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The Digestibility of *Pennisetum purpureum* Cv. Thailand Concerning Cowdung Supplementation and *Arbuscula Mycorrhizal* Inoculation

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Abstract: Manure usually consists of a mixture of livestock feces and urine, which also contains macro elements such as Nitrogen (N), Phosphate (P), Potassium (K), Magnesium (Mg), and Calcium (Ca) as well as the microelement Manganese (Mn). This study aims to determine the *in-vitro* influence of the digestibility of dry matter, digestibility of organic matter, crude protein digestibility, and crude fiber digestibility on elephant grass (*Pennisetum purpureum*) cv. Thailand supplemented with cow feces and inoculated with AMF on ultisol soil. We used a Completely Randomized Design (CRD) with 5 treatments and 4 replications. The treatments were A administration of cow feces fertilizer (5 tons/ha) + fertilizer N, P, K, B cow feces fertilizer (5 tons/ha) + AMF (10 g/clump), C cow feces fertilizer (10 tons/ha) + AMF (10 g/clump), D cow feces fertilizer (15 tons/ha) + AMF (10 g/clump) and E cow feces fertilizer (20 tons/ha) + AMF (10 g/clump). We observed some parameters such as the digestibility of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), and Crude Fiber (CF) *in-vitro*. The results showed that the administration of cow feces fertilizers gave significantly different results ($p<0.05$) on digestibility (DM, OM, CP, and CF). Average DM digestibility (57.15-57.69), OM digestibility (57.38-58.72), CP digestibility (60.85-61.13), and CF digestibility (60.08-61.03%). We concluded that the application of 5 tons/ha of cow feces fertilizer +10 g of AMF resulted in relatively the same digestibility of DM, OM, CP, and CF so that it could replace 100% of N, P, and K fertilizers.

Keywords: Elephant Grass Cv. Thailand, Cow Feces, AMF, Digestibility of DM, OM, CP, and CF

Introduction

Feed is an indicator of the success of raising livestock. High-quality feed should meet the basic needs of life, growth, and productivity of livestock. The biggest cost in raising livestock comes from the feed with 60-70% of the total cost. Forage is the main feed used by ruminant livestock. The lack of forage availability will negatively influence production and reproduction.

Forage can be grasses, legumes, and food crops by-products. Forage plays an important role in providing ruminants with a source of fiber, carbohydrates, protein, minerals, vitamins, and other nutrients that are beneficial for their survival. One of the forages that have good potential is Elephant Grass cv. Thailand which is a cross between elephant grass (*Pennisetum purpureum* Schumach) and pearl millet (*Pennisetum glaucum*). Elephant grass cv. Thailand is known as Pakchong grass. According to (Liman *et al*., 2022), Pakchong grass is a hybrid of elephant grass (*P. purpuruem* and *P. americanum*) which was first developed in Thailand by Dr. Krailas Kiyotthong. Pakchong grass has leaves that resemble the size and length of King Grass *P. purpurhoides*, the plant stems are not tough, and morphologically neither the stems nor leaves grow fine hairs which can reduce the palatability. The Pakchong grass contained crude protein of 16-18% and dry matter of 63-87 tons/ha/year which has a maturity stage at the age of 60 days (Liman *et al*., 2022).

At this time, the availability of land for cultivating forage is lower, so cultivation businesses use marginal land such as ultisol soil. Previous researchers reported that ex-gold mining land also has the potential to develop grass but still has high heavy metal contents (Putra *et al*., 2022), so it is necessary to use another alternative land, especially ultisol soil. Ultisol soil is soil that has a heavy clay texture which results in low permeability. In addition, acidic pH and low macronutrient levels (N, P, and K) affect the level of plant productivity (Prather *et al*., 2020).

One of the efforts that can correct the ultisol soil deficiencies is fertilization. Fertilizers are divided into 3 namely organic, inorganic, and biological fertilizers. Organic fertilizers or manure contribute to improving soil structure and texture, increasing water absorption, improving living conditions, and a source of food for plants. Cattle feces are solid waste from cattle farming and in the disposal, process is often mixed with urine and gases, such as methane and ammonia. Organic fertilizer increase yield and N use efficiency in Chinese intensive rice cropping systems (Zhang *et al*., 2018). Inorganic or mineral fertilizers are fertilizers that contribute one or more inorganic compounds (Kumar *et al*., 2019). The main function of inorganic fertilizers is to add nutrients such as Nitrogen (N), Phosphorus (P), and Potassium (K).

