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Dietary Protein Levels in the Small Intestine and Carcass Traits of Cross-Breed Chickens

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ABSTRACT

Protein is a source of nutrients that plays a significant role in biological processes. The current study aimed to evaluate the effects of feed with different protein levels on the pH and viscosity of the small intestine, ileum characteristics, and carcass traits of cross-breed chickens. A total of 160 cross-breed unsexed chicks aged 2 days were divided into three treatments (T1-T3), each consisting of 6 replications with 9 chicks per replication, consisting of a diet with protein level (T1; starter 18% and finisher 16%;), a diet with protein level (T2; starter 20% and finisher 18%), and a diet with protein level (T3; starter 22% and finisher 20%). Cross-breed chickens were crossed between Bangkok males and Lohmann laying hens. The variables were analyzed, including pH and viscosity of digesta, ileum characteristics consisting of total villous, height of villous, and depth of crypt. The carcass percentages consisting of the carcass, breast, thicks, wings, and back were measured. The research was analyzed using a completely randomized design. The results indicated that different protein levels in treatments were significantly different in total villous, height of villous, and depth of crypt but did not indicate a significant difference in pH and viscosity. Furthermore, the treatments have a significant difference in the carcass percentage and thick percentage but did not significantly affect the breast, wings, and back. It can be concluded that 22% crude protein in the starter and 20% crude protein in the finisher produced the greatest results in the intestinal characteristics and also in the carcass percentage of cross-breed chickens.

Keywords: Carcass, Cross-breed chicken, Feed, Intestinal characteristic, Protein

INTRODUCTION

Indonesian cross-breed local chickens have emerged, including Kampung Unggul Balitbangtan chicken (KUB) and Jowo Super chickens (joper). However, the cultivation of cross-breed chickens needs to be supported by appropriate feed. According to Sarjana et al. (2010), the nutrients that must be available in feed are protein, energy (carbohydrates and fat), minerals (calcium and phosphorus), vitamins, and water. Proteins and amino acids have a biological role in tissue biosynthesis in the body Alagawany et al. (2021). Protein is the most expensive nutrient in chicken feed, necessary for growth acceleration. Excessive protein in the feed reduces body fat accumulation, increases blood uric acid levels, and is excreted as nitrogen. Conversely, a lack of protein will increase fat accumulation in the body. This is because chickens convert excess energy into fat, whereas a lack of protein can reduce 6-7% of body weight per day. Singarimbun et al. (2013). Amino acids play a role in the metabolic processes of cells in the body Devignes et al. (2022). Essential amino acids such as lysine, methionine, threonine, and tryptophan are critical functions in the metabolic processes. Lysine has functions in muscle development, especially in chicken breast muscles.

Methionine in broiler chickens acts as a cysteine precursor, a source of sulfur, and an integral part of body protein (Kalbande et al., 2009; Ramadan et al., 2021). The availability of amino acids is more efficient in supplementing the lack of crude protein.

The healthy intestinal characteristics of cross-breed chickens can increase the maximum nutrient absorption process through improved villi height, number of villi, and crypt depth. According to Günther et al. (2012), proteins have an important role in cell formation, replacing dead cells and forming body tissue. Small intestinal epithelial cells are some of the cells and tissues formed by proteins. The wider surface area of villi maximizes nutrient absorption, leading to improved chicken performance and carcass quality. Protein intake in feed can be utilized in the process of forming muscle in chickens. As a result, the present study was initiated to evaluate the potential effects of different protein levels in intestinal characteristics and carcass traits of cross-breed chickens.

MATERIALS AND METHODS

Ethical approval

Ethical approval for the present experiment was given by the Ethic Clearance Commission under Universitas Brawijaya No. 118-KEP-UB-2022.

