Running title: *M. oleifera* leaf to substitute soybean meal in young growing meat goats

***Moringa oleifera* leaf for replacing protein portion of soybean meal in the diet of young growing meat goats**

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**ABSTRACT**

*In vitro* ruminal feed fermentability test and feeding experiment were conducted to clarify the substitution of soybean meal with *Moringa oleifera* leaf powder (MOlp) in diet of young growing goats. Five *M. oleifera* based feeds were studied in the test of *in vitro* ruminal fermentability: 0% MOlp, 25% MOlp, 50% MOlp, 75% MOlp and 100% MOlp. All feeds were designed to be isoprotein and isoenergy, containing 20% crude protein and 71 - 75% TDN. In the feeding experiment, three dietary experiments were offered to young growing Jawarandhu goats, aged 3 – 5 months with average body weight of 10 kg. The dietary experiments were 0% MOlp, 25% MOlp and 75% MOlp. All dietary experiments were designed to be isoprotein and isoenergy, containing 20% crude protein and 71 - 75% TDN. Result of the *in vitro* ruminal feed fermentability showed that feed dry matter and crude protein digestibilities, ruminal concentrations of total VFA and NH3, and ruminal total protein production increased (p<0.05) in line with the level of MOlp in the experimental feeds. Result of feeding experiment revealed that nutrient intakes and body weight gain were not different (p>0.05) among treatment groups. Blood concentrations of protein, urea, ammonia, glucose, triglycerides, and cholesterol were similar in all groups. The dietary treatment did not effect significantly (p>0.05) on some hematological parameters. *M. oleifera* leaf could replace protein portion of soybean meal in the diet of young growing goats without negative effect on productive performances.

*Keywords: Dietary protein, M. oleifera, Productive performance, Young goats*

**INTRODUCTION**

Goats are known to be active in selecting feed through browsing and grazing in extensive system, but good quality feed must be provided in an intensive management. Soybean meal is commonly used as a source of protein concentrate for ruminant feed. The cost and availability of soybean meal, pose a significant challenge to animal producers, particularly those who are involved in small farms. Consequently, there is a need for substitute ingredients that have a high protein content, a balanced amino acid profile, and are reasonably priced.

Some studies are attempted to substitute protein portion of soybean meal in the diet of goats. Peanut cake from biodiesel waste does not significantly change nutrient intake and after feeding blood metabolites levels, although peanut cake is not equal and complete substitute for soybean meal in diet of goat kids (Mariniello Silva et al., 2015). De Asis et al. (2018) reported that nutrient utilization and growth performance in goat kids is not altered when soybean meal portion in diet is replaced with cotton seed flake. Soybean meal portion could be replaced with dried distiller grains in the ration of lactating goats throughout the first four month lactation period, because feed intake and digestibility and some blood metabolites are unchanged (Pontes et al., 2020). Soya waste could be total substitute for soybean meal in the ration of goat kids, because it does not affect feed intake and digestibility, and productive performance (Rahman et al., 2020).

The soybean meal replacement is purposed to create more economical dietary formulation, because soybean meal represents a large proportion of the feeding cost and majority of the production cost. The seek for lower-cost alternatives to replace the common feedstuff is importance for gaining the profit. Although the crude protein content is lower than that of soybean meal, *M. oleifera* leaf has an advantage because of its content of bioactive substance. In addition, *M. oleifera* is widely distributed in the tropics and the leaf production is available throughout the year. Its amino acid composition is almost complete, and *M. oleifera* is also known to contain growth promoters, which are suitable for young ruminants (Soliva et al., 2005). However, there is a little data available on *M. oleifera* as a protein source of diet in young growing ruminant. The study was purposed to make assessment of soybean meal substitution for *M. oleifera* leaf in the diet of young growing meat goats.

**MATERIALS AND METHODS**

*In vitro* study and feeding experiment were carried out to assess the substitution of soybean meal protein with *M. oleifera* leaf powder in the feed of young growing goats. As the recommendation of Titi et al. (2000) and Atti et al. (2004), the experimental diet in this study was designed to contain 20% crude protein and 71-75% TDN, so that goat kids aged 3-5 months may achieve their optimum growth.