Biofertilizers contain biological agents that are beneficial for soil fertility and growth both vegetatively and generatively, for example, *Arbuscular Mycorrhizal* Fungi (AMF). The AMF has a symbiotic nature of mutualism with plant roots, where the roots become hosts for AMF which produce white hyphae to increase the absorption of more nutrients. According to (Diagne *et al*., 2020) using *Arbuscular Mycorrhizal* fungi can increase plant growth such as plant height, number of leaves, stem diameter, and dry weight.

Grass supplemented to the livestock will be digested by ruminal microbes and nutrient contents will be degraded so that the digestibility can be measured. According to (Ayaşan *et al*., 2020) Ismail 201, digestibility is the difference between the food substance consumed and excreted in the feces considering the absorbed ones, so digestibility is a reflection of the number of nutrients that can be utilized by livestock. The digestibility and fermentation can be studied via the *in-vitro* method.

The *in-vitro* method is a study of feed digestibility in the laboratory by imitating the processes that occur in livestock (Duijsens *et al*., 2022). According to (García-Yuste and Pérez-Barbería, 2020), the *in-vitro* method has many advantages including shorter conduction time, low cost, testing small sample sizes, ease of control, and the capability of evaluating more than one kind of material digestibility.

Materials and Methods

The tools and materials used in this study were knives, hoes, sickles, buckets, labels, Urea (N), SP-36 (P), KCl (K) as a control, and manure. (cow feces) and AMF, 640 Pakchong grass cuttings, equipment to measure digestibility in the *in-vitro* such as pipettes, analytical balances, fermenter tubes, Erlenmeyer, centrifuges, incubators, filter paper, stirrers, ovens, furnaces, glass filters, porcelain dishes, vacuum pumps, beaker glass, as well as tools for taking rumen fluid in the form of hot water flasks, filter cloths, rumen fluid from slaughterhouses (RPH), Mc Dougall's buffer solution and materials for proximate analysis.

Research Design

The current laboratory experiment was carried out using a 5×4 Completely Randomized Design (CRD) with 5 different treatments and 4 replications.

The treatments were as follows:

Treatment A = 5 tons/ha of cow manure + N, P and K (control)
Treatment B = 5 tons/ha of cow manure + AMF 10 g/clump
Treatment C = 10 tons/ha cow manure +10 g/clump of AMF
Treatment D = 15 tons/ha of cow's manure +10 g/clump of AMF
Treatment E = 20 tons/ha of cow manure +10 g/clump of FMA

Statistic Analysis

The statistical analysis was carried out using the model (Steel and Torrie, 1962) as follows:

$$Y_{ij} = \mu + \tau_j + \Sigma_{ij}$$

Information,
Y_{ij} = Result of observation of i^{th} repetition and j^{th} treatment
μ = General mean value
τ_j = Effect of the j^{th} treatment
Σ_{ij} = Error/remaining/random error of the i^{th} repetition and j^{th} treatment

Agronomic Preparation for Planting Pakchong Grass

After clearing the land, land cultivation was carried out by plowing to break up the layers of soil and then leaving it for a few days before drilling for the mineralization process for obtaining faster activity of bio-organisms. Loosening was carried out by destroying lumps from the remaining wild plant roots and the land was divided into 4 groups consisting of 5 plots (plots), each group measuring 4×3.5 m² and the distance between plots was 1 m ×1 mL, beds were made and cow manure was applied according to the treatment, then rake the soil. At the harrowing, Urea (N) 200 kg/ha, SP-36 (P) 150 kg/ha, and KCL (K) 100 kg/ha with 2 times administration at 14 HST and 28 HST. Then planting by cuttings and supplying with the AMF in the planting hole according to the treatment, then maintenance by cleaning weeds, eradicating pests, and harvesting at 60 HST.

Rumen Fluid Collection

The flask was filled with hot water at 39°C and closed. The rumen fluid was collected at the abattoir, then squeezed out with warm water using gauze and put into a warm thermos. Before being used as a rumen fluid container, the hot water in the thermos was removed. To keep the rumen fluid in an anaerobic condition, the thermos must be tightly closed and CO_2 was flushed before use.

In the results of the initial research conducted at the ruminant nutrition laboratory, faculty of animal husbandry Andalas University, the nutritional value of the Pakchong grass plant was obtained. The nutritional value of Pakchong grass is in Table 1.

