Research Article

Effects of the Replacing of Fish Meal with Freshwater Snail Flesh (*Pomacea paludosa*) Treated with Bromelain Enzyme on Growth Performance, Relatively Organ Weight, and Digestibility of Alabio Ducks (*Anas plathyrhyncos Borneo*)

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Abstract | This study aimed to determine the effects of the replacing of fish meal with freshwater snail flesh (*Pomacea paludosa*) treated with bromelin enzyme (FSFIBE) on growth performance, relatively organ weight, and digestibility of Alabio ducks (*Anas plathyrhyncos borneo*). A total of 210 one-day old ducks male Alabio with an average body weight of 45.26±2.59 g were placed in colony-shaped pen, and each pen contained seven ducks. The trial design used was completely randomized design with five treatments and six replications for each one, consisting of seven ducks. The treatment diet was formulated as follows: T0 basal diet + 100% fish meal (10% in diet) (10:0), T1 : basal diet + 75% fish meal (7.5% in diet)+ 25% FSFIBE(2.5% in diet) (7.5:2.5), T2 : basal diet + 50% fish meal (5% in diet)+ 50% FSFIBE(5% in diet) (5:5), T3 : basal diet + 25 % fish meal (2.5% in diet)+ 75% freshwater snail flesh incubated bromelain enzyme (7.5% in diet) (2.5:7.5), T4 : basal diet + 0 % fish meal (0% in diet)+ 100% FSFIBE(10% in diet) (0:10). The parameters observed was growth performance and digestibility. Result were analyses using general linear model and if a significant effect was identified, least significant differences (LSD) then applied. The result showed using freshwater snail for fish meal give significant difference ($p < 0.05$) on the apparent metabolizable energy-n corrected (AMEn) and reduce feed conversion ratio (FCR). It can be concluded using fresh water snail can be used as replacement for fish meal on Alabio ducks without any adverse effects.

Keywords | Alabio Duck, Bromelain enzyme, Carcasses, Fish meal, Growth performance

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INTRODUCTION

Poultry is one of the livestock commodities whose products are widely consumed by the community so it becomes an opportunity to become a resource-based industry because feed raw materials, technology, and support services are available (Adli, 2021). Waterfowl, such as Alabio ducks, are mostly kept traditionally to produce eggs and flesh and are sub-systems and can grow to reach sexual maturity only relying on local feed ingredients. Feed has the largest proportion in the livestock business, which is around 70-80% of production costs. Currently, the feed used in the cultivation of Alabio duck farms is mostly factory feed whose constituent materials are still imported,

so the cost is very high. This condition forces farmers to make various efforts to minimize feed costs so that Alabio its farm business can survive. One of the local feed ingredients that can be used is freshwater snail flesh (*Pomacea paludosa*). In order to fulfill the protein requirement, using swamp snail might can be recommended as an alternative feed (Siddiqui et al., 2022).

The freshwater snail, *Pomacea paludosa*, is one of the most common species of snail in the swamps of South Kalimantan. In addition, *Pomacea canaliculata* and *Pomacea insularum*, which are abundant in waters, rivers, ditches, and rice fields (Dharmawati et al., 2021). Although, the availability of freshwater snails is abundant, their use is not optimal as a replacement for fish meal in feed formulation for Alabio. The use of freshwater snails is not optimal due to low digestibility and unbalanced nutritional content. One of the causes of the low protein digestibility of swamp snails is that swamp snails contain heavy metals that hinder the absorption of nutrients in poultry (Dharmawati et al., 2021) and the presence of tannins that block protein absorption (Saputri and Pertiwi, 2021). The use of various types of enzymes in animal feed has long been developed, to increase protein digestibility. Gauthier (2007) stated approximately 20-25% of the protein in feed ingredients is undigested. Angelovicova et al. (2005), reported the use of enzymes (xylanase and protease) was able to increase weight gain and reduce feed conversion, preventing the loss of endogenous amino acids (Plumstead and Coieson, 2008). Using bromelain enzyme would be advantages increasing the protein digestibility in the snail, which is consisted low pH (Dharmawati et al., 2021). The use of freshwater snail flesh that has been incubated with the bromelain enzyme in Alabio duck feed has an important meaning with the aim of increasing feed efficiency and replacing the use of the fish meal. Specifically, bromelain works to break down proteins found in feed ingredients into simpler forms. Bromelain works more actively on animal protein (Taqwdasbriliani et al., 2013). This study aimed to determine the effects of the replacing of fish meal with freshwater snail flesh (*Pomacea paludosa*) treated with bromelin enzyme on growth performance, relatively organ weight, and digestibility of Alabio ducks (*Anas plathyrhyncos borneo*).