Experimental design

The present study was conducted between Juli to December 2021. This experiment used 162 Day Old Chicks (DOCs) unsexed cross-breed chickens using open-house colony systems. The cross-breed chicken was a cross between Bangkok Male and Lohmann laying hens produced by Berline Farm, Malang, East Java, Indonesia. The chicken was a new strain we called cross-breed chickens. The DOCs were vaccinated with ND-AI, Gentamicin, and ND-IB, and then in the first and second weeks, were vaccinated with the gumboro vaccine. The study was conducted for 60 days, divided into starter (0–30 days) and finisher (31–60 days) phases. The cages used were 18 open-house litter cages with length, width, and height dimensions, namely (1 m x 1 m x 2 m) every cage has nine cross-breed chickens. The composition of the basal diet was bran separator, soybean meal, yellow corn, Dried Distillers grains (DDGS), fish meal, copra meal, concentrate, coconut oil, salt, and methionine. The composition was formulated according to the poultry-balanced diet from the nutrient requirement of poultry NRC (1994). The composition of feed formulation and nutrient contents can be indicated in Table 1. During the experiment, chickens were given feed and drink *ad libitum*. The method was *in vivo*, using a completely randomized design with three treatments (T1-3) and six replications, where every replication consisted of nine chicks. The treatment provided includes feed with protein level (T1; starter 18% and finisher 16%), feed with protein level (T2; starter 20% and finisher 18%), and feed with protein level (T3; starter 22% and finisher 20%).

The variables measured in this study included pH, viscosity, intestinal characteristics, and carcass percentage.

Table 1. The feed composition and nutrient content of the diet in cross-breed chicken per treatment

Treatment* / Feed	T1		T2		T3	
	Starter (%)	Finisher (%)	Starter (%)	Finisher (%)	Starter (%)	Finisher (%)
Bran separator	20.57	18.33	14.69	12.33	9.91	6.45
Soybean meal	4.43	0.57	10.31	4.57	16.19	10.45
Yellow corn	38	45	38	45	37	45
Dried Distillers grains	10	10	10	10	10	10
Fish meal	10	8	10	10	10	10
Copra meal	5	5	5	5	5	5
Concentrate**	10	10	10	10	10	10
Coconut oil	0.43	1.64	0.44	1.55	0.55	1.56
Salt	0.19	0.12	0.1	0.1	0.21	0.1
Premix	1.3	1.34	1.46	1.45	0.98	1.44
Methionine	0.08	-	-	-	0.16	-
Nutrient Content***						
Dry Matter	87.64	90.88	87.97	90.33	86.80	91.21
Crude Protein	18.80	15.90	20.06	18.80	22.38	20.41
Extract Eter	6.07	6.85	5.48	6.61	5.01	6.43
Crude Fiber	6.18	5.42	5.04	5.55	5.82	5.11
EM (Kkal/g)	2814	2944	2834	2914	2822	2905
Lysin	1.08	1.10	1.18	0.92	1.00	1.06
Methionine	0.46	0.35	0.35	0.35	0.46	0.35

The composition was formulated according to the poultry-balanced diet NRC (1994). * T1: feed with protein level (starter 18% and finisher 16%), T2: feed with protein level (starter 20% and finisher 18%), T3: feed with protein level (starter 22% and finisher 20%). ** Nutrient composition of concentrate Crude Protein (min 20 %), extract eter (min 5 %), crude fiber (min 5 %), ash (max 8 %), Ca (0.8-1.1 %), P (min 0.5 %), enzyme phytase and aflatoxin max 50 µg/kg. ** Nutrient content was analyzed using proximate analysis

pH and viscosity

After 60 days of experiments, the cross-breed chickens were slaughtered by cutting using a knife in the jugular vein and carotid artery. Internal organs were removed from the body, especially the small intestine. Digesta obtained from the duodenum, jejenum, and ileum was taken and put into pot film. The digesta was used as a sample for the analysis of pH and viscosity. One gram of digesta was diluted with 10 mL of distilled water then immediately measured pH using a digital pH meter. Therefore, the mixture of 1 gram digesta and 10 mL distilled water was then centrifuged at 3000 rpm for five minutes. After that, the supernatant was taken and the viscosity was measured using a Brookfield viscometer. The measurement procedure for pH and viscosity was conducted following the method of Zhang et al. (2022).

