



Poultry By-Product Meal as a Potential Protein Source in Broiler Diets with Exogenous Protease Supplementation

Heshani Amalka Vithana¹, Shemil Priyan Macelline², Shan Randima Nawarathne³, Dinesh Darshaka Jayasena⁴, Myunghwan Yu³, Eunsoo Seo³, Mangala Amarsinghe⁵, Maleeka Nadeem Nambapana^{6†}, Jin Ho Cho^{7†} and Jung Min Heo^{8†}

¹Researcher, Department of Animal Science, Uva Wellassa University, Badulla 90000, Sri Lanka

²Researcher, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camperdown 2006, Australia

³Researcher, Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Republic of Korea

⁴Professor, Department of Animal Science, Uva Wellassa University, Badulla 90000, Sri Lanka

⁵General Manager, Nelna Farm (Pvt) Ltd, Hathduwa Estate, Meethirigala 11742, Sri Lanka

⁶Senior Lecturer, Department of Animal Science, Uva Wellassa University, Badulla 90000, Sri Lanka

⁷Professor, Department of Animal Science, Chungbuk National University, Cheongju 28644, Republic of Korea

⁸Professor, Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Republic of Korea

ABSTRACT The objective of this study was to investigate the effect of exogenous protease supplementation in diets formulated with poultry by-product meal on growth performance, small intestine magnitudes, and meat physiochemical characters in broiler chickens from 21 to 35 days post-hatch. A total of 120, one-day-old "Arbor Acres Plus" broiler chickens (male: female - 50:50) were allocated one of two dietary treatments to give six replicates and ten birds per cage. Two dietary treatments included a control diet (CON) and a diet supplemented with protease (CON+Pro). At day 35, body weight and feed intakes were measured to calculate the feed conversion ratio for the entire experiment period. Two birds from each pen were euthanized to measure the relative lengths and relative weights of three small intestine sections and meat samples were collected for physiochemical characteristic analyses at 35 days post-hatch. Exogenous protease supplementation did not influence ($P>0.05$) growth performance but showed a tendency to improve FCR ($P=0.082$). Protease supplementation showed a tendency to reduce proximal small intestine length ($P=0.091$). Broilers offered dietary treatments influenced minced meat color where protease supplementation resulted in lower CIE b^* ($P<0.001$) colorimetric value for yellowness and showed a significant trend ($P=0.059$) on reducing meat redness CIE a^* . In conclusion, the addition of exogenous protease to a broiler diet formulated with poultry by-product meal did not affect the growth performance, small intestine magnitudes, and meat physiochemical parameters (except CIE b^*) in broiler chickens.

(Key words: broiler chickens, growth performance, poultry by-product meal, protease, meat color)

INTRODUCTION

Soybean meal retail price in the global market has increased by 12.0% from 2021-July to 2022-Jan (526 versus 470 USD/MT) (www.indexmundi.com). Therefore, the interest in finding efficient cheap protein sources for broiler diet formulations has increased, especially in countries reliance on imported soybean meal as their main protein source in broiler diets. Moreover, land clearance caused by soybean cultivation negatively impacts global biodiversity and does not help sustainable chicken meat production (Greenhalgh et al., 2020). However, to date, soybean meal remains the dominant protein source for broiler chickens over

other protein sources due to its favorable protein content and amino acid profile (Beski et al., 2015). However, a gradual diminution of soybean meal inclusion in broiler diets is warranted and this can be achieved by introducing alternative protein sources for broiler diet formulation.

Poultry by-product meal (PBM) is tested as an alternative protein source in previous studies for broiler chickens (Aimiwu and Lilburn, 2006; Mahmood et al., 2016). PBM is produced by rendering the inedible waste products in poultry carcasses at poultry processing units (Senkoylu et al., 2005; Mahmood et al., 2016) and also supports efficient removal of poultry waste from processing plants and provides additional revenue. The nutrient composition of PBM can be

[†] To whom correspondence should be addressed : maleekanam@uwu.ac.lk, jincho@chungbuk.ac.kr, jmheo@cnu.ac.kr

inconsistent due to the diversity in poultry carcass components used to produce PBM; for instance, Ravindran and Blair (1993) reported that PBM has 600 g/kg of crude protein and 170 g/kg of crude fat concentrations whereas Mahmood et al. (2016) reported that 560 g/kg of crude protein and 340 g/kg of crude fat concentrations. Moreover, Li and Wu (2020) reported that broiler by-product meal has 694 g/kg of crude protein and 136 g/kg of crude fat concentrations. Even though PBM has a relatively higher crude protein content, lysine and methionine concentrations are limited for broiler chickens (Jackson et al., 1971; Bhargava et al., 1975). Glycine, arginine, and leucine are the prominent amino acids in PBM as reported by Ravindran and Blair (1993) and Mahmood et al. (2016).

