Evaluation of dietary Pancreatin as an exogenous enzyme on growth performance, gene expression, immunological responses, serum immunoglobulins, and intestinal morphology in cockerels

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ABSTRACT

The current study evaluated the inclusion of Pancreatin enzyme on growth performance, intestinal morphology, endogenous enzyme activity, and immunological responses in cockerels. A total of 480 d-old-Hy-line cockerels were randomly divided into 5 treatments, with 6 replicates, 16 birds per cage. Birds were given a standard corn-soybean-based (CD) starter and grower diet. Exogenous Pancreatin enzyme was supplemented at 0: 250; 500; 750, and 1000 mg/kg. Results demonstrated that Pancreatin supplementation did not affect (P > 0.05) the growth performance and duodenal enzyme activity of birds. However, the addition of Pancreatin enzyme at 500 mg/kg increased (P < 0.05) jejunal villus height at 42 d; duodenal crypt depth and jejunal crypt depth at 70 d. Pancreatin supplementation except 1000 mg/kg increased (P < 0.05) serum IgM but not IgA and IgG. Furthermore, Pancreatin supplementation had the potential to decrease jejunal pH, spleen gene expression, and antibodies titers against NDV. In conclusion, Pancreatin enzyme inclusion had no effect on cockerels’ growth performance despite the variation found on the gut morphology. Pancreatin enzyme at intermediary amounts (500 and 750 mg/kg) showed a satisfactory serum immunoglobulin result but had the potential to modulate differently on the antibody titers against NDV and gene expression.

Introduction

Enzyme supplementation in the poultry industry has proven beyond its ability in reducing anti nutritional factors to obtaining better productivity and health benefits. At present, exogenous enzymes are increasingly used in a corn-SBM-based diet for poultry although, these diets have low concentrations of soluble non-starch polysaccharides (NSP) (Hafeez et al. 2020). Commercial essential enzymes (protease, amylase) are mostly used with non-essential enzymes (carbohydrases, phytase, glucanases, etc.) to augment, target and metabolize undigested feed components to improve nutrient utilization (Saleh et al. 2018). These enzymes on a corn-SBM-based diet have been reported to improve growth parameters and immune responses by up-regulating the expression of nutrient transporters, humoral immunity, and reduction in serum a-toxin antibodies, lesions in the intestine (Liu et al. 2017; Attia et al. 2020). Thus, the possible mechanism to stimulate the immune response is reported to be by the exogenous enzyme involvement in dietary nutrient hydrolysis, which changes the internal organs weight and intestinal characteristics to modulate the gut microbiota to increase utilization of dietary nutrients for maintenance (Attia et al. 2020; Jiang et al. 2020).

Despite the recognition and beneficiary use of enzyme complex, there are still some discrepancies in outcomes due to the differences in bird type and age (Cowieson and Kluenter 2019). Also, the rate of enzyme response in birds depend on the enzyme components and addition levels (Walk and Poernama 2019; Attia et al. 2020). However, a recent review from Cowieson and Kluenter (2019) indicated that appropriate supplementation of an enzyme product with lipase, amylase, and protease in birds would increasingly affect the intestine and pancreatic tissue and secretions to proportionately increase birds weight. Thus, amylase improves starch digestibility via hydrolysis and releasing energy (Aderibigbe et al. 2020), protease increases not only amino acid digestibility but also provide extra-essential effects (Jabbar et al. 2021a), lipase elevates the protein-sparing effect by mediating the hydrolysis of proteins and improving intestinal health through fat digestion (Liu et al. 2016).