Intestinal characteristics

Samples for intestinal characteristics analysis using an ileum. A 4-centimeter sample of the ileum was taken and then cleaned using distilled water. Ileum samples were placed in a pot film containing a 10% Neutral Buffered Formalin (NBF) solution to be made into preparations using a hematoxylin-eosin (HE) procedure Jamilah (2014). Measurement of the total number of villi, villi length, and crypt depth was measured using a light microscope (DIC Olympus BX51TF, Japan), 4x magnification connected to the Optilab application. Measurements of villi length and crypt depth were carried out using the Image Raster application.

Number of villi

The number of villi was counted by all the villi in each field of view with a four-time microscope magnification Emma et al. (2013).

Villi height

The height of the villi was carried out by initial measurement from the apical villi to the crypts of Lieberkuhn. Villous height was measured in 4 fields of view using a microscope magnification of 4 times Amri et al. (2022).

Depth of crypt

Crypt depth was seen in 4 different fields of view according to 12, 3, 6, and 9 clockwise directions. Crypt depth was calculated from the base to the base of the villi using 4x magnification Setiawan et al. (2018).

Carcass percentage

Variable measurements were carried out by taking samples from four chickens from each replication. The life bird chicken was weighed by the experimental chickens sampled in each experimental unit. Carcass weight was obtained by weighing the weight of the chicken after slaughter and subtracting the blood, feathers, head, feet, and internal organs except the lungs and spleen. The abbreviation of carcass percentage according to Formula 1 (Subekti et al., 2012).

(Formula 1)

$$\text{Carcass percentage} = \frac{\text{Carcass weight (g/bird)}}{\text{Live weight (g/bird)}} \times 100\%$$

Breast weight was obtained by weighing the chest area scapula to the sternum (g). The percentage of chest weight was calculated according to Formula 2 (Subekti et al., 2012).

(Formula 2)

$$\text{Breast percentage} = \frac{\text{Breast weight (g/bird)}}{\text{Carcass weight (g/bird)}} \times 100\%$$

The thigh weight was obtained by weighing the exact part in the area of the lower thigh joint to the knee (g). The percentage of thigh weight was calculated according to Formula 3 (Subekti et al., 2012).

(Formula 3)

$$\text{Thight percentage} = \frac{\text{Thick weight (g/bird)}}{\text{Carcass weight (g/bird)}} \times 100\%$$

The back weight was obtained by weighing the spine to the pelvic bone (g). Percentage back weight was calculated according to Formula 4 (Subekti et al., 2012).

(Formula 4)

$$\text{Back percentage} = \frac{\text{Back weight (g/bird)}}{\text{Carcass weight (g/bird)}} \times 100\%$$

The wing weight was obtained by weighing the joints between the upper arm and with scapula. Percentage wing weight was calculated according to Formula 5 (Subekti et al., 2012).

(Formula 5)

$$\text{Wings percentage} = \frac{\text{Wings weight (g/bird)}}{\text{Carcass weight (g/bird)}} \times 100\%$$

Statistical analysis

A statistical analysis was conducted using analysis of variance using SPSS version 27. An error was expressed as a standard deviation. In the end, probability values were subjected to the Duncan Multiple Range Test. The following formula was used.

(Formula 6)

$Y_{ij} = \mu + T_i + e_{ij}$

Where, Yij denotes the parameters observed, μ is the overall mean, Ti indicates the effect of different levels of protein, and eij signifies the amount of error number.

The treatment provided included a diet with a protein level (starter 18% and finisher 16%; T1), a diet with protein level (starter 20% and finisher 18%; T2), and a diet with protein level (starter 22% and finisher 20%; T3). One-way ANOVA was used to compare the means of pH, viscosity, total villous, height of villous, depth of crypt, carcass percentage, thicks percentage, wings percentage, and back percentage. The significance difference was established at ($p < 0.05$). All analysis was carried out in three replications and the significant difference was defined as the 5% level ($p < 0.05$). Finally, probability values were subjected to the Duncan Multiple Range Test (DMRT).

RESULTS

The results of the different levels of protein in the small intestine of cross-breed chickens including pH, viscosity, number of villi, length of villi, and depth of crypts were presented in Table 2.

pH and digesta viscosity

The results obtained that different levels of protein diet had no significant effect ($p > 0.05$) on the intestinal pH of cross-breed chicken. It can be seen that the pH value at T1 obtained a more acidic value than T2 and T3.

