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The effect of protected soybean groats and soybean oil as feed supplement on total gas production

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Abstract. The study evaluated the influence protected soybean groats and soybean oil as feed supplement on total gas production. Protected feed supplement was produced from soybean groats protected by formaldehyde and soybean oil, through two protection methods, namely saponification and microencapsulation. The experiment consisted of three treatments i.e. P0: basal diet (control); P1: basal diet + 5 % protected feed supplement and P2: basal diet + 10 % protected feed supplement. Each treatment was repeated 10 times. The observed variables were total gas production for 48 hours, gas production from fraction a and b, and degradation rate of fraction b (c value). Data were analyzed by analysis of variance. Protected soybean groats and soybean oil decreased of total gas production for 48 hours P<0.05), but did not affect gas production from fraction a, fraction b and the degradation rate of fraction b (c value). It can be concluded that the effect of supplementation of soybean groats and soybean oil decreased total gas production in the rumen and did not affect gas production from fraction a, fraction b and degradation rate of fraction b (c value) and could strategically be used to decrease methane emission from rumen fermentation in vitro.

1. Introduction

Energy and protein are the main nutrients needed for hormone synthesis, tissue growth and repair, milk synthesis and other physiological functions to meet basic life needs, growth, milk production and animal reproduction [1]. Increasing energy intake in ruminants can be done by increasing feed energy using fat (oil), while protein intake can be increased by providing protected proteins that can escape degradation by rumen microbes [2]. Soybean oil and soybean groats are potential sources of protein. They both have the advantages such as high protein content, digestibility, and ration palatability. However, soybean groats without protection degraded in by rumen microbes. Therefore, protection for soybean groats was needed to make bypass protein. Soybean oil protection was needed to prevent unsaturated fatty acids from biohydrogenation process by rumen microbes [1]. Protection is also useful to eliminate the negative effects of high concentration of unsaturated fatty acid supplementation, and reduction in fiber degradability [3]. The aim of this study was to evaluate protected soybean oil and soybean groats as feed supplement on total gas production.
2. Material and methods

2.1 Sample preparation
The ration was used in this research consisted of elephant grass, basal concentrate (rice bran, tofu waste, coconut meal, coffee pulp, pollard, vitamins, cassava waste, minerals, and husk), protected soybean oil through two protection methods namely saponification and microencapsulation and soybean groats protected using formaldehyde as much as 1% of the dry matter for soybean groats. Rumen fluid was used as a microbial source. Rumen fluid was obtained from male SimPO (Simmental Ongole Cross Breed) which were slaughtered at the Jagalan Surakarta Slaughterhouse.

2.2 Experimental design
This study was designed as completely randomized design with 3 treatments with 10 replicates. The treatments were P0: 40% elephant grass + 60 basal concentrate; P1: 40% elephant grass + 50 basal concentrate + 10% protected soybean oil and soybean groats; P2: 40% elephant grass + 45 basal concentrate + 15% protected soybean oil and soybean groats.

2.3 Data analysis
The data obtained in this study were analyzed using analysis of variance to determine the effect of treatment on the variables observed. If there is a treatment effect then proceed with Duncan's Multiple Range Test (DMRT) to determine the differences between treatments.

3. Result and discussion

3.1 Total gas production for 48 hours
Total gas production describes microbial activity in degrading the rations in the rumen [5]. The results of the analysis of variance showed that the protected soybean groats and soybean oil in the diet reduced of total gas production in the rumen (P <0.05, Table 1).

| Group | Treatment | P0 | P1 | P2 |
|-------|-----------|----|----|----|
| 1     | P0        | 22.738 | 24.298 | 22.071 |
| 2     | P0        | 27.252 | 24.298 | 23.492 |
| 3     | P0        | 27.169 | 27.314 | 29.094 |
| 4     | P0        | 29.927 | 28.822 | 29.261 |
| 5     | P0        | 37.785 | 33.011 | 30.432 |
| 6     | P0        | 29.760 | 29.492 | 29.094 |
| 7     | P0        | 31.265 | 28.319 | 31.769 |
| 8     | P0        | 25.720 | 25.303 | 24.886 |
| 9     | P0        | 29.844 | 29.157 | 28.592 |
| 10    | P0        | 34.525 | 30.665 | 31.1 |
| Mean  | P0        | 29.598 | 28.068 | 27.862 |

\[a, b\] Different superscript in the same row show significant effect on treatment (P <0.05)

