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Growth performance of broiler chickens fed diets supplemented with amylase and protease enzymes individually or combined

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Abstract

Background: Feed additives that increase nutrient availability in feeds have gained a lot of interest.

Aim: An experiment was conducted to determine whether amylase, protease, and their combined supplementation affected broiler performance.

Methods: Two hundred eighty broiler chicks were selected and distributed randomly into 28 replicate pens with four treatment groups and seven replicates under a completely randomized design. A total of four diets were developed, having 0, 100, 100, and 100 + 100 g of control (AP0), amylase (A1), protease (P1), and amylase + protease (AP1)/ton of feed, respectively. Four replicates of each treatment were fed each diet. Each diet was randomly allotted to each group. *Ad-libitum* feeding was provided to the birds. The feeding program had starter and finisher diets. Upon completion of the experiment, three birds from each pen were slaughtered to analyze the carcass characteristics and organ weight.

Results: Differences were insignificant between 100 g/ton of amylase supplementation and FI, body weight gain (BWG), or feed conversion ratio (FCR) ($p > 0.05$). Supplementation with 10 0g/ton of protease did not significantly affect FI, BWG, and FCR ($p > 0.05$). Similarly, 100 + 100 g/ton of amylase + protease addition had no significant effect on FI, BWG, and FCR ($p > 0.05$). None of the treatments significantly affected carcass weight, abdominal fat percentage, dressing percentage, drumstick, wings, breast, and thigh weights ($p > 0.05$). In addition, there were no significant effects ($p > 0.05$) on the weight of the heart, liver, gizzard, and spleen.

Conclusion: In conclusion, amylase, protease, and their combined supplementation at a rate of 100 g/ton of feed did not influence BWG, FI, FCR, carcass characteristics, or organ weight.

Keywords: Animals, Amylase, Protease, Broiler, Growth performance.

Introduction

One method of increasing nutrient availability is to add supplemental enzymes to the feed. The feed enzyme industry currently generates over $1 billion in annual revenue, with phytases, proteases, and carbohydrases accounting for most of this (Barletta, 2010; Hashim *et al.*, 2023). The pH of the proventriculus is acidic, 2.5–3, where protein digestion occurs. They improve the energy value of feedstuff, improve the digestion, assimilation, and usage of carbohydrate, protein, fat, and phosphorus from undigested crop residues, and reduce the excretion level of P & N in nondigestible

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nutrients in the surrounding, resulting in decreased environmental degradation (Dosković *et al.*, 2013; Dersjant-Li *et al.*, 2021).

Enzymes are substances or biological catalysts that accelerate biochemical reactions without being consumed. Most enzymes are protein, and as such, they exhibit protein features. Exogenous enzymes are those supplied to an animal's diet to generate the desired result (Alabi *et al.*, 2019). Different enzymes are widely used for the betterment of the poultry industry. Although the usefulness of carbohydrases (amylase, xylanase, amylase, beta-glucans, and so on), proteases (bromelain, ficin, papain, and so on), and phytases (allzyme, Natuphose, Ronozyme, and so on) in poultry nutrition was very well-validated, still there is a lot of complexity at how exogenic enzymes function.

The broiler will not even be able to utilize high-fiber diets since they are challenging to digest fully. A rich fiber diet has an anti-nutritional effect, as seen through wet excrement and inadequate feed efficiency. As a result, including enzymes as a supplement in diet will improve their productivity while lowering feces volume and wet dropping. In addition, enhanced nitrogen and starch digestion and protein, carbohydrate, and fat absorption (Kiarie *et al.*, 2014). Phytase, protease, and other enzyme combinations are used to enhance nutrient maintenance and use in growing chickens. There is not much information about employing one enzyme alone or in combination with another to increase birds' productivity (Bedford, 2000). However, the efficacy of enzyme addition can be affected by various variables, including feed composition, avian age, environmental conditions, enzyme type and dosage, and interactions with other feed additives.