The statistical data presented in Table 2 indicated that the addition of protein at different levels to cross-breed chickens did not have a significant effect ($p > 0.05$) on the digesta viscosity. The results of this research indicated that there was a positive correlation between the level of protein in T3 and the resulting viscosity value.

Total, length and crypt depth villi in the small intestine

Table 2 indicated that different levels of protein had a significant effect on the number of villi cross-breed chickens in T1, compared to T2 and T3. ($p < 0.05$) The lowest number of villi was in treatment T1 (50.61 per transversal cut), while the most effective value was in treatment T3 (56.61 per transversal cut). An increase in the level of crude protein in the feed is followed by an increase in the number of villi. The results provided that the effect of different levels of protein on cross-breed chickens had a significant effect ($p < 0.05$) on the length of villi between T1 and T2 compared with T3. Where the lowest average value was T1 (467.86 μm) and the highest was T3 (506.89 μm).

The data revealed that the different levels of protein in the crypts of the intestines of cross-breed chickens had a significant difference ($p < 0.05$) between T1 and T3 (Figure 1; Table 2). The treatment with the lowest average value was T1 (124.27 μm), and the treatment with the highest average value was T3 (134.05 μm).

Carcass percentage

The results demonstrated that feeding cross-breed chickens with different protein levels had a significant effect on carcass weight ($p < 0.05$) in the T1, T2, and T3 (Table 3). The group with the highest treatment had the highest carcass weight, while the lowest weight was in the medium treatment group. The breast, wings, and back percentages of cross-breed chicken were not significantly different among the treatment groups ($p > 0.05$); however, the thick percentage significantly differed between T1 and T3 with the T2 ($p < 0.05$). In the thick percentage, medium crude protein (T2) decreases the thick percentage of cross-breed chicken. Although T3 had a higher percentage of carcass, breast, wings, thick, and back on cross-breed chicken, it yields different protein levels.

Table 2. Effect of different level protein diet on pH, viscosity, number of villi, length of villi, and depth of crypts in the finisher phase of cross-breed chickens

Variable / Treatment*	pH	Viscosity (cP)	number of villi (per transversal cut)	Length of villi (μm)	Crypts Depth (μm)
T1	6.22± 0.13	11.17 ± 1.17	50.61 ± 3.74^{a}	467.86 ± 29.26^{a}	124.27 ± 5.90^{a}
T2	6.39 ± 0.26	11.83 ± 3.19	55.50 ± 3.82^{b}	479.30 ± 12.22^{a}	129.19 ± 4.13^{ab}
T3	6.58 ± 0.69	12.50 ± 2.07	56.61 ± 2.76^{b}	506.89 ± 13.68^{b}	134.05 ± 7.54^{b}

Notes:* T1: feed with protein level (starter 18% and finisher 16%), T2: feed with protein level (starter 20% and finisher 18%), T3: feed with protein level (starter 22% and finisher 20%). Different superscript letters $^{(a,b)}$ in the column showed significant differences across the treatments ($p < 0.05$).

Table 3. Effect of different level protein diet on carcass percentage in the finisher phase of cross-breed chickens

Treatment*	Carcass (%)	Breast (%)	Thick (%)	Wings (%)	Back (%)
T1	56.344±0.82[b]	11.766±1.42	17.882±0.78[b]	8.976±0.37	14.606±1.47
T2	53.482±0.94[a]	11.846±1.08	15.956±0.81[a]	8.856±0.77	14.356±1.55
T3	58.872±2.73[c]	13.34±0.84	17.892±0.85[b]	9.522±0.57	16.182±1.06

Notes: *T1: feed with protein level (starter 18% and finisher 16%), T2: feed with protein level (starter 20% and finisher 18%), T3: feed with protein level (starter 22% and finisher 20%). Different superscript letters [(a,b)] in the column showed significant differences across the treatments ($p < 0.05$).

