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Full Length Research

Effect of multi-enzyme complex and feed form on growth performance, slaughter characteristic, total tract nutrient digestibility, and energy utilization in broiler chickens

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ABSTRACT: This study was conducted to investigate the effect of dietary supplementation of exogenous multi-enzyme complex and feed form on growth performance, slaughter characteristics, nutrient digestibility, phosphorus (P), calcium (Ca), and nitrogen-corrected apparent metabolizable energy (AMEn) utilization in broilers fed corn–soybean meal diets. A total of 312 male one day-old broiler chicks (Ross 308) were allocated to 6 dietary treatment groups with 13 replicates (4 birds each) in a 2×3 factorial arrangement of treatments in a randomized design, which includes mash (M) or pellet (P) diets supplemented with three levels of a multi-enzyme complex (0, 125, or 175 mg/kg) for 42 days. The results indicated that the addition of 175 mg/kg multi-enzyme complex to pelleted feeds (P175) significantly increased ($p<0.05$) the body weight and feed intake of the broilers during the grower period. The feed conversion ratio (FCR) was significantly improved ($p<0.05$) by adding the multi-enzyme complex to the pelleted feeds. The feed form * multi-enzyme interaction was significant on d0-21 for BW and FI and all three periods in FCR ($p<0.05$). Mash feed decreased the European Production Efficiency Factor (EPEF) ratio compared to that of the pelleted feed, and it was observed that EPEF increased ($p<0.05$) linearly with an increase in the multi-enzyme complex added to both the mash and pelleted feeds. Although there was no difference among the groups regarding the carcass yield and relative organ weights, the gizzard weight increased significantly in chickens fed with mash feeds ($p<0.05$). In addition, the difference between treatment groups in terms of total tract nutrient digestibility does not differ ($p>0.05$) significantly, while the highest ($p<0.05$) AMEn utilization was found in the P175 group. As a result, the pelleted feed form and the increased level of multi-enzyme supplementation improved the performance and efficiency of broiler production.

Keywords: Multi-enzyme, feed form, performance, digestibility, AMEn, broilers.

INTRODUCTION

The poultry feedstuff prices constitute almost 70-80% of the cost of broiler production. Corn, wheat, and soybean meal are the main feedstuff that provides energy and protein in broiler diets. However, the feed value of these feedstuffs is limited by anti-nutritive factors such as non-starch polysaccharides (NSPs) in the polymeric structure they contain (Denstadli *et al*., 2010). This is because broiler chickens lack the enzymatic capacity to degrade NSPs. These high water-soluble NSPs, such as cellulose, xylan, arabinoxylan, and β-glucan content in cereal-based diets, increase the viscosity of the digestive system, resulting in less digestibility of nutrients, which leads to

deterioration of performance in broilers (Nahas and Lefrançois, 2001; Józefiak *et al*., 2004). Furthermore, the decrease in nutrient digestibility is due to the high viscosity limiting the activities of endogenous enzymes and damaging the intestinal mucosal surface (Nahas and Lefrançois, 2001), resulting in lower productivity. In addition, Austin *et al.* (1999) reported that excess water-soluble NSPs in diets increase the water consumption in broilers and cause wet and sticky excreta welded litter problems. These adverse effects can be overcome by adding exogenous enzymes to the broiler diet (Lázaro *et al.,* 2004). It was determined that adding β-glucanase and xylanase enzymes to the broiler diets increased broiler chicks' daily body weight gain (BWG) and improved the feed conversion ratio (FCR). Besides, it was reported that these enzymes decrease the intestinal content viscosity and increase contact between endogenous enzymes and nutrients, thus improving nutrient digestibility (Munyaka *et al.,* 2016). Likewise, Barasch and Grimes (2021) reported that heat-stable exogenous xylanase increases BWG and nitrogen-corrected apparent metabolizable energy (AMEn) and decreases digesta viscosity. Some other studies showed that dietary phytase added to a broiler diet increases nutrient digestibility and mineral material availability (Żyła *et al.,* 2001; Santos *et al*., 2008; Walters *et al*., 2019). In addition, several studies have shown synergistic effects of adding phytase and NSPs enzymes on growth performance, nutrient digestibility, and viscosity. Peng *et al.* (2003) reported that the combination of phytase and xylanase significantly increased phytate digestibility by 77.8% and improved FCR by 7.3% compared to phytase alone. Lee *et al*. (2010) informed that in broilers fed corn, wheat, and soybean-weighted diets, a multi-enzyme complex containing phytase and NSPs enzymes improves growth performance and the mineral content of the tibia. It was also reported that improved performance is associated with decreased intestinal viscosity from adding the dietary multi-enzyme complex.

