Heating Effect on Rumen Digestion of Protein Feeds Fermented by Lactid Acid Bacteria

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Abstract. This study aimed to measure the in vitro digestibility of protein feeds as a product of Lactic acid bacteria (LAB) fermentation with different levels of molasses addition and incubation time. The LAB fermentation of protein feeds experiment had treatments given in the form of addition of molasses with levels of 0%, 3%, and 5% as well as at different incubation times of 0, 1, and 2 weeks. The best result of LAB fermentation was then protected from the rumen digestion by a heating treatment, carbohydrate, and fat addition. The parameter observed includes levels of pH and lactic acid production for LAB fermentation of protein feeds experiment and dry matter (DMD), organic matter (OMD), and crude protein digestibility (CPD) for protected rumen digestion experiment. Result showed that the lowest pH were 4.65±0.02, reached at the treatment with one week incubation (p<0.05) and 0% molasses addition (p<0.05). The highest lactic acid content were 1.82±0.10 mg/g fermentation feed based on treatment one weeks incubation (p<0.05) and 0% molasses addition (p>0.05). The highest content of protein showed at the treatment with two weeks incubation (p>0.05) and 5% molasses addition (p<0.05). Heating treatment had no significant compared to control, with the result respectively DMD 42.48±4.08%; 38.62±6.31%, OMD 53.21±4.74%; 49.71±5.62%, and CPD 38.15±4.71%; 34.88±2.72% (p>0.05). Fermentation could improve the nutrient quality of concentrate, but further research is needed to find ways to protect fermented concentrate.

1. Introduction
The feed is an essential component in the livestock industry. However, Indonesia's humid climatic conditions have the potential to cause the growth of destructive microbes or pathogens in the feed so that the feed will not only be quickly damaged but also cause a decrease in feed quality. The fermentation is expected to improve the quality, palatability, and bioavailability nutrient of feed. Fermentation by lactic acid bacteria would produce bacteriocin compounds, organic acids, and hydrogen peroxide, which can inhibit the growth of damaging microbes in a feed [1]. The use of yeast tape in ruminants could increase milk production by an average of 4.3% and an average body weight gain of 8.7% [2]. Other research showed that the fermentation process in kiambang concentrate using tempeh yeast could reduce levels of crude fiber and increase levels of crude protein, while also being able to increase the biomass of broilers [3].
A feed with high-quality nutrients needs to be protected from the fermentation process in the rumen and can be optimally utilized by the host animals in ruminant animals. One treatment for protection is by heating. The heating process causes a Maillard reaction involving sugar and amino acids so that it escapes the fermentation process in the rumen. Research by Moshtaghi Nia and Ingalls [4] reported that heating treatment of canola flour with a temperature of 127°C for 15 minutes could reduce the level of amino acid degradation in the rumen and increase the availability of amino acids for degradation in the small intestine with a level of Lysine availability of 75%, Histidine 154% in the rumen incubation process for 16 hours.

Based on the results of various studies, it is necessary to conduct research on fermentation with the addition of molasses and incubation time at different levels in high protein source concentrates using a combination of tempeh yeast, tape yeast, and lactic acid bacteria to obtain the highest quality concentrate, both in terms of nutrients and storage capacity. The fermentation results are then given a heating treatment to determine the level of degradation in the rumen.

2. Materials and methods

2.1. Feed fermentation
The fermented protein supplement mixture consists of three types of feed ingredients, i.e., fish meal, cornflour, and soybean meal with predetermined proportions. The composition of fermented concentrate presented in Table 1.

| Feedstuffs          | Treatment |
|---------------------|-----------|
|                     | I         | II        | III        |
| Fish meal (kg)      | 0.5       | 0.5       | 0.5        |
| Soybean meal (kg)   | 0.5       | 0.5       | 0.5        |
| DDGS meal (kg)      | 2         | 2         | 2          |
| Water (ml)          | 1.450     | 1.430     | 1.400      |
| Molasses (ml)       | 0         | 90        | 180        |
| LAB inoculants      | 50        | 50        | 50         |
| Yeast tape          | 50        | 50        | 50         |
| Yeast tempeh        | 50        | 50        | 50         |

The fermented concentrate was then dried at 55°C and then analyzed levels of lactic acid, pH, dissolved protein (Lowry protein), DM, OM, and CP. The results then used as a basis for the selection of fermented concentrates on in vitro Tilley and Terry one stage.

