Original article

Effect of dietary incorporation of peanut and linseed meals with or without enzyme mixture on physiological performance of broilers

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A B S T R A C T

The present study aimed to evaluate the impact of feeding peanut meal and linseed meal (LSM) with or without enzyme mixture on growth, plasma metabolites, muscle amino acid (AA) profile, nutrient digestibility, and expression of nutrient absorption-related genes in broilers. A total of 560 one-day-old Cobb-500 male broiler chicks were distributed into eight experimental treatments (7 replications of 10 chicks each) as follows: Group 1 (C) control fed the basal diet without supplements, Group 2 (C + E) is control group fed on 350 g/ton enzyme mixture, Group 3 (C + PNM100) is control group fed 100 kg/ton peanut meal, Group 4 (C + E + PNM100) is a control group fed on 350 g/ton enzyme mixture and 100 kg/ton peanut meal, Group 5 (C + LSM100) is a control group fed on 100 kg/ton linseed meal, Group 6 (C + E + LSM100) is a control group fed on 350 g/ton enzyme mixture and 100 kg/ton linseed meal, Group 7 (C + PNM50 + LSM50) is control group fed on 50 kg/ton peanut meal and 50 kg/ton linseed meal, Group 8 (C + E + PNM50 + LSM50) is a control group fed on 50 kg/ton peanut meal and 50 kg/ton linseed meal. Each gram of the enzyme mixture contains 11,000 U Xylanase, 6000 U Cellulase, 700 U b-Mannanase, 1500 U Phytase, 5 mg a-Amylase, and 2 mg Protease. No differences in Body weight, Bodyweight gain, Feed intake, and carcass parts were noticed among experimental groups, while abdominal fat (%) and FCR were reduced (P < 0.05) in PNM50 + LSM50 + E and LSM100 groups. Plasma metabolites were not altered except total cholesterol, triglyceride, and LDL, reduced (P < 0.01) in treated birds. Dietary inclusion of 100 kg PNM or LSM reduced (P < 0.05) methionine concentration in muscle, while all remaining AA and ammonia concentrations were unaffected. Hepatic MDA contents were reduced (P < 0.001) in treated groups. Nutrient digestibility was not altered among groups except for protein digestibility, which was elevated (P < 0.05) in PNM50 + LSM50 + E, E, and PNM100 + E groups. The highest mRNA expressions of PepT1, APN, SGLT1, HMGCR, GHr, and IGF-1 genes were noticed in PNM50 + LSM50 + E. Conclusively, PNM and LSM can efficiently substitute corn and soybean meal in broiler diets, particularly when fortified with exogenous enzymes, without negative impacts on broiler performance.

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1. Introduction

The limited amounts and continuous rising prices of high-quality feed ingredients are real challenges facing poultry producers. This enormous challenge remains the most significant deter-
minant of profit margins in poultry production (Ibrahim et al., 2020). Soybean and corn are the most expensive ingredients in traditional poultry rations. Nevertheless, must be many African, Asian, and European countries. External fluctuation influences their prices (Abdel-Moneim, Sabic et al., 2020). Therefore, constant evaluation of alternative and locally available feedstuffs is required to minimize feed costs.

Peanut is one of the legumes plants cultivated in tropical and subtropical regions mainly used as an oilseed, and its meal can be used as a protein source in animal diets (Sarbaz et al., 2018). Peanut meal (PNM) is considered fibrous since it contains about 10% crude fibre and multiple skins and shell residues (Davis and Dean, 2016). Due to the comprehensive extraction procedures, the residual oil content is highly variable, ranging from 3% or higher for a solvent-extracted meal to 10% in mechanically extracted cakes. More than 90% of the peanut oil's fatty acids (FA) are unsaturated FAs, mainly linoleic and oleic (Bera et al., 2020). However, several disadvantages of incorporating PNM in broiler diets include a lack of lysine, threonine, and methionine (Piva et al., 1995; Zhang and Parsons, 1996) the risk of aflatoxin contamination (Kana et al., 2013). Mineral deficiency is another limiting factor for using PNM in broiler diets. However, mineral availability has been found to improve with the addition of phytase enzyme, increasing metabolizable energy. It’s worth noting that PNM cannot be used as a single protein source in broiler diets, but when combined with other protein sources, it can be used efficiently at levels up to 15% (Costa et al., 2001; Diaw et al., 2010; Ghadge et al., 2009). Therefore, by balancing the diet and detoxifying peanut meal, the pre-existing restrictions can be removed (Driver et al., 2006).