DISCUSSION

pH level in the small intestine was an effective way of determining the effectiveness of feed digestion. The findings indicated that the lowest or highest protein level in the feed did not affect the pH of the small intestine of cross-breed chickens. Increasing crude protein content was in line with increasing pH value in the small intestine, especially in the ileum. Based on the findings of Hafsah et al. (2021) and Zhang et al. (2022) the normal pH level of digestion in the small intestine of native chickens varies depending on the location. The pH level of the poultry in the duodenum was 5-6; in the jejunum was 6.5-7; and in the ileum was 7-7.5. The study indicated that the high levels of protein in T3, particularly 22% starter and 20% finisher, had an alkaline pH value. This finding did not follow the previous research. Simpson et al. (1976) stated that high protein levels increase H+ ions, leading to decreased pH levels in broiler chickens. According to Liu et al. (2010) an increase in H+ ions caused protein hydrolysis into amino acids. However, amino acids were mainly absorbed in the upper of the small intestine namely the duodenum and the jejunum in poultry as mentioned by Truong et al. (2017)Therefore, amino acids in the ileum were the same as those in the rest of the duodenum and jejunum, and protein hydrolysis into amino acids did not affect the pH level of the ileum.

The ability of crude protein level on feed can increase digesta viscosity of cross-breed chickens. This research indicated that 22 % crude protein in the starter phase and 20 % in the finisher phase improved the viscosity. According to previous research by Olfati et al. (2021), dissolved solid components, like amino acids and proteins, can increase viscosity in the small intestine of chickens. Dissolved proteins can contribute to an increase in viscosity (Damodaran, 2008; Saenphoom et al., 2013) indicating that when the digesta viscosity increases, there is also an increase in enzyme activity, amino acid content in the small intestine, protein digestibility, and metabolic energy in broiler chickens. The previous finding indicated that the viscosity of broiler chickens value ranged from 10.48% to 17.64% Amerah et al. (2008). The acquired viscosity value of cross-breed chickens in this research was lower when compared to the findings of earlier research. It has been observed that excessively high viscosity can reduce feed digestibility, while excessively low viscosity can accelerate digestion and limit nutrient absorption. According to a study by Khempaka et al. (2011) adding feed containing crude protein has been indicated to impact intestinal significantly in broiler chickens. The result of this study aligns with previous findings conducted by Khattab et al. (2021) who argue that increasing viscosity can slow down digestion, leading to a more efficient absorption process in ducks. However, previous research by Natsir (2016) stated that increased viscosity in the small intestine of broiler chickens can decrease digestive efficiency due to the slowing down of the diffusion rate of endogenous enzymes that react with nutrients.

Villus height and crypt depth were markers for gut health and can be used to assess intestinal characteristics. The higher protein level in cross-breed chicken indicated a higher villus height and crypt depth (Figure 1). Higher villi indicated an increase in the surface area available for nutrient absorption from the gut. An increase in height has been indicated to improve nutrient transport across the villus surface as reported by El Sabry and Yalcin (2023). Furthermore, intestine development can be monitored by measuring villus morphology and enzymatic activity, as well as determining the expression of genes involved in nutrient transport. A deeper crypt suggested a higher turnover of enterocytes, which in turn required more protein and energy. The crypt depth in the intestinal is related to the absorptive and secretory cells. The secretory cells are responsible for producing mucins, which are the primary component of the protective mucous layer in the intestines Nguyen et al. (2021). According to Devignes et al. (2022), protein plays a role in cell formation, forming

body tissue, and assisting in the growth of body organs, including small intestinal epithelial cells.