Pancreatin enzyme is a biotechnological complex enzyme product obtained from the porcine pancreas. Mainly, it is defined as a substance that contains α-amylase, lipase and protease enzymes that show pharmaceutical importance at sub-therapeutic doses. Pancreatin enzyme stimulates nutrient absorption and improves serum nutritional parameters by restoring pancreatic digesting function in the gastrointestinal tract (D’Haese et al. 2014). Watanabe et al. (2017) highlighted Pancreatin’s role in immune responses against the formation...
of gut microbiota. Furthermore, Pancreatin enzyme has also been shown to modify the homeostatic proliferation of commensal microbiota by altering the nutritional content inside the gastrointestinal tract (Nishiyama et al. 2018). Although Pancreatin is known to play a crucial role in enzyme replacement therapy, there is a paucity of information regarding the impact of Pancreatin enzyme supplementation on physiological and immune responses in chicken. In addition, very few reports have focused on the inclusion levels that could mimic the endogenous pancreatic enzyme to optimum feed intake and growth rate in poultry. Therefore, this study aimed to determine the appropriate inclusion of Pancreatin enzyme as an exogenous enzyme on growth performance, immunological response, serum immunoglobins and intestinal morphology in cockerels fed corn-SBM-based diet.

Materials and methods

Ethical statement and exogenous pancreatin

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

The exogenous Pancreatin enzyme was provided by Shanghai Honest Biological Technology Co. Ltd. According to the supplier, the Pancreatin enzyme provided per gram contained 561 units of protease; 3061 units of amylase and 4352 units of lipase.

Birds, vaccinations and management

A total of 480-day-old cockerels (Hy-Line Silver-Brown), with an average body BW 39.58 ± 0.24 g, was placed in 0.064 m² floor space for a 70-d trial. Chicks were randomly assigned into 30-floor pens with wood shavings serving as litter material. The experimental room was disinfected by fumigation (mixing formalin 40% with potassium permanganate powder). The room temperature was well controlled (maintained at 33°C during the first week and was decreased gradually to suit the birds by the end of the experiment). The birds’ house was properly ventilated, with the relative humidity observed between 50 and 70%. At the start of the trial, an intermittent lighting program (23 h) using a bright light (40 lux) was set up. Each pen was equipped with one round bottom plastic feeder and a manual drinker through which feed and water were provided ad libitum. Chicks were fed twice a day (morning and evening). The birds were vaccinated against Marek’s disease at the hatchery on 1 d, Newcastle (Nobilis ND clone 30) on 14 d, and infectious bursal disease (Intervet) on 28 d by eye drop following manufacturers’ recommendations.

Diet formulations

All ingredients except the premix were obtained from a commercial feed mill and were formulated at the Yangzhou University feed mixing plant. Birds were fed on a control diet thus a typical corn-SBM-based diets fed in 2 phases (starter and grower) without Pancreatin. The control diet (CD) had 11.98 MJ/kg of metabolizable energy (ME); 18.40% of crude protein (CP) at starter and 11.85 MJ/kg of ME; 17.48% of CP at grower (Table 1). The other four treatment groups were supplemented with Pancreatin at 250, 500, 750, 1000 mg/kg on the CD diet, respectively. The enzyme was added to the diets in powder form, and all diets were fed as mash.

| Table 1. Composition and nutrient levels of the basal diet. |
|------------------------------------------------------------|
| Items | Starter (1-42 d) | Grower (42-70 d) |
|-------|------------------|------------------|
| MAIZE | 66.10            | 66.25            |
| soybean | 29.00            | 26.10            |
| wheat bran | 0.20            | 3.00             |
| methionine | 0.20            | 0.20             |
| lysine | 0.20             | 0.15             |
| salt | 0.30             | 0.30             |
| limestone | 1.30            | 1.30             |
| Ca(H2PO4) | 1.70            | 1.70             |
| premix | 1.00             | 1.00             |
| calculated analysis % | | |
| metabolizable energy, (MJ/kg) | 11.98 | 11.85 |
| crude protein | 18.40 | 17.48 |
| crude fiber | 2.78 | 2.80 |
| calcium | 1.08             | 1.07             |
| total phosphorus | 0.67 | 0.68 |
| available phosphorus | 0.42 | 0.43 |
| lysine | 1.13             | 1.01             |
| methionine | 0.47 | 0.45 |

Pancreatin was supplemented at 0, 250, 500, 750, and 1000 mg/kg on the Control diet (CD).
The premix was sourced from the Yangzhou University Feed Company (Yangzhou, China). The premix contained both Vitamin and trace mineral which per kg of feed contained the following: 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; retinol, 1200,000 IU; cholecalciferol, 400,000 IU; α-tocopherol, 1,800 IU; 2-methyl-1,4-napthoquinone, 150 mg; thiamine, 90 mg; riboflavin, 800 mg; pyridoxine, 320 mg; cobalamin, 1 mg.