Linseed is an oily crop that contains a considerable level of protein (25–35%) and a high source of ω-3 polyunsaturated fatty acids (PUFA) and produces linseed meal (LSM) as a by-product after oil extraction. However, several factors limit the incorporation of LSM in poultry diets in high proportions, including low content of lysine and methionine compared to other oilseeds, relatively high fibre content, and anti-nutritional factors (Ji et al., 2017). The presence of anti-nutritional factors, such as, e.g., cyanogenic glycosides, phytic acid, mucilage, anti-pyridoxine factor, allergens, and goitrogens, in PNM and LSM can bind to water and increase digesta viscosity. The high viscosity of digesta leads to the formation of a biofilm on the intestinal epithelium. It increases the fermentation processes and the proliferation of pathogenic microbes, elevating the risk of diseases and decreasing the productive performance of birds (Pirmohammadi et al., 2019; Rodriguez et al., 2001; Schöne et al., 1996). To the best of our knowledge, minimal studies have investigated the nutritional patterns of feeding PNM and LSM in the amino acid profile of broiler meat. It is worth noting that dietary protein is the primary source providing the essential amino acids required for muscle growth, tissue repair, and maintaining vital physiological functions (Toomer, Livingston, et al., 2020). Plant or animal protein sources can be used to formulate the diets; however, plant-based feedstuffs are typically included in broiler diets because of their availability and low costs (Babatunde et al., 2021).

Fortunately, recent studies have reported that adding exogenous enzymes to the diets containing linseed or peanut meal improved birds’ growth performance, nutrient digestibility, and gut health (Driver et al., 2006; Pirmohammadi et al., 2019). However, limited studies have investigated the dietary incorporation impacts of PNM and LSM with exogenous enzymes on broiler performance and nutrient digestibility. Therefore, and for the first time, this study was conducted to evaluate the effects of feeding PNM and LSM with or without enzyme mixture on muscle amino acids profile and expression of nutrients absorption related genes in broilers.

2. Material and methods

2.1. Ethical statement

The study was approved by the Ethics Committee of Local Experimental Animals Care Committee and conducted following Kafrelsheikh University, Egypt (Number 4/2016 EC) guidelines. All precautions were followed to decrease suffering during the entire experimental period.

2.2. Birds and experimental design

Five hundred sixty one-day-old male Cobb-500 broiler chicks (average initial weight of 41 ± 0.6 g) were allocated into eight experimental treatments (7 replications of 10 chicks each). Birds were housed in floor pens littered with wood shavings and supplied with a chain feeder and automatic nipple cup drinker systems. All birds were reared under the same uniform management conditions. The experimental diets were fed on three phases (starter, 0–10 d; grower, 11–24 d; and finisher, 25–35d), isocaloric and isonitrogenous and formulated to meet or exceed the nutrient requirements of Cobb-500 following the guidelines of the strain (Table 1). The eight experimental diets were as follows: Group 1 (C) control fed the basal diet without supplements, Group 2 (C + E) is control group fed on 350 g/ton enzyme mixture, Group 3 (C + PNM100) is control group fed 100 kg/ton peanut meal, Group 4 (C + E + PNM100) is a control group fed on 350 g/ton enzyme mixture and 100 kg/ton peanut meal, Group 5 (C + LSM100) is a control group fed on 100 kg/ton linseed meal, Group 6 (C + E + LSM100) is a control group fed on 350 g/ton enzyme mixture and 100 kg/ton linseed meal, Group 7 (C + PN M50 + LSM50) is control group fed on 50 kg/ton peanut meal and 50 kg/ton linseed meal and Group 8 (C + E + PN M50 + LS M50). Feed and drinking water were offered ad libitum. Each gram of the enzyme mixture (Natuzyme, BioProton Europe Oy, Finland) contains 11,000 U Xylanase, 6000 U Cellulase, and 700 U β-Mannanase (Trichoderma longibrachiatum), 1500 U Phytase (Escherichia coli), 5 mg α-Amylase (Bacillus subtilis), and 2 mg Protease (Aspergillus niger).