Feed form was also known to significantly affect growth performance and feed intake in broiler chickens (Dozier *et al.,* 2010). Many researchers also noted that BW is increased in broilers fed with pelleted feed than in those fed with mash feed, while the FCR is also improved (Chewning *et al*., 2012; Lv *et al*., 2015). Pelleted feeds have advantages such as reducing feed waste, preventing selection feed, eliminating pathogens, improving flavour, and improving nutrient digestibility (Lv *et al.,* 2015). Moreover, interactions between dietary form and content with enzyme supplementations have been investigated in many studies. Indeed, Waititu *et al.* (2014) stated that diets containing different types of cereals and industrial by-products with multi-enzyme mixture interaction improved FCR in broilers. Also, Barasch and Grimes (2021), investigating the enzyme efficacy after the pelleting process, reported that adding xylanase to crumble feeds improved BWG and FCR linearly compared to mash feeds. However, the temperature during the pelleting process has disadvantages regarding the adverse effects on the enzyme structures. Thus, the present study was conducted to evaluate the effectiveness of heat-stable multi-enzyme on growth performance, nutrient digestibility, and apparent metabolizable energy in broilers fed mash or pelleted feeds.

MATERIALS AND METHODS

Experimental design and procedures

In this study, 312 one-day old male broiler chicks (Ross-308) were purchased from a commercial hatchery. The chicks were allocated to 6 treatment groups (13 replicates with 4 birds per replicate). The study was designed in the factorial experiment (2 x 3) in a completely randomized design of two different feed forms (mash, pellet) and three different multi-enzyme levels (0, 125, and 175 mg/kg feed). The dietary group consists of 6 treatments, **M**; mash feed without any enzyme additives (control), **M125**; 125 mg multi-enzyme added to each kg of mash feed (50 Kcal ME/kg less from M Diets), **M175**; 175 mg multi-enzyme added to each kg of mash feed (50 Kcal ME/kg less from M Diets), **P**; pelleted feed without any enzyme additives (control), **P125**; 125 mg multi-enzyme added to each kg of pelleted feed (50 Kcal ME/kg less from P Diets), **P175**; 175 mg multi-enzyme added to each kg of pelleted feed (50 Kcal ME/kg less from P Diets). The multi-enzyme complex (Endofeed® DC, EU Register No:4a1601, Annex 1, Spain) used in the broiler diets contains endo-1,3(4)-beta-glucanase (min. of 1100 units/g) and endo-1,4-beta-xylanase (min. of 1600 units/g) from the fermentation product produced by a non-genetically modified strain of *Aspergillus niger* NRRL 25541. The feed raw materials used in the study were obtained from a commercial feed factory, and the diets of the experiment were prepared at the feed production facility in the Department of Animal Science, Dicle University. Pellet feeds were formed by pressing the mashed feed produced under dry airflow at 72°C. The disc temperature was measured at 90°C while the mashed feed was in the matrix. The mash feed was measured at 72°C after the 4 mm outlet and at 50°C when it reached 2 cm. Approximately 20 minutes after the application of hot, dry airflow, the temperature of the pellet feed is reduced to 25°C and humidity to 12% in 15 minutes. Enzymatic recovery in mash form was 100%, and in pellet form was 91% for β-glucanase and 87% for xylanase. Then the pelleted and also mash feed were stored in 25 kg bags. The nutrient contents of the mixed feed in mash and pellet form to be used in the experiments are prepared mainly in corn and soybean meal according to the nutrient requirements of broiler chickens reported in NRC (1994) and Aviagen (2014). During the experiment, broiler chicks were fed a grower diet from day 0 to 21 and a finisher diet from day 22 to 42. The ingredients and chemical composition of experimental diets used in the study were given in Table 1. Wood shavings was used as the litter in the experiment, and the research was carried out in a full

Table 1. Ingredients and chemical composition of experimental diets (as-fed basis).

