



Research article

Evaluation in Alabio duck (*Anas platyrhincos* Borneo) of soluble protein, protein digestibility, metabolizable energy and nitrogen retention of freshwater snail flesh (*Pomacea paludosa*) incubated with bromelain enzyme for different times and concentrations

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Article Info

Article history:

Received 17 December 2022

Revised 22 May 2023

Accepted 23 May 2023

Available online 30 June 2023

Keywords:

Alabio duck,
Digestibility protein,
Nitrogen retention,
Soluble protein

Abstract

Importance of the work: Metabolizable energy of feed is the remaining energy from feed after losing in urine and feces. As such, it is a useful parameter for improving feed performance. However, duckspecific information regarding the metabolizable energy and protein digestibility of freshwater snails is still limited.

Objectives: To evaluate in Alabio duck the soluble protein and protein digestibility, nitrogen retention, and metabolizable energy of freshwater snail flesh incubated with bromelain enzyme in difference incubation and concentration.

Materials and Methods: Freshwater snail flesh was incubated with bromelain enzyme (1%, 2%, 3%, or 4%) for varying durations (1, 2, 3, or 4 hours). The treated flesh was then fed to Alabio ducks using a factorial design in a completely randomized design with five replications (one duck/replication)

Results: The highest values for soluble protein ($6.58 \pm 1.87\%$), protein digestibility ($87.79 \pm 0.06\%$), nitrogen retention ($90.35 \pm 0.01\%$), apparent metabolizable energy ($3,492.86 \pm 9.48$ kcal/kg) and N-corrected metabolizable energy ($3,491.03 \pm 9.47$) were obtained in snail flesh incubated with 3% bromelain enzyme for 3 hr.

Main finding: Bromelain enzyme at 3% concentration with 3-hour incubation demonstrated superior outcomes, including increased soluble protein, protein digestibility, nitrogen retention, apparent metabolizable energy, and N-corrected metabolizable energy. This enzyme mixture holds promise for application in poultry feed formulations.

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<https://doi.org/10.34044/j.anres.2023.57.3.02>

Introduction

Species of the golden apple snail, specifically *Pomacea canaliculata*, *P. scalaris* and *P. maculata*, has been reported in several parts of Southeast Asia and several regions of South America, including Argentina (Cowie et al., 2006). Suharto et al. (2006) reported three species had entered Indonesia (*P. canaliculata*, *Pomacea paludosa* and *Pomacea insularum*) as part of the aquatic fauna readily found in and around rice paddy fields, swamps, irrigated areas and rivers. Often, the apple snail (*P. paludosa*) is considered a pest that can reduce cultural production in Indonesia, with damage due to snail pests in the range 10–40%, requiring effective management or removal (Mualim et al., 2013).

Currently, there is a lot of research on snails, especially as a source of animal feed, although such applications are inefficient and irregular. For example, freshwater snails are an excellent source of nutrition for poultry since they have a high protein content (50–60%), reproduce quickly and can survive in extreme situations, such as low oxygen, metal-polluted waters and various cropping conditions (Cowie et al., 2006). According to assays from other snail species, such as the channeled apple snail (*P. canaliculata*) their chemical contents include 62% crude protein (on a dry matter [DM] basis, for the flesh, excluding shell) and 14% DM, as well as being a good mineral source, as indicated on a DM basis by their calcium (35% in the shell), phosphorus (1.2%) and energy (13.94 MJ kg⁻¹) contents (Kaensombath, 2005). Despite having a high protein content as a feed item, freshwater snails cannot substitute for fish meals due to their low protein digestion and unbalanced amino acids (Jintasataporn et al., 2004 ; Ghosh et al., 2017).

The low protein digestibility of freshwater snail flesh is due to heavy metals that inhibit the absorption of nutrients in poultry. Heavy metals, such as lead (Pb), in feed can cause poisoning and reduce body weight in poultry (Erdoğan et al., 2005) and contaminate the digestive tract, especially the gizzards and liver, of poultry (Hossain et al., 2022). Snails are a class of mollusk that feeds by filtering food from their environment, making them susceptible to accumulating heavy metals. For example, the results of research by Dharmawati et al. (2022), showed that freshwater snails contained heavy metals, such as Pb, Hg, Fe and Cu. Protein and metabolizable energy are very important elements in preparing poultry feed (Ghazi et al., 1997). Data on the protein metabolizable energy and digestibility of freshwater snails flesh, especially *P. paludosa*, is still very limited, with most information being about the habitat, production and reproduction of these

snails. Information about metabolizable energy and protein digestibility is very important in the formulation of duck feed (Dharmawati et al., 2023). The snail flesh is commonly used in poultry; however, there is only limited information on the response of Alabio ducks fed with freshwater snail flesh, especially information related to protein digestibility, nitrogen retention and apparent metabolizable energy (AME).

