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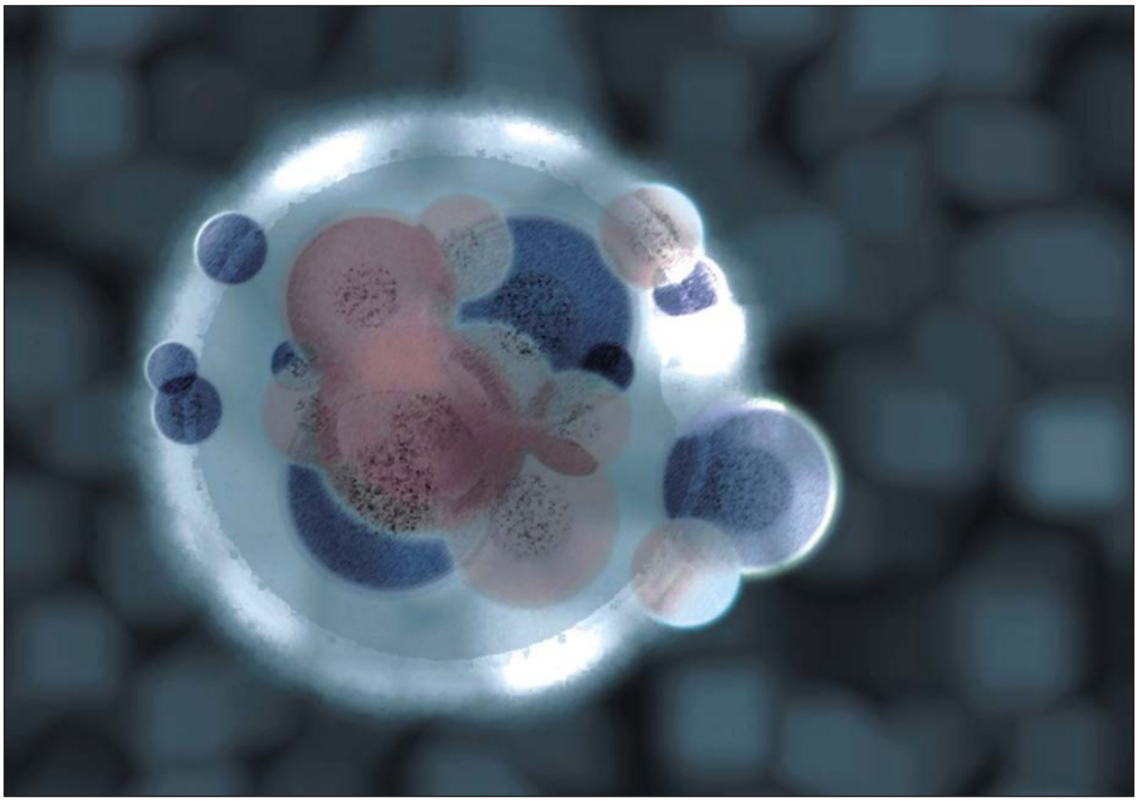

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Research Article

Digestibility of Goat Rations Containing Fermented Oil Palm Fronds by *Phanerochaete chrysosporium* Supplemented With Phosphorus, Sulfur and Magnesium

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Abstract

Background and Objective: The utilization of oil palm fronds (OPFs) as feed is limited due to their high lignin content. The biodelignification of OPFs with *Phanerochaete chrysosporium* plus the elements Ca and Mn can break down lignocellulose and lignin hemicellulose bonds. Supplementation of fermented oil palm fronds (FOPFs) with P, S and Mg results in the highest microbial protein synthesis and VFA concentration. The objective of this study was to evaluate the effect of supplementation with phosphorus, sulfur and magnesium in goat rations containing fermented oil palm fronds (FOPFs; fermented by *Phanerochaete chrysosporium*) on nutrient digestibility. **Materials and Methods:** This research was carried out using a randomized block design with 5 treatments and 3 replications. The following treatments were performed: A = 40% Napier grass (NG)+0% FOPFs+60% concentrate; B = 20% NG+20% FOPFs+60% concentrate; C = 0% NG+40% FOPFs+60% concentrate; D = 20% NG+20% FOPFs +60% concentrate plus P, S and Mg and E = 0% NG+40% FOPFs+60% concentrate plus P, S and Mg. The data were analyzed using a one-way analysis of variance (ANOVA) and differences between treatments were tested using Duncan's multiple range test (DMRT).The observed parameters were digestibility of the nutrients. **Results:** Supplementation with phosphorus, sulfur and magnesium in goat rations that contained fermented oil palm fronds by *Phanerochaete chrysosporium* at concentrations of 40 and 60% resulted in the highest digestibility of the nutrients. **Conclusion:** Supplementation with P, S and Mg in goat rations containing FOPFs is indispensable for the growth and activity of rumen microbes.