Parameters	Grower (d10-21)		Finisher (d21-42)	
	Control	Treatments*	Control	Treatments*
Ingredients (g/kg)				
Corn	505.00	515.00	570.00	580.00
Soybean meal (CP 46%)	195.00	195.00	200.00	200.00
Full fat toasted soybeans	250.00	250.00	155.00	155.00
Sunflower oil	10.00	-	40.00	30.00
Dicalcium phosphate	21.00	21.00	17.50	17.50
DL-Methionine	2.00	2.00	1.00	1.00
L-Lysine HCl	1.50	1.50	1.00	1.00
Limestone	10.00	10.00	10.00	10.00
NaCl	3.00	3.00	3.00	3.00
Vitamin and Mineral premix[1]	2.50	2.50	2.50	2.50
Multi-Enzyme[2]	-	0.125-0.175	-	0.125-0.175
Analysed nutrient composition, or calculated[3]				
Dry matter, %	90.02	90.26	89.80	90.14
Crude ash, %	5.62	5.80	5.75	5.67
Crude protein, %	22.50	22.50	20.05	20.05
Ether extract, %	7.62	6.90	9.57	9.05
Starch, %	36.22	36.79	39.05	38.85
Sugar, %	4.76	4.32	4.85	4.90
ME, Kcal/Kg[3]	3052	3002	3237	3187
Ca, %[3]	1.00	1.00	0.90	0.90
Available P,%[3]	0.46	0.46	0.40	0.40
L-Lysine, %[3]	1.34	1.34	1.11	1.11
Methionine+Cystine, %[3]	0.90	0.90	0.73	0.73
Na, %[3]	0.15	0.15	0.15	0.15

*ME values of the treatment group diets with enzyme addition were formulated to be 50 kcal/kg less than the control groups.
[1]Providing per 2.5 kg of diet: vitamin A, 12000000 IU; vitamin D3, 1500000 IU; vitamin E, 4000g mg; vitamin K_3, 5000 mg; vitamin B_1, 3000 mg; vitamin B_2, 7000 mg; vitamin B_6, 5000 mg; vitamin B_{12}, 30 mg; CAL-D pantothenate, 10000 mg; Biotin, 75 mg; Folic acid, 1000 mg; Niacin amide, 4000 mg; choline chloride, 400000 mg; Mn sulphate, 80000 mg; Fe (II) sulphate, 60000 mg; Cu (II) sulphate, 5000 mg; Zn sulphate, 60000 mg; Ca iodide, 1000 mg; Na selenite, 150 mg and CaCO3, 1135000 mg.
[2]Added 125 or 175 mg multi-enzyme complex/kg of the control diet
[3]Calculated value.

environmental controlled closed experiment coops set in the Department of Animal Science, Faculty of Agriculture, Dicle University. In the first 5 days of the experiment, the in-coop ambient temperature was between 33-34°C, gradually decreasing to 21-22°C on the other days of the study. Group feeding was applied for chickens in each pen (0.4 m x 1 m = 0.4 m^2), and feed and water were presented *ad libitum*. During the experiment, 23 hours of light and 1 hour of dark plan were applied. The experiment lasted for 6 weeks.

Feed analysis

Determination of the nutrient content of the mixed feed used in the experiment (excluding crude cellulose) was made according to the Weende analysis method (Naumann and Bassler, 1993). The determination of the crude fibre was done according to the Lepper method (Bulgurlu and Ergül, 1978). Additionally, metabolizable energy content was calculated using the regression equation recommended by Turkish Standards Institute standard No. 9610 (TSI, 1994).

Data collection

Performance parameters and measurement of organ weights

Chickens were weighed individually prior to and every week of the experiment, and the body weights (BW) were recorded. Feed intake (FI) for each sub-group was recorded based on replications to determine the growth performance. The feed conversion ratio (FCR) was calculated by dividing the feed intake values determined weekly for each replication by the body weights obtained in the periods of the study. The dead chickens were

recorded daily, and necessary corrections were made considering the FCR. At the end of the experiment, 10 chickens randomly selected from each group were weighed and killed by neck dislocation and bleeding. The chickens were then dissected and de-feathered. The hot carcasses, liver, gizzard, proventriculus, intestine, and abdominal fat were weighed. The slaughter characteristics results were given in proportion to the pre-slaughter BW in terms of proportional values (g/100g BW). Hot carcass yield was calculated by rating the hot carcass weight to pre-slaughter body weight. At the end of the study, The European Production Efficiency Factor (EPEF) was calculated according to Huff *et al.* (2013) using the following formula:

$$\text{EPEF} = \frac{\text{livability d0} - 42\ (\%)\ \text{x BW d42 (kg)}}{\text{Age (d) x FCR d0} - 42}\ \text{x } 100$$