It has been discovered that the incorporation of amylase into poultry diets improves the utilization of dietary carbohydrates, resulting in enhanced growth performance, feed efficiency, and body weight gain (BWG) (Ibrahim *et al.*, 2023). Yuan *et al.* (2018) reported amylase supplementation in combination with protease, or glucoamylase improves the digestibility of starch and the biodiversity of the intestinal microbes and increases the growth rate of broilers. Furthermore, it has been demonstrated that incorporating protease into poultry diets improves the utilization of dietary protein, thereby increasing the avian's access to amino acids. According to Mahmood *et al.* (2017), the supplementation of protease to the starter and finisher poultry diet increased BWG and feed conversion ratio (FCR) during the starter period of growth. Cowieson *et al.* (2017) found that the supplementation of protease to soybean meal had significant effects on the performance of broiler poultry, such as an increase in nitrogen digestion in a main protein-based ration and an increase in jejunal villus height.

According to Anwar *et al.* (2023), the combination of amylase, protease, and xylanase enhances the feed intake (FI), and BWG and improves the FCR in the overall phase. In addition, supplementation of exogenous xylanase and phytase alone or in combination enhances the nutrient digestibility and reduces the digesta viscosity. Amylase and protease can work better together than they do separately in broiler diets. Improved protein and carbohydrate digestion and absorption leads to better nutrient use, which generally enhances broiler growth performance. The main objective of this study is to observe the broiler's productive performance by adding amylase, protease, or a combination of these enzymes to their diets, in order to improve its production perfprmance.

Materials and Methods

Experimental station

The research was conducted at Sind Feed and Allied Products R&D broiler farm, Karachi, Pakistan.

Experimental birds

Two hundred and eighty birds were purchased from the market and all chicks were inspected for abnormalities. Birds were distributed into four treatments, AP0, A1, P1, and AP1, under Completely Randomize Design. Each treatment was divided into seven replicates and each replicate had ten birds.

The housing of birds and management

The poultry shed was washed, cleaned, and then disinfected. After the cleaning process fumigation was done by using potassium permanganate and formalin. After placing litter material and utensils, the replicates were distributed in the brooding pan. A total of 280-day-old broiler chicks were purchased from the market and raised at the broiler R&D farm Sindh Feeds (Karachi). The shed's temperature in the first week is 35°C and decreases by 2°C weekly. Biosecurity was maintained throughout the experimental time. Potassium permanganate was kept at the entrance of the shed. The drinkers and feeders were cleaned daily with potassium permanganate.

Vaccination schedule

Chicks were vaccinated against New Castle disease (ND), infectious bronchitis (IB), and infectious bursal

Table 1. Vaccination schedule.

Age (Days)	Vaccine	Route	Name
5	New castle disease + Infectious bronchitis	Eye drop	LASOTA
10	Gamboro	Drinking water	Gamboro 228
13	Infectious bronchitis	Drinking water	H120
15	New castle disease	Drinking water	LASOTA
25	New castle disease	Drinking water	LASOTA

disease (IBD) in this experimental trial according to the schedule given in Table 1.

Experimental diet and feeding

Four experimental diets were formulated (Tables 2 and 3), i.e., controlled, 100 g per ton of amylase, 100 g per ton of protease, and 100 g amylase + 100 g protease per ton of feed. A weighing of the birds was performed at the beginning and the end of the experimental period. FI, BWG, and FCR were also recorded weakly.

Data collection

Body weight

An electrical weighing balance was used to determine the initial weight of the chicks when they arrived. Chicks were weighed weekly in each replicate after that.

Table 2. Composition of experimental diets.