The recommended inclusion level of PBM to broiler diets varies from 50 to 100 g/kg (Mahmood et al., 2016) due to its inconsistent protein quality and lower amino acid digestibility which could be triggered by higher rendering temperature (Mahmood et al., 2017). Moreover, it has been reported that higher ash concentration in meat and bone meal depress amino acid digestibility coefficients in broiler chickens (Macelline et al., 2020) where PBM may have higher ash content when subjected to the inclusion of tibia and femur bones to the rendering process.

Exogenous protease has proven its ability to improve amino acid utilization and growth performance in broiler chickens as reported by Ghazi et al. (2003) and Freitas et al. (2011), respectively. Therefore, exogenous protease would be beneficial in diets containing poorly digestible raw materials.

The poultry population in Sri Lanka has increased from 6.3 million to 41.97 million during the last four decades (Livestock statistical bulletin, 2020) which means there is a high potential to produce PBM as a cheap protein source in Sri Lanka. However, there is a lack of studies performed to identify the efficient usage of PBM in broiler diets in the Sri Lankan context. Therefore, the present study is designed to determine the impact of protease enzyme supplementation in a diet formulated with PBM on growth performance, carcass parameters, and meat quality in broiler chickens from 21 to 35 days post-hatch.

MATERIALS AND METHODS

The Research Ethics Committee of the Uva Wellasa University of Sri Lanka has reviewed and approved (UWU/REC/2023/007) the complete experimental procedure of the current study. All bird rearing, caring, handling, and sampling were conducted following the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 4th edition (2020).

1. Bird Management and Experimental Design

A total of 120 Arbor Acres Plus broiler chickens (as hatched) were randomly allocated to two dietary treatments and each treatment contained six replicate cages (10 birds/pen). The average body weight of day-old chicks was 42 g and the cumulative variance of weight distribution between replicate pens was 2.84%. Broilers were raised in floor pens (30 feet × 5 feet × 5 feet) consistent with the Arbor Acres Plus Broiler Management Guide (2018). Birds were offered a common starter diet from 1 to 20 days post-hatch. Birds were offered experimental diets from 21 to 35 days post-hatch on an *ad-libitum* basis and had free access to fresh drinking water.

2. Diet Preparation

Experimental diets were formulated to meet or exceed the breeder nutrition requirement guidelines and diets were based on rice, wheat, and soybean meal (Table 1). Both experimental diets included poultry by-product meals as an alternative protein source. Both control (CON) and protease (Proactive) supplemented diets (CON+protease) included phytase and xylanase. Diets were steam pelleted at a temperature of 80°C and used in the experiment for two weeks. The diets were stored at a properly maintained facility upon their usage.

3. Data and Sample Collection

On days 21 and 35, individual body weights were recorded whilst pen basis feed intake was measured as the feed disappearance of the feeders in each pen. The weight difference between day 21 and day 35 was calculated as

Table 1. Diet composition and analyzed nutrient concentrations in experimental diets (as-fed basis, g/kg)

Item	Treatment	
	Control	Control+protease
Rice (80 g/kg of CP)	320.00	320.00
Wheat (110 g/kg of CP)	300.00	300.00
Soybean meal (480 g/kg of CP)	210.00	210.00
DDGS ¹ (260 g/kg of CP)	60.00	60.00
Poultry by-product meal	30.00	30.00
Vegetable oil	50.00	50.00
Di-calcium phosphate	5.00	5.00
Limestone powder	10.00	10.00
Salt	3.50	3.50
DL-Methionine	2.50	2.50
L-Lysine	3.00	3.00
L-Threonine	1.00	1.00
L-Glutamic acid	0.10	0.10
Vitamin premix	1.00	1.00
Mineral premix	1.00	1.00
Xylanase	0.10	0.10
Phytase	0.10	0.20
Coccidiostat (Diclazuril 0.5%)	0.20	0.10
Butyric acid (30%)	0.50	0.50
Choline chloride	1.00	1.00
Emulsifier	1.00	0.95
Protease	-	0.05

¹ Distiller's dried grains with soluble.