Measured Parameters

Digestibility of Dry Matter (DM)

Weigh 1 g of sample (*a*) and put it in a cup whose weight is known (*b*). Bake the sample at 100-105°C for 8 h or until constant weight, then cool in a desiccator for 10 min and weigh (*c*).

$$kadar\ Air\,(\%) = \frac{a+b-c}{c} \times 100$$

Note,
a = Sample weight
b = Weight of the cup after being in the oven and
c = Weight of the sample after being in the oven

$$Kecernaan\ BK = \frac{(Brt\ sml \times BK) - (Brt\ Resilen)}{Brt\ sml \times BK}$$

Digestibility of Organic Matter (OM):

$$Ash\ content\ (\%) = \frac{(\mathrm{c}-\mathrm{a})}{b} \times 100$$

BO (%) = % dry matter-% ash content:

$$Kecernaan\ Bo = \frac{(Brt\ sml \times BO) - (Brt\ Reie' \times BO - Brt\ Blanko \times BO) \times 100\%}{(Brt\ sample \times BO)}$$

Information,
a = Weight of cup (g)
b = Weight of the sample (g)
c = Cup Weight + sample weight (g)

Crude Protein Digestibility (CP, Kjeldahl Methods)

Add 25 mL of catalyst (Selenium Se), concentrated H_2SO_4, then destruct until the solution is clear. Cool the sample and add 150 mL of distilled water. Take 10 mL of the filtrate and put it in the distillation tube. Add 25 mL of 0.3 N NaOH and 75 mL of distilled water and boiling stones. The distillation was collected in 25 mL of H_2SO_4 which had been given 3 drops of methyl red indicator.

$$Kadar\ protein = \frac{(y-x) \times NaOH \times C \times 0.014 \times 6.25}{Brt\ sample} \times 100$$

Information,
y = Volume of *NaOH* 0.1 N titrant blank
x = Volume of *NaOH* 0.1 N painter sample
n = Normality of *NaOH* used
C = Dilution

$$Kecernaan\ PK = \frac{(berat\ sample \times BK\ sample \times PK\ sample) - (beraague)}{Home} \times 100$$

Table 1: Chemical composition of Pakchong grass given several doses of cow feces fertilizer with AMF inoculation on ultisol soil

Content treatment nutrition	(% BK, Except dry matter) A	B	C	D	E
Dry material	17.27	16.03	16.19	16.20	17.42
Organic ingredients	91.60	93.21	93.52	92.56	91.56
Coarse fiber	30.58	31.34	31.18	30.87	30.54
Crude protein	12.08	10.95	10.99	11.10	12.64
Ash content	8.40	6.79	6.48	7.44	8.44
NDF	71.28	71.51	71.46	71.37	71.20
ADF	45.36	45.94	45.74	45.55	45.15
Cellulose	35.38	35.61	35.76	35.55	35.19
Hemicellulose	25.92	25.57	25.72	25.82	25.05
Lignin + Silica	9.98	10.33	9.98	10.00	9.96

Source: Ruminant nutrition laboratory (2022)

Crude Fiber Digestibility (CF)

To determine the digestibility of crude fiber in Pakcong grass, namely:

$$Serat\,kasar\,(\%) = \frac{b - c - ax}{x} \times 100$$

Information,
a = Weight of filter paper
b = Weight of cup + filter paper + filter
c = Weight of cup + ashes
x = Weight of sample

$$CF\ digestibility = \frac{(beraaguensample \times Bk\ sample\,eggsample) - (beraague)}{Home} \times 100$$

Place and Time of Research

The current study was conducted from February to May 2022 and was carried out on ultisol land located at the Edufarm Faculty of Animal Husbandry Andalas University, Padang, as well as the Ruminant Animal Laboratory, Faculty of Animal Husbandry Andalas University, Padang.

Results

The influence of AMF-inoculated cow manure on the digestibility of dry matter, organic matter, crude protein, and crude fiber can be seen in (Table 2).

Effect of Treatment on Digestibility of Dry Matter (BK)

The effect of treatment on the digestibility of Pakchong grass Dry Matter ranged from 57.15-57.69%. The treatment had no significant effect on the digestibility of Dry Matter (DM). This is because the use of 5 tons/ha of cow feces fertilizer inoculated with 10 g of AMF in treatment B alone can offset the digestibility of dry matter in treatments A, C, D, and E.