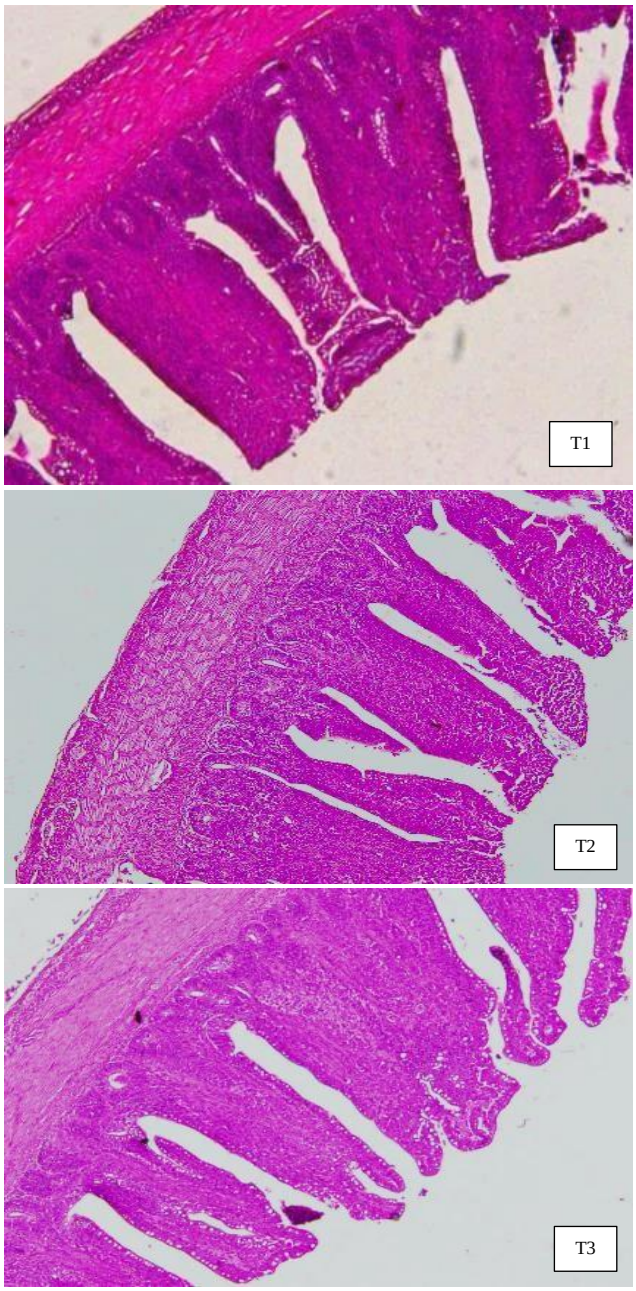

Figure 1. Ileum of cross-breed chickens in different treatment groups (T1, T2, and T3; 4x magnification).

Gu and Li (2004) reported that the growth and development of the small intestine, including the villi, in chickens occurs early in life and depends on nutrient intake at an early age which can be related to protein intake and villi development. The best result was obtained by using the T3 treatment (22% starter and 20% finisher) compared to other treatments on the cross-breed chicken. Protein feed was utilized by Lactic Acid Bacteria (LAB) as a source of nitrogen that forms the cell biomass. It is important to note that LAB in the gastrointestinal needs amino acids, vitamins, pirin, and pirimidin to grow (Nazzaro et al., 2012).

Feeding nutrients like protein and fat play a role in tissue formation and stimulate the proliferation of small intestine cells Wang et al. (2014). The larger protein content (T3) can increase the villi's size, making it longer and the intestinal lumen larger. Research by Nazzaro et al. (2012) reported that high protein levels resulted in more LAB colonies. Lactic acid bacteria require proteins based on nitrogen requirements. The nitrogen requirement for LAB was obtained from several sources including protein (organic nitrogen) in the feed. The growth of pathogenic microbes in the intestine can be suppressed by producing short-chain fatty acids (SCFA), lactic acid, and antimicrobial compounds of LAB. Conditions of the small intestine, such as high villi in the small intestine, represent an area for wider nutrient absorption. An increase in the height of the villi in the small intestine of broiler chickens was closely related to an increase in digestive and absorption functions due to the expansion of the absorption area which was an expression of a smooth nutrient transport system throughout the body and benefits the host (Tejeda and Kim, 2021).