***In vitro* Study**

Five feeds based on *M. oleifera* leaf powder (MOlp), which were tested for *in vitro* ruminal fermentability, namely 0-MO (0% MOlp), 25-MO (25% MOlp), 50-MO (50% MOlp), 75-MO (75% MOlp) and 100-MO (100% MOlp). All feeds were designed to be isoprotein and isoenergy, containing 20% crude protein and 71 - 75% TDN (Table 1). The *in vitro* test was carried out using the batch culture technique, using goat rumen fluid and buffer solution with volumes of 10 ml and 40 ml, respectively (Harahap et al. 2019). Each feed treatment was repeated five times. Ruminal fermentability parameters include feed dry matter and protein digestibility, rumen VFA and NH3 concentrations, total rumen protein production (Harahap et al. 2019).

**Feeding Experiment**

Local growing female Jawarandhu goats, average age and body weight of 3-5 months and 10 kg respectively, were used in the feeding experiment. The tested feeds included MO-0 (0% MOlp), MO-25 (25% MOlp) and MO-75 (75% MOlp). These three dietary treatments were designed to be isoprotein and isoenergy, containing 20% crude protein and 71 - 75% TDN (Table 2). The concentrate feed was offered at morning feeding, and each goat also received 140 g (DM basis) elephant grass (*Pennisetum purpureum*) at afternoon feeding. Each treatment group contained eight goats, placed in individual pens and drinking water was provided continuously. After a two-month adaptation period to the dietary treatment, each treatment goat was observed regarding feed consumption for ten days, then ended with weighing their body weight and taking blood samples via the jugular vein. Blood samples for analysis of the content of some metabolite and hematological parameters.

**Chemical Analyses**

In the *in vitro* study, rumen liquid concentrations of total VFA and NH3 were estimated according to methods of steam distillation and Conway, respectively. Rumen protein production was measured using method of tricarboxylic acid and salysilic acid precipitation. In the feeding experiment, feed concentration of proximate components were analyzed by the method of AOAC. Blood protein concentration was measured using Lowry method, blood ammonia and urea were determined using analytic kits (DiaSys Diagnostic Systems, GmBH, Holzeim). Blood glucose, triglycerides and cholesterol concentrations were assayed using analytic kits (PT Bavaria Combinindo, Jakarta). Haematological parameters were estimated using impedance electrodes (Auto Analyzer Animal Blood Counter Vet 18p, Germany).

**Statistical Analyses**

The treatments of experimental feed and experimental diet in *in vitro* study and feeding experiment, respectively, were alotted according to the completely randomized design. The respective data from *in vitro* study and feeding experiment were analyzed using one way anova.

**RESULTS AND DISCUSSION**

***In vitro* Ruminal Feed Fermentability**

The increasing level of *M. oleifera* in the feed enhanced the ruminal feed fementability, because dry matter and crude protein digestibilities, ruminal VFA and NH3 concentrations, and rumen total protein production increased (p<0.05) according to the increasing level of *M. oleifera* (Table 3).

Protein portion of soybean meal could be replaced by M. oleifera leaf in the diet of goats. Jayanegara et al. (2010) found that M. oleifera can be a potential alternative supplement to replace conventional concentrate in ruminant diets, as it showed in the in vitro ruminal fermentation pattern. The *in vitro* ruminal feed fermentability test is aimed at assessing whether M. oleifera leaf-based feed can support the rumen environment, especially to support optimum rumen microbial growth. It is well known that rumen microbial proteins have great support to the protein synthesis of ruminant products. The concentrations of rumen tFVA and NH3 were ranged 90-110 and 16-25 mM, respectively (Table 3). The M. oleifera based diet in this study may promote ruminal tVFA and NH3 and concentrations adequate for optimal microbial growth as observed by Kanyinji et al. (2009), Kazemi, M. and A. Mokhtarpour (2021) and de Evans et al., (2022). Soliva et al. (2005) found that Moringa leaves have a high crude protein content and can support microbial protein synthesis in the rumen. Melesse et al. (2013) compared the in vitro nutrient digestibility of M. stenopetala and Moringa oleifera and found that M. oleifera leaves had higher effective utilisable crude protein than M. stenopetala leaves.

**Feeding experiment**

M. Oleifera contains almost no anti-nutritional factors that can affect its palatability (Soliva et al., 2005), therefore the dry matter and several nutrients intakes did not differ among treatment groups (Table 4). In addition, the dietary treatments were designed to be isoprotein and isoenergy (Table 2). Kholif et al. (2015) reported that replacing sesame meal partially with M. oleifera increased nutrient intake. The decrease in dietary intake was influenced by replacing soybean meal with peanut cake (Mariniello Silva et al., 2015). Replacing soybean meal with cotton seed cake did not affect intake (de Asis et al., 2018). Replacing soybean meal with dried distiller grain with soluble did not affect intake (Pontes, 2020). Rahman et al. (2020) reported a decrease in intake as a result of replacing soybean meal with soy waste. Substitution of soybean meal with lower quality feed ingredients results in reduced consumption of dry matter and some nutrients. In other words, nutrient quality of M. oleifera and soybean meal are almost similar.