MATERIALS AND METHODS

Ethical approval

Ethical approval for the study was given by the Animal Care and Use Committee, University of Islam Kalimantan Muhammad Arsyad Al Banjary, No. 25/KEP/UNISKA/2021.

Experimental design

A total of 210 birds one-day-old duck male Alabio with an average body weight of 45.26±2.59 g were placed in colony-shaped pens, and each pens contained seven ducks. The trial design used was completely randomized design with five treatments and six replicated for each one, consisting of seven ducks. The treatment diet was formulated as follows: T0 basal diet + 100% fish meal (10% in diet) (10:0), T1 : basal diet + 75% fish meal (7.5% in diet)+ 25% FSFIBE(2.5% in diet) (7.5:2.5), T2 : basal diet + 50% fish meal (5% in diet)+ 50% FSFIBE(5% in diet) (5:5), T3 : basal diet + 25 % fish meal (2.5% in diet)+ 75% freshwater snail flesh incubated bromelain enzyme (7.5% in diet) (2.5:7.5), T4 : basal diet + 0 % fish meal (0% in diet)+ 100% FSFIBE(10% in diet) (0:10). The freshwater snail flesh used in this study was freshwater snail flesh which was incubated with bromelain enzyme 3% for 3 hours (Dharmawati et al., 2021). Freshwater snails flesh incubated are given in the form of paste was mixed with other feed ingredients. Feeding starts from the starter phase using freshwater snail flesh that has been incubated with the bromelain enzyme. The diets were given every morning and evening according to the requirement of each maintenance, and were allowed *ad-libitum* access water through adjustable nipple drinkers. The formulated feed consisted yellow maize, rice bran, soya bean meal, palm kernel meal, fish meal, freshwater snail treated with bromelain enzyme, coconut oil, custom mineral mix, and custom vitamin mix. Representative of the formulated feed were analysed for metabolizable energy (Kcal / kg); fat (%); crude fiber (%); calcium (%); available phosphorus (C_{av}) (%); Lysine (%); and methionine (%). All of formulated feed was formulated by following ISO energy (2900 kcal/kg) and iso protein (21%) by following procedure of (AOAC, 2000). The composition of formulated feed can be seen in Table 1.

Freshwater snail flesh preparation

First, freshwater snails were carefully chosen and separated from it shells. Second, the freshwater snail flesh soaked in liquid blend consisted ten percent of rice husk and charcoal for 12 hours to remove mucus and heavy metals by following (Dharmawati et al., 2021) procedures. Furthermore, the freshwater snail flesh was washed with running water. The incubation was subjected by following Dharmawati et al. (2021) using 3% bromelain enzyme and soaked for 3 hours using a water bath at 72^0C with buffer solution consisted pH 5.8.

Growth performance

The Alabio ducks were individually weighed at the end of the experiment (eight weeks' periods) by following (Sjofjan et al., 2021a) procedures. Feed intake was calculated the amount of feed given with total refusal feed and dividing by total eight weeks' periods by following (Sjofjan et al., 2021a) method. Furthermore, feed conversion ratio (FCR) expressed feed intake (g/bird/day) divided by body weight