Growth performance

Feed intake (FI) by pen was measured daily, and BW was recorded on 1, 42, and 70 d of age. The average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G) were calculated from 1–42 d and from 42–70 d of age, and mortality was recorded as it occurred. Calculations:

ADFI = feed consumption/feeding days in the whole period
ADG = total weight gain/feeding days in the whole period
F/G = ADFI/ADG

Intestinal pH analysis, intestinal morphology and endogenous enzyme activity

On 42 and 70 d, 2 birds, based on the average body weight per pen, were randomly selected, leg-banded, and weighed individually. Birds fasted for 8 h were humanely killed by electrically stunned, exsanguinated, and manually eviscerated. The pH measurements were performed using a pen-type pilotage positive meter. The probe was inserted into the duodenum’s distal...
sections (from the gizzard outlet to the end of the pancreatic loop) and jejunum (from the pancreatic loop to Meckel’s diverticulum).

Approximately 5 cm of the middle portion of the duodenum (apex section) and jejunum (between the entry of bile ducts and Meckel’s diverticulum) were cut, gently flushed with 0.9% saline solution, and placed in 10% neutral buffered formalin. The sections were prepared, dehydrated, cleared, and made immovable using conventional paraffin embedding techniques. Samples were sectioned at a thickness of 5 μm using a rotary Leica RM 2016 microtome machine (Leica Instrument Co. Ltd., Shanghai, China.), placed on a glass slide. Slides were dyed with a standard hematoxylin–eosin solution and covered with a slip. Histological indices were measured using 10X magnification, computer-aided light microscope (Nikon YS 100 microscope, Nikon Corporation, Tokyo, Japan). Villus height was measured from the tip (lamina propria) of the villus to the base (villus-crypt junction), while the crypt depth was measured from the villus-crypt junction to the distal limit of the crypt. Three sections from each part (upper, middle, and lower) of the duodenum and jejunum (3 villi/sections/segment/bird) were measured and calculated.

At 70 d, approximately 0.3 g duodenal samples were collected aseptically from sacrificed birds for endogenous enzyme activity. The digesta were homogenized using 4 ml of 0.9% sodium chloride (NaCl/ice-cold saline). The digesta were collected in a 5 ml screw-capped tube and immediately frozen and stored at −80°C for further analysis. Trypsin and chymotrypsin enzyme assay methods were adopted following the description of Lhoste (1993). Samples were not stored for more than 20 days.

**Immunological test**

On 35 d, blood samples were randomly collected by heart puncture using an insulin syringe from 2 birds per replicate into anticoagulant EDTA tubes. Blood samples were allowed to coagulate, then immediately centrifuged at 3500 rpm for 15 min. The obtained serum was stored at −20°C until analysis. Antibody (Ab) titers against Newcastle Disease virus (NDV) were measured using the hemagglutination inhibition (HI) test as described by Cunningham (1971). Commercial test kits for this assay were purchased from Elisa Co., Ltd., China. The titers were described as log2 based on the inverse of the highest concentration of HI identified.

**Determination of serum immunoglobulins**

On 42 and 70 d, blood samples were randomly collected from 2 birds per replicate from the wing ulnar vein into EDTA tubes before slaughtering. The samples were left to coagulate at room temperature. Blood samples were centrifuged at 3500 rpm for 15 min to obtain serum. The serum samples were carefully transferred and stored in Eppendorf tubes at −20°C until analysis. Serum biochemical parameters levels, IgG, IgM, and IgA, were determined spectrophotometrically using commercial diagnostic kits purchased from Nanjing Construction Bioengineering Research Institute. Co. Ltd.

