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In Vitro Analysis of Parijoto (*Medinilla speciosa*) Wild Fruit Extract Feed Digestibility in *Capra hircus*

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SUMMARY

The purpose of this study was to determine the digestibility value of parijoto fruit extract supplemented in feed based on the values of Dry matter digestibility (DMD), organic matter digestibility (OMD), volatile fatty acid (VFA), and ammonia (NH_3) given to Etawah crossbreed goats in vitro. A total of 12 Etawah crossbreed goats were used in this study. Parijoto fruit extract was added to the feed as much as 0% (T0), 5% (T1), 10% (T2), and 15% (T3) of the total dry matter of the basal diet. The basal diet was prepared to meet the nutritional needs of lactating dairy goats with 11–12% crude protein and 55% total digestible nutrients consisting of elephant grass (60%) and concentrate (40%). The basal diet was composed of soybean pulp (34, 88%), rice bran (23, 26%), coconut meal (23, 26%), onggok (11, 63%), and crude palm oil (CPO) (6, 96%). This study used a completely randomized design consisting of 4 treatments and 3 replications. The treatments applied were as follows: T0 = basal diet, T1 = basal diet + 5% parijoto fruit extract, T2 = basal diet + 10% parijoto fruit extract, and T3 = basal diet + 15% parijoto fruit extract. The parameters calculated in this study were DMD, OMD, NH_3, and total VFA. The results showed that the addition of parijoto fruit extract to the feed had a significant effect ($P < 0.05$) on the value of DMD, OMD, and NH_3 and was not significantly different ($P > 0.05$) for the value of VFA. The conclusion obtained based on the results of the study is that the higher the provision of parijoto fruit extract, the lower the values of OMD and NH_3. However, the value of DMD and VFA has the highest value in parijoto fruit extract supplementation at a dose of 150 mg compared to other doses and controls.

Keywords: In Vitro, Fermentation, *Capra hircus*, Digestible, Parijoto

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INTRODUCTION

Feed is one of the important factors in determining the level of animal productivity, especially in reproductive activities. Reproductive disorders can reduce animal productivity, such as low heat quality, resulting in a decrease in fertility. Free radicals can damage membranes and reduce the function of reproductive organs. The age factor that is too old also causes organ function to be impaired. One of the factors that affect fertility quality is the provision of rations with good nutritional value (Halmemies et al., 2018). Rumen performance, microorganism populations, and fermentation patterns are influenced by the type of feed derived from concentrates and forages (Wanapat, 2000). According to Niwińska and Andrzejewski (2011), the digestive system of

ruminants is characterized by microbial fermentation before entering the stomach and intestines. Ruminants have four stomachs: the rumen, where microbial fermentation occurs; the reticulum and omasum, which function as filters; and the abomasum, where enzymatic digestion occurs. In addition, the type of feed given also affects the digestibility value of feed ingredients, which affects the level of synthesis of feed ingredients given to livestock. The addition of feed that has good nutritional quality and can cure reproductive disorders that are safer, environmentally friendly, and affordable.

Parijoto fruit is one of the herbal plants with local wisdom that has the potential to increase fertility, which includes aphrodisiac plants. Aphrodisiac plants are plants that are used as sexual stimulants and increase low libido. The content of parijoto fruit is flavonoids, saponins, terpenoids, tannins, cardenolon, and beta-carotein (Maria et al., 2012). Flavonoids have potential as antioxidants that function in counteracting free radicals (Kumar and Pandey, 2013). According to Arowolo and He (2018), the addition of feed supplementation in the form of tannins, saponins and a combination of tannins and saponins has a higher organic matter digestibility (OMD) value compared to feed that is not given any supplementation (control). Sheep supplemented with the saponin content of 6.0 g per day in *Yucca schidigera* did not have a significant effect ($P > 0.05$) in reducing the Dry matter digestibility (DMD) value (Canul et al., 2014). The digestibility value is influenced by the factor of active compounds given in the feed (Chura et al., 2018). Supplementation of parijoto fruit extract in animal feed has never been done before. So, it is expected that this study can determine the effect of parijoto fruit extract if given to animal feed by looking at the digestibility value (OMD and DMD), VFA and NH_3 in vitro in Etawah crossbreed goats.