To improve the quality of freshwater snail flesh as a food source for poultry, processing has been reported based on incubation using bromelain enzymes. For example, Taqwadasbriliani et al. (2013) found that the bromelain enzyme is more active on animal protein, whereas papain is more active on vegetable protein. Bromelain enzymes cannot hydrolyze all tissues like keratin; however, bromelain enzymes can hydrolyze milk proteins and collagen. In other work, the snail flesh of *Pila ampullacea* was hydrolyzed using 1% bromelain enzyme for 15 hr to produce 4.18% soluble protein. In addition, Puspitasari et al. (2022) reported that *P. ampullacea* hydrolyzed with 15% bromelain enzyme for 15 hr yielded 9.03% soluble protein. Liang et al. (2012) reported that Fe and Cu were the main limiting factors and significantly reduced bromelain enzyme activity.

The bromelain enzyme does not directly increase metabolizable energy but works explicitly to break down the proteins in feed ingredients into simple forms (Rosida et al., 2022). Currently, the metabolizable energy value of local feed ingredients has not received optimal attention; however, this information would be very useful in the preparation of poultry feed. The metabolizable energy value of feed is widely used because of its practical use in poultry nutrition to support the main life goals, growth, production, physical activity and maintaining body temperature (Sjofjan et al., 2021). Until now, specific information regarding the metabolic energy of freshwater snail flesh has been limited to the protein content contained in these snails. Information on the metabolizable energy of freshwater snails is very important as a benchmark for the amount used in feed. The quantity of metabolizable energy is affected by feed digestibility and N retention (Sjofjan et al., 2021). One of the feed additives that can increase feed digestibility is the enzyme bromelain.

Bromelain enzymes are glycoprotein enzymes that contain oligosaccharides in each molecule bound covalently in a polypeptide chain (Ha et al., 2012). They can dissolve very thick mucus and wash away the accumulation of parasites and worms on the organ walls, as well as having anti-microbial activity and anti-worm (Steppek et al., 2005) and anti-candida (Brakebusch et al., 2001) effects. These important properties are very useful for increasing the nutritional value of feed, specifically freshwater snail flesh because this flesh has several

limiting factors, such as containing collagen and having viscosity that is higher than sea cucumber flesh (Purwaningsih and Triono, 2019) which contains pathogenic bacteria and worms (Hairani and Fakhri, 2017) and heavy metals (Widowati et al., 2019; Dharmawati et al., 2022; Mauriza et al., 2020). Thus, the purpose of the current study was to improve the quality of freshwater snail (*P. paludosa*) meat using the bromelain enzyme, based on its concentration and incubation time, so that it could be used optimally as a source of protein in duck feed.

Materials and Methods

Ethics statements

All experimental procedures were approved by the Animal Care and Welfare Committee of the Islamic Kalimantan University of South Kalimantan Banjarmasin (no. 25-KEP–UNISKA–2022).

Materials and methods

The freshwater snails (*P. paludosa*) were obtained from Hulu Sungai Village in South Kalimantan, Indonesia. The commercial bromelain enzyme was bought from Limited Company Bromelain Enzym Lampung Indonesia. The chemicals used were aquadest, boric acid 5%, phosphate-buffered saline (PBS), bovine serum albumin (BSA), tyrosin, trichloroacetic acid (TCA), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, K-Na-tartrate, NaOH 0.2 N and HCl 1N. The 80 male Alabio ducks aged 2.5 mth with an ($\pm$ SD) weight of $1,400 \pm 2.56$ g were obtained from the Livestock Breeding Center, Tanah Laut Regency, South Kalimantan, Indonesia.

