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#### Research article

# The effect of tropical alfalfa (*Medicago sativa* L. cv Kacang Ratu BW) supplementation on performance, intestinal histomorphology, and nutrient digestibility in hybrid ducks

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#### **Abstract**

Tropical alfalfa is a legume nutritionally complete and palatable to poultry. Hybrid ducks are aquatic birds with a higher tolerance to fiber-sourced feed than other breeds. This study aimed to determine the effects of tropical alfalfa (Medicago sativa L. cv Kacang Ratu BW) or KRBW supplementation on growth performance, histomorphology, and nutrient digestibility. Seventy-five hybrid ducks were used in this study. The treatments included T1 (basal feed or control), T2 (4% KRBW supplementation in basal feed), T3 (8% KRBW supplementation in basal feed), and water was provided ad libitum. The KRBW supplement was prepared in powder. Data were collected based on performance including feed intake, final body weight gain, feed conversion ratio (FCR); digestible dry matter (DDM), digestible organic matter (DOM), digestible crude fiber (DCF), and digestible ether extract (DEE); and the histomorphology of the intestine (duodenum, jejunum, and ileum). All data obtained in this study were analyzed using a completely randomized design. Data were analyzed using SPSS version 27 software. The results of this study showed that KRBW supplementation significantly decreased body weight gain. In contrast, control and 4% KRBW supplementation rates had no effects compared with 8% KRBW supplementation on feed consumption. However, KRBW supplementation had no significant effect on the FCR. Supplementation with 4% and 8% significantly decreased the DDM, DOM, DCF, and DEE. Furthermore, KRBW supplementation affected the histomorphology of the villi surface area in the duodenum, jejunum, and ileum. It could be concluded that the KRBW supplement reduced performance, intestinal histomorphology, and nutrient digestibility in hybrid ducks.

Keywords: Digestibility, Hybrid duck, Kacang Ratu BW, Legume, Villi surface area

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## INTRODUCTION

Broiler hybrid breed ducks are a poultry commodity widely cultivated by farmers. Hybrid ducks are the result of crossing between Peking ducks with Khaki Campbell or Peking ducks with Mojosari ducks, having superior duck traits of growth and adaptation that are better than local ducks (Rini et al., 2019; Muthmainnah and Jalali, 2022). Quality feeding contributes to supporting the genetic potential of hybrid ducks. Hybrid ducks have the genetic potential to be developed. Through quality feeding, the nutrient requirements of hybrid ducks can be well met during growth (Addini et al., 2020; Suwignyo et al., 2021c; Suwignyo et al., 2022a). Feeding a well-balanced ratio provides the animal's nutrition needs because the appropriate and complete nutrient components can support the animal's metabolic processes (Sjofjan et al., 2020).

The balanced diet formulation considers the nutritional requirements of poultry such as protein, fiber, metabolic energy, fat, and minerals (He et al., 2021). The high nutritional requirements of animals are achieved and obtained from feed ingredients with high nutrient content (Kisworo et al., 2017). One of the feed ingredients that contain good nutrients can be sourced from forage, legume forage at several different levels is useful as a feed supplement for hybrid ducks (Suwignyo et al., 2021c; Suwignyo et al., 2023a; Umami et al., 2023). Legumes, such as alfalfa could be used as a supplement in the hybrid duck's diet to supply the nutritional requirements of the animals.

Nowadays, research and development on forage feed ingredients such as alfalfa have been carried out extensively. The implementation of forage as a feed supplement in poultry Suwignyo et al. (2021c); Suwignyo et al. (2023a) and Rahmandriani et al. (2023) developing alfalfa (*Medicago sativa* L.) in the tropics to produce a new variety (*Medicago sativa* L. cv Kacang Ratu BW) well known as Kacang Ratu BW which contains superior macro and micronutrients and secondary metabolite content and has become one of the best feeds. Furthermore, Suwignyo et al. (2020a) described that alfalfa could be given to ruminants and non-ruminants as a functional feed containing secondary metabolites, vitamins, and organic matter. Then, Suwignyo and Sasongko (2019) showed that supplementation with 6% fresh alfalfa provides better performance (body weight gain and feed consumption) than alfalfa hay and it reduced the feed cost (Suwignyo et al., 2020b). Thus, alfalfa could be an additive to improve the growth performance and feed efficiency in poultry.

