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Original Article

Dietary Inclusion of Pancreatin Enzyme on the Ileal and Fecal Digestibility of Nutrients in Layer-Type Cockerels

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■Keywords

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ABSTRACT

The current experiment aimed to evaluate the effects of Pancreatin supplementation at different levels on ileal and fecal digestibility in layer-type cockerels. A total of 480-day-old silver brown Hy-Line male chicks were randomly allocated into 5 treatments, 6 replicates (16 birds per pen) arranged in a completely randomized design. Pancreatin enzyme was supplemented on a basal corn-soybean meal-based diet at 0, 250, 500, 750, and 1000 mg/kg and was fed in two growth phases (starter and grower). The results indicated that at the end of the starter stage, except for 1000 mg/kg, dietary Pancreatin supplementation levels increased (p<0.05) the ileal crude protein (CP). Similarly, addition of Pancreatin increased (p< 0.05) apparent ileal amino acids (AA) digestibility (AIAAD) total means of AA (MTAA), means of indispensable AA (MIAA) and dispensable AA (MDAA) with the optimum performance on 250 mg/kg and 500 mg/kg. However, except for histidine and alanine which were negatively affected (p<0.05), and MIAA, MDAA, MTAA which were also positively affected, the addition level at 1000 mg/kg did not affect most of the AIAAD compared to the non-supplemented. Further, Pancreatin supplementation had no effect (p>0.05) on nitrogen digestibility (ND), nitrogen retention (NR), digestible energy (DE), apparent metabolizable energy (AME), dry matter digestibility (DMD), dry matter retention (DMR), and apparent metabolizable energy corrected for nitrogen (AME-n) on fed starter diet. On the other hand, at the end of the grower stage, dietary Pancreatin enzyme supplementation reduced (p<0.05) the ileal CP, MIAA, MDAA, MTAA, AIAAD, AME, AME-n, ND, NR, DE, DMD, and DMR in a dose-dependent manner. The rate of reduction was more marked on Pancreatin addition level 1000 mg/kg. In conclusion, Pancreatin supplementation at 250 mg/kg, 500 mg/kg, and 750 mg/kg improved AIAAD and ileal CP, especially at the young age. The rate of pancreatin enzyme effect was dependent on enzyme supplement level to the ileal CP and individual amino acid.

INTRODUCTION

Enzyme supplementation has been an innovative technology to maximize the growth of birds with increasing nutritional, environmental, and economical sustainability. Exogenous enzymes increase the growth of birds through their ability not only to reduce the adverse effects of anti-nutritional factors (non-starch polysaccharide) but also to decrease cell membrane integrity, changes the gut microflora which consequently improves utilization of protein solubility and energy digestibility (Olukosi *et al.*, 2015; Singh *et al.*, 2017). Several commercially produced enzyme products composed of pure single digestive enzymes (protease, amylase, and lipase) have been used technically to improve nutrient digestibility and growth by augmenting the insufficient quantities of enzymes

produced by the animal (Cowieson *et al.*, 2019; Jabbar *et al.*, 2021a). Although, numerous studies have indicated that cocktails of these enzymes deliver more significant improvements in inherent digestibility, such as apparent metabolizable energy (AME), amino acids (AA) digestibility, nutrient availability, and to a lesser extent, reduced nutrient excretion, their result in corn soybean meal (SBM)-based diet has been inconsistent (Romero *et al.*, 2013; 2014; Kaczmarek *et al.,* 2014; Adebiyi & Olukosi, 2015).

Pancreatin is a multiple-enzyme product usually extracted from animals' pancreas. The enzyme has been used efficaciously with proven evidence in enzymatic deficiencies or replacement therapy in fish (Souza *et al.,* 2020), humans (Trang, 2014), pigs (Rengman *et al.*, 2010), cats and dogs (Xenoulis, 2020). In humans, this enzyme has been used for the treatment of pancreatic insufficiency with related conditions such as chronic pancreatitis, cystic fibrosis, and pancreatic carcinoma, which affect nutrient digestion (Trang 2014). Pancreatin enzyme is beneficial in mimicking its natural enzymatic properties in re-establishing the digestive conditions. Thus, pancreatin enzymes are used as a pivotal substitute for the physiological function of the pancreas to enhance digestion and absorption of nutrients passing through the intestines without being digested. Rengman *et al.* (2010) indicated that prepared oral pancreatin enzyme, with digestive properties, stimulated nutrient assimilation and anabolic processes in young fast-growing pigs. Similarly, the addition of pancreatin could improve the nutritional state by altering the intestinal microbiota in mice (Nishiyama *et al.,* 2017).