2.3. Nutrients composition of peanut and linseed meals

The chemical analysis of PNM and LSM (Table 2) was conducted at the feed analysis laboratory of Kafrelsheikh University, Egypt, following the AOAC International procedures (AOAC, 2003). The metabolizable energy of PCM and LCM has calculated the following equation: Metabolizable energy = 26.7 × dry matter (%) + 77 × ether extract (%) ÷ 51.22 × crude fibre (%). The study was approved by the Ethics Committee of Local Experimental Animals Care Committee and conducted following Kafrelsheikh University, Egypt (Number 4/2016 EC) guidelines. All precautions were followed to decrease suffering during the entire experimental period.

2.4. Growth performance, carcass parts, and sampling

At 35 d, all birds were individually weighed, and final body weight (BW), weight gain (WG), and feed intake (FI) were recorded on a replication basis. The feed conversion ratio was calculated as FCR = Feed consumed (g) ÷ Weight gain (g).

Seven birds per group (1 bird/replicate; 56 birds total) were randomly chosen, slaughtered, and dissected to evaluate carcass parts. Fresh weights of the hot carcass, breast and thigh muscles, heart, gizzard, spleen, liver, and abdominal fat were recorded and expressed as relative weights to live body weight (Abdel-Moneim, Selim, et al., 2020; Abdel-Moneim, Elbaz, et al., 2020).

Blood samples were gathered into heparinized test tubes for biochemical analysis at slaughter. Samples of the liver, superficial pectoral muscle, and ileum were collected appropriately for...
Table 1
Composition of the experimental starter, grower, and finisher diets.

| Ingredient, g/kg | Starter, (1–10 days) | Grower, (11–25 days) | Finisher, (26–35 days) |
|-----------------|----------------------|----------------------|-----------------------|
|                 | Control | PNMI00 | LSM00 | PNMI00 + LSM00 | Control | PNMI00 | LSM00 | PNMI00 + LSM00 | Control | PNMI00 | LSM00 | PNMI00 + LSM00 |
| Yellow corn     | 549.0  | 483.5  | 485.5 | 482.5  | 570.0  | 531.0  | 529.0 | 530.5  | 616.5  | 579.0  | 575.0 | 575.5 |
| Soybean meal, 46% | 360    | 332    | 360   | 348    | 358    | 280    | 312   | 296    | 305    | 226    | 259   | 244   |
| Corn gluten meal, 62% | 33    | -      | -     | -      | 0      | -      | -     | 0      | -      | -      | -     | -     |
| PCM, 23%        | -      | 100    | -     | 50     | -      | 100    | -     | 50     | -      | 100    | -     | 50    |
| LCM, 36%        | -      | 100    | 50    | 50     | -      | 100    | 50    | 50     | -      | 100    | 50    | 50    |
| Soya oil        | 17.0   | 41.5   | 13.0  | 27.7   | 35.0   | 49.0   | 21.0  | 35.0   | 43.0   | 57.0   | 29.0  | 43.0  |
| Premix*         | 3      | 3      | 3     | 3      | 3      | 3      | 3     | 3      | 3      | 3      | 3     | 3     |
| NaCl            | 3      | 3      | 3     | 3      | 3      | 3      | 3     | 3      | 3      | 3      | 3     | 3     |
| NaCO3           | 1      | 1      | 1     | 1      | 1      | 1      | 1     | 1      | 1      | 1      | 1     | 1     |
| Monocalcium phosphate | 15.8 | 16.2   | 16.0  | 16.0   | 14.0   | 14.4   | 14.0  | 14.0   | 12.8   | 12.8   | 13.0  | 13.0  |
| K2CO3           | 15.5   | 15.0   | 15.0  | 15.0   | 14.0   | 13.5   | 14.0  | 13.5   | 13.0   | 12.7   | 13.0  | 12.8  |
| -Lysine HCl, 98% | 1.3    | 2.5    | 1.5   | 1.8    | 0.1    | 2.3    | 1.4   | 2.1    | 0.7    | 2.9    | 1.9   | 2.4   |
| DI Methionine, 99% | 1.4   | 2.3    | 2.0   | 2.0    | 1.8    | 2.8    | 1.6   | 1.9    | 2.5    | 2.4    | 2.1   | 2.2   |

Table 2
Chemical analysis of peanut meal and linseed meal as dry matter (% basis).

| Nutrients               | Peanut meal | Linseed meal |
|-------------------------|-------------|--------------|
| Moisture, %             | 7.30        | 8.70         |
| Metabolizable energy, kcal/kg | 3670    | 1850         |
| Crude protein, %        | 23.00       | 36.00        |
| Crude fat, %            | 11.40       | 8.50         |
| Crude fiber, %          | 21.00       | 8.70         |
| Lysine, %               | 0.30        | 0.40         |
| Methionine, %           | 0.20        | 0.15         |
| Calcium, %              | 0.10        | 0.20         |
| Phosphorus, %           | 0.10        | 0.10         |
| Aflatoxin, mg/kg        | 7.30        | 3.10         |
| Ochratoxin, mg/kg       | 2.00        | 1.70         |

Further biochemical and real-time PCR analyses (Saleh, Shukry, et al., 2021).