Total tract digestibility analysis and AMEn calculation

For *in vivo* digestibility, 8 chickens per treatment group were placed in individual cages. FI values and total excreta were recorded daily between d17-21 in the grower phase and d38-42 in the finisher phase of the experiment. The excreta picked up from each cage was weighed, and 1/5 of the excreta was kept in a sample cup by adding 3–5 drops of chloroform (Kong and Adeola, 2014). Then the excreta samples were dried at 65°C until constant weight, and the excreta samples of the groups were pooled and labeled. In addition, the diet and excreta were ground to pass through a 1-mm screen and stored at -4°C for analysis. Dry matter (DM), gross energy, ether extract (EE), calcium (Ca), and phosphorus (P) contents of the feeds and excreta samples were analyzed by AOAC International (2005). The crude protein (CP) contents were analyzed using an automatic N/CP analyzer (Leco FP-528, USA). Organic matter (OM) content was calculated by subtracting the crude ash results from DM. Calculating CP digestibility, the uric acid content in the excreta was removed from the total nitrogen in the feces (Marquardt, 1983). DM, OM, CP, and EE digestibility were calculated following the nutrient digestibility (%) = [(nutrient intake - nutrient in excreta)/nutrient intake)x100] equation presented by Nkukwana *et al.* (2014). All values are expressed in dry matter. Nitrogen-corrected apparent metabolizable energy (AMEn) was calculated using the following formula reported by Adeola *et al*. (1997) and Sibbald (1988).

AMEn/g yem= [(YxBEy) - (DxBE$_D$) - (ATxK)]/Y

Where: Y = the amount of feed intake (g); BE$_Y$= gross energy of feed intake (kcal); D = the amount of excreta (g); BE$_D$ = gross energy of excreta (kcal); AT = (YxA$_Y$)-(DxA$_D$); A$_Y$ = nitrogen content in diet (g/g); A$_D$ = nitrogen content in excreta (g/g); K = 34.4 KJ/g; (1 gram of uric acid is fermented (kcal).

Statistical analysis

Data were analyzed using the two-way variance (ANOVA) procedure of SPSS 22.0 software (SPSS, 2013) in factor experimental with a model containing the fixed effects of feed form and multi-enzyme supplementation and their interaction terms. Means were separated by Tukey multiple range test. The data were assumed to be statistically significant when $p<0.05$. Mortality was analyzed by using the non-parametric Kruskal-Wallis and Mann-Whitney U tests.

RESULTS AND DİSCUSSİON

Broiler performances

The effect of dietary supplementation of multi-enzyme to mash or pelleted feed in various levels on performance and EPEF value in broilers is presented in Table 2. The difference in BW and FI of the treatment groups in the grower phase (d0-21) were recorded significantly ($p<0.05$), and the highest BW and FI values were observed in group P175. The lowest BW and FI values were found in groups M and P125. The BW and FI values of the groups between d22-42 and d0-42 were determined to be similar ($p>0.05$) across the treatments. Analyzing the groups in terms of FCR, the differences between the FCR values belonging to all periods were found to be a significant ($p<0.05$) difference. Accordingly, the FCR value between d0-21 attained the highest level in group M and M125, respectively, while the lowest level was observed in group P. The FCR value between d22-42 was similar ($p>0.05$) and higher ($p<0.05$) in groups M, M125, M175, and P compared to other groups. The best ($p<0.05$) FCR value was observed in group P175. The FCR value between d0-42 was at the highest ($p<0.05$) level in groups M and M125, while the lowest level ($p<0.05$) was determined in groups P125 and P175. The interaction between the feed form and multi-enzyme was found to be significant between d0-21 for BW and FI and in all periods for FCR ($p<0.05$). However, it was observed that broilers fed pellet-form diets had higher ($p<0.05$) levels of BW and FI and better FCR compared to those fed mash-form diets with the same composition (Table 2). Indeed, Barasch and Grimes (2021) reported that broiler feed in crumble form increases BW gain and FI and improved FCR in d21 compared to the mash feed, however; enzyme (xylanase) and feed form interaction did not affect the performance ($p>0.05$). Jensen *et al.* (1962) stated that this is an expected outcome and based the provision of pellet-form feed instead of mash-form feed upon the broilers expending less energy for the consumption of the equal amount of feed and spending less time for feeding. Nevertheless, it was determined that adding multi-enzyme to the feed at various levels did not affect the performance values ($p>0.05$). It was reported in the studies conducted in similar ways that the addition of multi-enzyme to the broiler diets did not affect BW, FI, and FCR (Hussein *et al*., 2020; Mohammadigheisar *et al*., 2021)

Table 2. Effect of dietary multi-enzyme supplementation and feed form on BW, FI, FCR, Mortality and EPEF in broiler chickens.