Blood concentrations of protein, urea, ammonium, glucose, triglycerides and cholesterol were unaffected by level of M. oleifera in diets (Table 4). These are in line with facts that nutrient intakes of young goats were unchanged after adaptation to experimental diets. Kholif et al. (2015) reported that replacing sesame meal with M. oleifera increases blood urea and cholesterol concentrations in lactating dairy goats, because nutrient intakes are also increased. Mariniello Silva (2015) stated that replacing soybean meal with peanut cake do not effect on blood urea and glucose concentrations in goat kids, although the dietary intake decreases. Supplementation of M. oleifera extract enhances nutrient utilization in lactating goats and increases blood glucose concentration, and decreases blood trglycerides and cholesterol concentrations (Kholif et al., 2018). Subtitution of soybean meal with dried distiller grain with soluble unaffects feed intake in lactating goats, and blood glucose and urea concentratios remains unchanged (Pontes et al., 2020). Chanjula et al. (2022) reported that dietary intake in lactating goats is not affected by replacing soybean meal with fermented palm kernel cake, and blood glucose and urea are also unaffected.

Goats were in good health condition during experimental period, because some hematological parameters were unaffected by soybean meal substitution with M. oleifera leaf (Table 4). It is well known, white blood cells are composed of granulocytes (neutrophils, eosinophils, and basophils) and non-granulocytes (lymphocytes and monocytes). White blood cells are a major component of the body's immune system. Zaher et al. (2020) reported, replacing daily concentrate feed with M. oleifera leaves to a level of 100% do not affect the blood hematologic profile of growing goats, although replacement starting at a level of 50% reduces feed intake.

**CONSLUSION**

Replacing protein portion of soybean meal with M. oleifera meal unaffects ruminal feed fermentability, productive performances, and hematological parameters in young growing goats. It is suggested M. oleifera leaf may have similar nutritive quality to that of soybean meal.

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Table 1. Ingredients and chemical composition of experimental feeds in the *in vitro* study (% of dry matter)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Experimental feeds | | | | |
| 0-MO | 25-MO | 50-MO | 75-MO | 100-MO |
| Ingredients |  |  |  |  |  |
| Cassava waste | 18.50 | 5.00 | 1.00 | 1.00 | 00.00 |
| Molase | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Maize grain | 14.00 | 32.00 | 40.00 | 36.00 | 34.00 |
| Soybean meal | 45.00 | 33.75 | 22.50 | 11.30 | 0.00 |
| Rice bran | 18.50 | 10.00 | 1.00 | 1.00 | 0.00 |
| *M. oleifera* leaf powder | 0.00 | 15.56 | 31.10 | 46.70 | 62.30 |
| Mineral-vitamin premix | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
|  |  |  |  |  |  |
| Chemical composition |  |  |  |  |  |
| Crude protein, % | 20.24 | 20.16 | 19.95 | 19.60 | 19.27 |
| Total digestible nutrient1, % | 75.48 | 75.06 | 73.40 | 71.22 | 69.20 |

1Calculated total digestible nutrient based on Harris *et al*. (1972).

0-MO: feed with 0% of *M. oleifera* leaf powder protein. The 25-MO, 50-MO, 75-MO, and 100-MO experimental feeds contained 25%, 50%, 75%, and 100% of *M. oleifera* leaf powder protein, respectively.

Table 2. Ingredients and chemical composition of experimental diets in the feeding experiment

|  |  |  |  |
| --- | --- | --- | --- |
|  | Experimental diets | | |
|  | MO-0 | MO-25 | MO-75 |
| Ingredients |  |  |  |
| Cassava waste | 18.00 | 5.00 | 1.00 |
| Molase | 2.00 | 2.00 | 2.00 |
| Maize grain | 14.00 | 32.00 | 36.60 |
| Soybean meal | 45.00 | 33.50 | 11.30 |
| Rice bran | 18.50 | 10.00 | 1.00 |
| *M. oleifera* leaf powder | 0.00 | 15.50 | 46.70 |
| Mineral-vitamin premix | 2.00 | 2.00 | 2.00 |
|  |  |  |  |
| Chemical composition |  |  |  |
| Dry matter, % | 88.69 | 87,49 | 88,09 |
| Crude protein, % | 20.24 | 20.05 | 19.60 |
| Ether extract, % | 3.96 | 3.52 | 2.77 |
| Crude fibre, % | 10.05 | 10.68 | 16.82 |
| Nitrogen free extract, % | 57.24 | 58.13 | 53.69 |
| Total digestible nutrient1, % | 75.48 | 74.82 | 71.22 |

1Calculated total digestible nutrient based on Harris *et al*. (1972).

