29-d37) (TND/kg)	0,7249	0,7104	0,7149
Cost of consummed feed (d29-d37) (TND/bird)	**1,202**	**1,187**	**1,159**
Total cost of consummed feed (d1-d37) (TND/bird)	3,011	3,001	2,938
Final live body weight (d37) (kg)	2,495	2,497	2,420
Cost of kg live body weight (TND/kg)	**1,207**	**1,202**	**1,214**

[1] C: standard diet based on corn and soybean meal formulated as a control
[2] D1: standard diet with a 60 Kcal/kg AME reduction and supplemented with Rovabio Excel at 0.05 g/kg
[3] D2: standard diet with a 120 Kcal/kg AME reduction and supplemented with Natuzyme at 0.1g/kg
1 USD was equal to about 2.77 Tunisian dinar (TND)

decreased along the days of experiment, independently of litter material, as expected due to the increase in the humidity produced by the birds and their manure.

Economic analysis

The data relative to the economic analysis revealed that the use of reduced energy diet supplemented with Rovabio Excel (D1) decreased the cost per kilogramme of live body weight by 0.44% compared to that of control diet, while that of reduced energy diet and supplemented with Natuzyme (D2) increased this parameter by 0.60% (Table 6).

DISCUSSION

The present paper aims to check whether the individual incorporation of two commercial enzyme products into low energy diet would compensate the reduced energy amount claimed by the producer for broilers fed standard corn-soybean meal based diet. Thus, the two reduced energy diets and supplemented with enzyme products were individually compared to a standard diet (without energy reduction) with respect to mortality, performance parameters and litter quality.

Mortality

Regarding mortality, there was no significant effect of the dietary enzyme inclusion on this parameter. Zanella *et al.*[15] and Hanumantha Rao *et al.*[16] also found that enzymes supplementation of corn-soybean diets did not affect mortality. Similarly, it has been shown that enzyme supplementation of barley-based diet had no significant impact on mortality[17]. However, our finding is not in line with those of Strelec *et al.*[18] and Khan *et al.*[19] who reported that dietary supplementation of broilers with exogenous enzymes decreased mortality rate considerably. In the study conducted by Khan *et al.*[19], broilers were fed a sunflower based diet containing a high fibre level (crude fibre >5%) that caused increasing incidence of pasting vents and wet litter resulting in more coccidiosis cases in control birds. Indeed, without enzymes, indigestible fibre promotes the growth of pathogenic microorganisms but with the enzyme, the fibre is broken down and promotes the growth of beneficial microorganisms.

Growth performance

With respect to growth performance, we investigated whether dietary enzyme supplementation could enable broilers fed reduced energy diets to restore their performance to levels equal to those obtained with a nutritionally adequate diet based on corn and soybean meal (standard diet).

In the current study, no significant differences were found in weight gain, feed intake and FCR of broilers fed diet containing 60 Kcal/kg less ME than the control diet and amended with the Rovabio Excel during all growing phases and over the entire rearing period. This result indicates that birds were able to maintain a weight gain comparable to that of control birds while consuming the same amount of feed but with 60 Kcal/kg less ME than the control diet. Our results are partially consistent with those of Nadeem *et al.*[20] who stated that Rovabio dietary supplementation (0.05 g/kg) of a diet having 50 Kcal/kg less ME than the control diet had no significant effect on weight gain but significantly increased feed intake and decreased FCR during the starter (1-28 days) and overall (1-42 days) growing periods. However, these authors did not observe significant differences in these parameters during the finisher (29-42 days) phase. Recently, in a study with more energy reduction (-100 Kcal/kg diet) than that used in the present study, Govil *et al.*[21] found that when the broilers were fed for 42 days the low energy diet supplemented with NSP degrading enzymes (xylanase at 0.05 g/kg + mannanase at 0.05 g/kg) plus amylase (0.04 g/kg), weight gain and FCR improved significantly, while feed intake was not changed. The study of Khan *et al.*[19] also showed that Rovabio dietary supplementation (0.05 g/kg) significantly improved weight gain and FCR of chicken fed sunflower meal (8%) - corn based diet but did not affect feed intake. The authors also observed a significant improvement in the digestibility of all nutrients in the enzyme-supplemented diet. These findings confirm that the beneficial effects of NSP-degrading enzymes might be somewhat higher with low-digestible feedstuffs like sunflower meal (14-18% crude fiber), than with high-digestible feedstuffs.