2.5. Plasma biochemical analysis

To separate plasma, blood samples were centrifuged at 4 °C for 20 min (3000g), and collected samples were stored at −20 °C pending analysis. Using spectrophotometric analysis and following the manufacturer instructions, triglycerides, total cholesterol, high- and low-density lipoprotein (HDL and LDL), glucose, albumin, total protein, creatinine, uric acid, alanine, and aspartate transaminases (ALT and AST) were calorimetrically measured using commercial kits (Diamond Diagnostics, Egypt).

2.6. Muscle amino acids profiles and hepatic MDA

Collected muscle samples were used to analyze the amino acid using gas-liquid chromatography (GLC) following the procedure described by Ahmed Ali Saleh (2013). In brief, after homogenizing 10 g of tissue in 40 mL of 0.1 N HCl for 45 s at 4 °C, the sample was centrifuged at 15,000 g for one hour minutes at 4 °C Celsius. Filtration and analysis of the supernatants were carried out (GC-4 CM-PFE, Shimadzu gas chromatograph, Tokyo, Japan) outfitted with a flame ionization detector (FID).

Concentrations of malondialdehyde (MDA) in hepatic tissues were also measured using a commercial test kit (Bio-Diagnostic kit, Giza, Egypt) according to Ohkawa et al. (1979). A 100 μl aliquot of tissue homogenate was combined with thiobarbituric acid reagent, incubated at 95 °C for 30 min, then centrifuged for 3 min at 10,000 r.p.m. At an absorbance level of 534 nm, the clear pink supernatant (thiobarbituric acid reactive products) was detected.

2.7. Nutrients digestibility

Four days before slaughtering, seven chicks per group were weighed, allocated individually in metabolic cages with free access to water and feed, and kept for 24 h adaptation period. Then, for three consecutive days, faeces samples were gathered. The final body weight of birds was recorded to ensure maintaining their weight. Diets and dried excreta were analyzed for crude fiber (#978.10), ether extract (#920.29), and crude protein (#954.01) following the (AOAC, 2003). According to Jacobsen et al. (1996), faecal nitrogen was estimated using trichloroacetic acid.

2.8. Gene expression

RT-PCR was used to determine the mRNA expression of duodenal and hepatic genes. In brief, total RNA extraction was conducted using TRIzol reagent from roughly 50 mg of tissue (Invitrogen, Life...
The effect of feeding PNM and LSM with or without enzyme mixture on growth performance of broilers at 35 days of age is presented in Table 3. No significant differences were observed among experimental groups in the BW, WG, and FI. FCR was reduced (P < 0.05) in PNM50 + LSM50 + E and LSM100 groups compared to the control group. The PNM50 + LSM50 + E groups recorded the highest BW and WG and the lowest FCR.

Feeding on PNM and LSM with or without enzyme mixture did not affect the relative weights of carcass, breast and thigh muscles, liver, spleen, gizzard, and heart among experimental groups (Table 4). The relative weight of abdominal fat was decreased (P < 0.01) in LSM100 (0.553) and PNM50 + LSM50 + E (0.642) groups compared to the control group.

As presented in Table 5, dietary supplementation of PNM and LSM with or without enzyme mixture did not alter plasma concentrations of HDL, total protein, creatinine, globulin, albumin, AST, ALT, uric acid, and glucose. However, total cholesterol, triglyceride, and LDL were reduced (P < 0.01) in all treated birds compared to the control group. Total cholesterol, triglycerides, and LDL in the E group were significantly lower than in the control group except for total cholesterol and LDL in the E group.