**RNA isolation, cDNA synthesis and quantitative real-time PCR**

At 70 d, an appropriate size of the spleen was rapidly collected in a tube and immediately flash-frozen in liquid nitrogen and stored at −80°C. Total mRNA was extracted from the spleen and homogenized in TRizol Reagent (Invitrogen, Carlsbad, CA, USA) using a Speed Mill PLUS homogenizer (Analytik Jena, Jena, Germany). Total RNA was further transcribed reversely by the UNiQ-10 Row Extraction Kit (BS11321) and reagent following the manufacturer’s instruction. Quantitative reverse-transcription polymerase chain reaction (PCR) was performed by diluting the cDNA and detected using 2X SG Fast qPCR Master Mix (B639271) and Light Cycler 480 II type fluorescence quantitative PCR instrument (Roche, Rotkreuz, Switzerland). PCR was performed initially at 45 cycles at 95°C for 15 s; denaturation at 95°C for 5 s; annealing at 60°C for 30 s. The primers were designed using their sequence-based ( Primer Premier 5.0 software), synthesized by Sango Biotechnology Co., Ltd. (China) (Table 2). The relative IgM and IL-2 primer pairs were designed such that β-actin primer pairs were set to normalize the mRNA expression. The relative gene expression was evaluated by multiplying the threshold cycle (CT) value of the internal reference gene and gene’s CT value to be tested by 2−ΔΔCT.

**Experimental design and statistical analysis**

A completely randomized design (CRD) with 5 treatments, 6 replicate, 30 pens, and 16 birds per pen was used. Data were analyzed as a one-way analysis of variance (ANOVA) of SPSS 16.0 statistical software. Polynomial contrasts (linear and quadratic) were conducted to evaluate the effect of increasing Pancreatin levels. Statistical significance claims were based on P < 0.05 by Duncan’s multiple range tests whiles a tendency was also set at 0.05 ≤ P ≤ 0.10 unless specified otherwise.

**Results**

**Growth performance**

The results of growth performance of cockerel during the 70-d experiment are shown in Table 3, Pancreatin enzyme

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**Table 2.** Primer pairs for β-actin, IL-2 and IgM genes from birds.1

| Target Gene | Gene Sequences (5’–3’) | Length of fragment (base pair) | Gene Code |
|-------------|------------------------|--------------------------------|-----------|
| β-actin     | F: GACAACTGAACGGCAACCA R: ATACCTTTTCTGCCTGGGCTT | 183 | NC_006101.5 |
| IL-2        | F: CAGTGTACATGCTGGGAGAAGTGG R: GCCCAAGATCTGTGGCATCTAC | 58 | NC_006091.5 |
| IgM         | F: GACAACTGAACGGCAACCA R: TGTGACCGATTGGCTTTGG | 158 | NC_006091.5 |

1Primers were designed with the Primer Premier 5.0 software design.

2IL-2; interleukin-2, β-actin; Beta actin; IgM; immunoglobulin M.
supplementation did not affect \((P > 0.05)\) the live BW, ADG, and F/G at 1–42 d and 42–70 d. Overall, from 1–70 d, Pancreatin enzyme on corn-SBM-based diet did not affect \((P > 0.05)\) cockerel growth performance.

**Gut morphology**

As seen in Table 4., at 42 d, quadratically improvement \((P = 0.016)\) was observed in duodenal crypt depth with a decreased \((P = 0.042)\) in duodenal villus/crypt ratio with increasing level of Pancreatin. Further, jejunal villus height was increased \((P = 0.051\), quadratic; \(P = 0.020\)) in fed diet 500 mg/kg. However, duodenal villus height, jejunal crypt depth, and villus/crypt ratio were not affected \((P > 0.05)\) by dietary treatment.

At 70 d, there was a tendency difference \((P = 0.087)\) with quadratic increase \((P = 0.035)\) on duodenal crypt depth associated with increasing level of Pancreatin. Additionally, a quadratic increase \((P = 0.041)\) was observed in jejunal crypt depth. Increasing dietary Pancreatin supplementation led to quadratic decreases \((P = 0.051)\) in the jejunal villus/crypt ratio. On the other hand, Pancreatin enzyme supplementation had no effect \((P > 0.05)\) on duodenal villus height, villus/crypt ratio and jejunal villus height.