Ingredients (g)	Period (days)											
	Starter				Grower				Finisher			
		Treatments				Treatments				Treatments		
	Control	100 g/ ton Am.	100 g/ ton Pr.	100 g/ ton AmPr.	Control	100 g/ ton Am.	100 g/ ton Pr.	100 g/ ton AmPr.	Control	100 g/ ton Am.	100 g/ ton Pr.	100 g/ ton AmPr.
Maize	930	930	930	930	1,035	1,035	1,035	1,175	1,175	1,175	1,175	1,175
Soybean meal	590	590	590	590	420	420	420	420	270	270	270	270
Full fat soya	60	60	60	60	150	150	150	150	220	220	220	220
Rice polish	200	200	200	200	195	195	195	195	60	60	60	60
Sunflower meal	100	100	100	110	0	0	0	0	0	0	0	0
Canola meal	0	0	0	0	20	20	20	20	90	90	90	90
PBM	50	50	50	50	80	80	80	80	80	80	80	80
MBM	0	0	0	0	60	60	60	60	70	70	70	70
Chips limestone	25	25	25	25	8.2	8.2	8.2	8.2	0	0	0	0
MDCP	8.9	8.9	8.9	8.9	0	0	0	0	0	0	0	0
PREMIX*	10	10	10	10	10	10	10	10	10	10	10	10
Soda	1.7	1.7	1.7	1.7	1.6	1.6	1.6	1.6	1.35	1.35	1.35	1.35
Salt	5.4	5.4	5.4	5.4	4.8	4.8	4.8	4.8	4.7	4.7	4.7	4.7
Lysine sulphate	8.6	8.6	8.6	8.6	7.8	7.8	7.8	7.8	7.6	7.6	7.6	7.6
DL-Meth	6.7	6.7	6.7	6.7	6.8	6.8	6.8	6.8	5.7	5.7	5.7	5.7
L-threonine	2.5	2.5	2.5	2.5	2.3	2.3	2.3	2.3	1.3	1.3	1.3	1.3
L-isoleocine	0.02	0.02	0.02	0.02	0	0	0	0	0	0	0	0
L-valine	0.02	0.02	0.02	0.02	0	0	0	0	0	0	0	0
L-Arginine	0	0	0	0	0	0	0	0	0	0	0	0
Phyzyme	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
L-tryptohan	0	0	0	0	0	0	0	0	0	0	0	0
Choline 70%	2	2	2	2	2	2	2	2	2	2	2	2
Total	2,001.31	2,001.31	2,001.31	2,001.31	2,003.4	2,003.4	2,003.4	2,003.4	1,998.05	1,998.05	1,998.05	1,998.05

*Supplied the following per kg of diet: Vitamin A (IU): 1,500, Vitamin D3 (IU): 200, Vitamin B1 (mg): 1.0, Vitamin B2 (mg): 5.5, Vitamin B6 (mg): 2.2, Vitamin B12 (mg): 0.02, Vitamin E (IU): 8.0, Vitamin K3 (mg): 2.0, Folic acid (mg): 0.50, Pantothenic acid (mg): 13, Niacin (mg): 37, Choline chloride (mg): 500, Manganese (mg): 66, Zinc (mg): 55, Copper (mg): 6, Potassium (mg): 3.2, Iodine (mg): 1.2, Iron (mg): 27.5 and Biotin (mg): 0.10.

Table 3. Nutrient composition of diets.

Nutrients	Inclusion %	
	Starter diet (0–21 days)	Finisher diet (0–35 days)
Crude protein	22.4	19.7
Metabolize energy	3,000 kcal/kg	3,170 kcal/kg
Lysine	1.28	1.15
Sodium	0.18	0.18
Chloride	0.21	0.21
Calcium	0.95	0.90
Phosphorous	0.48	0.42

Feed intake (FI)

Total FI was measured per replicate weekly. The feed offered was subtracted from the amount refused to calculate the amount of FI. The FI per bird per day was calculated using the information obtained.

$$\text{FI (gram/bird)} = \text{feed offered (g)} - \text{refused feed (g)}$$

Feed conversion ratio

FCR was calculated from FI during the week and BWG per replicate weekly by using a formula:

$$\text{FCR} = \frac{\text{Total feed in take}(\text{g})}{\text{Total weight gain}(\text{g})}$$

Carcass measurement

Upon completion of the fifth week, three birds were randomly selected from each treatment and individually weighed. After slaughtering carcass birds had bled out sufficiently, the weight was taken before the feathers and skin were plucked. The birds were eviscerated after de-feathering. The abdominal fat and liver were taken and weighed during evisceration. The carcass was split into sections (breast, thigh, liver, gizzard, and heart) to measure each item individually.

$$\text{Dressing percentage} = \frac{\text{carcass weight}(\text{g})}{\text{live weight}(\text{g})} \times 100$$

Statistical analysis

The experiment was a completely randomized design. All the experimental data were subjected to the analysis of variance using SPSS (SPSS, 2019). Where statistical significance was observed, the mean values were separated using Duncan's multiple-range test (Duncan, 1955).