The meat of freshwater snails was separated from the shells and soaked in 10% rice husk charcoal for 12 hr to remove mucus and heavy metals. Then, the flesh was washed under running water, weighed into 360 g samples of snail flesh and mixed with aquadest (2:1), before mashing in a blender. The freshwater

snail flesh was incubated using bromelain enzymes with the following treatments: bromelain enzyme usage levels of L1 = 1% (1 g/100g, enzyme/snail flesh), L2 = 2% (2 g/100 g), L3 = 3% (3 g/100 g), and L4 = 4% (4 g/100 g) and incubation times of T1 = 1 hr; T2 = 2 hr; T3 = 3 hr; and T4 = 4 hr in a water bath at 52 °C in buffer solution (pH 5.8). To stop the enzymatic process, the incubated freshwater snails were dripped with 30% TCA.

Soluble protein

The incubation filtrate was used to test the soluble protein using the biuret method (Sudarmadji et al., 2010), measured using a visible spectrophotometer at 640 nm. The filtrate was obtained by passing through Whatman filter paper no. 41. The soluble protein concentration was determined using the standard curve for protein of BSA.

Digestibility, dry matter and protein digestibility, metabolizable energy and N retention

Before the experiment, the ducks were checked thoroughly and any dirt adhering to their plumage and feet webs was removed. A 3 d adaptation period (Abdollahi et al., 2021) was followed by placing the birds into digestibility cages with the different feeds; water was made available during this period. The feeding and excreta collection techniques for the ducks were developed by Adeola et al. (1997) and Hong et al. (2002) and modified by Widodo et al. (2020).

In the experiment, 80 Alabio ducks were used with one duck per cage. The 16 treatment combinations comprised of four different bromelain enzyme concentrations (1%, 2%, 3% and 4%) and four different incubation times (1 hr, 2 hr, 3 hr and 4 hr), each with five replications. The ducks were fed with the bromelain treated freshwater snail flesh at 120 g/d. Excreta that had been sprayed with 5% boric acid were collected according to treatment and dried in an oven at 50 °C for 48 hr. The detailed experiment procedure is presented in Table 1.

Table 1 Experimental protocol (developed by Adeola et al. (1997); Hong et al. (2002) and modified by Widodo et al. (2020)).

Day/Time (hr)	Operation
0 hr	Feed withdrawal: Alabio ducks weighed and assigned to feedstuffs
Day 1–4 (96 hr)	Alabio ducks were placed in a metabolizable energy cage for adaptation, The first day the feed is provided for 10 hours, the second day the feed is provided for 6 hours, the third day the feed is provided for 3 hours, and the fourth day the feed is provided for 1 hour, so the ducks are able to finish the feed in just 1 hour.
Day 5–6 (48 hr)	Ducks were fed with 120 g/day of freshwater snail flesh according to the treatment
Day 5–6 (48 hr)	Excreta collection was carried out, with excreta sprayed using 5% boric acid to avoid nitrogen evaporation
Day 5–6 (48 hr)	Excreta collected and stored in freezer in separate plastic bags.
Day 7–8 (48 hr)	Two ducks/treatment were randomly chosen, fasted twice for 24 hr, while still being given drinking water. 1× first 24 hr to empty the digestive tract and 1×24 hr, excreta collected to obtain endogenous protein values. The excreta were dried in an oven at 50 °C for 48 hr.
Day 9	Excreta ground and filtered using a 0.55 mm sieve prior to analysis. Dry excreta were analyzed for levels of DM, nitrogen using the Kjeldahl method (AOAC, 2006), while gross energy was measured using an adiabatic bomb calorimeter.

Digestibility of dry matter

DM in the freshwater snail flesh was measured according to Regar and Kowel (2021), calculated using Equation 1:

$$DCDM = \frac{\text{Dry matter consumed} - \text{dry matter excreta}}{\text{Dry matter consumed}} \times 100\% \quad (1)$$

Metabolizable energy

The apparent metabolizable energy (AME, measured in kilocalories per kilogram) was calculated following Sjöfjan et al. (2021) using Equation 2:

$$AME = \frac{\text{GE intake} - \text{GE excreta}}{\text{GE intake}} \quad (2)$$

where the GE intake is the gross energy intake (gDM) × GE in feed (kcal/kg), GE excreta is the GE weight of excreta (in grams DM) × gross energy excreta (in kilocalories per kilogram), intake is the feed consumption (in grams DM).