**Intestinal pH**

The effect of the Pancreatin enzyme on intestinal pH is shown in Table 5. There was no significant impact \((P > 0.05)\) of Pancreatin supplementation on intestinal digesta pH (duodenum and jejunum) at 42 d. However, at 70 d, Pancreatin enzyme tended to decrease \((P = 0.094)\) on jejunum pH among treatments. In addition, there was a linear tendency difference \((P < .1)\) on dietary Pancreatin enzyme on intestinal pH.

**Serum immunoglobin**

Data on serum immunoglobins are shown in Table 6. At 42 d, supplementing Pancreatin at 250 mg/kg, 750 mg/kg and Table 3. Effect of supplemented Pancreatin on growth performance.\(^1\)

| Items                  | CD\(^2\) | 250  | 500  | 750  | 1000 | SEM\(^3\) | Pancreatin | Linear | Quadratic |
|-----------------------|---------|------|------|------|------|-----------|------------|--------|-----------|
| live bodyweight (g/bird) |        |      |      |      |      |           |            |        |           |
| 1d                    | 39.34   | 39.35| 39.49| 39.83| 39.55| 0.11      | 0.679      | 0.289  | 0.965     |
| 42d                   | 532.34  | 540.2| 557.5| 541.5| 545.72| 4.36      | 0.573      | 0.264  | 0.360     |
| 70d                   | 1032.57 | 1081.87| 1093.00| 1054.67| 1047.67| 11.92    | 0.644      | 0.790  | 0.161     |
| 1–42 d                |         |      |      |      |      |           |            |        |           |
| ADFI (g)              | 30.91   | 30.91| 31.14| 31.04| 31.34| 0.17      | 0.584      | 0.274  | 0.361     |
| ADG (g)               | 11.75   | 11.94| 12.19| 12.19| 12.05| 0.10      | 0.356      | 0.311  | 0.151     |
| FCR (g/g)             | 2.63    | 2.60 | 2.53 | 2.55 | 2.60 | 0.002     | 0.365      | 0.311  | 0.151     |
| 42–70 d               |         |      |      |      |      |           |            |        |           |
| ADFI (g)              | 63.30   | 65.23| 65.61| 65.36| 64.67| 0.40      | 0.389      | 0.211  | 0.116     |
| ADG (g)               | 17.85   | 19.32| 19.13| 17.96| 17.94| 0.38      | 0.615      | 0.864  | 0.167     |
| FCR (g/g)             | 3.57    | 3.43 | 3.47 | 3.67 | 3.66 | 0.06      | 0.749      | 0.561  | 0.388     |
| 1–70 d                |         |      |      |      |      |           |            |        |           |
| ADFI (g)              | 43.87   | 44.64| 44.93| 44.77| 44.67| 0.27      | 0.656      | 0.238  | 0.340     |
| ADG (g)               | 14.19   | 14.89| 15.05| 14.50| 14.40| 0.17      | 0.643      | 0.797  | 0.161     |
| FCR (g/g)             | 3.10    | 3.02 | 2.99 | 3.10 | 3.11 | 0.03      | 0.673      | 0.840  | 0.200     |

\(^1\)Data represent mean values of 6 replicates per treatment (16 birds per pen).

\(^2\)SEM: standard error of the mean; CD: Control diet; ADFI: average daily feed intake; ADG: average daily gain; FCR: feed conversion ratio.