The decrease in total gas production at P1 and P2 was due to the use of formaldehyde to protect proteins. Protein protection by using formaldehyde caused the formation of methylene bonds with proteins that surround the outer layer of the protein matrix, thus proteins bound to formaldehyde are not easily soluble in water. The chemical bond decreased substrate degradation by rumen microbes, led to decrease in gas production.

Declining total gas production was due to the use of protected soybean oil in the P1 and P2 rations resulting in low methane gas. This finding was consistent with previous research that fat significantly reduces the percentage of methane in total gas [5]. Fat reduces methane emissions through several
mechanisms, namely reducing the fermentation of organic matter and reducing the activity of methanogens and the number of protozoa. Especially fats rich in fatty acid content, the mechanism for reducing methane emissions is not saturated through the reaction of hydrogenation groups in unsaturated groups as hydrogen acceptors [8]. This result is in line with the previous research where addition of 6% soybean oil to Ongole breeders reduced methane gas by 37.43% [7].

![Figure 1](image-url)  
**Figure 1.** Total gas production with 48 hours incubation time

### 3.2 Fraction a

Fraction a is a fraction that is easily soluble and degraded by microbes [8]. The results of the analysis of variance showed that the mixture of soybean and protected soybean oil in the ration had no significant effect on the fraction a.

| Group | Treatment | P0       | P1       | P2       |
|-------|-----------|----------|----------|----------|
| 1     |           | -2.290   | -3.658   | -2.508   |
| 2     |           | -4.644   | -4.851   | -5.219   |
| 3     |           | -2.613   | -2.266   | -3.571   |
| 4     |           | -5.268   | -3.206   | -3.254   |
| 5     |           | 0.121    | 1.949    | 3.262    |
| 6     |           | 1.869    | -0.251   | 0.442    |
| 7     |           | -2.856   | -2.360   | -3.170   |
| 8     |           | -3.124   | -3.506   | -3.66    |
| 9     |           | -1.745   | -1.747   | -1.423   |
| 10    |           | -1.367   | -0.23    | -0.003   |
| Mean  |           | -2.192   | -2.013   | -1.910   |

The result of fraction a did not differ between treatments because the used of soybean groats and soybean oil in the ration P1 and P2 did not affect differences in the solubility properties of fraction a in. Soluble ingredients contain most soluble carbohydrates and proteins that are easily degraded in the rumen. Carbohydrates and proteins are divided into four fractions based on the nature of solubility in the rumen, one of which is a fraction which consists mostly of sugar, nitrate, ammonia, amines, amino acids, and nucleic acids. The fraction a is mostly found in the nitrogen-free extract (NFE) fraction [9]. Carbohydrates are divided into two components namely crude fiber and NFE. The NFE contains substances mono, di, tri and polysaccharide and all of them are easily soluble and have a high digestibility. The fraction a value describes nutrients that are easily soluble and degraded in the rumen.
which is the contents of plant cells, the higher the content of plant cells in a feed will also have a high fraction [10]. The fraction of each treatment produces a negative fraction value a. A negative value can occur due to the phase lag when rumen microorganisms degrade feed [11].

3.3 Fraction b
Fraction b is a fraction that is insoluble and slow degraded by rumen microbes, especially structural carbohydrates composed of cellulose, hemicellulose, and lignin [11]. The results of the analysis of variance showed that the protected of soybean groats and soybean oil in the ration had no significant effect on the fraction b.