Ethical approval

The research was conducted at Sind Feed and Allied Products R&D broiler farm, Karachi, Pakistan, following the Animal Care Ethics.

Results

FI, BWG, and FCR as affected by dietary amylase, protease, or their combined supplementation are presented in Table 4. Amylase, protease, and their combination did not significantly affect the birds' FI, BWG, and FCR in the overall period ($p > 0.05$).

In the same context, the effects of dietary amylase, protease, or their combined supplementation on carcass characteristics and organ weight in broilers are presented in Table 5. The analysis of variance showed supplementation of amylase, protease, and their combination have no significant impact on live weight, carcass weight, dressing percentage, thigh weight, drumstick, abdominal fat%, breast weight, and wings weight ($p > 0.05$). In addition, amylase, protease, and

Table 4. Effect of dietary addition of amylase, protease, and their combination on growth performance during experimental periods.

Items	Periods	Treatment				SEM	*p*
		Con	Am	Pr	AmPr		
FI	Starter phase (1–21 days)	1,369	1,366	1,389	1,389	20.872	0.5595
BWG		1,021	1,031	1,047	1,034	11.433	0.2333
FCR		1.34	1.32	1.32	1.34	0.0178	0.6371
FI	Finisher phase (22–35 days)	3,519	3,503	3,553	3,514	44.541	0.715
BWG		2,387	2,394	2,346	2,343	56.452	0.718
FCR		1.47	1.46	1.51	1.50	0.0444	0.654
FI	Overall (0–35 days)	2,149	2,137	2,164	2,124	36.913	0.7409
BWG		1,366	1,362	1,299	1,309	54.245	0.5018
FCR		1.58	1.57	1.66	1.62	0.0748	0.5481

The terms Con, Am, Pr, and AmPr refer to diets containing 0, 100, 100, and 100 + 100 g of amylase, protease, and amylase + protease per ton of feed. The SEM stands for SE of the mean. The probability value is represented by *p*. Feed intake, BWG, and FCR are referred to as FI, BWG, and FCR, respectively.

Table 5. Effect of dietary addition of amylase, protease, and their combination on carcass characteristics and organ weight of broiler.

Items	Treatment				SEM	*p*
	Con	Am	Pr	AmPr		
Live weight (g)	2,462	2,337	2,300	2,250	70.341	0.0566
Carcass weight (g)	1,637	1,562	1,525	1,562	82.443	0.5950
Dressing percentage	66	67	66.29	69.5	2.7813	0.6294
Breast weight (g)	799	780	785.5	790	23.331	0.067
Thigh weight (g)	304.2	281.2	283	272.2	15.134	0.2409
Drumstick (g)	107.5	104	102.5	93.7	6.6482	0.2533
Wings (g)	69.5	68.5	69.2	67.5	4.9149	0.065
Abdominal fat %	40.25	38.5	37.6	35.9	2.5890	0.4440
Heart	12.25	14.50	12.25	11.50	1.15	0.11
Liver	56.50	56.25	58.25	61.00	6.00	0.84
Gizzard	31.25	32.00	30.00	30.25	2.11	0.76
Spleen	2.20	2.17	2.17	2.14	0.08	0.93

The terms Con, Am, Pr, and AmPr refer to diets containing 0, 100, 100, and 100 + 100 g of amylase, protease, and amylase + protease per ton of feed. The SEM stands for SE of the mean. The probability value is represented by *p*.

their combined administration into broiler feed have no significant effect on the organ weight of the birds ($p > 0.05$).

Discussion

Results of the present study confirmed that using amylase, protease, or they are combined at different periods did not show any effect on FI, BWG, and FCR. The results of FI in the present study were in line with Liu *et al.* (2020), who described no significant effect of amylase addition in the broiler diet on FI. In contrast, Lin *et al.* (2019) observed no significant effect in FI with protease supplementation in a broiler diet.