Table 4. Effect of Pancreatin enzyme supplementation on intestinal morphology of birds.\(^1\)

| Items                  | CD\(^2\) | 250  | 500  | 750  | 1000 | SEM\(^3\) | Pancreatin | Linear | Quadratic |
|-----------------------|---------|------|------|------|------|-----------|------------|--------|-----------|
| Duodenum (\(\mu m\)) |         |      |      |      |      |           |            |        |           |
| Villus height         | 1475.18 | 1540.28| 1525.82| 1593.27| 1539.25| 28.27     | 0.778      | 0.336  | 0.621     |
| Crypt depth           | 187.42  | 215.65| 217.65| 202.90| 187.18| 5.33      | 0.193      | 0.973  | 0.016     |
| Villus:Crypt ratio   | 8.47    | 7.28 | 7.19 | 8.15 | 8.41 | 0.25      | 0.304      | 0.942  | 0.042     |
| Jejunum (\(\mu m\))  |         |      |      |      |      |           |            |        |           |
| Villus Height         | 853.67a | 932.84ab| 1081.37b| 907.11a| 878.80a| 26.40     | 0.051      | 0.605  | 0.020     |
| Crypt Depth           | 165.31  | 174.63| 194.95| 167.29| 148.74| 6.53      | 0.263      | 0.33  | 0.205     |
| Villus:Crypt ratio   | 5.16    | 5.34 | 5.55 | 5.42 | 5.95 | 0.17      | 0.297      | 0.287  | 0.269     |
| Jejunum (\(\mu m\))  |         |      |      |      |      |           |            |        |           |
| Villus Height         | 1321.14 | 1351.75| 1410.79| 1292.46| 1281.55| 28.74     | 0.637      | 0.458  | 0.253     |
| Crypt Depth           | 246.06  | 282.62| 300.92| 255.88| 267.32| 6.89      | 0.087      | 0.701  | 0.035     |
| Villus:Crypt ratio   | 5.37    | 4.78 | 4.69 | 5.29 | 4.94 | 0.15      | 0.611      | 0.495  | 0.390     |

\(^a\)Means within a row without common superscripts differ at \(P < 0.05\).

\(^1\)Data represent mean values of 6 replicate per treatment (2 birds per pen).

\(^2\)SEM: standard error of the mean; CD: Control diet.
1000 mg/kg significantly decreased \((P < 0.05)\) serum IgG and IgA. However, supplementing Pancreatin at 500 mg/kg and 750 mg/kg increased \((P < 0.05)\) serum IgM while 1000 mg/kg was significantly decreased \((P < 0.05)\).

At 70 d, serum IgM modulation was higher \((P < 0.00)\) in fed diets 750 mg/kg and 500 mg/kg compared to CD. An increased in serum IgA \((P = 0.031)\) and IgG \((P = 0.006)\) was also observed in fed diet 500 mg/kg.

**Immunological test**

The effect of Pancreatin supplementation on Ab titers of NDV in chicks is shown in Table 7. The results indicated that although not statistically significant, marginal decreased \((P = 0.078)\) on Ab titer against NDV among treatments was observed. Additionally, a linear decrease \((P = 0.042)\) was also observed on 750 mg/kg Ab titer against NDV compared to CD.

However, spleen gene expression (IL-2, IgM) tended to decrease \((\text{linear}, \ P < .1)\), as dietary Pancreatin enzyme level increased.