The Effect of Treatment on the Digestibility of Organic Matter (OM)

The mean of the results of the analysis of variance showed that the treatments had no significant effect on the digestibility of organic matter from Pakchong grass. It can be seen that the average organic matter digestibility of Pakchong grass grown on ultisol soil with the administration of cow feces fertilizers and AMF showed yields ranging from 57.38-58.72%. It can be concluded that using 5 tons/ha of cow feces fertilizer inoculated with 10 g of AMF in treatment B alone can balance the digestibility of organic matter compared to A, C, D, and E.

Organic matter digestibility was in treatment E with a yield of 58.72% and the lowest was in treatment B with a yield of 57.38%. The high average digestibility of organic matter from Pakchong grass in treatment E was due to using higher doses of cow feces inoculated with AMF compared to treatments A, B, C, and D. However, treatment B resulted in a lower average digestibility than the other treatments.

Effect of Treatment on Digestibility of Crude Protein (CP)

The results showed that the treatments had no significant effect on the digestibility of crude protein from Pakchong grass. The average crude protein digestibility of Pakchong grass grown on ultisol soil with different doses of cow manure and AMF showed yields ranging from 60.85-61.13%.

The highest crude protein digestibility was recorded in treatment E with a yield of 61.13% and the lowest was in treatment B with a yield of 60.85%, the difference in results was suspected because treatment B used a small amount of cow dung.

Effect of Treatment on Digestibility of Crude Fiber (CF)

The results showed that the treatments had no significant effect on the digestibility of crude fiber from Pakchong grass. The average crude protein digestibility of Pakchong grass grown on ultisol land with different doses of cow manure and AMF showed results ranging from 60.08-61.03%, with the highest percentage of crude fiber digestibility in treatment E, while the lowest digestibility was in treatment B.

Table 2: The average digestibility of dry matter, organic matter, crude protein and crude fiber in pakchong grass

No	Treatment	KcBK (%)	KcBO (%)	KcPK (%)	KcSK (%)
1	A	57.58	58.50	61.09	60.71
2	B	57.15	57.38	60.85	60.08
3	C	57.20	57.44	60.98	60.15
4	D	57.35	58.08	61.07	60.42
5	E	57.69	58.72	61.13	61.03
	SE	0.35	0.41	0.55	0.44

Description: Between treatments showed no significantly different effect ($p>0.05$), SE: Standard Error

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Discussion

The treatment utilizing 5 tons/ha of cow feces fertilizer and 10 g of AMF consistently demonstrated its effectiveness in enhancing dry matter digestibility across all treatments. This can be attributed to the nutrient composition of cow feces fertilizer, which contains vital elements such as Nitrogen (N), Phosphorus (P), and Potassium (K) crucial for the growth of Pakchong grass. Furthermore, the presence of AMF facilitated improved nutrient absorption in the soil.

Moreover, the application of manure and AMF not only positively influenced vegetative growth and nutritional content in Pakchong grass but also enhanced the fertility of ultisol soil. Ultisol soil is typically lacking in nutrients, but through the addition of manure and AMF, essential nutrients were provided, simultaneously improving the physical, chemical, and biological properties of the soil. Etesami (2020) highlighted that AMF plays a role in nutrient absorption from the soil and can be utilized as a biological fertilizer to reduce the reliance on conventional fertilizers.

The application of manure and additional AMF not only helps the process of vegetative growth and the nutritional content of Pakchong grass but also helps the fertility of ultisols planted with Pakchong grass. Ultisol soil is poor in nutrients and with the addition of manure and AMF, it can provide nutrients and improve the physical, chemical, and biological properties of the soil. Physically manure made the soil loose so that aeration becomes smooth. Chemically, manure provides nutrients for N, P, and K and biologically it can add organic matter to the soil which helps decompose organic matter so that the soil becomes fertile. The results agreed with those of (Liu *et al*., 2020) who stated that manure has a positive effect on the physical, chemical, and biological properties of soil.

The study revealed a positive relationship between organic matter digestibility and soil fertility, as indicated by Ahmed and Al-Mutairi (2022). Increased soil fertility contributes to improved plant growth and root development, resulting in higher vegetative biomass and enhanced digestibility. However, it was observed that treatment B exhibited a lower average digestibility compared to the other treatments. This decline can be attributed to inadequate nutrient absorption by the plants, leading to limited nutrient availability for both plant growth and the development of *arbuscular mycorrhizal* fungi (AMF). Consequently, the suboptimal development of AMF resulted in reduced efficiency in nutrient exchange with the plants.

