The Effect of Bromelain from Pineapple (Ananas comosus) on Increasing Protein Digestibility of Milk Replacer for Lamb

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Abstract. The research aimed to determine the addition of bromelain for increasing the protein digestibility of milk replacer for lamb. Bromelain extraction from pineapple (Ananas comosus) was precipitated by ammonium sulphate with different concentrations which were 30\%, 40\%, 50\%, 60\%, and 70\%; then those samples were dialyzed to obtain the best of specific enzyme activities. Milk replacer formulated by mixing skim powder and soybean meal. The research consisted of three replications for each treatment. The treatments were P0 or milk replacer without enzyme (control), P1 or milk replacer with 5\% bromelain addition, and P2 or milk replacer with 10\% bromelain addition. Specific enzyme activity from each precipitation, dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), and crude fibre digestibility (CFD) analysed by ANOVA according to one way complete randomized design and continued by Duncan multiple range test for significant differences due to treatments. The result showed that bromelain from a 50\% saturation level of ammonium sulphate had the best of specific enzyme activity compared to other treatments. The addition by 5\% of bromelain enzyme increased DMD, OMD, and CPD (P<0.05). In contrast, the addition of bromelain had no significant effect on CFD compared to the control (P>0.05). To conclude, the addition of purified bromelain in terms of the milk replacer increased the nutrient digestibility of milk replacer.

1. Introduction
The necessity of a newborn lamb to consume colostrum is expected to increase immunity. The amount of milk needed by a lamb during the weaning period is quite high so that it can cause problems if the ewe is unable to produce milk to supply the needs of its offspring, especially if the ewe gives birth twins 2, 3, or even 4. The rumen in pre-ruminant phase has not yet developed. Milk obtained by pre-ruminant from its ewe is directly digested in the abomasum, without going through digestion in the rumen [1]. In this phase, abomasum provides a vital role in the supply of nutrients. Many efforts were made to maintain the life of a lamb and increase its growth, one of which is the feeding of milk replacer that contains various types of feed ingredients used as a substitute for ewe’s milk. The obstacle experienced in substitute milk which is composed of vegetable protein is the value of crude protein digestibility is lower than the milk protein material so that the needs of lambs may not be fulfilled. An alternative is needed to increase the digestibility value of crude protein by adding the protease enzyme. One type of
protease enzyme is bromelain. Bromelain is an inexpensive alternative protease derived from pineapple plants. The enzymatic process of bromelain is influenced by several factors, such as enzyme specifications, the level protein denaturations as a substrate according to substrate and enzyme concentrations, pH, temperature, and the presence of inhibitors [2].

Based on this description, it is necessary to research the addition of protease enzymes to milk replacers which are expected to be able to increase the digestibility value of nutrients, especially the constituent proteins in milk substitutes. The research aimed to determine the addition of bromelain in increasing the protein digestibility of milk replacer for lamb by the in vitro method.

2. Materials and methods

2.1. Bromelain Extraction
Pineapple as a source of the bromelain enzyme and distilled water with a ratio of 1:1 blend for three minutes, then filtered. The filtrate obtained was centrifuged at 10,000 rotation per minute (rpm) for 20 minutes at 4°C. The supernatant is crude bromelain extract [3].

2.2. Partial Purification
The crude bromelain extract was precipitated using ammonium sulfate on different levels (30%, 40%, 50%, 60%, and 70%). The samples were stirred on cold conditions and kept on the refrigerator for 24 hours until the precipitate was formed [4][5]. The samples from partial purification then dialyzed. The protein content and the enzyme activity of the pellets obtained were tested.

2.3. Determination of Milk Replacer Feedstuff nutrients by Proximate Analysis
Proximate analysis performed includes analysis of crude protein (Kjeldahl method), crude fiber analysis, dry matter analysis, and analysis of organic matter [6].

2.4. Making Milk Replacer
Milk replacer consists of 20% crude protein content from the mixture of soybean meal and milk skim. The design of this study used a complete randomized design with different levels of enzyme addition to milk replacer, which presented in Table 1.

| Table 1. The composition of milk replacer |
|------------------------------------------|
| Milk replacer   | The levels of bromelain enzyme (%) |
|                | 0  | 5  | 10 |
| Soybean meal (gram) | 0.22 | 0.22 | 0.22 |
| Skim milk (gram)   | 0.28 | 0.28 | 0.28 |
| Bromelain enzyme   | 0  | 0.025 | 0.05 |

2.5. Determination of Milk Replacer Digestibility by In Vitro Method
The analytical procedure was a 0.5-gram sample (dry matter) into the in vitro tube then each sample was incubated with 15 ml of 0.1 N HCl containing 2000 U pepsin/ml for 45 minutes at 40°C. Then, added 5 ml of 1 M NaHCO₃ containing 2 mg pancreatin/ml and incubated for 2 hours at 40°C, then filtered using filter paper. Residues obtained were used for the analysis of dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), and crude fiber digestibility (CFD).