Key words: *Phanerochaete chrysosporium*, fermented, digestibility, rumen microbes, lignin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oil palm fronds (OPFs) are a waste product of oil palm production and can potentially be used as animal feed. The nutritional contents of oil palm fronds areas follows: Dry matter (46.02%), organic matter (94.50%), crude protein (2.67%), crude fiber (50.00%), [neutral detergent fiber (NDF) (81.91%)], [Acid Detergent Fiber (ADF) (70.00%)], hemicellulose (11.91%) and cellulose (39.63%)[1]. The utilization of palm fronds as feed is limited because of their high lignin content[2,3]. *Phanerochaete chrysosporium* is a white rot fungus from class *Basidiomycetes* that has the ability to degrade lignin[4]. *P. chrysosporium* growth is affected by the availability of the minerals calcium[5] and manganese[6]. Supplementation of calcium (Ca) and manganese (Mn) during oil palm frond fermentation by *P. chrysosporium* results in greater digestibility of dry matter, organic matter, NDF, ADF and cellulose and produces the highest concentration of VFA[3].

Phosphorus (P) is needed for an animal's tissues, bones and rumen microbes. A low concentration of phosphorus in the rumen will decrease the growth of rumen microbes and will thus decrease the degradation of cellulose[7]. Sulfur (S) is a part of the amino acid cysteine and its dimer cystine and the essential amino acid methionine[8] and is a precursor for the other S-containing amino acids[9]. Magnesium (Mg) is an essential mineral; many physiological and biochemical functions depend on Mg[10]. Magnesium plays an important role as an extracellular ion for nerve transmission[11] and is an essential mineral in ruminants[12]. The biodelignification of OPFs with *P. chrysosporium* plus the elements Ca and Mn can break down lignocellulose and lignin hemicellulose bonds and supplementation of FOPFs with P, S and Mg results in higher levels of microbial protein synthesis and increased VFA concentrations[2].

The nutritional content of Napier grass is as follows: Dry matter (20.52%), crude protein (13.45%), ADF (39.67%), NDF (69.15%) and lignin (6.24%)[1]. Napier grass can be used as a forage substitute in ruminant rations as some researchers have reported for deer[13], dairy cows[14] and goats[15].

Fermented oil palm fronds (FOPFs) produced using *P. chrysosporium* can be used as a substitute for elephant grass up to the level of 40% in ruminant rations[16]. Ammoniated palm leaves supplemented with the minerals S and P as well as cassava leaf flour can replace 100% of the grass fed to beef cattle and provide better performance[17]. The purpose of this study was to evaluate the effect on nutrient digestibility of supplementation with phosphorus, sulfur and magnesium in goat rations containing fermented oil palm fronds by *P. chrysosporium*.

MATERIALS AND METHODS

The present study was conducted at Faculty of Agriculture and Animal Sciences, State Islamic University Sultan Syarif Kasim, Riau, Indonesia during the period extended from November, 2014-February, 2015.

Based on the research of Febrina *et al.*[18], this study used 15 goats aged 1 year with initial body weights ranging from 11.03-13.92 kg. Goats were placed in individual cages and supplied with feed and water. The study was conducted over three periods, namely, the periods of adaptation, introduction and collection. The adaptation period lasted 15 days and aimed to allow the goats to adjust to the experimental environment. The introductory period lasted 15 days and aimed to eliminate the influence of the previous rations fed to the animals. The collection period lasted 7 days, during which data were obtained via faeces collection. Faecal outputs were weighed and representative samples were collected for nutrient analysis. The proximate components were determined as described by the AOAC[19] using FOSS equipment. The predominant fibre residues (hemicellulose, cellulose and lignin) were determined according to the method of Van Soest *et al.*[20] using a FOSS Fibretec system.