Shapiro and Nir (1995) noted no effect of combined supplementation of amylase and protease on FI. Supplementing poultry feeding soybean-meal diet with protease in the feed had no significant influence on FI (Rehman *et al.*, 2017; Law *et al.*, 2018). Cowieson *et al.* (2016) mention no beneficial impact of protease on FI in the broiler diet. Similarly, Angel *et al.* (2011) reported no significant improvement in FI. Furthermore, irrespective of whether the diet had low amounts of metabolizable energy, Cardoso *et al.*, 2011 found no positive effect on feed consumption of the birds provided diets with the administration of enzymes. No significant impact on FI was seen in broiler-fed diets based on corn with reduced salt-soluble protein, which also points to physiological symptoms because amylase administration was shown to suppress pancreatic amylase releases (Gehring *et al.*, 2012). Lourenco *et al.* (2020) observed no significant impact on FI by supplementing protease into decreasing crude protein (CP) levels in a broiler diet; therefore, protease supplementation has no beneficial impacts in a low CP diet.

In contrast to these findings, Oko *et al.* (2018) noted increased FI by supplementing amylase and protease. Jiang *et al.* (2008) supplemented amylase into a broiler diet, which improved FI. Yu and Chung (2004), Castro *et al.* (2019), and Perz *et al.* (2023) reported increased FI with amylase supplementation. Including protease in the soybean meal-based diet has enhanced feed consumption, leading to a significant diet-protease interaction (Cowieson *et al.*, 2016). Huang (2014) supplemented amylase into a broiler diet, which resulted in decreased FI, while Costa *et al.* (2006) reported decreased FI by supplementing the higher level of amylase. When an enzymatic complex, including the enzyme amylase, was used, it was shown that chicks that included enzymes had decreased diet consumption (Sorbara *et al.*, 2009). Castro *et al.* (2019) concluded that a higher level of amylase (250, 500, 750, and 1,000 g/ton) may lead to decreased FI. Iji *et al.* (2003) reported an increase in FI when maize with high moisture content was used in poultry feed with oven drying at 95°C, reducing the amylose concentration and leading to improved grain quality. Dietary protein and amino acid concentration, grain size, and dietary energy concentrations could all influence consumption. Birds fulfill their energy requirement by reducing feed consumption when their energy levels rise (Oko *et al.*, 2018).

Like the present study, Wang *et al.* (2020) supplemented amylase in a broiler diet which showed no significant improvement in production performance and BWG. Using varying levels of supplemental amylase, Gracia *et al.*, 2003 observed no clear improvement in growth performance. Kaczmarek *et al.* (2014) and Ghazi *et al.* (2002) found no difference in growth and yield in broiler chicks administered protease-supplemented feed, which may result from the higher rate of starch digestion in the upper gastrointestinal tract. Maize has been discovered to contain protease and some other inhibitors (Sharma *et al.*, 2022). The poly functional Hageman factor inhibitor derived from maize inhibited endogenous protease and amylase activity, which resulted in poor growth performance (Choct *et al.*, 2006). Amerah *et al.* (2017) discovered that amylase supplementation in broiler feed had no significant impact on production performance. Stefanello *et al.* (2017) also found that amylase did not affect BWG from 1 to 40 days. The growth performance of the chicks offered a soybean meal-based diet was unaffected by supplementary protease in the feed because total tract digestibility was decreased. Similarly, Cowieson *et al.* (2016) found no influence of protease on chick BWG. According to Svihus (2014), differences in the outcome of supplementing chicken diets with amylase could be due to the diet qualities, grain source, inclusion level, and birds-related variables.