Treatment	BW, g/bird			FI, g/bird			FCR, g/g			Mortality, %	EPEF
groups[1]	d0-21	d22-42	d0-42	d0-21	d22-42	d0-42	d0-21	d22-42	d0-42	d0-42	d42
M	799.67^{b}	1919.17	2718.83	1054.83^{ab}	3138.68	4193.49	1.29^{b}	1.65^{b}	1.54^{c}	4.62	384.00^{c}
M125	817.55^{b}	1882.86	2700.43	1055.33^{ab}	3169.04	4224.35	1.30^{b}	1.63^{b}	1.54^{c}	3.08	388.25^{c}
M175	801.77^{b}	1890.26	2692.04	1025.97^{ab}	3155.71	4181.66	1.27^{ab}	1.65^{b}	1.53^{b}	3.08	434.13^{b}
P	901.27^{ab}	1913.84	2815.12	1034.71^{ab}	3222.99	4257.67	1.17^{a}	1.64^{b}	1.49^{ab}	1.54	402.13^{c}
P125	841.13^{b}	2013.05	2845.18	1011.97^{b}	3238.91	4250.85	1.24^{ab}	1.56^{a}	1.46^{a}	4.62	459.63^{ab}
P175	922.58^{a}	1964.75	2887.32	1119.93^{a}	3215.65	4320.17	1.18^{ab}	1.59^{ab}	1.46^{a}	3.08	470.75^{a}
SEM[2]	10.58	10.78	24.08	6.97	20.68	22.42	0.01	0.01	0.01	-	9.46
Feed form											
Mash	812.64^{b}	1892.45	2699.74^{b}	1044.86	3156.07	4193.66^{b}	1.28^{b}	1.64^{b}	1.56^{b}	3.59	402.13^{b}
Pellet	888.33^{a}	1963.88	2852.21^{a}	1044.29	3136.86	4283.42^{a}	1.18^{a}	1.59^{a}	1.47^{a}	3.08	444.17^{a}
SEM[2]	10.9	18.59	24.44	7.56	24.59	21.77	0.10	0.06	0.07	-	5.46
Multi-enzyme											
0	854.35	1911.00	2770.50	1040.23	3150.63	4222.97	1.22	1.65^{b}	1.52	3.08	393.06^{c}
125	829.34	1947.97	2777.30	1032.44	3149.38	4236.49	1.25	1.59^{a}	1.51	3.85	423.94^{b}
175	867.77	1930.61	2785.56	1052.07	3139.39	4241.88	1.23	1.61^{ab}	1.51	3.08	452.44^{a}
SEM[2]	10.93	18.59	24.44	7.70	24.60	21.77	0.01	0.01	0.01	-	6.69
Main effect	p-value										
Feed Form	<0.001	0.055	0.001	0.970	0.699	0.042	<0.001	<0.001	<0.001	0.317	<0.001
Multi-enzyme	0.350	0.724	0.970	0.564	0.980	0.937	0.449	0.001	0.597	0.368	<0.001
Feed form * Multi-enzyme	0.002	0.297	0.072	0.002	0.702	0.616	0.003	<0.001	<0.001	0.416	0.024

$^{a-c}$Means within a column with different superscripts are significantly different at ($p<0.05$). BW -Body weight; FI -Feed intake; FCR -Feed conversion rate; EPEF - European Performance Efficiency Factor.
[1]Each value represents the least square mean from 13 cages per each treatment. Abbreviations: M, mash feed (positive control); M125, mash feed+125 mg/kg enzyme; M175, mash feed+175 mg/kg enzyme; P, pellet feed (positive control); P125, pellet feed+125 mg/kg enzyme; P175, pellet feed+175 mg/kg enzyme.
[2]SEM═pooled standard error of mean.