Analyzed nutrient composition in basal diet: crude protein=207 g/kg; crude ash=56.8 g/kg; crude fat=76.0 g/kg; crude fiber=30.2 g/kg; calcium=12.4; total P=6.30 g/kg; salt=3.70 g/kg.

weight gain whereas the feed conversion ratio was calculated as the feed intake requirement to increase 1 g of body weight.

Two birds per replicate (12 birds per treatment) that were closer to the mean BW were selected, fasted, and euthanized by cervical dislocation for sample collection on day 35. Abdominal incisions were made on each sacrificed bird and the duodenum, jejunum, and ileum were separated from the gastrointestinal tract. Then the relative length (relative to the

body weight of the bird) and the relative weight (relative to the body weight of the bird) of each intestinal part were calculated. The right portion of breast meat was collected from each sacrificed bird to determine the physicochemical parameters of meat (i.e., pH, cooking loss, water holding capacity, meat surface color, and minced meat color).

4. Determination of Physicochemical Parameters of Meat

The pH values were determined according to the

methodology reported by Jung et al. (2011) using a calibrated pH meter (pH 700, Eutech Instrument, Ayer Rajah Crescent, Singapore) at room temperature. The mean value of three repeated measurements from each sample was used. For the determination of cooking loss, vacuum-packed meat samples (25 g) were cooked in a water bath (LWB-IIID, Daihan Labtech) at 80°C for 20 min and allowed to cool down to room temperature. The cooking loss was calculated as the weight loss of the sample during cooking as a percentage of the initial weight (Onenc et al., 2004; Bai et al., 2017). The WHC was determined by following the method stated by Hamm (1961) and Wilhelm et al. (2010) with slight modification. Initially, the meat samples were made into 2.0±0.10 g cubes and placed between two pieces of filter paper (No. 4; Whatman International). Then, a standard weight of 10 kg was placed on top of the filter paper for 5 min separately for each sample and the final weights were recorded. Finally, WHC was calculated using the following equation, where W_i and W_f are the initial and final weights of the sample, respectively.

$$\text{Water holding capacity} = 100 - [(W_i - W_f) \times 100 / W_i]$$

The meat surface color and color values of minced meat samples were determined by a calibrated colorimeter (CR-410, Konica Minolta, Osaka, Japan). The values of lightness (CIE L^*), redness (CIE a^*), and yellowness (CIE b^*) were obtained using the average value of three repeated measurements taken from different locations on the meat surface and on minced meat samples.

5. Statistical Analysis

The complete experiment was conducted according to a completely randomized design and experimental data were analyzed using the one-way ANOVA technique, General

Linear Model (GLM) in the SPSS software package (Version 26; IBM SPSS 2019). Significant differences between mean values were determined by using Tukey's multiple range test at a significance level of $P < 0.05$. A probability value between 0.05 and 0.1 was considered tendentious.

RESULTS

All birds showed adequate growth performances and remained healthy during the experimental period. Dietary treatments did not affect the mortality of birds and the rate was below 2% during the entire period.

The influence of protease supplementation on growth performance in broiler chickens from 21 to 35 days post-hatch is shown in Table 2. Protease supplementation did not significantly influence growth performance ($P > 0.05$). However, there was a treatment trend ($P = 0.082$) on FCR where protease supplemented group had improved FCR by 12.0% (1.489 versus 1.693) compared to the control group.

Table 3 showed the impact of dietary treatments on the relative weights and lengths of three small intestinal sections in broiler chickens at 35 days post-hatch. There was no significant treatment effect found on relative weights and lengths in the duodenum, jejunum, and ileum. Protease supplementation numerically reduced ($P > 0.05$) duodenum length by 14.8% (6.15 versus 7.22 cm/kg bird) and jejunum length by 5.71% (10.41 versus 11.04 cm/kg) compared to the control group. On the other hand, protease supplementation numerically increased ($P > 0.05$) ileum length by 9.41% (54.3 versus 49.6 cm/kg bird) than broilers offered a control diet.