Despite these hypothetical possibilities, Pancreatin enzyme has been one of the least studied in poultry nutrition even though it has been used commercially for more than two decades. Although studies on Pancreatin supplementation in pigs has been reported (Cervantes *et al.*, 2011), studies to determine appropriate Pancreatin enzyme supplementation in the diet of poultry has not been reported. Accordingly, the present study was designed to determine the effect of dietary pancreatin supplementation on the ileal and fecal digestibility of nutrients in layer-type cockerels fed corn -SBM-based diet.

MATERIALS AND METHODS

Ethical statement

The current study was conducted in the research unit at the College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu Province. All experimental procedures and conducts were approved by the Care Advisory Committee of Yangzhou University Animal Ethic of Practice, Council of State, People Republic of China.

Experimental birds and Husbandry

A total of 480-day-old male chicks (Hy-Line Silver), with an average body weight (BW) 39.58 ±0.24 g from the Hy-line Company, were randomly assigned to 30-floor pens of 16 birds with wood shavings serving as litter material. Each pen with a floor space of 0.064 m^2 was equipped with one round bottom plastic feeder and a round manual drinker. Before the birds' arrival, disinfection was done with fumigation (mixer of formalin 40% with potassium permanganate powder). During the first week, the room temperature was maintained at 33 °C and gradually decreased to suit the birds with adequate ventilation. Chicks were fed with fresh feed and water at ad libitum during the entire period. The birds were vaccinated against Marek's disease at day old, Newcastle (Nobilis ND clone 30) on 14 d, and infectious bursal disease (Intervet) on 28 d by eye drop following manufacturers' recommendations.

Diet formulations and Enzyme

The experimental diets typically of a corn-SBM-based diet was formulated at the Yangzhou University experimental feed mixing plan. The diets were fed in mash form for 70 days and in 2 phases based on growing period; Phase 1: Starter (1-42 d), and Phase 2: Grower (43-70 d). Treatment diets contained 11.98 MJ/kg of metabolizable energy (ME); 18.40% of crude protein (CP) at the starter phase and 11.85 MJ/kg of ME; 17.48 % of CP at the grower phase in a completely randomized design (Table 1). The Pancreatin enzyme extracted from pig pancreas was obtained from Shanghai Honest Biological Technology Co. Ltd. According to the supplier, the enzyme contained 561 U/g of protease, 3061 U/g of amylase, and 4352 U/g of lipase. The enzyme was added at 0, 250, 500, 750, and 1000 mg/kg, respectively, to the experimental diets. The dietary supplementation levels of the enzyme were considered based on the finding of Kim *et al.* (2018) that enzyme supplementation from 250-1000 mg/kg in animal diets increased the total tract digestibility. Silicon oxide was incorporated at a level of 0.5% into the diets as an indigestible marker [for calculating acid insoluble ash (AIA)] at the last week of each feeding phase.

Table 1 – Dietary formulations and nutrient concentration of the experimental diets (air-dry basis).

Ingredients (%)	Starter diet[1]	Grower diet[1]	Analyzed Nutrients	Starter diet[1]	Grower diet[1]
Corn	66.10	66.25	Dry matter	88.58	89.63
Soybean	29.00	26.10	Gross ME (MJ/kg)	16.00	15.75
Wheat bran	0.20	3.00	Crude protein	18.41	17.94
Methionine	0.20	0.20	Ash	4.71	6.00
Lysine	0.20	0.15	Acid insoluble ash	0.48	0.50
Salt	0.30	0.30	Calcium	1.05	1.04
Limestone	1.30	1.30	Phosphorus	0.74	0.76
CaHPO3	1.70	1.70	Arginine	0.71	0.22
Premix[2]	1.00	1.00	Histidine	0.18	0.81
Total	100	100	Isoleucine	0.55	0.38
Calculated Nutrients (%)			Leucine	1.10	0.61
Metabolizable Energy (MJ/kg)	11.98	11.85	Lysine	1.01	0.97
Crude protein	18.40	17.48	Methionine	0.46	0.42
Crude fiber	2.78	2.80	Phenylalanine	0.64	0.51
Calcium	1.08	1.07	Threonine	0.45	0.52
Total phosphorus	0.67	0.68	Tryptophan	0.50	1.21
Available phosphorus	0.42	0.43	Valine	0.59	0.07
Lysine	1.13	1.01	Alanine	0.64	0.71
Methionine	0.47	0.45	Asparagine	1.17	1.35
			Glutamine	2.25	2.59
			Glycine	0.50	0.59
			Proline	2.39	0.81
			Serine	0.58	0.67

Analyzed concentrations for basal diets represent the means of the 5 diets since they consist of the same amount of feed ingredients.