MATERIALS AND METHODS

The material used was 12 Etawah crossbreed goats. Parijoto (*Medinilla speciosa*) fruit was obtained from Muria, Kudus, and Central Java and extracted at Perjuangan University of Tasikmalaya and Diponegoro University. The potential of the Parijoto fruit bioconversion product as a feed supplement was evaluated by adding to the concentrate as much as 0% (T0), 5% (T1), 10% (T2), and 15% (T3) of the total dry matter of the basal diet. The basal diet was prepared to meet the nutritional needs of lactating dairy goats with 11–12% crude protein and 55% TDN consisting of corn straw (60%) and concentrate (40%). The basal diet was composed of soybean pulp (34.88%), rice bran (23.26%), coconut meal (23.26%), onggok (11.63%), and crude palm oil (CPO) (6.96%). All feed ingredients used in the in vitro fermentation basal diet have been analyzed for nutritional composition and dry matter content in the basal diet presented in Table 1. The experimental design used was a completely randomized design with 3 treatments and 4 replications. The treatments applied were as follows: T0 = basal diet, T1 = basal diet + 5% parijoto fruit extract, T2 = basal diet + 10% parijoto fruit extract, and T3 = basal diet + 15% parijoto fruit extract.

PARIJOTO FRUIT EXTRACTION

Parijoto fresh fruit as much as 7 kg in the oven at 30°C for 24 hours, then mashed to powder (simplisia). Simplisia, as much as 2000 g, was macerated with 20 l of 70% ethanol (1:10). Maceration was carried out for 5 days and stirred every day for 15

minutes, after which it was filtered to separate the pulp and filtrate. The filtrate was evaporated to obtain a thick extract (Wijayanti *et al*., 2017).

STAGE I: DIGESTION

One gramme of sample was mixed with eight millilitres of rumen fluid and twelve millilitres of McDougall's solution in a fermenter tube, which was then placed in a shaker bath at 39 degrees Celsius. The tubes were shaken for 30 seconds with CO_2 at a pH of 6.5-6.9, then covered with vented rubber and fermented for 24 hours. After 24 hours, the fermenter's rubber cover was opened, and 2-3 drops of $HgCl_2$ were applied to kill the bacteria. For 10 minutes, the fermenter tube was centrifuged at 4,000 rpm. The substrate was divided into two parts: silt at the bottom and a clear supernatant on top. The supernatant was collected for future analysis (NH_3 or VFA). The substrate was used for dry matter (DM) and organic matter (OM) digestibility analyses.

DRY MATTER AS WELL AS ORGANIC MATTER DIGESTIBILITY MEASUREMENTS

The centrifuge residue was mixed with 20 ml of a 0.2% pepsin-HCl solution after being spun at 4,000 rpm for 15 minutes. The mixture was then incubated for 24 hours without the use of a rubber lid. A hoover pump was used to filter the digestive waste through Whatman No. 41 filter paper. A porcelain cup was used to hold the filter. Drying in an oven for 24 hours produced the dry material. In addition, the material in the cup was burned or fumigated in an electric furnace for 6 hours at 450-600 ^oC. As a blank, the residue from the fermentation was used without a sample of feed ingredients.

VFA CONCENTRATION MEASUREMENT

The VFA concentration was measured by the steam distillation method. The same supernatant as the NH_3 analysis was taken as 5 ml and then put into a distillation tube. H_2SO_4 15% was added, then immediately covered with a rubber cap with a hole and connected to a cooling flask. Immediately after adding H_2SO_4 to the supernatant, the distillation tube was put into a distillation flask containing boiling water (heated continuously during distillation). The hot water vapour will urge the VFA and will be condensed in the cooler. The water formed is collected in an Erlenmeyer flask containing 5 ml of 0.5 N NaOH until it reaches 300 ml. PP (Phenolphthalein) indicator is added as much as 2-3 drops and titrated with 0.5 N HCl until the colour of the titrate changes from pink to colourless.

DRY MATTER DIGESTIBILITY (DMD) AND ORGANIC MATTER DIGESTIBILITY (OMD) IN VITRO

Digestibility of dry and organic matter using the method of Tilley and Terry (1963). A total of 1 g of the tested ration was put in a fermenter tube, along with 10 ml of rumen fluid and 40 ml of artificial saliva solution (McDougall) shaken with CO_2 to create an anaerobic atmosphere with a pH of 5–6.9. Incubated for 24 hours in a water bath at 39^oC. After 24 h, the lid of the fermenter tube was opened, and 0.2 ml of saturated $HgCl_2$ solution was added to kill the microbes (Al-Arif *et al*., 2017). The tube was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded, and the precipitate was added to 50 ml of a 0.2% pepsin solution in an acidic atmosphere.