2021). Moreover, the addition of multi-enzyme to the feed increased ($p<0.05$) the BW in d14-42 according to Alam *et al*. (2003), in d0-42 according to Dersjant-Li *et al.* (2015), and in d11-42 according to Taheri and Shirzadegan (2017) while it did not affect BW in d1-21 according to Zhu *et al*. (2014), in d1-28 according to Hajati (2010), and in d7-35 according to Mohammed *et al*. (2018). However, Yu and Chung (2004) reported that adding glucanase and xylanase to the feed increased BW in the hot season. Adding protease and amylase decreased it, and both enzymes were ineffective in the cold season. The results obtained from the study on the addition of feed form or enzyme, the effect on FI was found to agree with Ahmed and Abbas (2013), Shabani *et al.* (2015), Stefanello *et al*. (2015), and disagree with Alam *et al.* (2003), and Hajati *et al.* (2009); On FCR, agree with Jafarnejad *et al.* (2010), Torres *et al.* (2013), Stefanello *et al.* (2015), and disagree with Zakaria *et al.* (2010). Thus, it can be expressed that the enzyme addition used in the current study has multiple enzymatic activities. Its combination with the pellet-form diet has a more significant effect on the performance than its combination with the mash-form diet and single enzyme additions targeting a substrate.

Table 3. Effects of dietary multi-enzymes supplementation and feed form on carcass yield and organ weights (% of BW) in broiler chickens.

Treatment groups[1]	Carcass yield	Abdominal fat	Liver	Gizzard	Pro-ventriculus	Intestinal tract
M	73.14	0.69	2.01	2.36[a]	0.33	5.25
M125	72.29	0.76	2.03	2.34[a]	0.36	5.85
M175	73.70	0.92	1.96	2.21[ab]	0.39	5.60
P	72.32	0.83	1.92	1.74[c]	0.33	6.32
P125	73.60	0.81	1.91	1.89[bc]	0.32	5.29
P175	73.95	0.79	1.95	1.51[c]	0.34	5.48
SEM[2]	0.20	0.04	0.04	0.04	0.01	0.12
Feed Form						
Mash	73.22	0.77	2.00	2.31[b]	0.35	5.55
Pellet	73.28	0.80	1.93	1.72[a]	0.33	5.71
SEM[2]	0.19	0.03	0.03	0.08	0.01	0.12
Multi-enzyme						
0	72.83	0.73	1.95	2.10	0.33	5.71
125	73.20	0.79	1.98	2.11	0.34	5.57
175	73.81	0.84	1.97	1.82	0.37	5.62
SEM[2]	0.19	0.03	0.03	0.06	0.06	0.12
Main effects	p-value					
Feed Form	0.876	0.669	0.325	<0.001	0.120	0.515
Multi-enzyme	0.101	0.342	0.941	0.074	0.065	0.890
Feed Form * Multi-enzyme	0.190	0.369	0.470	<0.001	0.076	0.104

[a-c]Means within a column with different superscripts are significantly different at ($p < 0.05$).
[1]Each value represents the least square mean from 10 birds per each treatment. Abbreviations: M, mash feed (positive control); M125, mash feed+125 mg/kg enzyme; M175, mash feed+175 mg/kg enzyme; P, pellet feed (positive control); P125, pellet feed+125 mg/kg enzyme; P175, pellet feed+175 mg/kg enzyme.
[2]SEM=pooled standard error of mean.

EPEF value provides an overall perspective on the economic status of production, are a widely used index that indicates the performance efficiency in poultry (Huff *et al*., 2013; Murugan and Ragavan, 2017). The current study determined that adding multi-enzyme to mash- or pellet-form diets at various levels have significantly changed the EPEF value ($p<0.05$, Table 2). The lowest and highest EPEF value among the groups were found in group M and group P175, respectively. Accordingly, the addition of multi-enzyme and pellet-form feed influenced EPEF value. This increase in EPEF was associated with the higher levels of BW attained by the chickens fed multi-enzyme-added pellet-form feed. It was reported similarly to this result that adding multi-enzyme increased the broilers' production index (Attia *et al.,* 2020). Contrary to that, adding an enzyme cocktail in broiler feeds did not affect EPEF value (Alqhtani *et al.,* 2022). However, Al-Nasrawi (2016) reported that the feed form affected EPEF value, and the highest EPEF value was found in crumble-form feed, followed by pellet and mash forms. Consequently, the interaction of pellet feed and multi-enzyme based on the level increase may have improved FCR by ensuring the hydrolysis of the NSPs in broiler feeds and, correspondingly, increased EPEF value, hence the efficiency inside the broiler house.