1Pancreatin enzyme was supplemented (top-dressed) on control diets at 0, 250, 500, 750, and 1000 mg/kg, respectively. 0.5% of SiO2 was added as a marker additionally to each group diet, which exceeded 100%.

2Vitamin and trace mineral premix content (per kg of feed): vitamin A, 5000 IU; vitamin D3, 1500 IU; vitamin E, 10 IU; vitamin K3, 8 mg; vitamin B1, 8 mg; vitamin B2, 3 mg; vitamin B6, 15mg; vitamin B12, 9 mg; biotin, 0.2 mg; folic acid, 0.001 mg; choline, 5.7 mg; pantothenic acid, 45 mg; niacinamide, 50 mg; Iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.35 mg; copper, 8 mg; selenium, 0.15 mg.

Experimental design and procedure

A week before the end of each phase, four birds with average weight were transferred into a wire flooring metabolic cage (70 cm^2 per cage with 2 cm^2 holes) equipped with a manual plastic drinker and a plastic feeder. Underneath each cage was a plastic tray for the excreta collection. According to the replicated diet per treatment, one metabolic cage was assigned to a floor pen's unit cage. Fresh fecal samples were collected from each pen during the last 3 days of each feeding phase and immediately mixed, pooled by cage, and stored frozen at –20 °C for further analysis. At the end of each feeding phase (starter and growth), two birds per pen were selected and leg banded. Anesthetically, the birds were exposed to CO2 gas for approximately 30 s and euthanized before exsanguination. Ileal digesta was obtained from the intestine; thus, a section between Meckel's diverticulum and ileo-cecal junction, about 2 cm in between, pooled per cage and immediately stored at -20 °C for further analysis.

Sample collection and analyses

The experimental diets and fecal samples were air-dried in an oven at 60 °C. The ileal digesta samples were also freeze-dried. The dried diets, fecal and ileal digesta were ground in a coffee grinder (CBG5 Smart Grind, Applica Consumer Products Inc., Shelton, CT, USA) through a 0.5 mm screen for analysis. The analysis of the dried ground samples is as follows; diet: ME, CP, Ash, AIA, Ca, P, C, AA. Ileal: AIA, AA, CP, DMD, DE, ND fecal:AIA DMR, NR, AME, AME-n. Samples were oven-dried at ±105 °C for 16 hrs (method 934.01; AOAC, 2006) to determine the dry matter (DM) content. The CP content (N×6.25) was determined using the Kjeldahl method (method 984.13 A-D; AOAC, 2006) in an automated analyzer (Kjeltec 8400 Analyzer unit). Gross energy (GE) was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Samples for AA profiles were analyzed by HPLC (Hitachi L-8800 Amino Acid Analyzer, Tokyo, Japan) at University experimental laboratory followingmethod 982.30; AOAC (2006). The crude fiber determination method was done by sequential extraction with dilute acid and alkali (method 920.39; AOAC, 2006). The calcium (Ca) and phosphorus (P) analyses were as per method 975.03 B(b) and method 968.08, respectively, all of AOAC (2006). Silicon oxide concentration was determined using the technique described byDe Coca-Sinova *et al.* (2011).

Calculations

a) Ileal crude protein (CP) and amino acid digestibility (AIAAD) were calculated using acid insoluble acid (AIA) in the diets, digesta and excreta adopted from Ravindran *et al.* (1999):

$$AIAAD(\%) = \frac{\left[\left(\frac{AA}{AIA}\right) in\ diet - \left(\frac{AA}{AIA}\right) in\ ileal\right]}{\left(\frac{AA}{AIA}\right) in\ diet} \times 100 \quad (1)$$

b) Apparent metabolizable energy (AME) was calculated according to Lammers *et al.* (2008):

$$DMR = \frac{(AIA\ in\ excreta - AIA\ in\ diet)}{AIA\ in\ excreta} \quad (2)$$

$$AME\left(\frac{MJ}{kg}\right) = GE\ in\ diet - [(1 - DMR) \times GE\ in\ excreta] \quad (3)$$

where DMR is the dry matter retention, AIA is the concentration of acid insoluble ash (g/kg), GE is the gross energy in the feed (MJ/kg)

c) Apparent metabolizable energy corrected for nitrogen (AME-n), dry matter digestibility (DMD) and digestible energy (DE)were also determined according to Yang *et al.* (2020):

$$AMEn\left(\frac{MJ}{kg}\right) = GE\ in\ diet - \frac{(GE\ in\ excreta \times AIA\ in\ diet)}{AIA\ in\ excreta - \frac{34.39 \times N\ retained}{100}} \quad (4)$$

where DMR is the dry matter retention, AIA is the concentration of acid insoluble ash (%), GE is the gross energy (MJ/kg), 34.39 (MJ/kg) is the energy value of uric acid.