It is known that the nutritive value of feed influences the performance of an animal. The effect of supplementary feed can be determined by evaluating performance and digestive tract health (Tejeda and Kim, 2020). Suwignyo and Sasongko (2019) reported that 6% fresh alfalfa supplemented in a ration for hybrid ducks improved feed consumption performance and body weight gain. Lokapirnasari et al. (2022) showed that the weight gain of poultry is directly related to high feed consumption which is affected by the essential nutrient source for better performance. Alfalfa supplementation of 5%, 8%, and 10% reduces the feed conversion ratio (FCR), and mortality, and makes poultry feed more efficient (Zheng et al., 2019; Samur et al., 2020).

Moreover, for a performance evaluation based on feed consumption and body weight, gut health is crucial for digesting and absorbing nutrients. Nelson (2020) indicated that nutrient absorption occurs in the folds of the intestine, which have thin and small cells called villi tissue. Then, Tejeda and Kim (2020) reported that supplementing feed with 4% soybean hulls increases the size of duodenal villi based on intestinal histomorphology. Villi develop optimally if the nutrient requirements of the animal are met during growth. Then, the absorption of nutrients triggers the expansion of the villi (Suwignyo et al., 2021c). Therefore, the digestive tract could work well and could evaluate digestibility of feed ingredients.

This study was conducted to investigate hybrid ducks' response to tropical alfalfa cv Kacang Ratu BW (KRBW) supplementation. In an earlier study, Suwignyo



et al. (2021c) demonstrated that supplementing duck diets with 3% alfalfa meal showed increased consumption, increased intestinal villus height and depth, and better nutrient digestibility compared to diets supplemented with 6% alfalfa. In addition, Umami et al. (2023) demonstrated that supplementation of *Cichorium intybus* in the hybrid ducks' diet at 10% level improved performance, and carcass quality.

Accordingly, this study focused on the effect of varying levels of tropical alfalfa supplementation in the diets of hybrid ducks using a new variety Kacang Ratu BW (KRBW) was conducted in this study. This study aimed to determine the effect of KRBW supplementation on feed consumption, body weight gain, FCR, and; histomorphology of the duodenum, jejunum, and ileum (length, width, crypt villi, and villi surface area) of local hybrid ducks.

## MATERIALS AND METHODS

#### **Ethical clearance**

The research design and procedures were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine UGM, Yogyakarta (No: 055/EC-FKH/Ext./2022).

# Study period and location

This study was conducted from April to July 2022. The feeding experiment was conducted at the poultry house, Faculty of Animal Science, Universitas Gadjah Mada Yogyakarta, Indonesia. Proximate analysis of feed was conducted at the forage and pasture laboratory and the biochemistry laboratory of the Faculty of Animal Science, Universitas Gadjah Mada. The proximate analysis of the excreta was performed at the Balai Pengujian Mutu dan Sertifikasi Pakan laboratory, Bekasi. The histomorphological analysis of the hybrid duck intestine was conducted at the histology microanatomy laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

# **Experiment and animal management**

The study used 75 day-old duck hybrids (Peking  $\sigma$  >< Khaki campbell  $\mathfrak{P}$ ) distributed in five replications, each replication consisted of 5 cages (1.25 × 1.25 × 1.00 m) with carrying mixed-sex of ducks (consisted of three males and two females in each cage) and lasted for 42 days.

The animals were fed in the morning and evening and drinking water was provided ad libitum. Tropical alfalfa KRBW supplemented in the basal diet was in the form of powder obtained after drying and milling the fresh alfalfa leaves. This study was conducted in vivo using a completely randomized design. Hybrid ducks were divided into three treatments with five replications. The treatment feed was formulated from 100% basal feed without tropical alfalfa KRBW supplementation, as a control (T1); 96% basal feed formulation, and 4% tropical alfalfa KRBW supplementation (T2); 92% basal feed formulation and 8% tropical alfalfa KRBW supplementation (T3). The feed formulation and content are detailed in Table 1.