According to Owens and Basalan (2016), the digestibility of organic matter reflects the number of digestible substances, especially nitrogen compounds, carbohydrates, fats, and vitamins. The current results showed that the effect was not significantly different on the organic matter digestibility due to the relatively similar nutritional content contained in Pakchong grass, resulting in relatively the same digestible nutrients. In the opinion (Barrios, 2004), the digestibility of organic matter is closely related to the digestibility of dry matter, because most of the dry matter is organic consisting of crude protein, crude fat, crude fiber, and Nitrogen Free Extract (NFE). In addition, the decrease in organic matter is also closely related to the increase in the contents of the fiber fraction.

According to Koksharova and Safronov (2022), nitrogen (N), phosphorus (P), and potassium (K) are essential nutrients present in the soil and required by plants. N plays a crucial role in the formation of proteins, fats, and various organic compounds, while P contributes to the formation of certain proteins. Farhadi *et al*. (2022) argue that the availability of nitrogen in the soil significantly affects the content and composition of crude protein in Sorghum Silage, and a deficiency in nitrogen can inhibit plant synthesis.

Leite *et al*. (2019) emphasized the significance of nitrogen in forming crude proteins, which has a major impact on high-quality protein production and enhanced digestibility in grass. Moreover, nutrient availability influences grass growth, which, in turn, affects protein content and digestibility. As the grass matures, the digestibility of crude protein tends to decrease. Suhartanto *et al*. (2020) demonstrated that plant protein contents decrease with increasing plant age, while crude fiber and lignin contents increase.

Livestock requires protein as a vital component for their basic functions, growth, and production (Riznaya *et al*., 2019). The digestibility of crude protein did not exhibit a significant difference, which aligns with the digestibility of dry matter and organic matter. As explained by Mertens and Grant (2020), protein content is directly related to dry matter digestibility. When the digestibility coefficient of organic matter increases, the coefficient of crude protein also tends to increase.

The crude fiber contents in Pakchong grass have increased and decreased with the different treatments given. However, the results were not significantly different for each treatment. Mateos *et al*. (2019) recorded that crude fiber functions as a source of energy for ruminants (bulky or satiating) and increases the peristaltic motion of the digestive tract. Crude fiber contains carbohydrate components such as cellulose, hemicellulose, and lignin (Pond *et al*., 2004).

Fahey *et al*. (2019) discovered that feed ingredients with higher crude fiber content tend to have thicker cell walls, making them more resistant to digestion by fiber-digesting microorganisms and consequently leading to lower digestibility. On the other hand, feed ingredients with lower crude fiber content are more easily digested.

The cell wall composition includes lignin and silica, which can inhibit forage digestibility. According to Van Soest *et al*. (1991), lignin, a component of the plant cell wall, is indigestible and can even reduce the digestibility of other plant fractions. Furthermore, Li (2021) observed that lignin binds to cell wall proteins, rendering lignin insoluble in rumen fluid.

Conclusion

The treatment of 5 tons/ha of cow feces fertilizer inoculated with AMF on ultisol soil was relatively the same for the digestibility of dry matter, organic matter, crude protein, and crude fiber, as well as AMF was able to balance the use of cow feces fertilizer. The average digestibility of dry matter was 57.15%, organic matter 57.38%, crude protein 60.85%, and crude fiber 60.08%.

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Author’s Contributions

Evitayani: Encompass conceptualized, data collection, analysis, and interpretation. Additionally, she bears the responsibility of manuscript preparation, ensured adherence to ethical guidelines and academic standards.

Lili Warly: Contributed to data analysis, manuscript preparation and scientific collaboration.

Armina Fariani: Expertise in literature review, experimental design, result validation, and critical analysis of findings. The input strengthens the research's credibility and widens its impact on the scientific community.

Bela Putra: Serve as manuscript editors, conduct data analysis, and search for the latest.

Arni Amir: Ensured grammatical accuracy, proper referenced and control over field research in the manuscript.

Afriwardi: Provide intellectual insights, offered constructive feedback, and engaged in discussions to enhance the research methodology, analysis and overall interpretation of the results.

Ethics

The authors are committed to addressing any potential ethical issues that may arise after the publication of this manuscript. They will diligently respond to and resolve any concerns related to research ethics, ensuring the integrity and credibility of their work.

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