Incubate again in an aerobic atmosphere for 24 h. Then the precipitate was filtered with Whatman 41 paper and analysed for dry matter and organic matter content. As a blank, rumen fluid without treatment was used.

NH_3 MEASUREMENT TECHNIQUE

The technique of measuring NH_3 levels is read using spectronics, which is based on the catalysed reaction of indophenols to produce a stable blue compound. Spectronic readings are taken at a wavelength of 630 nm (Beigh and Ganai, 2014). The rumen fluid came from the rumen of goats taken from a goat slaughterhouse in the Semarang area. The collection of rumen fluid was done by taking the contents of the rumen and putting them in a thermos that previously contained hot water, but the hot water was immediately discarded when the contents of the rumen were put into the thermos. In the laboratory, the contents of the rumen were squeezed, and the liquid was filtered using gauze. The filter results were put into a thermos previously filled with warm water (warm water was discarded when the rumen liquid was put in the thermos) and continued to be supplied with CO_2.

DATA ANALYSIS

The data obtained were analysed with an ANOVA with SPSS software version 25.0 and if they were significantly different, then further tested with Duncan's test. P <0.05 indicates a significant correlation.

RESULTS AND DISCUSSION

Preliminary research was conducted by examining the dry matter (DM) content of the feed. The feed used was commercial feed and corn straw. This analysis was conducted to determine the composition of feed substances used in in vitro fermentation. The results of the analysis can be seen in Table 1.

Table 1: Feed substance composition of forage and concentrate used for in vitro fermentation.

Composition (50:50)	Feed Ingredients			
	Corn Straw (1)	Corn Straw (2)	Commercial concentrate (1)	Commercial concentrate (2)
DM	86.88	86.88	87.09	87.09
Ash	10.20	5.10	12.91	6.46
CP	10.42	5.21	12.39	6.20
CF	1.78	0.89	4.62	2.31
CFi	27.40	13.70	15.23	7.62
BETN	50.20	25.10	54.85	27.43
Ca	1.24	0.62	1.10	0.55
P	0.10	0.05	0.63	0.32
TDN	60.00	30.00	65.26	32.63

Description: DM (dry matter), CP (crude protein), CF (crude fat), CFi (crude fiber), BETN (extracted material without nitrogen), and TDN (total digestible nutrients).

Based on the previously conducted study, it was demonstrated that the contents of the Parijoto fruit in qualitative terms were flavonoid (+), saponin (+), tannin (+),

antioxidants (+), terpenoid (+), and beta-carotene (+). The contents were flavonoid (2.65% b/b), saponin (4.91% b/b), antioxidant activity (20.65 ppm), total polyphenols (12.68%), and tannin (11.70%) (Wijayanti and Ardigurnita, 2018).

FEED DIGESTIBILITY RATE IN GOAT RUMEN

This study involved giving commercial feed and wild parijoto fruit extract according to the dose given to goats (*Capra hircus*) in vitro. The results of the analysis of dry matter digestibility (DMD) and organic matter digestibility (OMD) in the rumen of goats given Parijoto fruit extract are presented in Table 2.

Table 2: Results of Dry matter digestibility (DMD) and organic matter digestibility values.

Digestibility	Dose of Parijoto Fruit (mg/head)			
	0	150	200	250
Dry matter (%)	45.45±5.43[a]	45.87 ±5.29[a]	33.67±1.78[b]	35.43±5.16[b]
Organic matter (%)	44.73±5.68[a]	43.32±5.16[a]	31.03±1.97[b]	30.69±4.43[b]

[a,b] Different superscripts in the same row indicate significant differences ($P < 0.05$).