In contrast to these findings, protease supplementation in a broiler diet improved BWG, FI, and FCR according to some other investigations (Angel *et al.*, 2011; Freitas *et al.*, 2011; Cowieson *et al.*, 2016; Mahmood *et al.*, 2017). Chimote *et al.* (2009) noted improvement in BWG because amylase and protease supplementation increase the rate of digestion of nutrients. According to Jiang *et al.* (2008), supplementing amylase in broiler diets resulted in a considerable increase in production performance. According to several other types of research, supplementing the broiler diet with amylase or enzyme combination, including protease, resulted in higher growth performance (Gracia *et al.*, 2003; iji *et al.*, 2003; Hashim *et al.*, 2023). Adding amylase improved BWG in broiler birds that provided low-energy feeds, mainly when 1,000 g tons of amylase was included. This increased BWG relative to broiler-provided diets with varied enzyme inclusion levels (iji *et al.*, 2003). According to Yin *et al.* (2018), amylase administration enhanced the size of villi and height-to-crypt ratios inside the duodenum of the birds administered corn-soybean meal diet, which could improve the absorption of nutrients and energy digestibility.

Comparable to the present study, Lin *et al.* (2019) noted no significant impact on FCR by adding protease into the broiler diet. Jiang *et al.* (2008) observed no positive effect on FCR by administration of amylase into a broiler diet. The FCR was not affected by adding a combination of amylase and protease (Slominski *et al.*, 2006). Yuan *et al.* (2015) demonstrated that using protease at any level in a broiler diet did not affect feed efficiency. Likewise, Cowieson *et al*. (2016) showed a reduced FCR using amylase in the broiler diet. According to Ghazi *et al.* (2003), the FCR was either negatively or not impacted depending on the protease dose applied. Results of the overall FCR were in line with Stefanello *et al.* (2017), who observed a reduction in FCR from 1 to 40 days by amylase supplementation in a broiler diet. The FCR was approximately 4.8 percent lower in broilers fed the amylase-enriched feed than any of those fed the negative control diet, and those findings correspond with Sorbara and Pamer (2019), who reported that the FCR was approximately 3.5 lower in the amylase-administered diet than in the control group. Lourenco *et al.* (2020) detect no changes in FCR with the addition of protease in the feed.

Pancreatic enzyme activity did not show a significant difference after oral digestive enzyme administration, according to Ritz *et al.* (1995a, 1995b) and Ezati *et al.* (2023), who speculated that distinctions in the physical structure of intrinsic animal enzymes versus those of bacterial or plant sources could prevent inhibitory effects of pancreatic enzymatic activity. However, when chicks were fed a diet supplemented with exogenous amylase and protease, Mahagna *et al.* (1995) discovered reduced intestinal protease activity. Jiang *et al.* (2008) found that as exogenous amylase levels increased, pancreatic amylase production dropped by 9%–33%, indicating a detrimental pancreatic enzyme production response that resulted in no significant impact on FI by enzyme supplementation.

Contrary to these results, Liu *et al*. (2020) observed a reduction in FCR from 1 to 4th weeks of age with amylase supplementation in feed. While Aksaka and Bilal (2002) and Lan *et al.* (2002), broiler chicks fed an enzyme-supplemented diet exhibited an enhanced FCR due to their enhanced feed consumption. Mehmood *et al.* (2017) also explained that adding protease in broiler feed improved FCR. During the starter period, protease administration improved feed conversion (Odetallah *et al.*, 2005). Furthermore, these findings corroborated with Bozkurt *et al.* (2006), who found that broiler-administered enzyme growth rate and FCR were equivalent to or better than those of broiler-fed conventional feed. A similar conclusion was reached by Kaczmerek *et al.* (2014), who found that adding amylase to chicken-fed finely ground maize improved FCR significantly, probably due to the increased digestion of starch in the upper gut. Amylase addition in the poultry diet boosted pancreatic amylase functions (Gracia *et al.*, 2003).

There was a significant relationship between cereal verification and enzyme addition in FCR, with the enzyme mixture improving FCR by 4% in maize-based diets but not in wheat-based or sorghum diets (Liu *et al.*, 2014). Weurding *et al.* (2003) found substantial changes in feed conversion in broiler birds fed either fast or slowly digested starch with enzyme addition, concluding that slowly digestible starch with enzyme improved FCR. Gracia *et al.* (2003) and Jiang *et al.* (2008) discovered that added amylase improved growth performance. Furthermore, when contrasted to 1,500 U/g α-amylase, 3,000 U/g α-amylase increased the

FCR. Similarly, α-amylase from *Bacillus subtilis* did not affect performance, but α-1, 6-isoamylase from *Bacillus* appeared to raise the FCR of birds. Therefore, it is concluded that the result may vary with the source and type of the enzyme supplemented on the FCR.