2.2. Chemical quality test
The fermented feed sample is opened and placed in a container, then mixed until evenly distributed. The pH, lactic acid, dissolved protein levels of the samples from the fermentation then examined, and proximate analysis is conducted.

2.2.1. The degree of acidity (pH)
The fermented pH value was measured by taking 1 g of sample and adding 10 ml of distilled water; then the pH value was measured using a pH meter that calibrated at pH 4 and 7. The electrodes on the pH meter were carefully washed and dried. The electrode dipped in the sample, and the pH value is determined until the number reaches a stable position.

2.2.2. Determination of protein content
The dissolved protein content analyzed using Lowry method [5]. The absorbance read at λ 750 nm. The protein content is calculated by entering the absorbance into the following standard curve equation:

\[ Y = 1.9058X + 0.0412 \]
Y: absorbance of the sample
X: protein content (mg/ml)
Protein content (mg/100ml) = (X. dilution factor)/(supernatant volume) x100

2.2.3. Determination of lactic acid levels
Preparation and determination of lactic acid levels were carried out by the Barker and Summerson methods [6]. The solution was to read absorbance using a spectrophotometer at a wavelength of 560 nm. Lactic acid levels are measured by the standard linear regression equation of lithium lactate:

\[ Y = ax + b \]

Y: absorbance
X: levels of lactic acid (mg/ml)

2.2.4. Proximate analysis
Fermented feed samples were taken and analyzed for chemical composition includes DM, OM, and CP [7].

2.3. Heating fermented feedstuff
The fermented concentrate chosen was the one that has the best quality, which has the highest lactic acid content. The concentrate was then taken by sampling and then divided into two groups, namely heating and without heating. The formulation for heating treatment presented in Table 2.

| Feedstuff       | Total (kg) | Proportion (%) |
|-----------------|------------|----------------|
| Fermented feed  | 4.85       | 63.81          |
| Rice bran       | 0.9        | 11.84          |
| Tapioca         | 0.9        | 11.84          |
| Palm oil        | 0.75       | 9.87           |
| Salt            | 0.1        | 1.32           |
| Premix mineral  | 0.1        | 1.32           |
| **Total**       | **7.6**    | **100**        |

2.4. In vitro digestion test
A modified Tilley and Terry [8] in vitro one-stage digestibility test was used to reflect digestion in the rumen. The process was carried out by incubation for 48 hours to describe digestion in the rumen. The digestion process in the artificial rumen lasts for 48 hours, then harvesting for DMD, OMD, and CPD analysis.

2.5. Data analysis
The data obtained include levels of lactic acid, pH, levels of dissolved protein (Lowry protein), DMD, OMD, and CPD. Data on the degree of acidity (pH), levels of lactic acid and dissolved protein were analyzed by factorial completely randomized design, continued by Duncan multiple range test for significant differences due to treatments. The DMD, OMD, and CPD were analyzed by the T-test. The application used to analyze the research design is the Statistical Product and Service Solution (SPSS) program version 16.0 for Windows.

3. Results and discussion
3.1. The degree of acidity (pH) of concentrate fermentation
Data pH of concentrate fermentation presents in Table 3. There was no effect of cinnamon bark meal added up to 4.5% on IVDMD and IVOMD.
Table 3. Effect of incubation time and molasses concentration on pH

| Molasses concentration | 0%         | 3%          | 5%          | Mean        |
|------------------------|------------|-------------|-------------|-------------|
| 0 week                 | 5.12±0.01  | 5.12±0.01   | 5.12±0.01   | 5.12±0.01   |
| 1 week                 | 4.65±0.02  | 4.74±0.02   | 5.06±0.24   | 4.82±0.22   |
| 2 weeks                | 4.75±0.10  | 5.10±0.15   | 5.23±0.02   | 5.03±0.23   |
| Mean                   | 4.84±0.22  | 4.99±0.20   | 5.14±0.15   | 4.99±0.22   |

ns = Different superscripts in the same column show real differences (p<0.05)
ab,c = Different superscripts in the same row show real differences (p<0.05)