Table 1: Experimental diet composition

Ingredients (% as is basis)	Expriment diets composition				
	T0	T1	T2	T3	T4
Yellow Maize	46.50	45.00	46.00	47.00	48.00
Rice bran	10.00	11.50	11.50	11.50	12.50
Soybean meal	15.00	15.00	14.00	13.00	13.00
Palm kernel	15.00	15.00	15.00	15.00	13.00
Fish meal	10.00	7.50	5.00	2.50	0.00
Freshwater snail treated with bromelain enzyme	0	2.5	5	7.5	10
Coconut oil	2.5	2.5	2.5	2.5	2
Mineral	0.5	0.5	0.5	0.5	0.5
Top Mix	0.5	0.5	0.5	0.5	1
Total	100	100	100	100	100
Calculated composition					
Ingredients					
Crude protein (%)	21.38	21.62	21.37	21.13	21.13
Crude fat (%)	9.25	9.37	9.25	9.13	8.65
Crude fiber (%)	5.41	5.61	5.65	5.69	5.55
Metabolizable energy (kcal/kg)	2919.49	2923.87	2951.74	2979.61	2968.83
Calsium (%)	0.92	0.96	0.89	0.81	0.73
Available phosporus (%)	0.44	0.33	0.36	0.39	0.42
Lysin(%)	0.83	0.81	0.78	0.75	0.74
Methionine (%)	0.39	0.40	0.41	0.42	0.43
Proximate composition					
Crude protein (%)	21.88	21.01	21.33	22.01	22.09
Crude fat (%)	9.31	8.33	8.23	8.11	7.98
Crude fiber (%)	5.67	5.66	5.31	5.11	5.01
Metabolizable energy (kcal/kg)	2879.45	2885.01	2890.77	2895.23	2892.22
Calcium (%)	0.89	0.90	0.85	0.80	0.71
Available phosporus (%)	0.40	0.31	0.35	0.34	0.39

gain (g/bird/day). At the end of rearing the selected Alabio duck were subjected fasting for 10 hours and slaughtered by following Sjofjan et al. (2021a) method.

Digestibility

At the end of the experiments (8 weeks old), a total of 80 male Alabio were removed into artificial-single pen. The feed intake of the male Alabio Duck was recorded for apparent metabolizable energy (AME) on weeks and apparent metabolizable energy-nitrogen corrected (AMEn) on eight weeks by following formulae:

AME = AME-ANR/FI)
AMEn = AME-(8.73xANR/FI)
Where: ANR is apparent Nitrogen retention, FI denotes feed intake. The correction factor is 8.73 (Sjofjan et al., 2021b). The apparent digestibility of protein is calculated using using the following formulate : Protein digestibility = (AxB)-(CxD)/(AxB)
Where: A is feed consumption, B signifies protein in feed, C accounts for the amount of excreta, and D denotes protein (%) in excreta (Sjofjan et al., 2021a:; 2021b).

Statistical analysis

A statistical analysis was conducted using analysis of variance using Proc Mixed with general linear model (GLM) using SAS studio for academics Online Edition (https://odamid-apse1.oda.sas.com/SASStudio/). An error was expressed as standard error mean (SEM). At the end, probabilities values were subjected in Duncan Multiple Range Test. The following model was used (Ardiansyah et al., 2022; Adli et al., 2022).

$Y_{ij} = \mu + T_i + e_{ij}$
Where Yij was parameters observed, μ was the overall mean, Ti the effect different the effect level of freshwater snail flesh incubated with bromelain enzyme, and eij the amount of error number.

RESULTS AND DISCUSSION

Effects of the replacing of fish meal with freshwater snail flesh (*Pomacea paludosa*) treated with bromelain enzyme on digestibility of Alabio ducks (*Anas plathyrhyncos Borneo*)