2.6. Data Analysis
ANOVA analysed data according to one way completely randomized design and continued by Duncan multiple range test for significant differences due to treatments [7].
3. Result and discussion

3.1. Enzyme activity of Pineapples bromelain extracts at different purification levels
Table 2 shows the protein content, bromelain activity, specific activity, purification factor, and bromelain recovery. There was a significant (P<0.05) increase in the specific activity of bromelain after every purification stage.

| Levels of Ammonium sulfate (%) | Total protein (mg) | Total activity (U) | Specific activity (U/mg) | Purification factor | Recovery (%) |
|--------------------------------|-------------------|-------------------|--------------------------|-------------------|--------------|
| 0                             | 69.75±1.19a       | 35.12±0.53a       | 0.50±0.01c               | 1                 | 100          |
| 30                            | 11.12±1.07d       | 9.90±0.88d        | 0.89±0.01b               | 1.78              | 28.19        |
| 40                            | 13.19±1.11c       | 12.14±1.20c       | 0.92±0.03b               | 1.84              | 34.57        |
| 50                            | 12.42±0.80d       | 16.64±1.17b       | 1.34±0.01a               | 2.68              | 47.38        |
| 60                            | 17.78±2.54b       | 17.95±1.55b       | 1.03±0.05b               | 2.06              | 51.11        |
| 70                            | 8.30±0.96d        | 7.63±0.28d        | 0.94±0.08b               | 1.88              | 21.73        |

a, b, c, d Different superscripts in the same column show significant differences (P<0.05)

Table 2 shows that the highest total protein and total activity in the saturated bromelain enzyme is in the fraction of 60% saturation, respectively 17.78 mg and 17.95 U. The highest specific activity of the dialyzed bromelain enzyme is found in the fraction with 50% saturation of 1.34 U/mg with a purification factor up to 2.68 times. The lowest total activity, specific activity, and purification factor were found in fractions with 70% saturation. The previous study [8] showed that the activity of the bromelain enzyme in the crude extract of pineapples without salting-out was 4.71 U/ml. The other study showed that the enzyme-specific activity in the bromelain was 1.521 U/mg [9]. The specific activity of bromelain enzyme which had undergone saturation, partial purification, and purification using filtration gel with 20-40% ammonium sulfate fraction was 79.2 U/mg whereas with 40 to 60% ammonium sulfate fraction was 164.7 U/mg [10]. Based on the result, the saturation level of ammonium sulfate which is suitable for salting out the bromelain enzyme from pineapples is 50%. The addition of 50% ammonium sulfate causes the separation of water molecules that are not bound to the protein enzyme, resulting in the precipitation of enzymes. Gacesa and Hubble [11] explained that solution of sulfate ions (SO\(_4^{2-}\)) would bind most of the water molecules and the amount of water bound to protein molecules will decrease.

3.2. In Vitro Digestibility of Milk Replacer
Table 3 shows the nutrients digestibility (crude protein, organic matter, dry matter, and crude fiber digestibility) of milk replacer by the addition of bromelain enzyme at different levels.

| Parameters                  | 0 (n=3)            | 5 (n=3)            | 10 (n=3)           |
|-----------------------------|--------------------|--------------------|--------------------|
| Crude protein digestibility (%) | 67.56 ± 0.16c      | 74.33 ± 0.60b      | 80.54 ± 0.18a      |
| Organic matter digestibility (%) | 65.28 ± 0.21c      | 70.50 ± 0.01b      | 76.58 ± 0.14a      |
| Dry matter digestibility (%) | 65.54 ± 0.14c      | 68.08 ± 0.02b      | 74.89 ± 1.40d      |
| Crude fiber digestibility (%) | 68.17 ± 0.09       | 67.75 ± 0.13       | 67.50 ± 0.24       |

a, b, c Different superscripts in the same line show significant differences (P<0.05)

The addition by 5% of bromelain enzyme could increase dry matter digestibility, organic matter digestibility, and crude protein digestibility (P<0.05). The highest digestibility of crude protein, organic matter, and dry matter was shown at the 10% level, with each value were 80.54%, 76.58%, and 74.89%, respectively. Meanwhile, the lowest digestibility of crude protein, organic matter, and dry matter was shown at 0% level (control), respectively 67.56%, 65.28%, and 65.4%. The previous study showed that the crude protein, organic matter, and dry matter digestibility of milk replacer contained 64% soybean
meal were 77.5%, 70.4%, and 65.7% [12]. Bromelain can hydrolyze proteins because it can recognize the carbonyl side of several amino acids, such as lysine, alanine, tyrosine, cysteine, and glycine [13]. The addition of 10% bromelain enzyme showed the best digestive response. Dry matter consists of organic substances (carbohydrates, fats, proteins, vitamins) and inorganic substances (minerals), so the digestibility value of organic matter is also expected to increase the digestibility value of dry matter. According to the previous study, the increase in the digestibility of organic matter is in line with the increase in the digestibility of dry matter [14].

In contrast, the addition of bromelain had no significant effect on crude fiber digestibility compared to the control (P>0.05). Fiber digestion almost does not occur in pre-weaned cattle because the rumen has not yet developed; as a result of the unavailability of enzymes to digest fiber. The other study explained that in pre-ruminant, carbohydrates ingested in the small intestine are limited because the rumen has not functioned. Carbohydrates that digested in the small intestine are only lactose. The addition of the enzyme amylglucosidase in milk replacer containing sorghum improves growth performance [1].

4. Conclusion
The addition of purified bromelain in terms of the milk replacer could increase the nutrient digestibility of milk replacer.

Acknowledgement
The authors acknowledge Universitas Gadjah Mada for financial support under the scheme of Rekognisi Tugas Akhir (RTA) under contract No.2129/UNI/DITLIT/DIT-LIT/LT/2019.

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