MO-0: diet with 0% of *M. Oleifera* leaf powder protein. The MO-25 and MO-75 experimental diets contained 25% and 75% of *M. Oleifera* leaf powder protein, respectively.

Table 3. Results of *in vitro* ruminal fermentability on experimental feeds

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Experimental feeds | | | | | Significant  value |
| 0-MO | 25-MO | 50-MO | 75-MO | 100-MO |
| DM digestibility, % | 64.28±2.44 | 70.04±2.61 | 72.57±4.47 | 74.81±2.92 | 77.11±2.51 | p<0.05 |
| CP digestibility, % | 46.66±2.74 | 52.32±3.13 | 57.18±2.22 | 61.55±1.66 | 64.52±3.13 | p<0.05 |
| Total VFA, mM | 89.20±2.77 | 95.00±5.29 | 99.00±1.00 | 105.80±4.38 | 111.20±4.15 | p<0.05 |
| NH3, mM | 16.40±1.29 | 18.44±0.37 | 19.21±0.73 | 21.34±1.21 | 25.36±1.78 | p<0.05 |
| Protein production | 298.70±2.10 | 302.63±3.09 | 309.47±4.49 | 320.77±5.03 | 332.26±14.77 | p<0.05 |

0-MO: feed with 0% of *M. Oleifera* leaf powder protein. The 25-MO, 50-MO, 75-MO, and 100-MO experimental feeds contained 25%, 50%, 75%, and 100% of *M. Oleifera* leaf powder protein, respectively.

Table 4. Nutrient intakes, some blood metabolite and hematological parameters, and body weight gain in experimental goats

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Experimental diet groups | | | Significant  value |
|  | MO-0 | MO-25 | MO-75 |
| 1Nutrient intakes, g.d-1 |  |  |  |  |
| Dry matter | 300.47±22.47 | 277.54±19.05 | 355.66±39.62 | NS |
| Crude protein | 60.82±4.55 | 55.65±3.82 | 69.71±7.77 | NS |
| Extract ether | 11.90±0.89 | 9.77±0.67 | 9.86±1.10 | NS |
| Crude fiber | 32.09±2.40 | 29.64±2.03 | 59.82±6.66 | NS |
| Nitrogen free extract | 172.03±12.91 | 161.28±11.08 | 191.01±21.29 | NS |
| Total digestible nutrient | 226.86±17.00 | 207.56±14.28 | 253.39±28.15 | NS |
|  |  |  |  |  |
| Hematological parameters, x103 |  |  |  |  |
| Total leucocyte | 11.49±0.50 | 11.31±0.45 | 11.68±0.35 | NS |
| Neutrophyl | 0.37±0.06 | 0.41±0.09 | 0.43±0.09 | NS |
| Eosinophlyl | 0.58±0.04 | 0.60±0.02 | 0.59±0.04 | NS |
| Lymphocyte | 10.51±0.44 | 10.27±0.44 | 10.43±0.43 | NS |
|  |  |  |  |  |
| Blood metabolite parameters | | | |  |
| Protein, g.dl-1 | 2.33±0.16 | 2.31±0.12 | 2.16±0.10 | NS |
| Ureum, mg.dl-1 | 60.74±0.64 | 58.48±1.58 | 58.39±1.70 | NS |
| Ammonium, mg.dl-1 | 1.33±0.13 | 1.43±0.09 | 1.38±0.05 | NS |
| Glucose, mg.dl-1 | 68.48±1.91 | 67.25±1.56 | 67.93±1.85 | NS |
| Triglycerides, mg.dl-1 | 106.63±1.96 | 108.37±1.48 | 109.60±4.82 | NS |
| Cholesterol, mg.dl-1 | 102.30±5.36 | 99.46±2.62 | 99.73±7.67 | NS |
|  |  |  |  |  |
| Body weight gain, g.d-1 | 45.44±5,25 | 44.82±5.63 | 51.47±7.83 | NS |

1Intakesof concentrate feed merely.

MO-0 diet group received 0% of *M. oleifera* leaf powder protein. The MO-25 and MO-75 groups received experimental diets containing 25% and 75% of *M. oleifera* leaf powder protein, respectively.

NS: not significant