The significant influence was because the crypt depth was closely related to the intestinal villi, where protein administration also had a significant influence on the number of villi and the length of the intestinal villi of cross-breed chickens. New epithelial cells and enterocytes were produced by the crypts, which then migrate to the villi (Bermudez-Brito et al., 2012). The T3 treatment (22% starter and 20% finisher) indicated the best result compared to the other treatment. It was because the results of protein hydrolysis in the form of nitrogen will be used by LAB as a nitrogen source. According to Marciniak et al. (2018), protein hydrolysis was influenced by the concentration of hydrolyzing ingredients, temperature and time of hydrolysis, and air pressure. Increasing the enzyme concentration will increase the volume of insoluble protein hydrolyzate into soluble nitrogen compounds. Nitrogen will be utilized by LAB as a source of nitrogen, which functions in cell biomass formation Ayivi et al. (2022). According to research by Mangisah et al. (2020), the addition of *Lactobacillus* sp. as a feed additive in ducks had a significantly different effect on the crypts' depth which greatly influenced the process of nutrient absorption in the small intestine in conditions that the absorption area was wider. The wider

villi improve nutrient absorption, making protein content more efficient in the growth of poultry (Tejeda and Kim, 2021). Protein is an important nutrient needed for the growth of cross-breed chickens. The finding of this experiment was aligned with the finding of Aftab et al. (2006), who reported that protein is the largest part of the carcass and is a very important element for muscle growth and development in chickens. The research study obtained the percentage of carcass cross-breed chickens affected by the different levels of dietary crude protein. This study implies that higher levels of crude protein, specifically at the starter phase (22%) and finisher phase (20%) for crossbred chickens, were necessary for optimal carcass and thigh percentage. The findings were not in agreement with Magala et al. (2012), which found no significant impact of dietary protein level (15-17%) on carcass yield in Ugandan cockerels. Carcass yield was influenced by various factors, such as genetics, feed, slaughtering conditions, live weight, and sex in broiler chickens (Havenstein et al., 2003; Kierończyk et al., 2017). In this study, protein deposition was characterized by carcass percentage which was determined by the rate of protein synthesis and protein degradation. A higher rate of protein synthesis or a lower rate of protein degradation can lead to increased quality of growth and protein deposition in the chicken Tesseraud et al. (2000). Chickens can adjust their feed intake to meet their protein requirements in the feed that has sufficient energy levels. These findings align with Kamran et al. (2008), who demonstrated that a low-protein diet with a better energy-to-protein ratio can increase energy consumption, which may be stored as fat after fulfilling energy requirements in broiler chicken. These results were consistent with Srilatha et al. (2018), who observed that broiler chickens fed lower protein levels (17.29%) at the finishing stage had more abdominal fat deposition. Tavaniello et al. (2022) stated that the order of percentage of broiler chicken carcass cuts from highest to lowest includes breast, upper thigh, lower thigh, wing, back, and front back. However, the research indicated that the carcass cut of cross-breed chickens from the highest to the lowest is a thicks, back, breast, and wings. The genetic nature of cross-breed chickens deposits the formation of meat on the thick and back. This finding indicated that the addition of a higher protein level diet of cross-breed chickens improved carcass cut compared to the lowest treatments. It disagreed with research before Eits et al. (2022) that there was no evidence that energy intake limited protein deposition at high amino acid intake. Limited amino acids did not depend on protein deposition in the carcass. However, this study finding proved that the higher protein also improves the carcass traits of cross-breed chickens.

CONCLUSION

The dietary addition of protein in the starter and finisher phases at levels of 22% and 20%, respectively, caused the best results for the intestinal characteristics and carcass percentage of cross-bred chickens. In the future, the expression gene analysis can evaluate the growth factor of cross-breed chickens.

DECLARATIONS

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Authors' contributions

Yuli Frita Nuningtyas contributed to doing the research, collecting data, data analysis, and preparing the manuscript. Osfar Sjofjan, Muhammad Halim Natsir, Aulanni'am, and Veronica Margareta Ani Nurgiartiningsih contributed to the research design, revised the manuscript, and supervised. Ahmad Furqon revised the manuscript grammatically. Suci Puji Lestari contributed to the research. All authors read and approved the final version of the manuscript and analyzed data in the present journal.

Competing interests

There is no potential conflict of interest relevant to the present article.

Ethical considerations

All ethical aspects, such as ethical issues, plagiarism, fabrication and falsification, double publication, and redundancy, have been thoroughly examined and addressed.

Availability of data and materials

All data generated or analyzed during the study are included in this article; supplementary information is available upon request.

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