diff = Not significant

Table 2 showed that the lowest pH is obtained at a combination of one week incubation time treatment and 0% molasses level. The higher addition of molasses level gives a higher final pH value (p<0.05). The lowest pH was found in fraction with incubation time one week, but the pH rises slightly in the second week (p<0.05). A previous study [9] reported that fermented soybean meal with Aspergillus and Lactobacillus produced a final pH of 4.5 ± 0.6. The addition of molasses level gives a higher final pH value, this is caused by the buffering capacity of molasses. The addition of molasses 5% did not decrease pH significantly, contrast with the treatment of adding molasses 1%. The decrease of pH fermentation by the addition of molasses gives insignificant results due to the buffer action of molasses [10]. The increase in pH at two weeks incubation due to NH₃ production by lactic acid bacteria. Several strains of Lactobacillus, Enterococcus, and Pediococcus were able to produce NH₃ from arginine [11].

3.2. Dissolve protein content of concentrate fermentation

Table 4. presents the dissolved protein content of the fermented concentrate treated with different levels of molasses and incubation time.

Table 4. Protein content in the fermented concentrate

| Molasses concentration | 0%          | 3%          | 5%          | Mean        |
|------------------------|-------------|-------------|-------------|-------------|
| Week 1                 | 33.94±8.29  | 87.31±6.52  | 109.53±7.47 | 76.92±33.55 |
| Week 2                 | 45.69±3.36  | 99.42±14.85 | 120.21±20.36 | 88.44±35.23 |
| Mean                   | 39.82±8.60  | 93.37±12.55 | 114.87±15.52 | 82.68±34.30 |

ab,c = Different superscripts in the same row show real differences (p<0.05)
s = Not significant

Based on Table 2 showed that the higher level molasses addition, in line with the dissolved protein content (p<0.05). The incubation time had no significant effect (p>0.05) on the dissolve protein content. The highest dissolve protein content was obtained by adding 5% molasses with levels of 114.87 ± 15.52 mg / g. Research from Teng et al. [12] reported that the content of dissolved protein in soybean meal fermented with Aspergillus oryzae was 24.15%, greater than the dissolved protein content of soybean meal without fermentation which was 20.22%. Other research reported that microbes in the fermentation process produced protease enzymes capable of degrading complex proteins into simple proteins with smaller molecular sizes and higher solubility [13].

3.3. Lactic acid levels of concentrate fermentation

Table 5 presents the lactic acid content of the fermented concentrate by treating different levels of molasses and incubation time.

Table 5. Effect of incubation time and molasses concentration on lactic acid levels

| Molasses concentration | 0%          | 3%          | 5%          | Average     |
|------------------------|-------------|-------------|-------------|-------------|
| 0 week                 | 0.40±0.13   | 0.52±0.04   | 0.72±0.20   | 0.55±0.19   |
| 1 week                 | 1.82±0.10   | 1.25±0.44   | 0.84±0.15   | 1.30±0.49   |

ab = Different superscripts in the same row show real differences (p<0.05)
Based on table 2, showed that the incubation time of one week and molasses level of 3% is the best treatment. One-week incubation gave the highest lactic acid fermentation results (p<0.05), a decrease occurred at 2 weeks incubation. The higher level of molasses addition can increase the production of lactic acid, but the production of lactic acid decreases at the level of 5% addition. These results are in line with the research of Dumbrepatil et al. [10] who reported that the addition of molasses to the level of 190 g/l was able to increase the production of lactic acid up to 0.95 g/g, but the production of lactic acid decreased to 0.94 g/g at the addition of 240 g/l molasses. This condition is caused by inhibition of the growth of lactic acid bacteria due to excess molasses. Research from Wee et al. [14] reported that the addition of molasses to the level of 267 g/L increase the growth of lactic acid bacteria, but the higher addition of molasses concentration actually decreases the growth of lactic acid bacterial cells.