The effect of protease supplementation on breast meat physiochemical parameters in broiler chickens at 35 days post-hatch is tabulated in Table 4. Neither meat pH, water

Table 2. Influence of protease supplementation on growth performance in broiler chickens from 21 to 35 days post-hatch

Treatment	Weight gain (g/bird)	Feed intake (g/bird)	Feed conversion ratio (g/g)
Control	976.69	1,648.43	1.69
Control+protease	1,053.93	1,559.23	1.49
SEM ¹	47.611	79.660	0.073
<i>P</i> -value	0.284	0.451	0.082

¹ Standard error of the mean.

Table 3. Influence of protease supplementation on relative weights and lengths of three different small intestine sections in broiler chickens at 35 days post-hatch

Treatment	Duodenum		Jejunum		Ileum	
	Length (cm/kg bird)	Weight (g/kg bird)	Length (cm/kg bird)	Weight (g/kg bird)	Length (cm/kg bird)	Weight (g/kg bird)
Control	7.22	2.48	11.04	2.48	49.62	16.36
Control+protease	6.15	2.48	10.41	2.99	54.29	17.41
SEM ¹	0.520	0.075	0.642	0.372	5.356	2.168
<i>P</i> -value	0.184	0.967	0.511	0.370	0.734	0.744

¹ Standard error of the mean.

Table 4. Influence of protease supplementation on physiochemical parameters in breast meat in broiler chicken at 35 days post-hatch

Treatment	pH	WHC (%)	Cooking loss (%)	Surface meat color			Minced meat color		
				CIE L*	CIE a*	CIE b*	CIE L*	CIE a*	CIE b*
Control	7.62	98.44	9.65	62.12	11.96	10.21	57.05	12.89	11.81
Control+protease	7.71	98.50	9.51	60.99	12.24	9.46	55.07	9.80	9.99
SEM ¹	0.070	0.061	0.592	0.660	0.502	0.551	1.040	1.084	0.292
<i>P</i> -value	0.378	0.453	0.869	0.239	0.698	0.344	0.264	0.059	<0.001

¹ Standard error of the mean.

holding capacity, cooking loss, or surface color were influenced by dietary treatments ($P>0.05$). Protease supplementation reduced ($P<0.001$) yellowness (CIE b*) in minced meat by 15.4% (9.99 versus 11.81) compared to meat obtained from the control group. Moreover, protease supplementation showed a tendency ($P=0.059$) to reduce meat redness (CIE a*) in minced meat by 24.0% (9.80 versus 12.89) compared to the control group.

DISCUSSION

Overall weight gain and feed intake in the present study were inferior to the Arbor Acres Plus Broiler Performance Objectives (2019) for 21 to 35 days post-hatch. Nevertheless, overall FCR was improved in the present study by 6.52% (1.591 versus 1.702) than performance objectives where birds offered protease-supplemented diets had 12.5% better FCR than breeder performance objectives (1.489 versus 1.702), and the control group showed comparable FCR with

performance objectives. However, it was noted that there is a marked feed intake reduction in the present study than the targeted feed intake in breeder objectives.

There was no significant treatment effect found on growth performance in the present study, but overall FCR was better than the performance objectives. Therefore, it indicates that 30 g/kg inclusion of PBM would be acceptable in broiler diets regardless of protease supplementation. These outcomes are consistent with previously published work as diets formulated with 30 to 60 g/kg of PBM showed similar growth performance in broiler chickens in comparison to a corn-soybean meal-based conventional broiler diet (Mahmood et al., 2017, 2018). Therefore, the present study confirmed that the low inclusion level of PBM, or approximately 16% of protein from PBM out of total dietary protein is acceptable for broiler diets. On the other hand, the influence of exogenous protease maybe not be prominent in older birds where it is reported that positive effects of exogenous protease on growth performance are more pronounced in

young broiler chickens (Erdaw et al., 2017). This may be due to the low secretion of endogenous protease in young broiler chickens and starter diets have higher crude protein contents than grower or finisher diets where protease has more substrates to react on.