Carcass yield and organ weights

It was also reported that the growth rate in broilers might be associated with visceral organ development and function (Singh *et al.,* 2021). Likewise, in the current study, the addition of multi-enzyme to mash- or pellet-form diets in various levels had no observable effect on the carcass yield and organ weights of the broilers in d42 apart from the gizzard weights ($p>0.05$, Table 3). The broilers fed pellet-form feed's gizzard weights were higher than those fed mash-form feed ($p<0.05$). Feed form and enzyme interaction was found significant only for the gizzard weight ($p<0.05$). Furthermore, the effect of feed form or the addition of multi-enzyme on the organ weights was found insignificant. The differences between the organ weights belonging to the groups were similar ($p>0.05$). It was reported in the studies conducted on broilers that the addition of multi-enzyme to the diets increased the carcass, duodenum, and jejunum weights (Hussein *et al*., 2020), while the addition of an exogenous enzyme cocktail improved the carcass yield and decreased the gizzard and intestinal weights (Alqhtani *et al*., 2022). Ahmed and Abbas (2013) reported that the feed form did not affect the carcass and organ weights. Lv *et al*. (2015) also suggested that feed form and size affected the gizzard weights. The

Table 4. Effects of dietary multi-enzymes supplementation and feed form on total tract nutrient digestibility, P, Ca and AMEn utilization in broiler chickens at the grower (d17-21) and finisher (d38-42) period.

Treatment	DM, %		OM, %		CP, %		EE, %		AMEn, kcal/kg		P, %		Ca, %	
groups[1]	Gro.	Fin.	Gro.	Fin.	Gro.	Fin.	Gro.	Fin.	Gro.	Fin.	Gro.	Fin.	Gro.	Fin.
M	72.56	71.53	80.41	84.76	82.19	85.20	85.39	88.69	2818.50^{b}	2946.86^{b}	59.45	56.77	41.32	43.5
M125	71.75	71.97	81.05	85.38	83.22	85.29	86.50	89.15	2858.93^{ab}	3042.54^{ab}	61.43	60.57	44.14	47.1
M175	71.31	72.28	81.02	84.21	83.04	85.23	86.98	89.49	2884.81^{ab}	3063.03^{a}	63.68	58.69	41.91	46.2
P	71.79	71.35	81.41	84.94	83.13	86.35	86.79	89.28	2855.25^{b}	3050.64^{ab}	61.02	60.67	42.43	44.0
P125	71.80	72.14	80.81	84.56	83.39	88.08	86.81	90.06	2880.45^{ab}	3112.80^{a}	63.03	62.80	41.67	45.0
P175	71.58	72.02	81.43	83.79	83.28	87.43	87.51	90.20	2929.40^{a}	3118.54^{a}	65.77	62.43	42.51	45.8
SEM[2]	0.29	0.27	0.27	0.18	0.41	0.38	0.21	0.16	8.68	13.50	0.49	0.81	0.47	0.69
Feed form														
Mash	71.85	72.02	80.82	84.77	82.83	85.29^{b}	86.29^{b}	89.11	2856.49	3014.25^{b}	61.55	58.62^{b}	42.51	45.5
Pellet	71.72	71.99	81.22	84.36	83.27	87.12^{a}	87.03^{a}	89.84	2888.27	3097.85^{a}	63.27	62.29^{a}	42.20	44.9
SEM[2]	0.29	0.27	0.28	0.19	0.42	0.47	0.25	0.83	8.69	13.28	0.54	0.54	0.47	0.45
Multi-enzyme														
0	72.14	71.44	80.90	84.85	82.69	85.78	86.09	88.99	2840.49^{b}	2998.75^{b}	60.28^{b}	58.72^{b}	41.91	43.7
125	71.78	72.23	80.93	84.93	83.31	86.37	86.65	89.60	2869.69^{ab}	3073.54^{a}	62.23^{ab}	62.10^{a}	42.90	46.0
175	71.45	72.36	81.22	83.92	83.16	86.48	87.24	89.84	2907.11^{a}	3094.35^{a}	64.73^{a}	60.44^{ab}	42.21	45.9
SEM[2]	0.29	0.27	0.28	0.19	0.42	0.38	0.71	0.16	8.69	13.28	0.54	0.54	0.47	0.45
Main effects	p-value													
Feed Form	0.831	0.959	0.483	0.279	0.605	0.015	0.047	0.834	0.066	0.001	0.110	<0.001	0.749	0.459
Multi-enzyme	0.633	0.336	0.877	0.051	0.824	0.730	0.054	0.071	0.005	0.005	0.002	0.032	0.685	0.052
Feed form * Multi-enzyme	0.920	0.927	0.929	0.214	0.980	0.171	0.108	0.623	0.009	0.001	0.605	0.200	0.258	0.515