#### **Performance**

Feed consumption performance data were recorded daily for each replicate cage. Final body weight gain is obtained from the difference between final body weight and initial body weight through random sampling and the FCR was calculated from the feed consumption and body weight gain.



Table 1 Nutrient content of treatment feed ingredients

Hama	Treatment feed formula		
Items	T1	T2	Т3
Ingredients composition			_
Tropical alfalfa cv Kacang Ratu BW	0	4	8
Pollard	8.2	3	4.5
Corn	44.5	46	47
Rice bran	17.2	18.5	10.8
Crude palm oil (CPO)	0	0	1.4
Soybean meal	23.5	20.1	20
Meat bone meal	5.5	8	8
Methionine	0.1	0.3	0.3
CaCO3	1	0.1	0
Total (%)	100	100	100
Chemical composition nutrient			
Crude protein (%)	20.87	21.14	21.37
Metabolizable energy (Kcal/Kg)	2798.45	2837.12	2856.17
Crude fiber (%)	3.61	4.18	4.77
Ether extract (%)	8.16	6.41	7.85
Ca (%)	1.09	1.08	1.11
P (%)	0.83	0.94	0.86
Available P (%)	0.52	0.65	0.57
Methionine (%)	0.39	0.60	0.60
Lyisin (%)	1.03	1.03	1.04

T1: control, T2: 4% KRBW in basal feed, T3: KRBW 8% in basal feed.

## **Histological preparation**

A duck was taken randomly from each cage and slaughtered following the halal slaughter procedures (Chao, 2022). Intestinal samples (duodenum, jejunum, and ileum) were immediately excised. Histological data were collected from  $\pm 2$  cm sections of the small intestine. The segments were placed in 10% neutral buffered formalin solution for 24 – 48 hours at room temperature (fixation), for the pressing (trimming), dehydration stages of 50% – 100%, clearing, embedding in paraffin (embedding-blocking), cutting to  $\pm 5~\mu m$  thickness and hematoxylin-eosin staining (staining), gluing (mounting) and labeling.

The preparations were observed at  $4 \times 10$  to  $10 \times 10$  magnification under a microscope OptiLab Viewer 2.2 (PT. Miconos Transdata Nusantara, Yogyakarta) connected to a computer monitor. The length and width of the intestinal villi crypts were measured using ImageRaster 4.0 image analysis software (PT. Miconos Transdata Nusantara, Yogyakarta). Villi length was measured from the basal or bottom coinciding with the top of the crypt and the point at the bottom was drawn toward a point at the top of the villi (Jiang et al., 2012a). The villi surface area calculation followed a previous method (Sakamoto et al., 2000).

# **Digestibility trial**

In vivo digestibility determinations was conducted on the birds during the last 6 days of the feeding trial. Digestible dry matter (DDM), digestible organic matter (DOM), digestible crude fiber (DCF), and digestible ether extract (DEE) were calculated based on the dry matter consumed and dry matter excreted. Twenty ducks were selected and adapted for 2  $\times$  24 hours in individual metabolic cages (55  $\times$  35  $\times$  60 cm) with excreta collection containers. Each cage contained one male duck based on the feed treatment. Animals were fasted for 6 - 12 hours after which they were provided drinking water ad libitum and fed the treatment diets base on the requirements of ducks. Furthermore, the excreta collection was carried out for 4 days.





The excreta was moved to a plastic clip ( $40 \times 30$  cm) on a tray. Excreta samples were wet-weighed and sprayed with insecticide to prevent fly infestation. The samples were air dried for 3-5 days in the shade. The samples were dried in an oven at 550C to constant weight. The total sample was crushed in a blender. The proximate analysis was conducted using the Association of Official Analytical Chemists and the gravimetric method (AOAC, 2005). The results of the proximate analysis of feed and excreta were used to determine the DDM, DOM, DCF, and DEE values.