The nitrogen-corrected metabolizable energy (NCME) was calculated according to Farrel (1978) using Equation 3:

$$NCME = \frac{\text{GE intake} - \text{GE excreta}}{\text{GE intake}} \times 8.73 \times \text{N retention} \quad (3)$$

where 8.73 is the correction factor and N retention (measured in grams) is calculated according to Sandi et al. (2018) using Equation 4:

$$\text{Nitrogen retention} = \text{N consumption} - (\text{N excretion} - \text{N endogenous}) \quad (4)$$

where N endogenous is the total endogenous excreta × % nitrogen endogenous excreta.

True protein digestibility

True protein digestibility (TDP) was calculated according to Regar and Kowel (2021) using Equation 5:

$$TDP = \text{CP intake with feed} - \frac{(\text{CP excreta and in faeces} - \text{CP in endogenous substances})}{\text{CP intake with feed}} \quad (5)$$

However, the calculation of TDP is not a strict measure of digestibility as urine is not separated from feces, while the total difference between intake and content in droppings represents the amount that is not only digested but also includes urinary loss.

Statistical analysis

A completely randomized design was used with two factors: the incubation time (1 hr, 2 hr, 3 hr or 4 hr) and the concentration of the bromelain enzyme (1%, 2%, 3% or 4%). The data were analyzed using a two-way analysis of variance in the SPSS statistic version 25 software (ibm.com/spss-statistics-software, USA). Significantly different results were further tested based on Duncan's multiple range test at the at $p < 0.05$ level.

Results

Composition of raw materials

The components in the raw freshwater snail flesh are presented in Table 2. The freshwater snail flesh contained 37.68% DM, 26.61% crude protein and 4.70% ash.

Table 2 Flesh composition (mean ± SD) of raw freshwater snail (*Pomacea paludosa*)

Component	Quantity (%)
Dry matter	92.12±0.11
Ash	11.55±0.10
Crude protein	67.35±0.03
L-Sistin	1.59±0.01
L-Metionine	2.97±0.01
L-Serine	5.39±0.01
L-Glutamic acid	12.52±0.02
L-Phenylalanine	4.88±0.01
L-Isoleucine	4.90±0.01
L-Valine	5.86±0.01
L-Alanine	7.05±0.01
L-Arginine	8.68±0.01
L-Glicyne	8.00±0.01
L-Lysine	4.66±0.01
L-Aspartic acid	7.94±0.02
L-Leucine	9.53±0.01
L-Tyrosine	3.99±0.01
L-Proline	5.02±0.01
L-Threonine	5.59±0.01
L-Histidine	1.43±0.01
Crude Fiber	1.84±0.22
Crude fat	3.84±0.03

Soluble protein, digestible protein, nitrogen retention and metabolizable energy

The results showed that the incubation time and the concentration of bromelain enzyme had significant effects on the soluble protein content, digestible protein, N retention and metabolizable energy of freshwater snail flesh (Table 3). Furthermore, DM matter consumption, nitrogen retention and energy retention were significantly affected. The metabolizable energy and N-corrected metabolizable energy of the freshwater snail flesh incubated with bromelain enzyme for 3 hr were the highest at 3,452.00 kcal/kg and 3,451.25 kcal/kg, respectively, with a decrease after 4 hr of incubation.

Regarding concentrations of bromelain enzyme, the metabolizable energy values for the freshwater snail samples incubated with 2 or 4 % enzyme were the highest (Table 3).

Notably, the interaction between bromelain concentrations and incubation times was statistically significant and showed that using 3% bromelain enzyme with an incubation period of 3 hr resulted in the highest dry matter digestibility (88.48±0.09%), protein digestibility (87.79±0.06%), nitrogen retention (90.35±0.01%), metabolizable energy (3492.86±9.48 kcal/kg) and N-corrected metabolizable energy (3491.03±9.47 kcal/kg) (Table 4). The lowest protein digestibility was with 1% enzyme with an incubation period of 1–4 hr with a digestibility range of 72.48±0.14–73.76±0.15%.