The intestinal organ dimensions may be indicative physiochemical functionality of the gastrointestinal tract (GIT) in broiler chickens and could be influenced by the diet composition (Mahardhika et al., 2021). Moreover, relatively larger intestinal organs negatively impact final dressing weights in chicken carcasses (Yuan et al., 2008). Particularly, relative dimensions of intestinal sections may represent their physiochemical functionality. The impacts of dietary protease supplementation on weights and lengths of different GIT sections in broiler chickens were inconsistent throughout the previous studies, such as Kalmendal and Tauson (2010) reported that protease supplementation did not alter relative weights and lengths of the duodenum, jejunum, and ileum; whereas Mahardhika et al. (2021) stated that protease supplementation significantly reduced ileum weight and jejunum lengths whilst other small intestine sections retained non-effected. Moreover, protease supplementation into diets formulated with 150 g/kg of DDGS resulted in 14.5% lower duodenum weight compared to the control diet whereas exogenous protease did not influence duodenum weights at 0 and 300 g/kg of DDGS inclusions (Barekatin et al., 2013). Moreover, it has been reported that 0.3 g/kg of protease inclusion significantly reduced small intestine weight in broiler chickens compared to 0.1 g/kg protease inclusion by 8.14% (Erdaw et al., 2017). Overall, it is indicated that the exogenous protease has a tendency to reduce small intestine weights and lengths which is lined with the outcomes of the present study for duodenum and jejunum lengths. Moreover, it has been reported that any changes in pancreases physiology and functionality would affect duodenum size (Erdaw et al., 2017) and the inclusion of exogenous protease could compromise endogenous protease production in pancreases, which can be resulted in smaller pancreases and duodenum by reducing its workload for enzyme production (Mahardhika et al., 2021). Moreover, it was described that reducing small intestine dimensions resulted in improved

body weight gain in broiler chickens which might be indicating that the exogenous protease has the potential to enhance physiological functions in the small intestine (Erdaw et al., 2017). Similarly, protease supplementation numerically reduced relative lengths of duodenum length (14.8%) and jejunum (5.71%) compared to the control group in the present study where their reduction percentages are presented in parentheses. Moreover, there was a treatment trend on duodenum+jejunum length (data not shown) where protease supplementation reduced the proximal small intestine length by 9.31% (16.56 versus 18.26 cm/kg; $P=0.091$). A different rationale was described by Cowieson et al. (2003) about the impact of exogenous protease on reduced GIT tract dimensions where exogenous protease reduces substrates availability to protein putrefactive microorganisms in GIT and compromised their capability on volatile fatty acid production. It was noted that some volatile fatty acids can stimulate intestinal cell proliferation and enlarge the small intestine.

The significant effect of dietary protease supplementation on meat color is noted in the present study where protease reduced redness and yellowness in minced breast meat at 35 days post-hatch. However, dietary protease supplementation has not influenced chicken meat color in previous studies (Xu et al., 2017; Lu et al., 2020; Sumanasekara et al., 2020; Wang et al., 2020). Higher meat pH value seems to be influential in generating darker or reddish color in meat (Fletcher, 1999; Wattanachant et al., 2004); however, it is not the case in the present study. Meat's red color is caused by the presence of higher myoglobin and haem pigments concentrations whilst yellow color results due to a higher accumulation of carotenoids and xanthophyll pigments in poultry meat, which would be influenced by the feed grain used in the diet (Smith et al., 2002) and supplementation of synthetic vitamin E (Nam et al., 2013). The dietary xanthophyll absorption in GIT in broiler chickens was investigated by Littlefield et al. (1972) who found that most of the xanthophylls are absorbed in the proximal small intestine. In the present study, protease supplementation reduced proximal small intestine length which may also reduce the surface area for dietary color pigment absorption. Interestingly, it is

deduced from the data from the present study that the duodenum+jejunum length has significant positive linear correction with meat redness ($r=0.482$, $P=0.032$) and showed a positive trend with meat yellowness. On the other hand, it has been reported that fat digestibility has a positive impact on xanthophyll and carotenoid absorption in humans (Pérez-Gálvez et al., 2005). Therefore, a significant reduction of duodenum length in response to protease supplementation may negatively be impacted fat digestion which might result in inferior color pigments and vitamin A absorptions in the present study.