## **Data analysis**

Data on feed consumption, body weight gain, FCR, histomorphology, and nutrient digestibility were analyzed by one-way analysis of variance using SPSS version 27 software (SPSS Inc., Chicago, IL, USA). Significant differences were detected using Duncan's new multiple-range test. A p-value  $\leq$  0.05 was considered significant.

## **RESULTS**

## Hybrid duck performance

The result of hybrid duck performance after KRBW supplementation (Table 2) had a significant effect on feed intake and body weight gain in hybrid ducks (p < 0.05), but the FCR was not affected. Feed consumption by the 4% KRBW treatment group was not different from the control.

Table 2 Effect of KRBW supplementation on hybrid duck performance

	KRBW Supplementation treatments			
Parameters	T1	T2	Т3	
FI (g/head)	2349.48±121.29 <sup>a</sup>	2305.60±38.81 <sup>a</sup>	2107.62±65.60 <sup>b</sup>	
FBW (g/ head)	1036.64±74.59 <sup>a</sup>	943.56±70.67 <sup>b</sup>	908.05±37.95 <sup>b</sup>	
FCR <sup>ns</sup>	2.28±0.25	2.45±0.17	2.32±0.09	

T1: control, T2: 4% KBW in basal feed, T3: 8% KRBW in basal feed; FI: feed intake, FBW: final body weight, FCR: feed conversion ratio; <sup>a,b</sup> Superscripts with different letters within the same row indicate significant differences (P < 0.05).

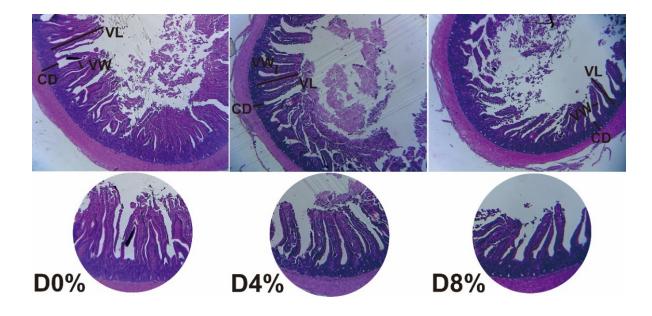
# Intestinal histomorphology

The intestinal histomorphology results on duodenum, jejunum, and ileum (Table 3) showed an effect on the length, width, crypt depth, and villi surface area. KRBW supplementation of 4% and 8% showed a significant effect (p < 0.05) on villus surface area. 4% KRBW supplementation on the villi surface area ( $V_{SA}$ ) of duodenal and jejunum showed no significant difference with control, but 8% KRBW supplementation on the ileal  $V_{SA}$  showed the highest development in the treatments of control and 4% KRBW supplementation (Table 3 and Figure 1, 2, and 3).

**Table 3** The effect of KRBW supplementation on the histomorphology of the duodenum, jejunum, and ileum in hybrid duck.