The goat rations consisted of Napier grass, rice bran, tofu and FOPFs. Rations contained protein from 12.04-12.64% and [total digestible nutrients (TDN) from 64.58-66.76%. The feeding was done twice a day at 08.00 and 17.00. Feeding was done according to NRC guidelines[21], i.e., adequate to sustain 4% body weight based on the dry matter. Drinking water was provided ad libitum. The ratio of concentrates to forage was 60: 40.

Oil palm frond fermentation: The substrate used in this research was oil palm fronds (OPFs) that had been cut and dried and then finely milled. The source of Ca was $CaCl_2$ and Mn was from $MnSO_4.H_2O$. *Phanerochaete chrysosporium* was maintained on potato dextrose agar (PDA) slants at 4°C and then transferred to PDA plates at 37°C for 6 days and subsequently grown on rice bran. The fermentation process was initiated by adding water to the OPFs so that the water level reached 70% and Ca and Mn were then added. Fermentation lasted for 10 days as described by Febrina *et al.*[3]; the resulting product was dried and P, S and Mg were added according to the specific treatment. The sources of P, S and Mg were Na_2HPO4, $Na_2 SO_4$, and MgO, respectively.

Experimental design and statistical analysis: The research used a randomized block design with 5 treatments and 3 replications. The differences among the treatments were

Table 1: Nutrient contents of rations for each treatment

	Treatments				
Nutrient contents	A	B	C	D	E
Dry matter (%)	56.29	65.39	71.30	65.39	71.30
Crude protein (%)	12.64	12.10	12.04	12.10	12.04
Crude fiber (%)	27.20	28.75	29.97	28.75	29.97
Ether extract (%)	5.10	4.50	3.53	4.50	3.53
Ash (%)	11.38	9.28	6.50	9.28	6.50
NDF (%)	50.80	55.99	62.38	55.99	62.38
ADF (%)	32.92	37.43	41.80	37.43	41.80
Hemicellulose (%)	27.17	25.95	25.12	25.95	25.12
Lignin (%)	6.25	9.16	11.92	9.22	11.92
Cellulose (%)	22.55	24.82	27.47	24.82	27.47
Organic matter (%)	87.62	89.72	92.50	89.72	92.50
TDN (%)	64.58	65.46	66.76	65.46	66.76
Ca (ppm)	3174.89	2297.44	5461.52	3011.23	1738.47
Mg (ppm)	2000.73	1721.04	2050.71	1725.17	1595.84
Fe (ppm)	149.07	232.74	592.95	598.34	767.91
S (ppm)	119.30	127.78	140.95	151.16	155.64
P (ppm)	1650.32	1620.26	1664.90	1615.90	1609.54

evaluated by one way ANOVA. Duncan's multiple range test was used to determine significant ($p<0.05$) differences among the mean values.

The following treatments were performed:

A = 40%NG+0% FOPFs+60%concentrate
B = 20% NG+20% FOPFs+60% concentrate
C = 0% NG+40% FOPFs+60% concentrate
D = 20% NG+20% FOPFs+60% concentrate plus P, S and Mg
E = 0% NG+40% FOPFs+60% concentrate plus P, S and Mg

The nutrient contents of the rations are presented in Table 1.

RESULTS AND DISCUSSION

Nutrient digestibility of all variables was lowest for treatment A, which contained 40% Napier grass and 60% concentrate; the differences were not statistically significant ($p>0.05$) from treatments B and C (except for digestibility of ether extract and crude fiber) but were significantly ($p<0.05$) lower than in treatment D (except for digestibility of ADF and hemicellulose) and treatment E (Table 2).

The low digestibility of treatment A is related to its physical properties such as particle size and density. Treatment A contained Napier grass for which the particle sizes ranges from 3-5 cm; Napier grass also has a low density[22]. Feeding grasses with low density causes the goats to quickly become satiated, thus reducing consumption and resulting in lower digestibility of nutrients. In treatments B, C and D the rations contained FOPFs; the smaller particle sizes (material was powdered) allowed the livestock to accelerate digesta flow, resulting in higher digestibility. Particle size influences digestibility and milk production[23,24].