The analysis showed that the DMD of feed from a mixture of commercial feed and parijoto fruit extract showed a significant difference ($P < 0.05$). Table 2 shows that *Capra hircus*species supplemented with parijoto fruit extract at a dose of 200 mg/head were not significantly different from the dose level of 250 mg/head, but the DMD value was significantly different ($P < 0.05$) with feed digestibility in the rumen of *Capra hircus species* that were not given parijoto fruit extract at a dose of 150 mg/head. The lowest feed digestibility value was 33.67% in the rumen of goats given parijoto fruit extract at a dose of 200 mg/head. The highest DMD value was 45.87% in the rumen of goats that were given additional feed parijoto fruit extract (150 mg/head) compared to goats (*Capra hircus*) that were not given additional feed parijoto fruit extract. Giving excessive doses of parijoto fruit extract can leave DM residues so that a lot of feed is not degraded completely. The higher DMD value of feed indicates that livestock hasto digest the feed given well. The value of feed digestibility in goats given parijoto fruit extract at 150 mg/head is not significantly different from goats that are not given parijoto fruit extract. This indicates that with a dose level of 150 mg/head, parijoto fruit extract is still safe to be given to goats. Parijoto fruit has secondary compounds such as flavonoids, saponins, tannins, terpenoids, and antioxidants (Wijayanti and Ardigurnita, 2018). The content of saponins and flavonoids in parijoto fruit helps in the process of feed digestion in the rumen if given at a safe dosage level.

The OMD value showed a significant difference ($P < 0.05$) between goats fed with parijoto fruit extract (200 and 250 mg/head) and goats fed with a dose level of 150 mg/head and goats without parijoto fruit extract. The digestibility of organic matter that has the highest value is in goats that are not fed with parijoto fruit extract, which is 44.736%. This is because there is no addition of active substances in the treatment, so there is no increase in water content. However, the control results are not much different from goats given parijoto fruit extract at a dose level of 150 mg/head (Table 2). The lowest value in the treatment of goats given additional feed with a dose level of 250 mg/head had an OMD value of 30.696%. The decrease in OMD value is caused because the parijoto fruit extract contains tannin compounds. The tannin content in

parijoto fruit extract is 11–70% (Wijayanti and Ardigurnita, 2018). According to research by Bhatta *et al*. (2013), the digestibility value of organic matter can decrease due to the addition of pure tannin at a level of 13% in the ratio. Tannins form complex bonds with proteins, carbohydrates (cellulose, hemicellulose, and pectin), minerals, vitamins, and microbial enzymes in the rumen (Barman and Rai, 2008.). The addition of parijoto fruit extract to the rumen of goats affects changing the digestibility values of DMD and OMD in the feed consumed by goats. The digestibility of organic matter shows the amount of nutrients such as fat, carbohydrates and protein digested by livestock (Noziere et al., 2010).

Ruminants are livestock that have a special stomach because four stomachs help the livestock digestion process. The rumen is a stomach where several rumen microbes assist in numerous fermentation processes. Without the microbes in the rumen, the feed digestion process does not run well. The feed consumed by goats has high SK, which is difficult for livestock to digest. However, the help of microbes in the rumen can help digest feed properly. Well-digested feed can directly produce a lot of food substances that are circulated in the bodies of livestock. Feed quality and digestibility value are important factors in livestock reproduction. Good-quality feed and the ability of livestock to digest feed can increase goat fertility. The higher the Body Condition Score (BCS) value, the better the reproductive ability of the livestock. Factors affecting the value of feed digestibility are the composition of feed ingredients and the nutritional content of feed (Shurson *et al*., 2021). The higher the content of the feed given, the higher the digestibility value of the resulting feed. Good feed digestibility values of DMD and OMD indicate that goats can digest feed well. According to Pinotti *et al*. (2021), the higher the digestibility value of a feed material, the higher the quality of the feed material.

The treatment of parijoto fruit extract had a significant effect on the digestibility of DM and OM ($P < 0.05$). According to Ebrahim and Negussie (2020), plant secondary compounds such as saponins and tannins at low levels do not cause interference with ration digestibility. When there is too much parijoto fruit extract in the feed, the digestibility value goes down. This is because the tannins in parijoto fruit make it harder for the animal to digest crude fibre and protein. Parijoto fruit extract in this study is a solution to inhibit the negative impact in the form of direct toxic effects on rumen microbes. It was said by Wijayanti and Ardigurnita (2018) that the extract of parijoto fruit has secondary compounds like terpenoites and phenolics that are antibacterial and antifungal. The digestibility value of DM in this study was higher than the digestibility of OM, presumably because the ration had a low insoluble ash content. Ash degradation in BK is low, and the ability of microbes to degrade BK components is higher, causing DMD to be higher than OMD (Jairath et al., 2023). The digestibility value of DM did not show a significant difference ($P > 0.05$) at a dose of 150 mg/head with the digestibility value of DM without the provision of parijoto fruit extract. This is due to the treatment with parijoto fruit extract, and the dose level given. The composition of forage in the research ration is quite high, so the rumen environmental conditions are stable for rumen microbial activity. Saponin and tannin compounds in parijoto fruit extract did not cause disturbances in ration digestibility.