Results indicated no statistically significant differences among treatments in carcass characteristics and organ weight of broiler. Results of live, carcass, thigh, organ, and abdominal fat%, breast weight, and wings weight were in line with other researchers who reported no significant effect of protease supplementation in broiler diet on the carcass, abdominal fat, and thigh, breast weight, and wing weight (Kamran *et al.*, 2008; Rada *et al.*, 2014). Similarly, Freitas *et al.* (2011) and Mehmood *et al.* (2017) reported no beneficial impact of protease addition in broiler diet on carcass yield, abdominal fat, and thigh, breast, and wing weight. Castro *et al.* (2019) detect no influence of amylase supplementation on carcass characteristics. Torres *et al.* (2003) and Bedford and Cowieson *et al.* (2012) found no variation in carcass characteristics when utilizing feed, including amylase. The findings of this study also correlate with those of Cardoso *et al.* (2011) and Fortes *et al.* (2012), who found no significant difference in carcass yield and broiler cuts at the end of the trial. According to Kermani *et al.* (2017), gelatin substitution of soybean meal protein in the diet by up to 22% did not influence carcass and breast meat yield as long as dietary amino acid balance was maintained in terms of protease effects. Amino acid availability is widely regarded as the primary determinant of carcass output.

In contrast with other findings, Rada *et al.* (2014) and Olfati *et al.* (2020) noted a significant impact of protease addition on carcass characteristics. Café *et al.* (2002) declared an increase in abdominal fat by supplementing amylase and protease into the diet. Govil *et al.* (2017) supplemented amylase into low-energy feed and noted a significant effect on overall carcass characteristics. Lower breast weight was observed when Lin *et al.* (2019) supplemented protease into the diet (Tougan *et al.*, 2013). The dressing % of the broiler-fed diet with 0.1% Avizyme was much greater. Avizyme-supplemented diets resulted in a significantly increased abdominal fat percentage in the chicks. This finding shows that the azymes-supplemented diets provided the birds with additional energy. Increased calorie-protein or calorie-amino acid ratio typically increases carcass or abdominal fat (Café *et al.*, 2002).

Correspondingly to the present study, Nastain *et al.* (2021) reported no significant effect of protease addition in the broiler on organ weight. Mehmood *et al.* (2017) also found no significant liver and heart weight improvement. Correspondingly, Rehman *et al.* (2017) reported that all organs, such as the heart, gizzard, liver, giblets, and spleen, were unaffected by the supplementation of distinct sources of protease. Onderci *et al.* (2006) reported that amylase addition in a broiler diet did not significantly affect liver weight. Gracia *et al.* (2003) also observed no significant improvement in all organ weight with amylase supplementation except pancreas weight. Córdova *et al.* (2020) supplemented amylase into a coarse corn-based diet, significantly improving liver weight. The supplementation of protease had no noticeable impact on the organ weight weights of the chicken (Ajayi *et al.*, 2015).

Unlike other findings, Yuan *et al.* (2017) reported significant improvement in liver weight with amylase provision into the diet. Wheat-based feed resulted in the smallest gizzard size, likely due to the soft grain texture and insoluble fiber. The particle diameter scale (Symes, 1965) of the 3 grains was 6 in corn, 9 in millet, and 12 in wheat, with the most minor score in maize indicating the hardest texture and the highest value in the wheat indicating the softest texture.

Conclusion

This experiment was conducted to determine whether amylase, protease, and their combined supplementation affected broiler performance. Results concluded that amylase, protease, and their combined supplementation at 100 g/ton of feed did not affect BWG, FI, FCR, carcass characteristics, and organ weight.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Data availability

The study's datasets are available upon reasonable request from the corresponding author.

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