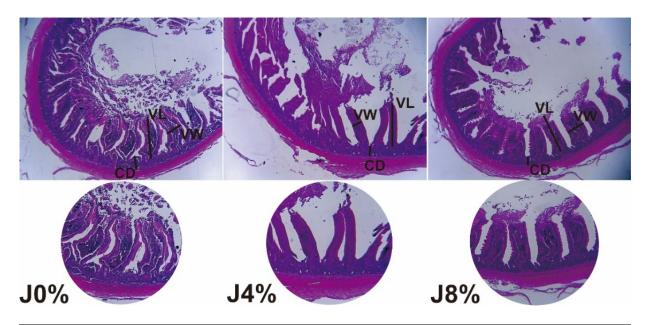
Dawanastana	KRBW Supplementation treatments			
Parameters	T1	T2	Т3	
Duodenum				
VL µm <sup>ns</sup>	466.91±82.82	452.87±91.87	404.48±52.36	
VW µm	114.96±17.15 <sup>ab</sup>	127.52±10.24 <sup>a</sup>	108.31±6.69 <sup>b</sup>	
$V_{SA} \times 10^3 \mu m^2$	170.99±48.63ª	180.96±37.45 <sup>a</sup>	137.84±21.19 <sup>b</sup>	
CD µm <sup>ns</sup>	172.51±13.88	165.52±8.571	161.03±5.55	
Jejunum				
VL µm	415.35±80.08 <sup>a</sup>	414.76±59.57 <sup>a</sup>	368.61±44.01 <sup>b</sup>	
VW µm <sup>ns</sup>	127.89±24.70	128.61±19.87	128.48±45.85	
$V_{SA} \times 10^3 \mu m^2$	171.75±63.62 <sup>a</sup>	167.40±31.71 <sup>a</sup>	152.82±74.32 <sup>b</sup>	
CD µm	55.08±2.16 <sup>a</sup>	39.60±7.99 <sup>b</sup>	43.95±4.41 <sup>b</sup>	
lleum				
VL µm <sup>ns</sup>	360.34±89.92	358.04±71.55	356.29±44.51	
VW μm	113.68±12.83 <sup>b</sup>	106.56±16.58 <sup>b</sup>	133.25±31.77 <sup>a</sup>	
$V_{SA} \times 10^3 \mu m$	129.15±39.44 <sup>b</sup>	121.50±41.03 <sup>b</sup>	147.94±31.53 <sup>a</sup>	
CD µm <sup>ns</sup>	50.92±6.38	43.97±7.15	44.51±9.29	

T1: control; T2: 4% KRBW in basal feed; T3: KRBW 8% in basal feed; D: duodenum; J: jejunum and I: ileum; VL: villi length; VW: villi width;  $V_{SA}$ : villi surface area and CD: crypt depth. <sup>a,b</sup> Superscripts with different letters within the same row indicate significant differences (P < 0.05).

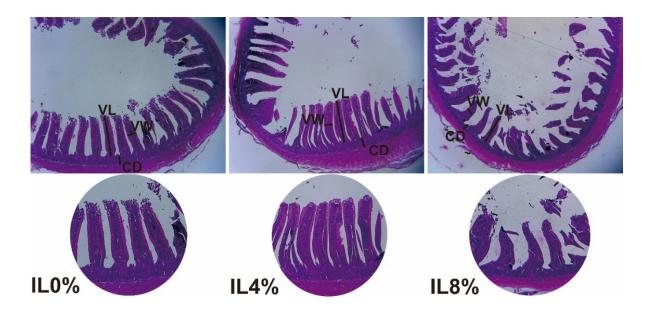


**Figure 1** The histology of the duodenal segment of the small intestine; D0%: duodenal segments of T1; D4%: duodenal segments of T2 (4% KRBW); D8%: duodenal segments of T3 (8% KRBW); VL: villi length; VW: villi width and CD: crypt depth.





**Figure 2** The histology of the jejunum segment of the small intestine; J0%: jejunum segments of T1; J4%: jejunum segments of T2 (4% KRBW); J8%: jejunum segments of T3 (8% KRBW); VL: villi length; VW: villi width and CD: crypt depth.



**Figure 3** The histology of the ileum segment of the small intestine; IL0%: ileum segments of T1; IL4%: ileum segments of T2 (4% KRBW); IL8%: ileum segments of T3 (8% KRBW); VL: villi length; VW: villi width and CD: crypt depth.

## **Nutrient digestibility**

A significant decrease in nutrient digestibility (p < 0.05) was observed in the 4% and 8% KRBW-supplemented hybrid ducks (Table 4). Organic matter digestibility in the 4% KRBW-supplemented group was not significantly different from the control group. However, the 8% KRBW-supplemented group was significantly different from the control group.

Table 4 The effect of KRBW supplementation on nutrient digestibility in hybrid ducks.