Replacing of freshwater snail flesh incubated with bromelain up to 7.5% to fish meal was slightly tends to increasing the digestibility of Alabio duck, even though insignificant differences if compared to control from 78.38% to 86.08% (Table 2). The AME and AME-N corrected compared to the control diet (Table 2), from 2879.41 Kcal/kg to 2895.26 Kcal/kg ($p < 0.05$). The trend was tending to elevate from 4.3 to 7.91%. This showed that the use of freshwater snail flesh incubated with the bromelain enzyme is easier to digest than the feed control. The condition indicated that metabolizable energy were directly proportional to the feed consumption of male Alabio ducks which utilized feed nutrients as energy. Factors that affect metabolizable energy are gross energy and the amount of feed energy used. Insignificant difference of the digestibility result may have correlated with several factors such as amount of samples collected, amount of animal being used, and technical factors during collecting the samples (Sjofjan et al., 2021a:; 2021b). Akit et al. (2019) reported that the use of bromelain enzymes in feed can increase protein digestibility. The bromelain enzyme is exogenous and mutually supports digestive enzymes (Yu et al, 2002). It was also explained that feed incubated with bromelain enzyme was able to reduce digesta viscosity in starter-period poultry (Meng et al., 2005). Increasing the viscosity of the digesta will limit the interaction of the enzyme-substrate it will reduce the digestibility of nutrients (Bedford and Schulze, 1988). The use of endogenous proteases also has a positive effect on nutrition and energy digestibility that can be metabolized in broiler chickens (Fru Nji et al., 2011). The increase in protein digestibility as a result of the bromelain enzyme will reduce the non-digestible protein substrate available to pathogenic bacteria will decrease (Yin et al., 2018)

Effects of the replacing of fish meal with freshwater snail flesh (*Pomacea paludosa*) treated with bromelain enzyme on growth performance of Alabio ducks (*Anas plathyrhyncos Borneo*)

Analysis of the feed intake, weight gain, feed conversion and live weight is shown in Table 3. In addition, carcass, carcass percentage with skin, weight liver weight gizzard, percentage gizzard and pH gizzard presented Table 3. Furthermore, replacing freshwater snail flesh incubated with bromelain enzyme effect on feed intake, weight gain, feed conversion and live weight affect male Alabio duck ($p >0.05$).

The average consumption of feed using freshwater snail flesh incubated with bromelain enzyme was lower than the consumption of control feed, where the replacing of freshwater snail flesh incubated as much as 2.5% of fish meal in the feed resulted in the lowest feed consumption (3734.70 g/heads) and highest in control feed (4263.66 g/heads). The highest average body weight gains and live weight of male Alabio ducks were found in the replacing of incubated freshwater snail flesh with bromelain enzyme 7.5% + 2.5% fish meal in the feed respectively 1428.35 g/head and 1476.92g/head, then the lowest conversion feed was at Freshwater snail flesh replacing incubation 5.0%-7.5% in feed (2.86). Replacing of freshwater snail flesh incubated with bromelain enzyme to fish meal at the level of 10% in duck feed was significanlty ($p < 0.05$) on feed conversion ratio of Alabio duck (Figure 1).

Replacing of freshwater snail flesh incubated with bromelain enzyme to fish meal at the level of 10% in duck feed was insignificantly ($p > 0.05$) on the body weight gain, live weight, final body weight, and carcass weight of male Alabio ducks at 8 weeks of age. The results of the analysis showed that male Alabio ducks that consumed feed containing freshwater snail flesh that had been incubated with bromelain enzymes tended to be lower than control diets (without using freshwater snail flesh). Because of the feed containing freshwater snail flesh incubated with the bromelain enzyme has a balanced nutritional quality and is more easily absorbed by male Alabio ducks, so the duck's nutritional needs, especially protein and energy, are more fulfilled. Physiologically, poultry will stop consuming feed if the needs for maintenance, growth, and production have been met (Sjofjan et al., 2021a). The use of freshwater snail flesh that has been incubated with the bromelain enzyme causes an overhaul of the protein sources contained in the feed mixture, in addition to the combination of fish meal with freshwater snail flesh also plays a role in complementing the protein and energy needs of male Alabio ducks. Angel (2010) stated that protease enzymes play a role in breaking peptide bonds, furthermore according to Sjofjan et al. (2019) that protein also functions as an energy source for poultry. Protein metabolism is not directly involved in energy production, but protein is involved in the production of enzymes, hormones, structural components, and blood proteins of body cells. Metabolizable energy derived from protein is preceded by the breakdown of protein into amino acids, then amino acid groups are released through oxidative deamination in liver cells. Deamination products will enter the Krebs cycle to form energy through the pyruvate and acetyl coenzyme A pathway before entering the Krebs cycle. This is in line with the results of this study where the feed consumption of male Alabio ducks fed a diet containing snail flesh from incubation was lower than the control diet. The average feed consumption of male Al