SUMMARY

Broilers offered a diet formulated with PBM with or without exogenous protease did not influence growth performance from 21 to 35 days post-hatch. Exogenous protease showed a trend of improving FCR. Exogenous protease showed a tendency to reduce relative duodenum+jejunum length which may be indicating the impact of exogenous protease on duodenum and pancreases functionality. Broilers offered a diet supplemented with exogenous protease generated lower colorimetric values for redness and yellowness in minced meat at 35 days post-hatch.

CONFLICT OF INTEREST

The author Mangala Amarasinghe is an employee of Nelna Farm (Pvt) Ltd. and declares no conflicts of interest.

ACKNOWLEDGMENTS

The authors wish to acknowledge Nelna Farm (Pvt) Ltd, for the funding and material support provided for the feeding study. Further, this research was supported by the “Regional Innovation Strategy (RIS)” through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (MOE) (2021 RIS-001, 1345356234).

ORCID

Heshani Amalka Vithana	https://orcid.org/0000-0002-8537-9878
Shemil Priyan Macellin	https://orcid.org/0000-0001-6771-3804
Shan Randima Nawarathne	https://orcid.org/0000-0001-9055-9155
Dinesh Darshaka Jayasena	https://orcid.org/0000-0002-2251-4200
Myunghwan Yu	https://orcid.org/0000-0003-4479-4677
Eunsoo Seo	https://orcid.org/0000-0002-0207-7381
Mangala Amarsinghe	https://orcid.org/0000-0003-4545-261X
Maleeka Nadeemmale Nambapana	https://orcid.org/0000-0001-6267-6821
Jin Ho Cho	https://orcid.org/0000-0001-7151-0778
Jung Min Heo	https://orcid.org/0000-0002-3693-1320

REFERENCES

- Arbor Acres Plus Broiler Performance Objectives. Aviagen. <https://eu.aviagen.com/brands/arbor-acres/products/arbor-acres-plus>. Access on November 14, 2021.
- Bai JJ, Lee JG, Lee SY, Kim S, Choi MJ, Cho Y 2017 Changes in quality characteristics of pork patties containing antioxidative fish skin peptide or fish skin peptide-loaded nanoliposomes during refrigerated storage. *Korean J Food Sci Anim Resour* 37(5):752-763.
- Beski SS, Swick RA, Iji PA 2015 Specialized protein products in broiler chicken nutrition: a review. *Anim Nutr* 1(2):47-53.
- Cowieson AJ, Acamovic T, Bedford MR 2003 Supplementation of diets containing pea meal with exogenous enzymes: effects on weight gain, feed conversion, nutrient digestibility and gross morphology of the gastrointestinal tract of growing broiler chicks. *Br Poult Sci* 44(3):427-437.
- Erdaw MM, Perez-Maldonado RA, Bhuiyan M, Iji PA 2017 Partial replacement of commercial soybean meal with raw, full-fat soybean meal supplemented with varying levels of protease in diets of broiler chickens. *S Afr J Anim Sci* 47(1):61-71.