**Discussion**

A specific multi-enzyme supplied on top of a nutrient-dense corn-SBM-based diet may increase the enzyme’s potential to improve growth performance through nutrient absorption (Abudabos 2012). The results presented in this study revealed no significant improvement in terms of growth parameters (BW, ADG, ADFI, and FCR) among dietary treatments. The lack of positive effects of Pancreatin treatments on cockerel performance are in agreement with some research studies on broilers using multi-enzyme product in fed corn-SBM-based diet (Gitoee et al. 2015; Hussein et al. 2020). Notably, broiler gains more under exogenous enzyme supplementation than to cockerel (Kianfar et al. 2013; Attia et al. 2020). However, the similarity in results could be attributed to the findings of Kim et al. (2004) and Zhu et al. (2014), indicating that enzyme supplementation might be difficult to detect due to the low activity of the enzyme in the gut or the lower level of soluble NSP and lesser viscosity in corn-SBM-based diet. Contrary to the results presented, a reduction in growth performance and nutrient digestibility in poultry and pigs using Pancreatin® and Pancreatic®, similar exogenous pancreatic enzyme (Al-
Marzooqi and Leeson 1999; Cervantes et al. 2011). These authors attributed the negative responses to the exogenous pancreatic enzyme mechanism on the endogenous pancreatic enzyme secretion and gastric environment. The differences in results could be attributed to high NSP ingredients (sorghum and Canola meal) used in those experiments. However, to our knowledge, the individual enzyme components (amylase, protease, and lipase) or combined use of amylase and protease have improved the growth performance of birds fed corn-SBM-based diet (Iji et al. 2003; Kaczmarek et al. 2014; Liu et al. 2016; Aderibigbe et al. 2020; Jabbar et al. 2021b). These enzymes could singly or work synergistically to change the digestibility of the feed ingredients in a way that was not easily accessible by the endogenous enzymes of the bird. Although, it was pointed out that the degree of response varies depending on the enzyme to substrates turnover, as well as the enzyme source and level of addition (Jha et al. 2015). Further, results on multi-enzyme products, i.e. amylase, xylanase, and protease, have shown marginal improvement in birds’ growth performance (Singh et al. 2017; Kim et al. 2018). However, these authors revealed that the extensive inclusion of carbohydrase (xylanase) in these multi-enzyme products could break down the cross-linkage between the NSP chains to disrupt the cell wall matrix, thereby causing easy access for other enzymes to proteolytic and cellulolytic entrapped nutrients. Either way, it is rather uncertain as to why Pancreatin couldn’t enhance growth performance since nutrients from corn-SBM-based diet can rapidly be extracted with exogenous enzymes supplementation (Romero et al. 2013). Observed differences in growth parameters among other works could be attributed to the unsuccessful degradation of NSP in the feed. Hence, it is plausible that growth performance with the use of exogenous enzyme may depend on the enzyme source, composition, and mode of action in the gut.

The duodenum and jejunum are largely responsible for the digestion and absorption of all major nutrients (Svihus 2014). Enzyme supplementation can cause substantial physicochemical changes on the intestinal lumen and wall (decreasing digesta viscosity, improving endogenous enzyme activity and pH of the gut digesta), and morphological changes (shortening and thickening the villus and increases in the depth of crypt in the intestinal wall) (Hashem et al. 2019; Jiang et al. 2020). Pancreatin enzyme also mitigated gut morphology changes by producing a higher jejunal villus length and crypt depth for greater absorptive capacity. Presented data on the comparison between villus height for the absorptive area in the jejunal segment of cockerels fed diets containing corn-SBM-based diet, increased crypt depth. The increased crypt depth indicated increased villus cell stimulation and thus an increase in nutrients being absorbed and utilized by the gastrointestinal tract but was not observed on the growth parameters. These data showed that feeding corn-SBM-based diet with Pancreatin enzymes could exert changes in the jejunum segment. In agreement, Kalantar et al. (2019) indicated that multi-enzyme addition increased the nutrient absorbent surface of the jejunum segment of broiler chickens.

In the present study, Pancreatin supplementation did not affect duodenal enzyme activities (trypsin and chymotrypsin). There was a marked tendency to decrease in jejunal and duodenal pH with increasing Pancreatin supplementation on 70 d. A major enzyme-mediated change in these parameters could have largely influenced the rate of digestion and nutrient absorption. This is because viscose digesta obstructs the action of digestive enzymes; more enzymes are required to complete digestion, resulting in increased intestinal enzyme activity and pH. In disagreement, Moftakharzadeh et al. (2018) reported that enzyme supplementation does not affect the intestinal pH contents and could decrease the secretion of enzymatic activities (Yuan et al. 2017).