Table 2: Effects of the replacing of fish meal with freshwater snail flesh (pomacea paludosa) treated with bromelain enzyme on digestibility of Alabio ducks (*Anas plathyrhyncos borneo*)

	T0	T1	T2	T3	T4	SEM
ME (Kcal/ kal)	2879.41	2886.75	2891.08	2892.74	2892.74	110.06
AMEn (Kcal/kal)	2879.15[a]	2886.79[ab]	2891.06[ab]	2895.06[b]	2898.11[b]	91.14
Digestibility	78.38	81.11	83.32	84.92	86.07	0.04

[a, b, c, d] Means with different superscripts in the row differ significantly ($p \leq 0.05$). AMEn – Apparent metabolizable energy; BWG – Body weight gain; FCR – Feed conversion ratio; FBW – Final body weight; FI – Feed intake. T_0 basal diet + 100% fish meal(10% in diet), T_1 : basal diet + 75% fish meal (7.5% in diet)+ 25% freshwater snail flesh incubated bromelain enzyme (2.5% in diet), T_2 : basal diet + 50% fish meal (5% in diet)+ 50% freshwater snail flesh incubated bromelain enzyme (5% in diet), T_3 : basal diet + 25 % fish meal (2.5% in diet)+ 75% freshwater snail flesh incubated bromelain enzyme (7.5% in diet), T_4 : basal diet + 0 % fish meal (0% in diet)+ 100% freshwater snail flesh incubated bromelain enzyme (10% in diet).

Table 3: Effects of the replacing of fish meal with freshwater snail flesh (pomacea paludosa) treated with bromelain enzyme on growth performance and relatively organ weight of Alabio ducks (*Anas plathyrhyncos borneo*)

	T0	T1	T2	T3	T4	SEM
FI (g/day)	4267.49	3733.06	3867.82	3932.28	3879.77	49.33
BWG (g/day)	1235.73	1272.28	1353.14	1426.50	1341.27	39.60
FBW (g)	1277.68	1316.86	1398.97	1473.82	1387.65	41.11
FCR	3.45[c]	2.93[b]	2.85[a]	2.86[a]	2.88[ab]	0.0001
Carcasses (g)	779.22[b]	849.24[c]	859.03[d]	881.73[e]	752.54[a]	1.09
Carcass (%)	61.74[b]	65.93[e]	62.72[c]	63.35[d]	58.41[a]	0.10
Liver (g)	22.77[a]	32.91[e]	28.82[c]	25.46[b]	31.03[d]	0.14
Liver (%)	1.76[b]	2.49[e]	2.00[c]	1.70[a]	2.24[d]	0.01