The use of an enzyme complex containing carbohydrases and phytase was suggested as a tool to decrease dietary concentration of nutrient, i.e. AME, P, CP/amino acids, and Ca in poultry feeds due to improved nutrient utilisation[9;10; 11;12]. In the present report, Natuzyme supplementation of a diet deficient in energy (-120 Kcal/kg) was successful in fully returning growth performance parameters to those obtained with a conventional diet without enzymes supplementation during all growing phases excepting for finisher 1 (d22-d28) phase where it resulted in significantly lower body weight gain and thus in significantly higher FCR. Our result regarding non-significant differences in feed intake between Natuzyme supplemented group and the control group is not consistent with those of Attia *et al.*[22] who showed that the supplementation of broilers standard diet with a combination of phytase and multienzyme preparation (containing NSP degrading enzymes and amylase) increased BWG by 4.9%, improved FCR by 6.6%, and decreased feed intake by 2.2%, as compared with those of broilers fed the standard diet without enzyme supplementation. Cowieson and Adeola[23] also reported 14% and 10% improvement in weight gain and FCR, respectively, after supplementing an enzyme cocktail (xylanase, amylase, protease, and phytase) to broilers fed a corn-soybean-based diet that was nutritionally marginal in terms of metabolizable energy (-180 Kcal/kg), Ca, and P. However, in no instance did this multienzyme preparation supplement result in growth performance equal to the nutritionally adequate diet as measured by FCR or BWG. According to authors, this was due more to the scale of the removal of energy and P from the nutritionally marginal diet and not to a lack of response to the supplemented enzymes. Zaghari *et al.*[24] reported that supplementing a corn-soybean meal based diet deficient in metabolisable energy, crude protein, non-phytate phosphorus and amino acids with 0.35 g/kg Natuzyme improved broilers body weight and FCR. However, this supplementation could not restore chick performance to the levels equal to a nutritionally adequate diet. In the same study, the authors estimated nutrient equivalency values of Natuzyme using increased inclusion levels (from 0.1 to 0.4 g/kg) and found that the enzyme product did not liberate nutrient equivalency values recommended by the producer for broilers fed corn-soybean meal based diet. They speculate that these recomended values may be appropriate in diets with high indigestible feedstuffs. In this respect, Makinde *et al.*[25] evaluated the performance of finisher broilers (4 weeks old Anak chickens) fed rice offal (as a replacement of dietary corn) under Natuzyme dietary supplementation. They observed that broilers fed diets containing 20-30% rice offal and supplemented with Natuzyme (0.25 g/kg) had similar final body weight, body weight gain and FCR than those fed corn-soybean meal based diet. Oliaei *et al.*[26] evaluated the effect of supplementing Natuzyme (0.35 g/kg) to broilers diet containing canola meal (6 or 12%) and reported a significant increase in body weight (by 7%) but no effect on feed intake and FCR as compared with the unsupplemented control diets. Recently, the inclusion of different levels of Natuzyme (0, 0.5, 0.75 and 1 g/kg) into a sorghum-based diet did not affect growth performance of Ross 308 broiler chicks, except for FCR which was significantly higher in chickens receiving the highest level of enzyme inclusion during starter phase as compared to control chickens[27]. In the latter study, the enzyme supplemented diets and unsupplemented diet were isocaloric and isonitrogenous.

Litter quality

Improving litter condition reduces ammonia in sheds and reduces the incidence of hock bums and breast blisters, and carcass downgrading in broiler chickens. Feed additives such as exogenous enzymes may have a positive influence in this regard. In the current study, exogenous enzyme dietary supplementation had no effect on litter quality in terms of litter humidity. It has been shown that the dry matter content of the litter of wheat or barley-fed broilers is improved (reduced sticky droppings) by adding NSP degrading enzymes to their diets[28;29]. Yuan *et al.*[30] also reported that NSP hydrolysing enzymes reduced excreta moisture of birds significantly. The results of recent studies showed that diet supplementation with feed enzymes (xylanase, amylase, and protease cocktail) in combination with probiotic bacteria decreased litter moisture and reduced the severity of foot pad dermatitis in broilers[31;32]. However, Cengiz *et al.*[33] reported that the supplementation of a corn-soybean diet with different enzyme preparations (with galactosidase, xylanase, protease, amylase, glucanase, or mannanase activity) had no effect on litter moisture. Similar results were observed when a mixture of NSP-degrading enzymes was added to the diet containing a high level of barley[34]. No improvement was also observed in terms of dry matter content of excreta and litter when carbohydrase complex was added in different inclusion levels in broilers diet[35].

CONCLUSIONS

The findings of this study suggest that supplementation of Rovabio Excel to diet with reduced energy level allowed for the total recovery of broiler growth performance and provided the best economic result. Natuzyme supplementation partially restored performance results. Dietary enzymes did not affect mortality and litter quality. Further studies are needed to consider the impact of summer management and heat stress on energy and nutrient levels with enzyme supplementation since the current study was carried during the spring season. Moreover, this experiment could be repeated in future studies while considering fecal or digesta sampling in order to have much more knowledge about enzyme supplementation effect on chicken gastrointestinal microbiota.

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