Parameters -	KRBW	KRBW supplementation treatments		
	T1	T2	Т3	
Digestible dry matter	72.71±6.25 <sup>a</sup>	66.74±2.72 <sup>b</sup>	64.93±2.06 <sup>b</sup>	
Digestible organic matter	77.23±6.64ª	72.56±2.96 <sup>ab</sup>	67.53±2.14 <sup>b</sup>	
Digestible crude fibre	60.29±6.74 <sup>a</sup>	49.25±3.58 <sup>b</sup>	36.47±4.58°	
Digestible ether extract	36.05±3.09 <sup>a</sup>	33.06±1.22 <sup>b</sup>	32.61±0.86 <sup>b</sup>	

T1: control; T2: (4% KRBW); (8% KRBW); a,b Superscripts with different letters within the same row indicate significant differences (P<0.05).

## DISCUSSION

## **Performance**

The results show that the 4% and 8% KRBW supplement decreased performance compared with a previous study by Suwignyo et al. (2021c) that used 3% and 6% alfalfa tropic supplementation in hybrid ducks which 3% tropical alfalfa supplementation increased feed intake, body weight gain. KRBW supplementation at 4% and 8% decreased feed consumption in the hybrid ducks, which was caused by low palatability due to the higher crude fibre content than in the control. High and low palatability can affect the level of feed consumption by animals (Suwignyo and Sasongko, 2019).

Furthermore, the decrease in consumption caused by the level and type of fibre in the feed is also explained by Suwignyo et al. (2021c) explained that the fibre content in 6% tropical alfalfa supplementation decreased consumption in hybrid ducks after 35 days. The decreased consumption may be related to the dietary fibre which represents non-starch polysaccharides and indigestible lignin (Kanjak et al., 2023). The fibre content in feed is a limiting factor in consumption because it increases bulk and reduces feed consumption (Rini et al., 2019). The composition of the fibre, such as cellulose and hemicellulose, fills the digestive tract space producing a bulky condition, which decreases feed consumption (Suwignyo et al., 2021d).

Decreased feed consumption leads to a decrease in body weight. It could be due to unbalanced nutrient intake in supporting the physiological and metabolic functions of animals (Suwignyo et al., 2021b). The 4% and 8% KRBW-supplemented groups exhibited decreased feed consumption, which affected the nutritional requirements for the growth of the hybrid ducks. High and low nutrient consumption will respond to metabolic hormones to balance nutrient needs through the mechanism of insulin and glucagon secretion (Liu, 2014; Qaid and Algaradi, 2021). The mechanism of insulin on carbohydrate metabolism occurs when glucose concentration is high, so insulin is secreted to promote glycolysis to lower glucose levels (Afifah, 2016; Marupanthorn et al., 2024). Furthermore, the condition of low blood sugar levels triggers the secretion of glucagon hormone to promote gluconeogenesis to increase blood glucose levels by the breakdown of glycogen and the release of glucose by the liver (Qaid and Abdelrahman, 2016; Marupanthorn et al., 2024). Therefore, in a situation of low nutrient intake, the insulin and glucagon hormone secretion will work together to maintain glucose homeostasis.

Although KRBW supplementation of 4% and 8% decreased body weight, hybrid ducks were still in good health. it is assumed that the compounds contained





in tropical alfalfa are beneficial for animal health. Suwignyo et al. (2023a) stated that tropical alfalfa KRBW contains secondary metabolite compounds such as saponins and flavonoids. Secondary metabolite compounds contained in tropical alfalfa tend to be an antioxidant activity for animals, especially monogastric (Rafińska et al., 2017). Flavonoids contained in tropical alfalfa KRBW include polyphenolic compounds as antioxidants and enzyme activities such as *glutathione peroxidase* and *glutathione S transferase pi*, which plays a role in detoxification, so it is safe for animal health (Rafińska et al., 2017; Kanjak et al., 2023).

Tropical alfalfa KRBW supplementation of 4% and 8% did not affect the FCR value. It is assumed that the efficiency of feed utilization is well converted by animals. Suwignyo et al. (2020c) reported that tropical alfalfa supplementation up to 6% level has no significant effect on the hybrid ducks FCR, so it was still within the allowed tolerance limit for poultry (5.95–8.39%). Lokapirnasari et al. (2022) explained that factors affecting FCR values are digestibility, feed nutrient content, quality, management, environment, and genetics. Balami et al. (2018) stated that increased feed intake and body weight gain, but low FCR values indicate good feed quality.