[a-c]Means within a column with different superscripts are significantly different at ($p < 0.05$). DM –dry matter; OM -organic matter; CP -crude protein; EE -ether extract; AMEn -nitrogen corrected apparent metabolizable energy; P – phosphorus; Ca – calcium. [1]Each value represents the least square mean from 13 cages per each treatment. Abbreviations: M, mash feed (positive control); M125, mash feed+125 mg/kg enzyme; M175, mash feed+175 mg/kg enzyme; P, pellet feed (positive control); P125, pellet feed+125 mg/kg enzyme; P175, pellet feed+175 mg/kg enzyme. [2]SEM=pooled standard error of mean. **Key:** Fin. = Finisher; Gro. = Grower.

gizzard weight decreased since the mechanical digestion periods of the pellet-form particles were shortened. Likewise, it was observed in this study that the broilers fed a pellet-form diet had lower gizzard weights compared to those fed a mash-form diet. Otherwise, Hajati *et al.* (2009) reported that adding a multi-enzyme did not change the organ weights. Zakaria *et al.* (2010) suggested that the ineffectiveness of adding multi-enzyme to organ weights can be associated with levels. Mohammadigheisar *et al.* (2021) also reported that adding multi-enzyme to broiler diets increased the gizzard weight, however; the connection between that increase and multi-enzyme is hard to explain, and the increase in weight is instead associated with the structural form of the feed.

Nutrient digestibility and energy utilization

No difference was observed among the treatment groups in the grower and finisher phases regarding DM, OM, EE, and CP digestibility and P and Ca utilization ($p>0.05$). In contrast, AMEn utilization significantly increased ($p<0.05$, Table 4). Moreover, the pellet-form feed significantly increased

EE digestibility in the grower phase. In contrast, it increased CP digestibility, AMEn, and P utilization in the finisher phase compared to the mash-form feed ($p<0.05$). In addition, multi-enzyme improved AMEn and P utilization in both grower and finisher phases in line with the level increase ($p<0.05$). The efficiency of the feed form and enzyme interaction on AMEn utilization was also significant ($p<0.05$). This interaction can be explained by the fact that there is less segregation in pelleted feeds, resulting in a more uniform feed intake in broiler chickens and, thus, more benefit from the multi-enzyme supplementation. Alqhtani *et al*. (2022) reported that the enzyme addition to broiler diets did not have an effect on DM and nutrient digestibility but improved AMEn utilization in d35-38. This improvement may be associated with the fact that multi-enzyme activity, main xylanase, decreased the viscosity of the intestinal contents (Bedford, 2000; Scheideler *et al.,* 2005), and glucanase, which is effective in cellulose degradation, broke down the 1-4 bonds in the glucan chain forming the cellulose microfibrils (Singh *et al*., 2016). In addition, Mitchell and Smith (1991) reported a correlation between visceral organ development and maintenance energy requirement and the relative amount of mucosa in the small intestine. Thus cell turnover both have a negative correlation with the growth rate of the birds. Accordingly, the significant increase, particularly in the gizzard weights of the broilers fed mash-form feed and the relative increase in the weights of the other organs, can be partially associated with the decrease in AMEn utilization. However, Barasch and Grimes (2021) determined that xylanase improved AMEn utilization in parallel with the level increase. Still, AMEn utilization was higher in the mash feed than in the crumble feed, contrary to the current study results. This difference between the results of the studies can be explained by the effect of heat treatment conducted on the crumble feed or the feed in the pelleting process on the enzyme activity. In addition, Johnson *et al*. (2014) reported that phytase addition did not change DM and CP digestibility, while Oliaei *et al*. (2016) stated that multi-enzyme addition increased them. In this regard, it is possible to express that the effect of the enzymes on the nutrient digestibility, P, Ca, and AMEn utilization changes according to their addition to the feed, either in single or mixture form.

Conclusion

In conclusion, the increase in the performance of the broilers was observed to be associated with the increase in nutrient digestibility and energy utilization based on the multi-enzyme concentration in addition to the pellet-form diets. For this reason, the multi-enzyme addition and pelleting process are recommended in broilers fed grain-based diets.

COMPETİNG İNTERESTS

The authors declare that they have no competing interests.

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