d) The N retained was calculated as:

$$NRetained = NR\ in\ diet - \frac{[N\ in\ excreta \times AIA\ in\ diet]}{AIA}\ in\ excreta \quad (5)$$

where N is nitrogen (g/kg),thus birds per kilogram of diet consumed.

e) The nitrogen retention (NR) in excreta was calculated as described by Lammers *et al.* (2008):

$$NR = \frac{\left(N\ in\ \frac{diet}{AIA}\ in\ diet - N\ in\ \frac{excreta}{AIA}\ in\ excreta\right)}{N\ in\ \frac{diet}{AIA}\ in\ diet} \quad (6)$$

f) The digestible energy (DE) was calculated, using AIA as indigestible marker, as shown below:

$$DE\left(\frac{MJ}{kg}\right) = GE\ in\ diet - [(1 - DMR) \times GE\ in\ excreta] \quad (7)$$

$$DMD = \frac{AIA\ in\ ileal - AIA\ in\ diet}{AIA}\ in\ ileal \quad (8)$$

where DMD is the dry matter digestibility, AIAis the concentration of acid insoluble ash (g/kg), and GEis the gross energy (MJ/kg).

g) The nitrogen digestibility (ND) was calculated as described by Lammers *et al.*(2008):

$$ND = \frac{\frac{N\ in\ diet}{AIA\ in\ diet} - \frac{N\ in\ ileal}{AIA\ in\ ileal}}{\frac{N\ in\ diet}{AIA\ in\ diet}} \quad (9)$$

where N represents nitrogen (g/kg) and AIA represents the concentration of AIA (g/kg).

Statistical analysis

The experimental data was initially processed by Microsoft Excel (Windows version 10) and were subjected to further analysis using ANOVA of SPSS 16.0 statistical software. Duncan's multiple range tests were used to determine significant differences among treatment means. Unless specified otherwise, multiple comparisons with statistical differences were considered significant at $p<0.05$. The effect of increasing the enzyme-graded levels was variably assessed into linear and quadratic components using orthogonal polynomial contrasts for potential differences.

RESULTS

Growth performance

The present study's growth performance data and discussion can be retrieved from Asare *et al.* (2021).

Ileal crude protein and amino acid digestibility

From table 2, data indicated that at the end of the starter phase, ileal CP digestibility was enhanced ($p<0.05$) by adding 250, 500, and 750 mg/kg of Pancreatin enzyme compared to the non-supplemented diet. Similarly, an increase ($p<0.05$) in AIAAD, total mean of AA (MTAA), and means of indispensable (MIAA) and dispensable AA (MDAA) was observed with dietary Pancreatin supplementation levels 250 mg/kg and 500 mg/kg. A linear increase ($p<0.05$) was observed on most of the AA under 250, and 500 mg/kg of Pancreatin enzyme except for histidine, leucine, phenylalanine, alanine, glutamine, proline, tyrosine and MDAA compared to the non-supplemented diet. Further, except for arginine, glutamine, and proline, 750 mg/kg of Pancreatin supplementation significantly increased ($p<0.05$) most of the ileal AA digestibility. In addition, with the exemption of histidine and alanine,

Table 2 – Influence of Pancreatin supplements level on the coefficient of apparent ileal digestibility (%) of amino acids (AA) fed starter diet [1, 2].