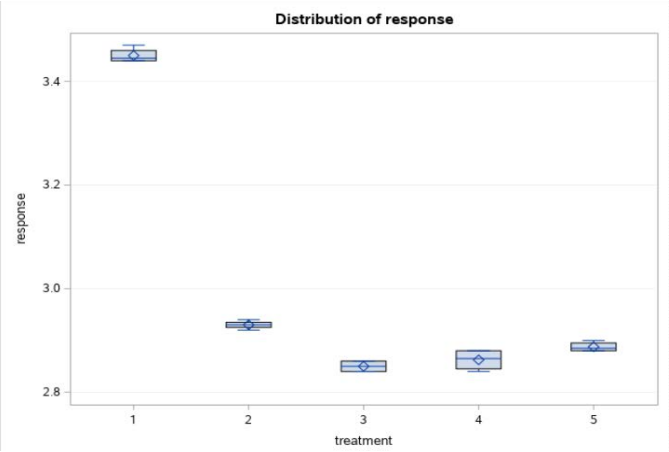

Figure 1: Distribution of response of feed conversion ratio of Alabio ducks

abio ducks in this study was higher than that of Matitaputty et al. (2011) where the average feed consumption ranged from 3444.57 – 3523.43 g/head at the same age. Replacing of freshwater snail flesh that had been incubated with bromelain enzyme as much as 75% (7.5% in diets) to fish meal (2.5% in diets) showed a better body weight gain of 1428.35g/head, but when fed substituted 100% with incubated freshwater snails (10% in diets) tended to reduce the body weight gain of male Alabio ducks. It is suspected that the feed protein is starting to become unbalanced due to the use of freshwater snails as a single source of animal protein in duck feed, so some of the protein components used for growth may be reduced. The use of feed incubated with bromelain enzymes at a certain level can improve poultry performance because it will stimulate nutrient digestibility which will be followed by increased digestibility of metabolizable energy and protein (Akit et al, 2019). In this study, the role of the bromelain enzyme in freshwater snail flesh is to eliminate anti-nutrients and pathogenic bacteria, worms commonly found in snail flesh, so that nutrient absorption is better. In addition, to feed ingredients that are incubated with bromelain enzymes when consumed, poultry will interact with endogenous enzymes found in the gastrointestinal tract and simultaneously together with digestive enzymes degrade proteins that are difficult to digest, thereby increasing intestinal solubility and digesta viscosity (Lee et al., 2013; Olukosi et al. al., 2015). The application of cysteine proteases in the animal feed industry can increase the digestibility, acceptable flavor and palatability ingredients (Hassan et al., 2014).

Freshwater snail flesh replacing incubated with bromelain enzyme resulted in lower feed conversion values compared to the control feed conversion (Table 3). The showed that the feed using freshwater snail flesh incubated with bromelain enzyme is more efficiently absorbed by the digestion of male Alabio ducks compared to feed that uses 100% fish meal (control). Because of the feed incubated with the bromelain enzyme peptide bond is simpler so it is easier for male Alabio ducks to digest (Sjofjan et al., 2021a;

2021b). In this study, the protein digestibility of freshwater snails flesh incubated with bromelain enzyme was higher than those not incubated with bromelain enzyme, namely 87.79% compared to 73.64% control feed. Digestion by protease enzymes were needed to convert protein into amino acids, especially protein source feed ingredients such as freshwater snails. Angelovicova et al. (2005) stated that the advantage of using protease enzymes is that they can help neutralize excess nitrogen in the small intestine and facilitate the decomposition of some nitrogen component molecules into smaller, looser molecules that are easier to absorb. The biological effects of bromelain enzyme are related to their proteolytic activity (Mazarra et al., 2018). Bromelain enzym is a sulfhydryl protease enzyme that can be hydrolyze proteins into simple water soluble amino acids (Gautam et al., 2010). The results also showed that the use of freshwater snail flesh incubated with bromelain as much as 100% (10% in feed) caused a decrease in the performance of male Alabio ducks. This happens, it is estimated that the amino acids in the feed become unbalanced because freshwater snail flesh is the only source of animal protein in the feed, in contrast to the use of freshwater snail flesh which has been incubated as much as 2.5%-7.5%, still uses fish meal., so they are complementary. This indicates that the use of freshwater snail flesh incubated with the bromelain enzyme as much as 7.5% has been maximized in male Alabio duck feed.

CONCLUSIONS

Replacing freshwater snail flesh incubated with bromelain enzyme on fish meal up to 7.5% in Alabio male duck diets improved on digestibility protein. The best feed conversion was obtained replacing of freshwater snail flesh incubated with bromelain enzyme at 5-7.5% in diets male Alabio duck.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

NOVELTY STATEMENT

Based on the literature review, the freshwater snail *Pomacea paludosa* mostly informs about the habitat, morphology, production and reproduction of these snails. Information regarding its performance and of this species of snail is still very limited and there is not much information on the use of the freshwater snail as feed for ducks, especially male Alabio ducks. Most of the literature informs research on the species *Pomacea canaliculata*. The novelty of this research is the use of *Pomacea paludosa* meat that has been incubated with the bromelain enzyme and used as a protein source in duck feed by looking at the performance and physiological response of duck digestion (liver and gizzard).

AUTHOR'S CONTRIBUTION

Siti Dharmawati contributed to collecting data, analysis of nutrient,data analysis and preparing the manuscript. Hartutik, Osfar Sjofjan, Muhammad Halim Natsir contributed to the research design, revised the manuscript and supervision. Danung Nur Adli supervised and revised the manuscript grammatically. All authors read and approved the final version ot the manuscript in the present journal

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