The lowest digestibility of nutrients (treatment A) is also related with dry matter content and rumen activity. Dry matter content in treatment A was the lowest at 56.29% (Table 1). The low dry matter content in treatment A resulted in the least amount of nutrients entering the digestive tract; consequently, low rumen activity caused the low nutrient digestion. On the other hand, the bulk density of Napier grass caused the low digestibility of nutrients; goats in treatment A were given 40% Napier grass (the highest percentage among the treatments). Digestibility in ruminants is related to the activity of rumen microbes and rumen fermentation[25]. Similar results were reported by Baete and Aregheore[15], who found that Anglo-Nubian x Fiji local goats that were given Napier grass in their rations had lower nutrient digestibility compared to when they were fed Napier grass supplemented with legumes (*Gliricidia sepium*, *Ipomea batatas* and *Leucaena Leucocephala*).

The biodelignification process causes the release of lignin from cellulose and hemicellulose so that they can be utilized by microbes as an energy source. The energy provided can be used by microbes for growth and the production of enzymes. The resulting enzymes in sufficient quantities will allow the microbes to optimally digest nutrients. Treatments B, C, D and E, in which the rations contained FOPFs, resulted in higher digestibility of nutrients than treatment A. This suggests that the biodelignification of oil palm fronds using *P. chrysosporium* can improve the digestibility of nutrients (Table 2). Pretreatment on lignocellulose compounds can improve the digestibility of lignocelluloses biomass[26,27]. Delignification of corn stover by *Ceriporiopsis subvermispora* improved the digestibility of hemicellulose[28].

Table 2: Effect of rations on the digestibility of nutrients (%)

	Treatments				
Digestibility (%)	A	B	C	D	E
Dry matter	42.19 ± 7.72^{b}	53.82 ± 6.71^{ab}	54.47 ± 10.01^{ab}	58.76 ± 2.33^{a}	63.08 ± 6.84^{a}
Organic matter	45.77 ± 7.24^{b}	55.58 ± 6.57^{ab}	56.53 ± 10.13^{ab}	61.41 ± 2.07^{a}	64.58 ± 6.47^{a}
Crude protein	52.22 ± 2.81^{b}	63.07 ± 10.26^{ab}	59.13 ± 7.11^{ab}	67.68 ± 2.30^{a}	70.91 ± 6.83^{a}
Ether extract	43.33 ± 7.57^{b}	70.87 ± 6.19^{a}	65.04 ± 6.70^{a}	72.18 ± 10.75^{a}	68.57 ± 5.82^{a}
Crude fiber	34.03 ± 9.46^{b}	54.48 ± 10.78^{a}	55.13 ± 3.57^{a}	57.00 ± 1.59^{a}	62.20 ± 3.38^{a}
NDF	19.66 ± 12.96^{b}	39.44 ± 15.75^{ab}	42.71 ± 8.83^{ab}	44.19 ± 3.24^{a}	54.70 ± 8.19^{a}
ADF	13.52 ± 6.19^{b}	24.37 ± 16.80^{ab}	30.74 ± 9.85^{ab}	30.99 ± 3.71^{ab}	43.94 ± 13.49^{a}
Cellulose	44.28 ± 18.84	57.50 ± 10.44	57.97 ± 3.57	59.18 ± 8.51	60.04 ± 6.35
Hemicellulose	68.21 ± 2.13^{b}	78.42 ± 9.77^{ab}	72.99 ± 5.73^{ab}	79.12 ± 1.67^{ab}	80.79 ± 4.71^{a}

All values are expressed as Means±SD, Means in the same row with different letters (a and b) are significantly different ($p<0.05$), A: 40% Napier grass (NG)+0% fermented oil palm fronds (FOPFs)+60% concentrate, B: 20% NG+20% FOPFs+60% concentrate, C: 0% NG+40% FOPFs+60% concentrate, D: 20% NG+20% FOPFs+60% concentrate plus P, S and Mg, E: 0% NG+40% FOPFs+60% concentrate plus P, S and Mg

Rations containing fermented cocoa pod husk improved the digestibility of crude fiber[29] and produced a nutrient digestibility that was higher than that of the cocoa pod husk without fermentation[30].