Item (%)	Control	Pancreatin supplement on control (mg/kg)				SEM	*p*-Value		
		250	500	750	1000		Pancreatin	Linear	Quadratic
Crude Protein	80.50^{a}	83.53^{b}	82.74^{b}	82.75^{b}	80.51^{a}	0.36	< 0.001	0.003	0.003
Indispensable AA									
Arginine	87.03^{a}	93.13^{b}	92.01^{b}	87.66^{a}	87.60^{a}	0.59	< 0.001	0.043	0.608
Histidine	85.59^{b}	91.91^{d}	90.02^{cd}	87.22^{bc}	79.75^{a}	0.90	< 0.001	0.209	0.087
Isoleucine	82.12^{a}	88.90^{b}	87.03^{b}	87.51^{b}	79.71^{a}	0.83	< 0.001	0.022	0.991
Leucine	85.97^{ab}	90.98^{c}	88.86^{bc}	87.50^{b}	83.65^{a}	0.62	< 0.001	0.347	0.899
Lysine	82.48^{a}	91.30^{c}	89.62^{bc}	87.61^{b}	82.96^{a}	0.79	< 0.001	< 0.001	0.360
Methionine	76.30^{a}	88.14^{b}	87.31^{b}	87.08^{b}	77.43^{a}	1.13	< 0.001	< 0.001	0.384
Phenylalanine	86.01^{ab}	91.31^{d}	89.57^{cd}	87.54^{bc}	84.22^{a}	0.62	< 0.001	0.192	0.895
Threonine	78.91^{a}	86.21^{b}	84.84^{b}	87.67^{b}	76.28^{a}	1.01	< 0.001	0.004	0.974
Valine	81.86^{a}	88.85^{b}	86.47^{b}	87.27^{b}	79.35^{a}	0.81	< 0.001	0.028	0.894
MIAA	82.92^{a}	89.99^{b}	81.22^{a}	87.457^{b}	88.41^{b}	0.78	< 0.001	0.027	0.519
Dispensable AA									
Alanine	84.62^{b}	88.85^{c}	87.05^{bc}	87.33^{bc}	80.98^{a}	0.70	< 0.001	0.319	0.537
Asparagine	84.54^{a}	91.40^{c}	89.21^{bc}	88.17^{b}	82.56^{a}	0.74	< 0.001	0.039	0.887
Glutamine	88.43^{a}	93.71^{b}	91.91^{b}	87.22^{a}	87.35^{a}	0.60	< 0.001	0.586	0.922
Glycine	80.57^{a}	87.07^{b}	85.30^{b}	87.18^{b}	77.01^{a}	0.93	< 0.001	0.025	0.752
Proline	88.41^{a}	93.73^{b}	91.92^{b}	87.18^{a}	87.36^{a}	0.60	< 0.001	0.583	0.940
Serine	83.84^{a}	89.84^{b}	88.21^{b}	87.72^{b}	81.95^{a}	0.71	< 0.001	0.042	0.989
Tyrosine	85.25^{ab}	90.47^{c}	88.68^{c}	87.38^{bc}	83.08^{a}	0.65	< 0.001	0.483	0.857
MDAA	85.09^{ab}	90.72^{d}	82.90^{a}	87.45^{bc}	85.90^{cd}	0.67	< 0.001	0.202	0.316
MTAA	83.87^{a}	90.31^{b}	81.95^{a}	87.45^{b}	88.63^{b}	0.73	< 0.001	0.067	0.427

MIAA, means of indispensable amino acid; MDAA, means of dispensable amino acid; MTAA, means of total amino acid.

[1]Data represent mean values of 6 replicates per treatment.

[2]Means within a row without common superscripts (a, b, c, d) differ at $p<0.05$. SEM: pooled standard error of means.

which were negatively affected ($p<0.05$), and MIAA, MDAA, MTAA which was also positively affected ($p<0.05$), the addition level of 1000 mg/kg did not affect most of the AIAAD compared to the non-supplemented diet.

On the other hand, at the end of the grower phase, dietary Pancreatin supplementation significantly decreased ($p<0.05$) the ileal CP digestibility, MIAA, MDAA, MTAA and AIAAD coefficients regardless of the supplementation level compared to the control diet. The rate of reduction was dependent on the type of amino acid to the Pancreatin supplementation level. However, a marked rate of reduction was observed on MIAA, MDAA, MTAA and AIAAD coefficients when Pancreatin enzyme was supplemented at 1000 mg/kg compared to the control diet. Similarly, a quadratic decrease was observed on histidine, proline and valine based on the level of Pancreatin supplementation (Table 3).

Ileal and fecal nutrient determination

As shown in Table 4, there was no significant effect ($p>0.05$) on DMR, NR, AME, and AME-n when Pancreatin was supplemented on the starter diet. However, the addition of Pancreatin on a corn-SBM-based diet significantly lowered ($p<0.05$) the utilization of DMR, NR, AME, and AME-n compared to the non-supplemented diet on fed grower diet. Comparatively, the magnitude of reduction appeared to be more marked on 1000 mg/kg of Pancreatin enzyme supplementation.

Additionally, there was no significant effect ($p>0.05$) on ileal DMD, DE, and ND when Pancreatin was supplemented at different levels on the starter diet. However, irrespective of Pancreatin supplementation level on the grower diet, a decrease ($p<0.05$) in DMD, DE, and ND was observed compared to the control. A similar pattern of diminishing marginal response was observed quadratically ($p=0.019$) on the ND under the Pancreatin enzyme supplemented groups at the grower diet (Table 5).