The mRNA expressions of PepT1, APN, SGLT1, and IGF-1 were significantly upregulated in E, LSM100 + E, and PNM50 + LSM50 + E groups compared to the remaining groups (Figs. 1 and 2). The mRNA expression of GHr was upregulated (P < 0.05) in all treated groups except the PNM50 + LSM50 group compared to the control (Fig. 2). HMGCGR mRNA expression was elevated (P < 0.05) in all treated groups except E and PNM50 + E groups (Fig. 3). There are no significant changes in SOD1, CAT, and FAS expression among the experimental groups (Figs. 3 and 4). The highest mRNA expression of the genes mentioned above was recorded in the PNM50 + LSM50 + E group.

The present study revealed non-significant changes in growth performance indices and carcass traits when broilers fed PNM and LSM with or without enzyme mixture. The effects of feeding PNM and LSM on the growth of broiler chickens are diversified among studies. The variations among the results of earlier studies may depend on dietary inclusion level, chemical structure and percent of oil residue, and the concentrations of the anti-nutritional factors in both meals (Abdel-Moneim, Selim, et al., 2020; Mridula et al., 2011; Toomer, Livingston, et al., 2020). In line with our findings, Mridula et al. (2011) reported that the growth performance of broiler chickens was not affected by dietary incorporation of 10% LSM while omega-3 fatty acid content in the meat yield was improved. Moreover, Meherunnisa et al. (2017) found that feeding 15% water-treated linseed meal can be used in broiler diets without any adverse effects. Contrarily, Toomer et al. (2019) demonstrated that broilers fed a high oleic-PNM diet recorded lower BW than the control group. In the present study, the highest BW and WG and the lowest FCR were recorded in the PNM50 + LSM50 + E group. This indicates that using various plant protein sources in poultry diets...
and exogenous enzymes efficiently enhances nutrient digestibility and improve utilization. This improvement might be attributed to the synergistic effect between different protein sources, which provide the essential amino acids required by the chicken growth.

| Item                        | Experimental Diets | SEM | P-Value |
|-----------------------------|--------------------|-----|---------|
| Carcass, %                  | C                  | 61.51 | 61.91 | 61.95 | 62.75 | 63.07 | 63.39 | 62.43 | 63.34 | 0.942 | 0.7797 |
| Breast muscle, %            | C                  | 23.72 | 23.22 | 23.45 | 23.54 | 23.93 | 23.34 | 22.22 | 23.39 | 0.825 | 0.9986 |
| Thigh muscle, %             | C                  | 16.89 | 16.85 | 16.67 | 16.80 | 16.16 | 16.13 | 16.48 | 16.18 | 0.595 | 0.9052 |
| Gizzard, %                  | C                  | 1.53  | 1.45  | 1.61  | 1.53  | 1.60  | 1.63  | 1.58  | 1.63  | 0.064 | 0.4585 |
| Heart, %                    | C                  | 0.353 | 0.591 | 0.583 | 0.588 | 0.508 | 0.525 | 0.503 | 0.502 | 0.042 | 0.5199 |
| Spleen, %                   | C                  | 0.163 | 0.185 | 0.205 | 0.175 | 0.182 | 0.190 | 0.180 | 0.197 | 0.016 | 0.7081 |
| Liver, %                    | C                  | 2.382 | 2.535 | 2.867 | 2.615 | 2.768 | 2.327 | 2.455 | 2.395 | 0.166 | 0.2487 |
| Abdominal fat, %            | C                  | 1.027 | 0.683 | 0.828 | 0.670 | 0.553 | 0.697 | 0.702 | 0.642 | 0.941 | 0.0096 |

\[a-b\] Means in each row with different superscripts are significantly different at \( P < 0.05 \). SEM; standard error of mean. \( 1C = \) control, \( E = C + 350 \) g/t enyme mixture, \( PNM100 = C + 100 \) kg/t PNM, \( PNM100 + E = C + E + PNM100 \), \( LSM100 = C + 100 \) kg/t LSM, \( LSM100 + E = C + E + LSM100 \), \( PNM50 + LSM50 = C + 50 \) kg/t PNM and \( 50 \) kg/t LSM, \( PNM50 + LSM50 + E = C + E + PNM50 + LSM50 \).