One major finding of the present study is that Pancreatin supplementation improved serum IgM on both 42 and 70 d. However, Pancreatin supplementation decreased serum IgG and IgA of cockerels on 42 d and did not affect on these plasma parameters on 70 d. Serum immunoglobulins expression and secretion are one of the end parts after nutrient absorption related to an organism’s health status (Eraud et al. 2005). IgM is a plasma-lymph fluid and the first antibody to be made by the body to fight a new infection through intra-cellular fluids (Sarker et al. 2000). The increasing level of IgM with Pancreatin supplementation will increase the bird’s ability to fight invaders. The improved plasma IgM of cockerels fed Pancreatin in the present experiment might be attributed to the immunomodulatory activities of Pancreatin enzyme. This study collaborates with Zou et al. (2006), who found an increase in serum IgM under enzyme supplementation. On the other hand, Attia et al. (2020) indicated that supplementation of multienzyme concentrations improved birds’ immunity by organ changes which increased the levels of serum immunoglobulins.

Chickens with low humoral antibodies may be more susceptible to diseases (Rengman et al. 2020). Enzyme supplementation improved chick immunity by increase the weight of the spleen and bursa of Fabricius to increasing Ab’s production for producing mucosal and cell-mediated immunity against

### Table 8. 70 d spleen gene expression and enzyme activity of birds fed Pancreatin supplement diets.1

| Parameters                  | Dietary Pancreatin level (mg/kg) | P-Value |
|-----------------------------|---------------------------------|---------|
|                             | CD2                             |         |
|                             | 250                             | 500     | 750     | 1000    | SEM2   | Pancreatin | Linear  | Quadratic |
| Spleen gene expression      |                                 |         |
| IL-2                       | 1.62                            | 1.44    | 1.20    | 1.13    | 1.09    | 0.09     | 0.403    | 0.057    | 0.875    |
| IgM                        | 2.36                            | 2.23    | 1.88    | 1.67    | 1.44    | 0.18     | 0.503    | 0.086    | 0.711    |
| Duodenal enzyme activity (U/g) |                                 |         |
| Trypsin                    | 4.96                            | 4.58    | 4.48    | 4.57    | 4.54    | 0.14     | 0.841    | 0.403    | 0.459    |
| Chymotrypsin               | 1.81                            | 1.86    | 1.85    | 1.74    | 1.91    | 0.03     | 0.532    | 0.706    | 0.797    |

1Data represent mean values of 6 replicate per treatment (2 birds per pen).
2SEM: standard error of the mean; CD: Control diet.
NDV (Lewis et al. 2019; Attia et al. 2020). In this study, the trend of decrease in immune titer (14.78%) on account of pancreatic addition (750 mg/kg) could be attributed to a reduction in the availability of serum immunoglobulin at 42 d. Contrary to this, Seidavi et al. (2017) demonstrated that supplementing enzyme mixture on fed corn-SBM-based diet had no effect on the humoral response against NDV on 42 d. Further, previous works have been reported that irrespective of the enzyme blend and graded level, there was no substantial effect on Ab titer after vaccination (Sateri et al. 2017; Saleh et al. 2018).

Interestingly, dietary Pancreatin addition in this present study did not affect the expression of IL-2 and IgM genes. However, there was a noteworthy tendency for a decrease, linearly, in these two determined genes. These results might give an insight into the humoral antibodies observed. Thus, the spleen acts as the leading site of lymphocyte differentiation and proliferation involved in producing humoral and cell-mediated responses for immunologic functions (Lewis et al. 2019). Increased expression of IL-2 and IgM could produce antibodies that could aid in the removal of apoptotic cells while rapidly producing follicles for antigen delivery (independent T cells) during or after vaccination (Table 5) (Baumgarth 2011). Contrary to the present results, Saleh et al. (2018) reported that enzyme supplementation could enhance the immunity of birds up-regulating the production of the intestinal IL-2 gene.

Conclusion
The inclusion of dietary Pancreatin in a corn-SBM-based diet for cockerels did not affect the growth performance and enzyme activity although, the gut morphology was affected positively. Given the potential impact, the addition of Pancreatin at intermediary amounts (500 and 750 mg/kg) mediated changes in the serum immunoglobulins. However, these change patterns of serum immunoglobulins were not consistent with the spleen gene expression and NDV Ab titer. This result suggested that Pancreatin enzyme supplementation is not an effective dietary tool to achieve optimal productive performance in cockerels. Further research is needed to explore Pancreatin evaluation in fast-growing broilers.

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