The biodelignification process on oil palm fronds (OPFs) using *P. chrysosporium* has a positive effect in degrading the lignin content by 25.77% (from 30.18-22.40%)[1] and supplementation of P, S and Mg in FOPFs can support bioprocesses in the rumen, thereby increasing the digestibility of nutrients and fibre fractions[2]. The supplementation of P, S and Mg in the rations (D and E) resulted in higher digestibility than in the treatments without supplementation with P, S and Mg (A, B and C) and the highest digestibility was observed in treatment E; the digestibility was significantly ($p<0.05$) higher than in treatment A (Table 2). The increase of nutrient digestibility in treatments D and E, which were supplemented with P, S and Mg, indicated that the rumen microbes needed minerals for growth and enzyme activity. Mineral supplementation improves rumen microbial growth, thereby increasing the digestibility of nutrients.

Sulfur supplementation in the rations will increase the activity of cellulolytic microbes. Increased activity of cellulolytic microbes will in turn cause increased digestibility of the fibre fraction. This indicates that sulfur minerals are essential for metabolic processes, increasing the microbial populations supporting cellulolytic activity and cell wall degradation, especially cellulose and hemicellulose. This is evident in the treatments D and E where sulfur supplementation in the rations resulted in higher digestibility than for rations without sulfur supplementation (treatments A, B and C). As reported by Bal and Ozturk[31], sulphur in sufficient supply can optimize the digestibility of cellulose through the stimulation of specific bacteria (cellulolytic); supplementation with sulfur in the rations can increase the microbial population and cellulose degradation in the rumen[32]. Sulfur supplementation in rations of dairy cattle can improve the digestibility of dry matter and NDF[33]; in ration of cattle it can stimulate microbial growth by increasing protein supply to livestock[34], improving the digestibility of dry matter, organic matter and ADF[35] thereby increasing the growth of livestock[36].

Feed that is derived from waste products such as oil palm fronds is often deficient in P, hence the addition of mineral P is necessary to support the growth of rumen microbes. One role of P is in the production of ATP, which produces energy through the citric acid cycle. The ongoing process of metabolism in rumen microbes will increase their population size and activity, leading to higher fibre digestibility as was seen in the treatments D and E where phosphorus supplementation in the rations resulted in higher digestibility of nutrients compared to treatments A, B and C. Phosphorus can be deficient in forage and thus, supplemental phosphorus generally is needed in forage-based diets[37]; supplementation with P at 0.227% in the rations resulted in dry matter and organic matter digestibility that were higher compared to 0.119% P supplementation[7].

Magnesium is an enzymatic cofactor of enzyme and Mg supplementation in ration supports the processes of microbial metabolism. If the metabolic process is going well, this will increase the population size and microbial activity such that the microbes can digest nutrients optimally. This suggests that Mg plays a role in the metabolism of rumen microbes and that Mg supplementation is required for microbial growth and activity. In the treatments D and E where the rations were supplemented with Mg, the level of nutrient digestibility was higher than in treatments without Mg supplementation.

Magnesium is an essential mineral and is required for the enzymatic catalysis and metabolism of carbohydrates, lipids and nucleic acids[38]. Increasing the Mg level, increased the digestibility of nutrients[39]. Treatment E, with supplementation of P, S and Mg with 40% FOPFs in the rations, resulted in nutrient digestibility that was significantly ($p<0.05$) higher than that of treatment A. This suggests that P, S and Mg supplementation in goat rations can lead to more efficient rumen function; the growth and activity of rumen microbes will then be close to optimal and produce the highest nutrient digestibility.

CONCLUSION

Phosphorus, sulfur and magnesium are indispensable for rumen microbes for their growth and activity. P, S and Mg supplementation in goat rations can lead to more efficient rumen function. Administration of 40% FOPF supplemented with phosphorus, sulfur and magnesium in goat rations resulting in the highest nutrient digestibility.

SIGNIFICANCE STATEMENTS

This study evaluated the effect of supplementation with the minerals P, S and Mg in goat rations that contained FOPFS fermented by *P. chrysosporium* on nutrient digestibility. The results indicated that supplementation with the minerals P, S and Mg is highly effective in rations derived from plantation waste products, such as oil palm fronds, which often lack sufficient minerals. This study will help researchers to realize that supplementation with the minerals P, S and Mg is important to support metabolic processes, growth and microbial activity so as to improve the digestibility of nutrients. Thus, this research could lead to a new theory on the importance of supplementation with the minerals P, S and Mg in rations derived from plantation waste products, such as oil palm fronds, which often lack minerals to support the growth and activity of rumen microbes and their effects on nutrient digestibility.

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