### 3.4 In Vitro Rumen Digestibility of Concentrate Fermentation

Table 6. presented the nutrient digestibility (DMD, OMD, CPD) of fermented concentrate which is given heating treatment and without heating.

|                  | Heating         | Non-Heating    |
|------------------|-----------------|----------------|
| DMD\textsuperscript{ns} | 42.48±4.08      | 38.62±6.31     |
| OMD\textsuperscript{ns} | 53.21±4.74      | 49.71±5.62     |
| CPD\textsuperscript{ns} | 38.15±4.71      | 34.88±2.72     |

\textsuperscript{ns} = not significant

Table 4 showed that the heating and non-heating treatments were not significantly different (p>0.05) to DMD, OMD, and CPD from the fermentation concentrate in the rumen. Some studies reported that soybean meal which is heated at 95 °C for one hour has a CP\textsuperscript{D} level in the rumen of 35.4% [15], DMD with heating 165 °C of 51.26% [16]. According to the references, the results showed that the digestibility of fermented concentrate treated with heating and without heating had almost the same value.

### 4. Conclusion

The fermentation process improves the quality of the nutrients of the feedstuff. Heating treatment had no significant effect on DMD, OMD, and CPD.

### Acknowledgments

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

### References

[1] Helander I M, Wright A V and Sandholm T M M 1997 Potential of lactic acid bacteria and novel antimicrobial against gram-negative bacteria J. Food Sci. and Technol, 8 146-150

[2] Wina E 2000 Pemanfaatan ragi (yeast) sebagai pakan imbuhan untuk meningkatkan produktivitas ternak ruminansia Wartazoa 9(2) 50–56

[3] Zaman Q, Suparno G and Hariani D 2013 Pengaruh kiambang (Salvinia molesta) yang difermentasi dengan ragi tempe sebagai suplemen pakan terhadap peningkatan biomassa ayam pedaging LenteraBio 2(1) 131-137

[4] Moshtaghi N S A and Ingalls J R 1995 Influence of moist heat treatment on ruminal and intestinal disappearance of amino acids from Canola Meal Journal of Dairy Science 78(7) 1552–1560

[5] Alexander R R and Griffith J M 1993 Basic Biochemical Methods (2nd ed.) (New York: Wiley-
[6] Oser B L 1965 *Hawk’s Physiological Chemistry* (14th ed.) (New York: McGraw-Hill)

[7] AOAC 2005 *Official Method of Analysis* (Washington DC: Association of Official Analytical Chemists)

[8] Tilley J M A and Terry R A 1963 A two-stage technique for the in vitro digestion of forage crops *J. Brit. Grassl. Soc.* 18 104-111

[9] Chen C C, Shih Y C, Chiou P W S and Yu B 2010 Evaluating nutritional quality of single stage-and two stage-fermented soybean meal *Asian-Aust. J. Anim. Sci* 23(5) 598-606

[10] Dumbrepatil A, Adsul M, Chaudhari S, Khire J and Gokhale D 2008 Utilization of molasses sugar for lactic acid production by *Lactobacillus delbrueckii* subsp. *delbrueckii* mutant Uc-3 in batch fermentation *Appl. Environ. Microbiol* 74(1) 333-335

[11] Rashid H, Togo K, Ueda M and Miyamoto T 2007 Identification and characterization of dominant lactic acid bacteria isolated from traditional fermented milk *Dahi* in Bangladesh. *World J. Microbiol. Biotechnol* 23 125-133

[12] Teng D, Gao M, Yang Y, Liu B, Tian Z, and Wang J 2012 Bio-modification of soybean meal with *Bacillus subtilis* or *Aspergillus oryzae* Biocatalysis and Agricultural Biotechnology 1 32-38

[13] Amodou I, Kamara M T, Tidjani A, Foch M B K and Guo-Wei L 2010 Phisicochemical and nutritional analysis of fermented soybean protein meal by *Lactobacillus plantarum* Lp6. *World J. Dairy & Food Science* 5(2) 114-118

[14] Wee Y J, Kim J N, Yun J S and Ryu H W 2004 Utilization of sugar molasses for economical *L(+)lactic acid production by batch fermentation of Enterococcus faecalis* Enzyme and Microbial Technology 35 568-573

[15] Castro S I B, Phillip L E, Lapierre H, Jardon P W and Berthiaume R 2007 Ruminal degradability and intestinal digestibility of protein and amino acids in treated soybean meal products *J. Dairy Sci.* 90 810-822

[16] Nowak W, Michalak S and Wylegala S 2005 *In situ* evaluation of ruminal degradability and intestinal digestibility of extruded soybeans *Czech J. Anim. Sci.* 50(6) 281-287