NH_3 AND VFA VALUES

Feed fermentation is seen in the values of NH_3 and VFA in the rumen of goats that have been given parijoto fruit extract at different dose levels. The results of feed fermentation can be seen in Table 3.

Table 3: NH_3 and VFA Values of Goats Treated with Parijoto Fruit Extract

Digestibility	Dose of Parijoto Fruit (mg/head)			
	0	150	200	250
NH_3	2.58±0.34[a]	1.55±0.17[b]	2.01±0.54[ab]	1.88 ±0.13[b]
VFA	86.66±.15.27	96.66±11.54	73.33±20.81	73.33±20.81

[a,b] Different superscripts in the same row indicate significant differences ($P < 0.05$).

The response of the combination of parijoto fruit extract supplementation with different dose levels to feed fermentability in vitro can be seen in Table 3. The response of feed fermentability in vitro includes the production of VFA and NH_3. Supplementation of parijoto fruit extract with a dose level of 150 mg/head is significantly different (P <0.05) with a dose level of 250 mg/head and on the value of NH_3, which is not given parijoto fruit extract. However, the NH_3 value with a dose level of 150 mg/head was not significantly different from the dose level of 200 mg/head. The lowest NH_3 production occurred in parijoto fruit extract supplementation, which amounted to 1.55 mM. The highest NH_3 value occurred in parijoto fruit extract, which was 2.58 mM. According to research by Sadi et al. (2019), feeding a mixture of white teak leaves and corn kernels to goats with a dose of R1 550.39 g/head/day is significantly higher ($P < 0.05$) than the consumption of goats R2 414.51 g/head/day, with NH values of R1 4, 27 mM, and R2 7, 16 mM. The decrease in NH_3 value supplemented with parijoto fruit extract is due to the content in parijoto fruit being an antimicrobial drug. According to McDonald et al. (2002), the process of microbial synthesis can be optimal if the value of the ammonia concentration is between 6 and 21 mM. The availability of carbohydrates in the ration as energy for protein formation is the main factor that affects ammonia.

VFA production due to treatment can be seen in Table 3. The provision of parijoto fruit extract did not have a significant effect on VFA production ($P > 0.05$). According to McDonald et al. (2002), the type of feed that livestock consumes affects VFA concentration. Total VFA production in this study has met the normal range, which is between 70 and 150 mM. The highest VFA value was 96.666 mM in supplementing parijoto fruit extract with a dose level of 150 mg/head. Supplementation with doses of 200 and 250 mg/head has the same VFA value of 73.3333 mM lower than untreated. The high VFA value is in line with the high DM digestibility and OM digestibility. The higher the level of parijoto fruit extract given, the lower the value of VFA. The results of research by Nurdin and Susanti (2015) show that the use of turmeric and cumin flour, which account for as much as 3% of body weight, contains flavonoids, phenolic compounds, and saponins. These herbal ingredients, which are anti-inflammatory and antioxidant, can improve the ecological balance of the environment by suppressing the number of pathogenic microbes. The addition of parijoto fruit extract to feed causes suppression of bacterial activity. This is due to an

increase in hydrogen accumulation, which results in a change in the reaction from pyruvate to lactate (Gao et al., 2015). The same thing was conveyed by Antonius et al. (2023), the saponin content in *M. oleifera, C. longa Linn.*, and *P. timoriana* are effectively used as a defaunator to reduce the protozoa population and increase the bacterial population in the rumen. It is suspected that the active substance works by forming bonds with sterols contained in the protozoa cell wall, thus affecting the surface tension of the protozoa cell membrane, which results in increased cell wall permeability and fluid from outside the cell entering the protozoa cell. Rumen bacteria that work well play a role in the fermentation process, thereby increasing feed utilisation.

CONCLUSIONS

The conclusion obtained based on the results of the study is based on the results of testing the addition of parijoto fruit extract: the higher the provision of parijoto fruit extract, the lower the OMD and NH_3 values. However, the DMD and VFA values in parijoto fruit extract supplementation at a dose of 150 mg are the highest compared to other doses and controls.

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