### Table 5
Effect of feeding peanut meal (PNM) and linseed meal (LSM) with or without enzyme mixture on blood biochemical analysis of broilers.

| Item                        | Experimental Diets | SEM | P-Value |
|-----------------------------|--------------------|-----|---------|
| Total lipid profile, mg/dl  | C                  | 156.75 | 141.25 | 125.50 | 127.50 | 130.25 | 133.25 | 133.25 | 125.50 | 0.942 | 0.0001 |
| Triglycerides               | C                  | 26.00  | 15.75  | 18.00  | 17.75  | 13.75  | 14.50  | 13.50  | 14.75  | 0.825 | 0.0001 |
| LDL-cholesterol             | C                  | 91.00  | 67.00  | 48.25  | 48.25  | 51.50  | 66.00  | 51.00  | 47.00  | 0.941 | 0.0001 |
| Protein fractions, g/dl     | C                  | 0.58   | 0.66   | 0.72   | 0.96   | 0.76   | 0.68   | 0.80   | 0.51   | 0.941 | 0.0001 |
| Lipid profile, mg/dl        | C                  | 3.03   | 3.25   | 3.28   | 3.30   | 3.38   | 3.15   | 3.43   | 3.48   | 0.941 | 0.0001 |
| Glucose, mg/dl              | C                  | 2.98   | 2.60   | 2.93   | 2.68   | 2.93   | 2.80   | 2.60   | 2.78   | 0.941 | 0.0001 |
| Essential AA, mg/100 g protein | C                  | 10.200 | 10.000 | 10.767 | 11.200 | 10.767 | 11.200 | 10.133 | 10.500 | 10.833 | 0.262 | 0.0641 |
| Arginine                    | C                  | 2.333  | 2.533  | 2.367  | 2.233  | 2.700  | 2.500  | 2.533  | 2.833  | 0.243 | 0.4147 |
| Non-essential AA, mg/100 g protein | C                  | 10.200 | 10.000 | 10.767 | 11.200 | 10.767 | 11.200 | 10.133 | 10.500 | 10.833 | 0.262 | 0.0641 |

\[a-b\] Means in each row with different superscripts are significantly different at \( P < 0.05 \). SEM; standard error of mean. \( 1C = \) control, \( E = C + 350 \) g/t enyme mixture, \( PNM100 = C + 100 \) kg/t PNM, \( PNM100 + E = C + E + PNM100 \), \( LSM100 = C + 100 \) kg/t LSM, \( LSM100 + E = C + E + LSM100 \), \( PNM50 + LSM50 = C + 50 \) kg/t PNM and \( 50 \) kg/t LSM, \( PNM50 + LSM50 + E = C + E + PNM50 + LSM50 \).
Table 7
Effect of feeding peanut meal (PNM) and linseed meal (LSM) with or without enzyme mixture on nutrients digestibility and hepatic MDA content of broilers.

| Item                              | Experimental Diets  | SEM P-Value |
|-----------------------------------|---------------------|-------------|
| Liver MDA, g/100 g bw             | C E PNM100 PNM100+E LSM100 LSM100+E PNM50+LSM50 PNM50+LSM50+E |
| Crude protein digestibility, %    | 13.047± 10.007± 6.432± 5.093± 10.447± 9.940± 7.463± 7.66± | 0.823 0.0001 |
| Crude fiber digestibility, %      | 62.667± 65.667± 63.667± 65.667± 63.000± 65.000± 64.667± 66.00± | 0.726 0.0348 |
| Crude fat digestibility, %        | 23.000± 24.333± 24.000± 23.667± 23.667± 23.667± 24.667± 24.333± | 1.000 0.9362 |
| MDA, g/100 g bw                   | 7.46± 7.66± | 1.000 0.9362 |

Means in each row with different superscripts are significantly different at P < 0.05. SEM, standard error of mean; MDA, malondialdehyde. C = control, E = C + 350 g/ton enzyme mixture, PNM100 = C + 100 kg/ton PNM, PNM100 + E = C + E + PNM100, LSM100 = C + 100 kg/ton LSM, LSM100 + E = C + E + LSM100, PNM50 + LSM50 = C + 50 kg/ton PNM and 50 kg/ton LSM, PNM50 + LSM50 + E = C + E + PNM50 + LSM50 + E.