### Table 3. Mean of fraction b (ml/200 mg CF) for 48 hours of incubation

| Group | Treatment | P0   | P1   | P2   |
|-------|-----------|------|------|------|
| 1     | P0        | 25.028 | 27.955 | 24.579 |
| 2     | P0        | 31.897 | 29.148 | 28.712 |
| 3     | P0        | 29.782 | 29.580 | 32.665 |
| 4     | P0        | 35.195 | 32.028 | 32.515 |
| 5     | P0        | 37.664 | 31.062 | 27.170 |
| 6     | P0        | 27.891 | 29.743 | 27.481 |
| 7     | P0        | 34.121 | 30.680 | 34.939 |
| 8     | P0        | 28.844 | 28.809 | 28.546 |
| 9     | P0        | 31.589 | 30.904 | 30.015 |
| 10    | P0        | 35.891 | 30.895 | 31.103 |

Mean 31.790 30.080 29.772

The addition of unsaturated fat affected fiber digestibility [12]. Barriers to fiber degradation take place through cloaking which inhibits direct contact of microbes or cellulytic enzymes with feed particles [13]. Fraction b value is suspected because the use of protected of soybean groats and soybean oil in P1 and P2 does not interfere with microbial activity in fiber degradation. This is consistent with the opinion that the protection of unsaturated fatty acids with saponification pathways through the formation of calcium salts can eliminate the negative influence of degradation of fiber [14]. The use of calcium soap-protected soybean at a level of 5% results in fiber digestibility that is not significantly different [15]. Fraction b is influenced by the content of fiber components, especially the cell wall fraction, the decrease in fraction b is in harmony with the crude fiber content of each treatment ration [16].

3.4 Degradation rate of fraction b (c value)
The c value is the rate of gas production from a fraction that is not dissolved and slowly degraded (fraction b). The results of the analysis of variance showed that the protected of soybean groats and soybean oil in the ration had no significant effect on the value of c.

The c value was not different between treatments because protected of soybean groats and soybean oil in the P1 and P2 rations did not cause changes in the constituent components of the fraction b of the treatment ration. Types of structural carbohydrates, especially the constituent components of the cell wall and physical form of ration which are relatively the same, can cause the fermentation pattern in the rumen to be the same so that the value of c is not different [17]. The cell wall component is divided into two easily digested fractions consisting of hemicellulose and difficult to digest fractions consisting of cellulose, lignin, and silica [18]. The rate of degradation is lower if the cell wall content is high. The rate of degradation of fraction b was influenced by the fiber content of the feed ingredients, the higher the cell wall content of a feed material could reduce the rate of degradation [19]. The rate of feed degradation was determined by several factors including nutrient composition, length of stay in the rumen and availability of substrate for microbial activity in degrading feed in the rumen [20]. The degradation rate is also determined by the characteristics of the constituent feed ingredients. The ration
which is dominated by the soluble fraction will give a different value of c with a ration that contains many degraded potential fractions. The high level of fiber fraction in feed and lignification at a later stage can also reduce the activity of food degradation by rumen microbes, the amount of lignin content will of course indirectly reduce the rate of degradation of fraction b [21].

| Table 4. Mean of c value (ml/hours) for 48 hours incubation |
|-------------------------------------------------------------|
| Group | Treatment |   |
|-------|-----------|---|
|       | P0        | P1 | P2 |
| 1     | 0.067     | 0.073 | 0.073 |
| 2     | 0.081     | 0.084 | 0.088 |
| 3     | 0.074     | 0.072 | 0.079 |
| 4     | 0.067     | 0.070 | 0.069 |
| 5     | 0.081     | 0.074 | 0.073 |
| 6     | 0.069     | 0.075 | 0.072 |
| 7     | 0.071     | 0.071 | 0.068 |
| 8     | 0.074     | 0.075 | 0.079 |
| 9     | 0.068     | 0.072 | 0.070 |
| 10    | 0.076     | 0.073 | 0.070 |
| Mean  | 0.073     | 0.074 | 0.074 |

4. Conclusion
Supplementation of soybean groats and soybean oil decreased the total gas production in the rumen but did not affect gas production from fraction a, fraction b and degradation rate of fraction b (c value) and could strategically be used to decrease methane emission from rumen fermentation in vitro.

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