Table 3 Soluble protein, protein digestibility, nitrogen retention, apparent metabolizable energy and N-corrected metabolizable energy of freshwater snail (*Pomacea paludosa*) flesh incubated using bromelain enzyme with different incubation times and concentrations fed to Alabio ducks

Incubation time (hr)	Soluble protein (%)	Dry matter digestibility (%)	Protein digestibility (%)	Nitrogen retention (%)	Apparent metabolizable energy (kcal/kg)	N-corrected metabolizable energy (NCME) (kcal/kg)
1	3.36±0.42 ^a	74.21±0.17 ^a	76.38±0.45 ^a	77.19±0.01 ^a	3329.13±3.68 ^a	3328.48±3.67 ^a
2	4.61±0.67 ^b	75.15±0.26 ^b	78.88±0.15 ^c	78.65±0.01 ^b	3418.98±5.02 ^b	3417.08±5.02 ^b
3	5.94±0.67 ^c	75.75±0.09 ^b	79.82±0.04 ^d	81.52±0.01 ^c	3452.00±4.27 ^c	3451.25±4.27 ^b
4	5.14±1.43 ^d	79.18±0.15 ^c	76.78±0.05 ^b	78.79±0.01 ^b	3332.14±3.76 ^a	3331.43±3.74 ^a
Bromelain enzyme concentration (%)						
1	4.61±2.03 ^a	70.01±0.09 ^a	72.79±0.21 ^a	74.39±0.01 ^a	3231.89±8.52 ^a	3231.27±8.50 ^a
2	5.80±0.75 ^b	73.11±0.46 ^b	74.87±0.30 ^b	74.22±0.01 ^a	3456.62±12.95 ^c	3444.76±12.95 ^c
3	6.13±1.22 ^c	80.08±0.18 ^c	82.99±0.28 ^d	83.41±0.01 ^b	3419.03±6.66 ^b	3405.72±6.66 ^b
4	5.64±1.58 ^b	81.09±0.25 ^d	81.20±0.28 ^c	84.12±0.01 ^c	3449.29±8.85 ^c	3446.67±8.84 ^c

Mean ± SD in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 4 Soluble protein, dry matter digestibility, protein digestibility, nitrogen retention, apparent metabolizable energy and N-corrected metabolizable energy of freshwater snail (*Pomacea paludosa*) flesh incubated with 16 combinations of bromelain enzyme concentrations and incubation time and fed to Alabio duck

Interaction (concentrations of bromelain enzyme and incubation time)	Soluble protein (%)	Dry matter digestibility (%)	Protein digestibility (%)	Nitrogen retention (%)	Apparent metabolizable energy (kcal/kg)	N-corrected metabolizable energy (kcal/kg)
1%, 1 hr	1.23±0.612 ^a	72.73±0.13 ^c	72.48±0.14 ^a	72.20±0.01 ^a	3158.99±3.95 ^b	3158.35±3.96 ^b
1%, 2hr	3.20±0.612 ^c	67.85±0.22 ^a	72.41±0.30 ^a	71.53±0.01 ^a	3324.15±9.24 ^d	3323.61±9.24 ^d
1%, 3 hr	5.28±1.936 ^e	67.30±0.28 ^a	73.76±0.15 ^c	78.63±0.01 ^f	3364.45±6.31 ^e	3363.77±6.31 ^e
1%, 4 hr	5.20±1.27 ^e	72.74±0.35 ^c	72.52±0.25 ^a	75.18±0.01 ^e	3079.97±14.55 ^a	3079.35±14.48 ^a
2%, 1 hr	3.05±0.500 ^c	69.84±0.08 ^b	73.08±0.21 ^b	74.02±0.01 ^{bc}	3466.12±5.70 ^{hi}	3465.49±5.70 ^{gh}
2%, 2 hr	4.48±0.612 ^f	72.59±0.68 ^c	75.38±0.33 ^c	74.69±0.01 ^{cd}	3436.52±7.35 ^g	3430.87±13.44 ^g
2%, 3 hr	6.43±0.500 ^h	80.22±0.11 ^h	76.61±0.41 ^f	73.43±0.01 ^b	3476.89±16.55 ⁱ	3476.16±16.54 ^h
2%, 4 hr	5.65±1.118 ^g	69.80±0.24 ^b	74.43±0.27 ^d	74.75±0.01 ^{cd}	3406.55±16.12 ^f	3406.55±16.12 ^f
3%, 1 hr	4.08±1.275 ^c	74.97±0.55 ^d	81.35±0.30 ^j	80.29±0.01 ^g	3205.13±5.56 ^c	3204.44±5.55 ^c
3%, 2 hr	5.56±0.935 ^e	77.11±0.09 ^c	83.18±0.42 ^k	82.32±0.01 ^h	3435.88±5.69 ^g	3435.18±5.69 ^g
3%, 3 hr	6.58±1.871 ^h	88.48±0.09 ^j	87.79±0.06 ^m	90.35±0.01 ⁱ	3492.86±9.48 ^j	3491.03±9.47 ⁱ
3%, 4 hr	5.23±1.000 ^g	79.74±0.17 ^g	79.65±0.32 ^h	80.68±0.01 ^g	3493.94±5.93 ^j	3492.23±5.93 ^j
4%, 1 hr	5.10±0.612 ^g	79.41±0.12 ^g	78.60±0.16 ^g	82.23±0.01 ^h	3486.27±2.53 ^j	3485.64±2.53 ^j
4%, 2 hr	5.20±1.000 ^g	85.53±0.87 ⁱ	84.57±0.34 ⁱ	86.07±0.01 ^k	3479.39±18.95 ⁱ	3478.67±18.94 ^h
4%, 3 hr	5.48±1.000 ^g	80.71±0.23 ^h	81.12±0.41 ^j	83.66±0.01 ⁱ	3474.79±5.27 ^{hi}	3474.03±5.28 ^h
4%, 4 hr	4.50±3.202 ^f	78.83±0.26 ^f	80.53±0.21 ⁱ	84.53±0.01 ^j	3349.11±8.65 ^e	3348.35±8.65 ^e