Table 8
Primers sequences and target genes for SYBR green RT-PCR.

| Gene     | Forward                      | Reverse                      | Accession number |
|----------|------------------------------|------------------------------|------------------|
| PepT1    | CCCCCGGAGGAGGATCACTGTTGCAGTT | CAAAAGACCACGCAACGA | NM_204365 |
| APN      | AATACGCGCTCGAGAAAACC         | AGCGGGTACGCCGTGTTGAGTGC     | NM_204861 |
| SGLT1    | GCCATGGCCAGGGCTTA             | ATAACCTGATCTGTGCACCA       | XM_415247 |
| GHR      | GATCCACCACCAACGAGCAA         | TGTCGGTGCTGAGACATTGT        | AH007350.1 |
| IGF-I    | GTGGGTCAAGTGGCTTTGATTGC     | CGTAGAGCCGCGCATGATTAGTG     | JN492578.1 |
| SOD1     | ATCCCTTTGCTGCATCAAT          | CTTATGACTACCAATACATTGTAGTT | NM205064.1 |
| CAT      | ACTGCAGGTTGCCAGAACAA         | CTATCTACATCGTCCAAACTTGC    | NM00103215.1 |
| HMGCR    | CGTCAGGCTGCAGCAAAAT          | TACGTATACATCGTCCAAACTTGC   | NM001199487.1 |
| GAPDH    | CAGGCTTATCCGGTTAGCAA         | AGTGCAGGCTCAAGTATTG         | NM204305 |

PepT1, peptide transporter 1; APN, aminopeptidase N; SGLT1, Na+−dependent glucose transporter 1; IGF-I, insulin-like growth factor 1; GHR, Growth hormone receptor; SOD1, superoxide dismutase 1; CAT, catalase; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Fig. 1. Effect of feeding peanut meal (PNM) and linseed meal (LSM) with or without enzyme mixture on mRNA expression of PepT1, APN and SGLT1. a-d Means with different superscripts are significantly different at P < 0.05. C = control, E = C + 350 g/ton enzyme mixture, PNM100 = C + 100 kg/ton PNM, PNM100 + E = C + E + PNM100, LSM100 = C + 100 kg/ton LSM, LSM100 + E = C + E + LSM100, PNM50 + LSM50 = C + 50 kg/ton PNM and 50 kg/ton LSM, PNM50 + LSM50 + E = C + E + PNM50 + LSM50.

Fig. 2. Effect of feeding peanut meal (PNM) and linseed meal (LSM) with or without enzyme mixture on mRNA expression of GHR and IGF-I. a-d Means with different superscripts are significantly different at P < 0.05. C = control, E = C + 350 g/ton enzyme mixture, PNM100 = C + 100 kg/ton PNM, PNM100 + E = C + E + PNM100, LSM100 = C + 100 kg/ton LSM, LSM100 + E = C + E + LSM100, PNM50 + LSM50 = C + 50 kg/ton PNM and 50 kg/ton LSM, PNM50 + LSM50 + E = C + E + PNM50 + LSM50.

Fig. 3. Effect of feeding peanut meal (PNM) and linseed meal (LSM) with or without enzyme mixture on mRNA expression of FAS and HMGCR. a-f Means with different superscripts are significantly different at P < 0.05. C = control, E = C + 350 g/ton enzyme mixture, PNM100 = C + 100 kg/ton PNM, PNM100 + E = C + E + PNM100, LSM100 = C + 100 kg/ton LSM, LSM100 + E = C + E + LSM100, PNM50 + LSM50 = C + 50 kg/ton PNM and 50 kg/ton LSM, PNM50 + LSM50 + E = C + E + PNM50 + LSM50.

Fig. 4. Effect of feeding peanut meal (PNM) and linseed meal (LSM) with or without enzyme mixture on mRNA expression of SOD1 and CAT. a-d Means with different superscripts are significantly different at P < 0.05. C = control, E = C + 350 g/ton enzyme mixture, PNM100 = C + 100 kg/ton PNM, PNM100 + E = C + E + PNM100, LSM100 = C + 100 kg/ton LSM, LSM100 + E = C + E + LSM100, PNM50 + LSM50 = C + 50 kg/ton PNM and 50 kg/ton LSM, PNM50 + LSM50 + E = C + E + PNM50 + LSM50.
without shortages or excesses (Pesti et al., 2003). Confirming this hypothesis, Driver et al. (2006) revealed that supplementation of an exogenous enzyme (phytase) to broilers' diet based on PNM increased their metabolizable energy. Musharaf and Latshaw (1991) showed that formulating a starter broiler diet (23% crude protein) composed of different protein sources, such as fish, meat, soybean, PNM, and sesame meals, resulted in an improvement in growth rate and FCR.