- Fletcher DL, Qiao M, Smith DP 2000 The relationship of raw broiler breast meat color and pH to cooked meat color and pH. *Poult Sci* 79(5):784-788.
- Greenhalgh S, Chrystal PV, Selle PH, Liu SY 2020 Reduced-crude protein diets in chicken-meat production: justification for an imperative. *Worlds Poult Sci J* 76(3):537-548.
- Hamm R 1961 *Biochemistry of Meat Hydration*. Pages 355-463 In: *Advances in Food Research*. Academic Press. Indexamundi. Soybean meal futures end of day settlement price. <http://www.indexamundi.com/commodities/?commodity=soybean-meal>. Access on February 24, 2022.
- Jung YK, Jeon HJ, Jung S, Choe JH, Lee JH, Heo KN, Kang BS, Jo CR 2011 Comparison of quality traits of thigh meat from Korean native chickens and broilers. *Food Sci Anim Resour* 31(5):684-692.
- Kalmendal R, Tauson R 2012 Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet. *Poult Sci* 91(6):1387-1393.
- Li P, Wu G 2020 Composition of amino acids and related nitrogenous nutrients in feedstuffs for animal diets. *Amino Acids* 52(4):523-542.
- Littlefield LH, Bletner JK, Shirley HV, Goff OE 1972 Locating the site of absorption of xanthophylls in the chicken by a surgical technique. *Poult Sci* 51(5):1721-1725.
- Livestock Statistical Bulletin-2020. http://www.daph.gov.lk/web/images/content_image/news_bulletins/livestock_statistical/2020/StatBulletin2020Print.pdf. Accessed on February 21, 2022.
- Lu P, Choi J, Yang C, Mogire M, Liu S, Lahaye L, Adewole D, Rodas-Gonzalez A, Yang C 2020 Effects of antibiotic growth promoter and dietary protease on growth performance, apparent ileal digestibility, intestinal morphology, meat quality, and intestinal gene expression in broiler chickens: a comparison. *J Anim Sci* 98(9):skaa254.
- Macelline SP, McQuade LR, McInerney BV, Moss AF, Selle PH, Liu SY 2020 Protein digestive dynamics of meat and bone meals in broiler chickens. *Anim Nutr* 6(4):521-528.
- Mahardhika BP, Ridla M, Mutia R, Naja M 2021 Response size of digestive organs of broiler that are fed containing Jack bean (*Canavalia ensiformis*) different levels of protein with protease enzyme supplementation. *Indones J Anim Sci* 31(2):133-139.
- Mahmood T, Mirza MA, Nawaz H, Shahid M 2018 Exogenous protease supplementation of poultry by product meal based diets for broilers: effects on growth, carcass characteristics and nutrient digestibility. *J Anim Physiol Anim Nutr* 102(1):e233-241.
- Mahmood T, Mirza MA, Nawaz H, Shahid M, Athar M, Hussain M 2017 Effect of supplementing exogenous protease in low protein poultry by-product meal based diets on growth performance and nutrient digestibility in broilers. *Anim Feed Sci Technol* 228:23-31.
- Önenç A, Serdaroglu M, Abdraimov K 2004 Effect of various additives to marinating baths on some properties of cattle meat. *Eur Food Res Technol* 218:114-117.
- Pérez-Gálvez A, Mínguez-Mosquera MI 2005 Esterification of xanthophylls and its effect on chemical behavior and bioavailability of carotenoids in the human. *Nutr Res* 25(7):631-640.
- Ravindran V, Blair R 1993 Feed resources for poultry production in Asia and the Pacific. III. Animal protein sources. *World Poult Sci J* 49(3):219-235.
- Senkoylu N, Samli HE, Akyurek H, Agma A, Yasar S 2005 Performance and egg characteristics of laying hens fed diets incorporated with poultry by-product and feather meals. *J Appl Poult Res* 14(3):542-547.
- Sumanasekara TD, Nayananjali WA, Ang L, Kumari MA 2020 Effect of protease supplementation on growth performances, carcass and meat quality characteristics of broiler chicken fed with low protein diets. *Sri Lankan J Agric Ecos* 2(2):122.
- Wang QD, Zhang KY, Zhang Y, Bai SP, Ding XM, Wang JP, Peng HW, Tian G, Xuan Y, Su ZW, Zeng QF 2020 Effects of dietary protein levels and protease supplementation on growth performance, carcass traits, meat quality, and standardized ileal digestibility of amino acid in Pekin ducks fed a complex diet. *Poult Sci* 99(7):3557-3566.
- Wattanachant S, Benjakul S, Ledward DA 2004 Composition, color, and texture of Thai indigenous and broiler chicken muscles. *Poult Sci* 83(1):123-128.

- Wilhelm AE, Maganhini MB, Hernández-Blazquez FJ, Ida EI, Shimokomaki M 2010 Protease activity and the ultrastructure of broiler chicken PSE (pale, soft, exudative) meat. *Food Chem* 119(3):1201-1204.
- Xu X, Wang HL, Pan L, Ma XK, Tian QY, Xu YT, Long SF, Zhang ZH, Piao XS 2017 Effects of coated proteases on the performance, nutrient retention, gut morphology and carcass traits of broilers fed corn or sorghum based diets supplemented with soybean meal. *Anim Feed Sci Technol* 223:119-127.
- Yuan J, Yao J, Yang F, Yang X, Wan X, Han J, Wang Y, Chen X, Liu Y, Zhou Z, Zhou N 2008 Effects of supplementing different levels of a commercial enzyme complex on performance, nutrient availability, enzyme activity and gut morphology of broilers. *Asian-Australas J Anim Sci* 21(5):692-700.

Received Jan. 23, 2023, Revised Feb. 27, 2023, Accepted Feb. 28, 2023