DISCUSSION

Increased digestible protein, carbohydrates, and other nutrients are necessary for the optimal growth in birds. Even though corn -SBM- based diet are suggested not viscous, the rapid passage of some

Table 3 – Influence of Pancreatin supplements level on the coefficient of apparent ileal digestibility (%) of amino acids (AA) fed grower diet [1, 2].

Item (%)	Control	Pancreatin supplement on control (mg/kg)				SEM	*p*-Value		
		250	500	750	1000		Pancreatin	Linear	Quadratic
Crude protein	82.13^{c}	72.72^{ab}	74.71^{ab}	74.05^{b}	76.87^{a}	1.24	0.001	< 0.001	0.210
Indispensable AA									
Arginine	84.07^{c}	78.50^{a}	80.70^{b}	81.13^{b}	77.56^{a}	0.48	< 0.001	< 0.001	0.288
Histidine	85.82^{d}	78.99^{ab}	80.13^{bc}	81.04^{c}	78.04^{a}	0.55	< 0.001	< 0.001	0.001
Isoleucine	66.89^{e}	53.60^{b}	62.47^{d}	58.93^{c}	49.57^{a}	1.24	< 0.001	< 0.001	0.306
Leucine	80.88^{d}	72.35^{b}	76.09^{c}	75.31^{c}	69.10^{a}	0.79	< 0.001	< 0.001	0.963
Lysine	85.30^{d}	78.01^{b}	81.31^{c}	80.70^{c}	74.89^{a}	0.68	< 0.001	< 0.001	0.649
Methionine	81.11^{d}	73.04^{b}	76.76^{c}	75.38^{c}	70.51^{a}	0.72	< 0.001	< 0.001	0.620
Phenylalanine	83.75^{c}	75.96^{a}	80.02^{b}	78.65^{b}	75.79^{a}	0.60	< 0.001	< 0.001	0.060
Threonine	76.32^{d}	63.60^{b}	70.96^{c}	69.10^{c}	60.56^{a}	1.09	< 0.001	< 0.001	0.802
Valine	82.85^{c}	62.21^{a}	78.04^{b}	76.27^{b}	60.78^{a}	1.68	< 0.001	< 0.001	0.020
MIAA	80.78^{d}	70.69^{b}	76.28^{c}	75.17^{c}	68.53^{a}	0.85	< 0.001	< 0.001	0.317
Dispensable AA									
Alanine	84.89^{d}	77.79^{b}	80.72^{c}	79.98^{c}	75.79^{a}	0.62	< 0.001	< 0.001	0.317
Asparagine	82.58^{c}	72.80^{a}	77.58^{b}	77.56^{b}	71.54^{a}	0.78	< 0.001	< 0.001	0.282
Glutamine	89.17^{d}	83.39^{b}	85.04^{c}	85.99^{c}	81.03^{a}	0.53	< 0.001	< 0.001	0.556
Glycine	79.11^{d}	68.63^{b}	74.29^{c}	72.44^{c}	64.30^{a}	0.99	< 0.001	< 0.001	0.342
Proline	88.40^{d}	83.03^{b}	83.83^{bc}	85.02^{c}	81.21^{a}	0.48	< 0.001	< 0.001	0.042
Serine	80.91^{d}	71.37^{b}	76.42^{c}	75.18^{c}	68.43^{a}	0.85	< 0.001	< 0.001	0.793
Tyrosine	85.49^{d}	78.52^{b}	81.44^{c}	81.07^{c}	76.88^{a}	0.59	< 0.001	< 0.001	0.268
MDAA	84.37^{d}	76.50^{b}	79.90^{c}	79.60^{c}	74.17^{a}	0.68	< 0.001	< 0.001	0.606
MTAA	82.35^{d}	73.24^{b}	77.86^{c}	77.10^{c}	71.00^{a}	0.77	< 0.001	< 0.001	0.804

MIAA, means of indispensable amino acid; MDAA, means of dispensable amino acid; MTAA, means of total amino acid.

[1]Data represent mean values of 6 replicates per treatment.

[2]Means within a row without common superscripts (a, b, c, d) differ at $p<0.05$. SEM: pooled standard error of means.

Table 4 – The effect of Pancreatin supplementation on fecal nutrient retention and metabolizable energy determination of cockerels[1, 2].