## Histomorphology

The morphometry of villus surface area ( $V_{SA}$ ) in the duodenum and jejunum was not significantly different in any intestinal segments from the control and 4% KRBW-supplemented groups (Table 3). However,  $V_{SA}$  in the ileal segment with 8% KRBW supplementation was higher than in the other groups. This may be the result of the fibre supplementation type that could have affected the difference in morphometry of the villi length and width in the duodenum, jejunum, and ileum (Figure 1 – 3). A healthy digestive tract is indicated by good digestive function through peristaltic activity and maximizing intestinal villi function to absorb micronutrients (Ariyadi et al., 2019; Suwignyo et al., 2021c; Suwignyo et al., 2021d). The feed fibre type in feed could affect epithelial morphology, depending on the characteristics of the soluble or insoluble fibre, the level of inclusions, the age of the animals, and the health of the digestive tract (Jha and Mishra, 2021).

The high level and type of fibre in the feed can increase contractions or peristalsis of the digestive tract muscles in poultry causing differences in the development of intestinal villi because pancreatic juice continues to digest in the duodenal segment (Figure 1). The segmental duodenum is in direct contact with the pancreas which secretes digestive enzymes and the gallbladder which secretes emulsifier fluid functioning to digest feed through gastroduodenal reflux during feed retention (Svihus, 2014; Qaid and Abdelrahman, 2016). Absorption of nutrients in the duodenal segment is not maximized by villi because it consists of macronutrients or large particles (Umami et al., 2023). Larger particles affect the permeability of digestive enzymes to digest large particles into smaller or micronutrient ones (Ariyadi et al., 2019). Additionally, wet or paste feed may affect the increase in villi and crypt depth of duodenum, jejunum, and ileum compared with dry feed (Jiang et al., 2012b).

The villi in the jejunum are more sensitive to nutrient absorption and, therefore, the type of crude fibre will affect villi morphometry (Figure 2). High villi increase the contact surface area between enterocytes and nutrients, thus increasing nutrient absorption (Suwignyo et al., 2021c). The increase in villi surface area ( $V_{SA}$ ) morphometry is directly related to the absorption and utilization of feed nutrients and animal performance. The digestibility and absorption of feed are affected by the surface area of the small intestine, so increasing animal productivity is closely related to increasing the nutrient absorption activity in the intestine (Jha and Mishra, 2021). The  $V_{SA}$  indicates maximum nutrient absorption and transportation to meet the nutrient requirements of hybrid ducks (Rahmatnejad and Saki, 2016; Umami et al., 2023). Furthermore, the jejunal segment absorbs starch nutrients, fatty acids, and amino acids, and the ileal segment as the last part of the small intestine functions to absorb water and minerals (Ariyadi et al., 2019).

The 8% KRBW supplementation showed greater ileal morphometry (Figure 3) development than the control and 4% KRBW supplementation, indicating that the types of fiber that are difficult to digest and remain in the cecum would be fermented and utilized by cecal microbes. The type of supplemented fibre stimulates volatile fatty acids produced by microorganisms through the fermentation process in the cecum which improves villi morphometry in poultry (Suwignyo et al., 2021c). V<sub>SA</sub> increases in response to the fermentation of shortchain fatty acids (SCFAs), such as butyric acid in the poultry cecum, by increasing cellular production in the small intestine (Umami et al., 2023).

One of the main products of SCFAs fermentation is butyric acid as an energy substrate for colonocytes and enterocytes, which support body development and homeostasis (Frampton et al., 2020; Ioniţă-Mîndrican et al., 2022). The mesenchymal cells trigger villi morphogenesis of the villi through vilification and intestinal epithelial differentiation is stimulated through the secretion of ligands so that intervillous proliferation becomes cryptic proliferation (Kwon et al., 2020). Butyric acid contributes to the proliferation of intestinal epithelial cells and improves intestinal mucosal morphology (Frampton et al., 2020). Consequently, different types and sources of fiber have multiple effects on gastrointestinal-physiology functions and animal health, depending on their physicochemical properties and chemical components.