Mean ± SD in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Discussion

The components of freshwater snail flesh reported herein were higher than the results Obande (2013) reported for *Pomacea canaliculata* that contained 23.68% DM, 10.67% protein and 5.54% ash. These differences could have been caused by differences in species, age, season, nutritional value and snail habitat. The ash content of the freshwater snail flesh was high in this study, indicating that the mineral content of the freshwater snail flesh was quite high. The high ash content was possibly due to their ability to absorb heavy metals in their habitat, including Fe, Cu, Hg and Pb (Dharmawati et al., 2022). In addition to the freshwater snails, the primary raw material essential in this study was the bromelain enzyme powder, as this is closely related to the bromelain enzyme activity in degrading the freshwater snail flesh.

The soluble protein of the freshwater snail flesh incubated using the bromelain enzyme was in the range 3.36–5.94%. The highest soluble protein content (5.94%) was produced after 3 hr of incubation and the lowest was at 4 hr of incubation. These amounts were higher than those reported by Rosida et al. (2022), who incubated the freshwater snail flesh (*P. ampulacea*) for 3–18 hr, producing 2.23–4.18% soluble protein. Putra et al. (2018) reported that golden snails incubated using papain enzyme produced 1.81% soluble protein. These differences in the soluble protein yield were related to the snail species used, habitat, age and the protein content of the snail flesh before incubation. There was a decrease in the amount of soluble protein with increasing incubation time.

The use of bromelain enzymes in freshwater snail flesh increased the amount of soluble protein; however, excessive (4%) resulted in a decrease. Rosida et al. (2022) reported that the higher the bromelain enzyme concentration added to the flesh of *P. ampulacea* snail, the lower the soluble protein. In the current study, the soluble protein produced was higher than reported by Rosida et al. (2022) where incubated snail flesh with 10% pineapple extract for 12 hr and produced 4.12% soluble protein. Ha et al. (2012) stated that the bromelain enzyme has higher activity and reacts more quickly to the peptide bond of flesh than the papain and actinidin enzymes.

The analysis of soluble protein used in this study used the biuret method, where the biuret reagent reacts specifically on proteins, not amino acids (Purwanto, 2014). The higher concentration of the bromelain enzyme used had a greater effect on hydrolyzed peptide bonds, resulting in the number of peptide bonds counted as soluble proteins being lower.

Likewise, the longer the incubation period, the longer the period the bromelain enzyme can be active in hydrolysis resulting in weaker peptide bonds and a lower the amount of soluble protein using the biuret method.

The highest amount of soluble protein (6.58%) was produced with a 3% concentration and incubation for 3 hr. Ha et al. (2012) stated that commercial bromelain enzymes begin to hydrolyze flesh proteins in the first hour, gradually stabilizing during the second hour and stabilizing in the third hour. They considered that based on protease enzymes, the hydrolysis ability for different enzymes was zingiberazine > bromelain > papain > actinidin. The bromelain enzyme works by opening the protein connective tissue, especially collagen (Nanda et al., 2020).