Item (%)	Control	Pancreatin supplement on control (mg/kg)				SEM	*p*-Value		
		250	500	750	1000		Pancreatin	Linear	Quadratic
Starter diet									
DMR (g/g)	0.87	0.87	0.87	0.86	0.88	0.003	0.247	0.287	0.468
NR (g/g)	0.76	0.79	0.77	0.75	0.79	0.006	0.212	0.527	0.987
AME (MJ/kg)	14.26	14.38	14.21	14.25	14.33	0.034	0.593	0.898	0.947
AME-n (MJ/kg)	13.49	13.58	13.43	13.49	13.54	0.030	0.638	0.975	0.937
Grower diet									
DMR (g/g)	0.78^{c}	0.74^{b}	0.72^{b}	0.76^{ab}	0.68^{a}	0.008	< 0.001	< 0.001	0.744
NR (g/g)	0.58^{d}	0.49^{bc}	0.45^{ab}	0.54^{cd}	0.37^{a}	0.018	< 0.001	< 0.001	0.691
AME (MJ/kg)	12.74^{d}	12.05^{bc}	11.76^{b}	12.33^{cd}	11.16^{a}	0.120	< 0.001	< 0.001	0.754
AME-n (MJ/kg)	12.18^{d}	11.56^{bc}	11.32^{b}	11.80^{cd}	10.80^{a}	0.103	< 0.001	< 0.001	0.770

DMR, dry matter retention; AME, apparent metabolizable energy; AME-n, N-corrected apparent metabolizable energy; NR, nitrogen retention; SEM, pooled standard error of means.

[1]Data represent mean values of 6 replicates per treatment.

[2]Means within a row without common superscripts (a, b, c, d) differ at $p<0.05$. SEM: standard error of the mean.

nutrients, especially digestible protein in the gut, may significantly affect the deficiency of innate enzymes. Noy & Sklan (1995) and Jin *et al.* (1998) indicated that specific enzymes like lipase, amylase, and protease are needed to spearhead the digestion and absorption of nutrients. The present findings support literature findings indicating that pancreatin supplementation at the young stage played a double role, and except for its digestive properties, it increased the digestibility of the ileal CP and AIAAD (Rengman *et al.*, 2010; Nishiyama *et al.,* 2017). The pancreatin enzyme was markedly more effective at improving AIAAD, on a dose equivalent basis, and at a lowest dose of 250 mg/kg achieved a greater improvement then to 500 and 750 mg/kg. However, 1000 mg/kg had no effect on CP and several AA except for MIAA and MTAA which

Table 5 – The effect of Pancreatin supplementation on ileal nutrient retention and digestible energy determination of birds[1,2].

Item (%)	Control	Pancreatin supplement on control (mg/kg).				SEM	*p*-Value		
		250	500	750	1000		Pancreatin	Linear	Quadratic
Starter diet									
DMD (g/g)	0.73	0.72	0.70	0.71	0.74	0.009	0.732	0.938	0.215
DE (MJ/kg)	14.02	14.13	13.99	13.92	14.20	0.04	0.221	0.583	0.399
ND (g/g)	0.67	0.66	0.61	0.69	0.67	0.01	0.381	0.981	0.263
Grower diet									
DMD (g/g)	0.74^{c}	0.59^{a}	0.69^{b}	0.66^{b}	0.56^{a}	0.013	< 0.001	< 0.001	0.530
DE (MJ/kg)	12.51^{c}	11.92^{b}	11.75^{b}	12.10^{bc}	10.91^{a}	0.121	< 0.001	< 0.001	0.202
ND (g/g)	0.77^{d}	0.64^{b}	0.66^{b}	0.70^{c}	0.60^{a}	0.009	< 0.001	< 0.001	0.019

DMD, dry matter digestibility; DE, digestible energy; ND, nitrogen digestibility.

[1]Data represent mean values of 6 replicates per treatment.

[2]Means within a row without common superscripts (a, b, c, d) differ at $p<0.05$. SEM: pooled standard error of means.