# **Nutrient digestibility**

The DDM, DOM, DCF, and DEE digestibility values in hybrid ducks decreased in the 4% and 8% KRBW-supplemented groups (Table 4). This was affected by the type and level of fibre in the ration causing low consumption and poor feed digestibility. The results of this study are supported by previous research by Suwignyo et al. (2021c) showed that 6% fresh tropical alfalfa supplementation decreases the digestible dry matter, digestible organic matter, digestible crude protein, and digestible crude fibre values in hybrid ducks. Jha and Mishra (2021) reported that the fibre type and level decrease dry matter digestibility and increase dry matter intake in excreta so that nutrient digestibility decreases with increasing fibre type and level. Fibrous components, such as cellulose, hemicellulose, and lignin in KRBW, reduce digestibility. Tejeda and Kim (2020) showed that fibre components, such as cellulose and insoluble fibre, affect the function of the digestive tract and digestive enzymes in digesting nutrients, thereby reducing digestibility in poultry.

Suwignyo et al. (2023a) described that tropical alfalfa as a legume containing crude fibre significantly affects the digestibility of animal feed with 40.45% – 44.90% neutral detergent fibre and 16.20% – 25.40% acid detergent fibre. In addition, the anti-nutrient content in animal feed can cause low digestibility of organic matter. Jha and Mishra (2021) reported that the fibre type contained in feed indicates the difficulty of enzymes in hydrolyzing, which reduces growth performance and retention of nutrients in the digestive tract of livestock. Decreasing digestibility of organic matter decreases the digestibility of crude fibre and the ether extract (Suwignyo et al., 2021c). The digestive tract would not absorb the undigested crude fibre fraction and it may be excreted from the body, resulting in a decreased digestibility value.

Furthermore, Suwignyo et al. (2021a) showed that the crude fiber type affects consumption intake and consequently becomes a limiting factor in meeting the nutrient requirements of hybrid ducks. Crude fiber that is not enzymatically digested is utilized in the cecum as a fermentation substrate by microorganisms. Additionally, Suwignyo et al. (2021c) reported that fiber supplementation in feed stimulates microorganisms to digest and produce SCFAs. Fiber content interacts positively with microorganisms in the health of the digestive tract. The presence of fermented microbes in the digestive tract produces fermentation products that decrease the pH and inhibit pathogenic bacteria (Ioniţă-Mîndrican et al., 2022).

Furthermore, supplementation with dietary fiber decreased DCF and DEE. Those could be indicated by the physicochemical properties of fiber and bile acid secretion in animals. Then, a negative association has been reported that fiber type decreased protein and fat digestibility (Jha and Mishra, 2021). Supplements of legumes contain secondary metabolites such as saponins, that reduce cholesterol content by inhibiting the reabsorption of bile acid and interfering with cholesterol synthetic activity (Kisworo et al., 2017; Frampton et al., 2020; Suwignyo et al., 2023b). Fat is an ingredient and precursor to the production of bile acids in emulsifying fats that are digested. The absorption of fat and cholesterol is disrupted because the fiber content in feed increases the viscosity of the intestinal lumen, which increases the excretion of bile acids bound by fiber, then, reabsorption of bile acids is inhibited (loniță-Mîndrican et al., 2022).

#### CONCLUSIONS

KRBW supplementation at 4% and 8% reduced feed consumption, body weight, and nutrient digestibility. Supplementation at 4% and 8% reduced the villi length and villi width in the duodenum and jejunum. However, at 4% and 8% KRBW-supplementation did not affect the FCR of hybrid ducks.

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## **AUTHOR CONTRIBUTIONS**

Makmun Murod: Executed the experiment, collected data, carried out laboratory analysis, analyzed the data, and drafted the manuscript. Bambang Suwignyo: Designed and supervised the experiment. Bambang Ariyadi: Drafted and modified the manuscript. All authors read and agreed on the final manuscript.

## CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

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