Bromelain enzymes function on a single substrate and catalyze reactions in a particular way; thus, reduced enzyme activity results from decreased enzyme substrate. The addition of excessive enzymes with limited substrates cannot increase enzyme activity due to the enzyme activity ceasing because of the lack of substrate (Khattak et al., 2006). Putra et al. (2020) stated that increasing the substrate concentration can increase enzyme activity until the maximum limit is reached. These conditions indicated that the substrate molecule and the enzyme molecule will combine at the enzyme's active site until all of the active sites are used up. In this case, the enzyme is said to be in maximum condition. Bromelain enzyme in freshwater snail flesh tended to affect the consumption by ducks of DM and N.

The use of bromelain enzyme up to 3% for 3 hr resulted in the highest DM retention and N retention of 309.79 kcal/kg (88.49%) and 33.08 g/DM (90.35%), respectively, with the lowest DM retention and N retention occurring with 1% enzyme and an incubation period of 1 hr. The level of N retention is influenced by feed energy; if the feed energy is lacking, then the process of utilizing N in the poultry body will be hampered, and the remaining N that cannot be used will be disposed of through excreta. The function of the bromelain enzyme in swamp snail meat, apart from loosening protein bonds, is also to eliminate anti-nutrients. Bromelain enzymes eliminate anti-nutrients in feed and can increase the digestive tract's ability to absorb nutrients, partially hydrolyze feed proteins, increase protein utilization, suppress endogenous protease secretion, and improve digestive system performance (Ghazi et al., 1997).

Several studies have examined the effectiveness of exogenous enzymes in poultry to increase nutritional value, especially in chickens (Adeola et al., 2004). In contrast, similar studies, especially in Alabio ducks, are still very limited.

Adeola and Bedford (2004) investigated duck body mass, and reported an average effect of 7,500 units of protease activity per kg/DM and 15,000 units of protease activity per kg/DM to increase energy metabolism and nitrogen retention, respectively in Pekin ducks. Ghazi et al. (2002) reported that supplementing protease enzymes to Soybean meal at 0.1 g/kg (3,300 units/g) increased the AME, energy retention and N retention, while reducing endogenous N loss. The use of 3%–4% enzyme concentration, with an incubation period of 1–4 hr, produced levels of higher metabolizable energy.

The decreases in the AME value with 4 hr of incubation and 4% bromelain enzyme were due to the decreases in the DM, protein digestibility and nitrogen retention of the freshwater snail flesh. The process of enzymatic hydrolysis begins with the breaking of peptide bonds by enzymes with the help of water. The hydrolysis of these peptide bonds will increase the number of ionized groups due to the opening of the protein molecule, so that the solubility increases and the viscosity decreases. The bromelain enzyme breaks peptide bonds to produce smaller peptide units that are easily soluble and digestible. Digestible dissolved protein is used for metabolic purposes in male Alabio ducks and the metabolic flow is based on the reactions of anabolism and catabolism. Metabolism begins by consuming freshwater snail flesh that has been incubated with bromelain enzymes (containing various large and small compounds), which are broken down in the digestive tract into smaller molecules. High or low metabolic energy values are closely related to protein digestibility and nitrogen retention (Sjofjan et al., 2021). Proteins are macromolecules made up of amino acids linked in the form of peptides. Protein functions as fuel for the body and acts as a building and regulatory substance. During hydrolysis, the conversion of insoluble protein to soluble nitrogen occurs, which is further broken down into simpler compounds such as peptides, amino acids, and ammonia so that they are more easily absorbed by the body.