were increased at the young age compared to the non-supplement diet. In addition, a diminishing response was exerted with increased inclusion level 1000 mg/kg on histidine and alanine on fed supplemented starter diet. In some experiments, a marked increase in ileal digestibility of CP and AA was attributed to supplementing enzyme at 1000 mg/kg in bird's diet. Published studies have shown that multi enzyme addition could be effective in improving the nutritive value of low- viscous, corn -SBM- based diet (Kim *et al.,* 2018; Saleh *et al.,* 2020). The current results were consistent with these authors indicating that multi enzyme addition at 250 to 750 mg/kg added to birds' diet has been efficient in enhancing the digestion of undigested protein passing through the gut. At the end of growth phase, adverse effects in ileal CP and AIAAD were found in response to Pancreatin supplementation at varied levels. Again, enzyme effects were dose-dependent, and at the highest pancreatin supplemented level (1000 mg/kg) compared to the control negatively affected the ileal CP digestibility, MIAA, MDAA, MTAA and AIAAD coefficients. Contrary to the present finding, Saleh *et al.* (2020) reported that AA and CP digestibility was increased by multi enzyme irrespective of the supplementation levels. Cowieson *et al.* (2019) indicated that although matured birds can secrete enough gastro-mucosal enzymes to enhance the consistent supply of digestible CP and AA stability, dietary supplementation with multi-enzyme complexes is noted to give high efficiency in improving nutrient digestibility in grow-finisher birds (Ravindran & Son 2011). The difference is probably due to the fact that the enzyme supplementation levels could have interfered with the digestive enzyme released and gut development to alter nutrient digestibility (Romero *et al.*, 2014; Amerah *et al.*, 2017). In terms of the release of individual AA by supplemented Pancreatin enzyme, all the AA were readily released from corn- SBM -diet under supplementation levels 250 and 500 mg/kg on fed starter diet. Kim *et al.* (2018) have reported an increase in CP digestibility in response to increasing supplementation levels of multi-enzyme in fed corn-SBM-based diet. The current study agreed well with those reported earlier of corn-SBM- based diet for broiler chicken that the effect of adding enzymes to broilers diet fed at the starter phase is more significant compared to that during the growth phase (Mohammadigheisar & Kim, 2018). This suggests that birds at the starter phase might be more receptive to supplementary enzymes because of the enzymes ability to augment the insufficient endogenous enzymes synthesized by their immature gut (Uni *et al.*, 1999; Dosković *et al.*, 2013). Therefore, one possible explanation for this difference in Pancreatin supplementation between the two growth phases could be attributed to bird's age.

To our knowledge, the usage of either exogenous protease, amylase, or lipase enzymes as "standalone" has a direct positive contribution to energy digestibility and nutrient retention from the main energy-yielding substrates at the ileal and total tract level in birds (Liu *et al.,* 2016; Amerah *et al.,* 2017; Jabbar *et al.,* 2021b). However, results have showed the synergistic and superior effect on the combination of these enzymes than protease, amylase, or lipase alone on the utilization of energy and nutrient retention. In the current study, Pancreatin supplementation did not affect the ileal DMD, DE, and ND or fecal DMR, NR, AME, and AME-n digestibility at the young age, irrespective of the supplementation level. Additionally, Pancreatin enzyme negatively affected both ileal DMD, DE, ND and fecal DMR, NR, AME, and AME-n digestibility at

the grower stage irrespective of the supplementation level. It had been assumed that the use of Pancreatin enzyme would indirectly alter the gut microflora or bacterial population in the digestive system to ferment substantially the long-chain carbohydrate molecules used by some bacteria in the digestive tract as bird ages (Nishiyama *et al.* 2018). However, the lack of effect on Pancreatin enzyme supplementation could be attributed to the fact that the enzyme levels could not disrupt the protein–starch interactions in the feed to short fragments digestible by innate pancreatic enzymes.

Contrary to the current work, Lee *et al.* (2018) reported a positive effect on the digestibility of canola co-products and reduced fermentation of the resulting undigested nutrients by exogenous enzyme, porcine pepsin, and pancreatin in an in vitro experiment. However, the findings on total tract dry matter, starch digestibility and AME-n content of the current study agree with those of Cervantes *et al.* (2011) that Pancreatin supplementation level could affect feed utilization on sorghum-SBM-based diets fed to growing pigs. Also, Kaczmarek *et al.* (2014) observed a reduction in nutrient digestibility in enzyme supplemented corn-SBM-based diet due to endogenous pancreatic enzyme inhibition required for starch digestion. Although, several factors including feed processing, particle size, starch content in the feed, and non–feed-related factors such as bird's genetics and age may account for the contradictory findings (Zaefarian *et al.,* 2016). The reduction in nutrient retention and metabolizable energy digestibility at the grower phase which was dependent on the supplementation level in the current study could have also been influenced by the limited endogenous pancreatic enzyme secretions due to the supplementation of exogenous enzymes (Kaczmarek *et al.,* 2014).

CONCLUSION

The present findings indicated that Pancreatin supplementation at 250-750 mg/kg could enhance the ileal crude protein and amino acid digestibility of cockerels at a young age. The magnitude of increase depended on the enzyme supplement level to the individual amino acid. The current study suggested that Pancreatin enzyme can be an effective tool in poultry production, especially at the young age, to improve amino acid digestibility. However, there is a need for further research to determine the effects of Pancreatin enzyme supplementation in broiler production.

CONFLICT OF INTEREST

Authors declare no conflict of interest with any organization regarding the materials discussed in the manuscript.

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