Furthermore, dietary inclusion of 0.10% threonine to PNM based-diet improved WG and FCR of broilers compared to the control (Costa et al., 2001). The authors also documented that the source of protein in the broiler diet did not significantly affect the carcass part's weights. In the same line, Pekel et al. (2009) reported significant changes in carcass yield and weights of carcass and breast in broilers fed flaxseed-based diets. Nevertheless, Toomer, Livingston, et al. (2020) and Toomer et al. (2019) found that carcass parts weights were decreased by providing on high oil PNM compared to the other treatments. However, Sarbaz et al. (2018) noticed higher carcass weight and lower abdominal fat pad when birds were fed 50 g/kg peanut pod. The reduction in abdominal fat deposition might be attributed to the increasing omega-3 fatty acids in PNM and LSM and the balance of amino acids, especially lysine, in the broiler diet (Ajuayah et al., 1991).

Inclusion of PNM and LSM with or without enzyme mixture in broiler diets did not affect hepatic and renal function biomarkers in the plasma. The lack of differences among hepatic and renal function biomarkers indicates the absence of any adverse health effects of dietary supplements at tested levels on treated birds (Abd El-Moneim and Sabic, 2019). Nevertheless, the hypocholesterolemic impacts of these supplements were well-presented as plasma levels of total cholesterol, triglyceride, and LDL were reduced in treated birds. These results align with Toomer, Livingston, et al. (2020), who demonstrated that feeding on diets fortified with high-oleic PNM recorded the lowest total cholesterol content compared to the remaining groups. Similarly, fat and cholesterol contents of meat were reduced when 100 g/kg LSM for at least three weeks (Kumar et al., 2019).

Additionally, dietary inclusion of PNM at 5.3% and 10.6% reduced egg yolk concentration of cholesterol (Lu et al., 2013). The reduction in cholesterol and triglycerides levels could be attributed to the high contents of unsaturated fatty acids of oil residues in PNM and LSM (Bayrak et al., 2010; Bera et al., 2019), which contribute to reducing plasma cholesterol levels and increasing LDL turnover in the liver by increasing hepatic LDL receptor number (Fernandez and West, 2005). Another potential reason for the hypocholesterolemic effect of PNM and LSM is their high phytostersols content (Awad et al., 2000; Han et al., 2015), which have a similar chemical structure to animal cholesterol; leading to decreasing blood cholesterol (Abdel-Moneim, Selim, et al., 2020). Furthermore, previous studies showed that the Linseeds might contribute to reducing plasma cholesterol levels and increasing LDL turnover in the liver by increasing hepatic LDL receptor number (Fernandez and West, 2005). Both metabolites affect the growth and several functions in the body. GHR gene is responsible for the translation of growth hor-
monoe receptor protein which binds to growth hormone triggering a signalling process that stimulates the division and growth of cells. Primarily by hepatic cells, this signalling also leads to the production of IGF-1 (Ketelslegers et al., 1995). IGF-1 is used as a marker to evaluate the nutritional status, and its mRNA expression is regulated quantity and quality of dietary proteins (Miura et al., 1992). In the present study, GHR and IGF-1 were upregulated in birds treated with dietary supplements and exogenous enzymes. As mentioned earlier, the highest mRNA expression of the genes was recorded in the PNM50 + LSM50 + E group. This indicates the importance of the enzyme mixture to enhance the nutritional potential of PNM and LSM by elevating the available nutrients in the intestine lumen for absorption, which enhances the expression of nutrients transportation- and growth-related genes leading to improved growth and health status of treated birds.

5. Conclusion

Feeding broilers with diets incorporated with 100 kg/ton PNM, LSM, or combination in equal amounts with or without enzyme mixture as substitutes for corn and soybean meal did not affect their growth performance and health status parameters. Hypocholesterolemic impacts of these supplements were noticed. Dietary incorporation of PNM or LSM did not affect the nutrient digestibility and amino acids profile of superficial pectoral muscle. Nutrient transportation-related genes were upregulated in the duodenum of treated birds. The finding of this study suggests the use of PNM and LSM in broiler diets, particularly their combination (50 kg/ton each) with enzyme mixture, as an efficient tool to use of PNM and LSM in broiler diets, particularly their combination (50 kg/ton each) with enzyme mixture, as an efficient tool to increase the protein quality of broiler chickens.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability statement

All data sets collected and analyzed during the current study are available from the corresponding author on reasonable request.

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