Overall, the metabolizable energy of freshwater snail flesh incubated with bromelain enzyme produced a fairly high metabolizable energy in the range $3,079.35 \pm 14.48$ – $3,492.23 \pm 5.93$ kcal/kg. These results indicated that Alabio ducks responded well to freshwater snail flesh incubated with bromelain enzyme. Feed adaptation factors and single feeding might play a role; thus, Alabio ducks are physiologically able to absorb the nitrogen and potential energy contained in freshwater snail flesh. Several studies have reported that metabolizable energy testing in ducks tends to produce higher metabolizable energy levels compared to chickens. Sumiati

et al. (2001) reported that Kayambang (*Salvinia molesta*) in ducks produced a metabolizable energy of 2823 ± 288.6 kcal/kg. Adeola et al. (2004) reported that soybeans with protease enzymes and wheat using test ducks, produced 3,974 kcal/kg and 3,731 kcal/kg metabolizable energy, respectively. Furthermore, Zakaria (2006) measured the metabolizable energy of bran using local duck test animals, resulting in metabolizable energy of 3,160–3,334 kcal/kg.

The highest protein digestibility of freshwater snail flesh was obtained at 3 hr of incubation ($79.82\% \pm 0.04$) and the lowest was at 1 hr of incubation (76.38%), as shown in Table 4. These results were different from Yu et al. (2002) reported that bromelain enzymes did not increase protein digestibility in either starter or finisher phase chickens, because the molecular weight of the digesta protein was not reduced so it was not able to improve protein digestibility in chickens. The inclusion of the bromelain enzyme in the study by Yu et al. (2002) of 65 GDU (Gelatin digestible unit)/kg, was probably not enough to produce the expected responses because of the short transit time for the digesta being insufficient to break down the substrate in the small intestine (Dosković et al., 2013). In this study, the use of the bromelain enzyme in the range 50–200 GDU/kg showed that 150 GDU produced the highest soluble protein, protein digestibility, nitrogen retention and metabolizable energy.

Differences in the physiology of the digestive system and small intestine between different livestock species will have different effects on exogenous enzyme activity (Hale, 2004; Dosković et al., 2013). Bromelain enzymes work specifically and are active on proteins, so they do not interfere with the function of endogenous enzymes such as lipase; on the contrary, they will complement the functions of endogenous enzymes in the digestive tract (Yu et al., 2002). Bromelain enzymes have anti-inflammatory effects in the digestive tract (Hale, 2004; Hale et al., 2005; Maurer, 2001). In addition, bromelain enzymes reduce digesta viscosity (Meng et al., 2005). An increase in digesta viscosity due to endogenous enzyme-substrate interactions can decrease digestibility (Bedford and Schulze, 1998). Bromelain enzymes have the ability to remove anti-nutrients (Erdaw et al., 2018). The use of bromelain enzymes in freshwater snail flesh will eliminate these anti-nutrients so that when consumed by ducks, they will not damage the intestinal wall. Several studies have reported the ability of the bromelain enzyme to reduce anti-nutrients in soybeans (Akit et al., 2019). The ability of enzymes to break down substrates will reduce indigestible protein substrates available for pathogenic bacteria commonly found in the large intestine (Yin et al., 2018).

The high protein digestibility with the use of 3% bromelain enzyme with an incubation period of 2–4 hr was closely related to the concentration of soluble protein and N retention in the treatment. The use of 3% enzyme with an incubation period of 3 hr resulted in higher soluble protein and higher N retention (Table 4) and higher DM retention (Table 4). The bromelain enzyme eliminates anti-nutrients in feed and can increase the ability of the digestive tract to absorb nutrients (Zheng et al., 2017), partially hydrolyze feed protein, increase protein utilization and suppress endogenous protease secretion, to improve digestive system performance (Ghazi et al., 1997). Bromelain enzymes in feed will reduce the pH of the digesta, especially in the gizzard, and increase the pH of the ileum (Yu et al., 2002). The decrease in digesta pH is caused by the release of H⁺ from the carboxyl group of the peptide when it is hydrolyzed if the bromelain enzyme has been included in the feed (Hsu et al., 1977). Yu and Tsen (1993) stated that the bromelain enzyme activity was close to zero at pH 2 and the activity increased by 80% at pH 3–4; in addition they reported that the pH of the intestine and gizzard was in the range 3–5, which is beneficial for the bromelain enzyme to function. Animal protein activity is mostly (70–80%) in the gizzard, while vegetable protein is mostly active in the jejunum and ileum (Makkink et al., 1994). The current results were in agreement with Akit et al. (2019) who reported that using bromelain enzymes in feed could improve protein digestibility and the performance of poultry intestines, especially in Alabio ducks.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The Ministry of Education, Culture, Research and Technology and Universitas Brawijaya provided research funding support for the Doctoral Student.

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