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The Effect of *Tithonia diversifolia* Substitution on The Digestibility of Livestock Nutrients

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Abstract. This study analyzed the substitution effect of *Tithonia diversifolia* as an alternative concentrate in dairy cattle feed to overcome the challenges of high feed costs and dependence on imported materials. Conventional concentrates generally consist of high-quality materials such as corn and rice bran, rich in protein and energy. However, their unstable availability and high prices are obstacles for farmers, especially in areas with limited access. Therefore, a more efficient and sustainable local feed alternative is needed. *Tithonia diversifolia*, or paitan, has a relatively high crude protein, mineral, and fiber content, so it has the potential to replace concentrates without sacrificing digestibility and nutritional efficiency. This study used a Randomized Block Design with four treatments, namely A (50% pakchong grass + 35% *Tithonia* + 14% concentrate + 1% mineral), B (50% pakchong grass + 40% *Tithonia* + 9% concentrate + 1% mineral), C (50% pakchong grass + 45% *Tithonia* + 4% concentrate + 1% mineral), and D (50% pakchong grass + 49% *Tithonia* + 1% mineral) which were each repeated four times. The variables measured included crude fiber content, crude fat, and digestibility of nitrogen-free extract. The results showed that the treatments significantly affected the three parameters ($P < 0.05$), where Treatment B significantly increased feed digestibility. In addition to having high nutritional value, *Tithonia diversifolia* is also easy to cultivate, grows quickly, and adapts to various environmental conditions, making it a more economical and sustainable local feed alternative. Thus, substituting *Tithonia diversifolia* in dairy cattle feed reduces production costs, increases the efficiency of local resource utilization, and supports a more environmentally friendly livestock system.

1. Introduction

Feed is the most important factor in the livestock industry because it can increase livestock productivity and ensure livestock welfare. Therefore, developing quality and efficient feed is the main focus of increasing livestock productivity. Farmers must know the balance of nutrients livestock need to support their basic needs to produce well [1]. A deep understanding of ration composition, livestock nutritional needs, and ration formulation technology is fundamental.

Various factors affect livestock rations' quality and composition, such as the availability of raw materials, feed formulation innovation, and processing technology. Diverse feed sources, including greens, grains, agricultural waste, and concentrates, continue to be research materials to expand efficient and quality feed sources. Efficient rations can help reduce livestock production costs, while environmentally friendly feed can help minimize the environmental impact of the livestock industry [2].

The use of feed concentrates in the livestock industry is a solution that continues to be developed to optimize livestock production economically and environmentally. However, the raw



material concentrate as a source of protein has experienced significant price changes, limited availability, and dependence on imports in several regions. Feed ingredients such as soybean meal and corn, which are often used in concentrate formulation, are increasingly expensive, and their supply is not always stable. In addition, the production and use of soybean and corn-based concentrates have environmental impacts, including a high carbon footprint and large land requirements. Therefore, alternative concentrate feeds that have high protein content, are easy to obtain, are cheaper, and are sustainable in the long term are needed.

One source of feed that has attracted attention is *Tithonia diversifolia*. This green plant has high nutritional potential and is widely available in tropical and subtropical regions, especially in Indonesia[3]. This plant shows strong potential as animal feed because of its high protein content (22.98%) and crude fiber (18.17%) [4]. *Tithonia diversifolia* is a high-protein feed ingredient that can be used as a partial or complete substitute for conventional concentrates [5] [6] [7] [8] [9]. In addition, this plant has agronomic advantages, such as rapid growth, tolerance to various environmental conditions, and high biomass production, making it more economical and easier to obtain than other feed sources.

However, *Tithonia diversifolia* also has challenges, such as high levels of phytic acid, around 0.79% [10], which can reduce its palatability due to the bitter taste it causes. However, with proper processing, such as fermentation or mixing with other feed ingredients, this plant can still be used as an alternative source of concentrate rich in protein. Therefore, using *Tithonia diversifolia* as a feed ingredient has attracted researchers' interest as a more sustainable and economical livestock feed solution than conventional concentrates. In this context, in-vitro research has become a popular method to illuminate the potential of alternative feed such as *Tithonia diversifolia* in improving nutrition in livestock. The main objective of this study was to improve understanding of the effect of *Tithonia diversifolia* addition on the digestibility ratio of crude fiber, crude fat, and nitrogen-free extract (NFE) in the in-vitro digestive system. Crude fiber digestibility is closely related to the crude fat content in feed ingredients, where high crude fiber content can reduce feed digestibility. On the other hand, NFE is a soluble organic material consisting of monosaccharides, disaccharides, and polysaccharides, so it has a high digestibility. Nutrient digestibility is the leading indicator of feed quality because dry matter digestibility includes crude fiber and NFE in ruminants, which reflects the high content of nutrients that can be effectively digested in the rumen.

Understanding how *Tithonia diversifolia* affects nutrient digestibility will provide insight into its value in developing more efficient and sustainable ration formulations. In addition, this study is expected to help farmers choose the right concentrate source to improve the health and performance of their livestock information. Based on the explanation above, it is important to research "**The Effect of *Tithonia diversifolia* Substitution on the Digestibility of Livestock Nutrients.**"

2. Materials and Methods

2.1 Ethical approval

In this research we do not use livestock as the object of research, therefore we do not need a ethical approval.

2.2 Research tools and materials

The tools utilized in this research are shaker water bath, soxhlet extractor, aluminum foil, filter paper, tissue, hot plate, organic material spray, porcelain cup, desiccator, funnel, lighter, erlenmeyer, measuring flask, analytical balance, suction pump, 10 mL measuring pipette, 50 mL measuring cup, 100 mL measuring cup, 500 mL goblet, distillation flask, 100 mL test tube with lid,

oven, furnace, kjeldahl boiling flask (50 mL), acid chamber, beaker glass, biuret distiller, plastic, autoclave.

The materials used for this study were tithonia plants, pakchong grass, corn, bran, cocoa husk, minerals, rumen fluid, and distilled water. Chemicals used for the manufacture of McDougall solution (NaHCO_3 , $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, NaCl , KCl , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, NaCl , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), distilled water, concentrated H_2SO_4 , Selenium and 0.3 N NaOH .

2.3 Research Design

The experimental design employed in this study was a Randomized Group Design consisting of four treatments (T1, T2, T3, and T4), with four replicates for each treatment. The feed ingredients utilized in the study include Pakchong grass (*Pennisetum purpureum* cv. Thailand), *Tithonia diversifolia*, and concentrates (bran, corn, cocoa husk). The total number of rumen used was 4, and there were four replicates, so the total number of samples was 16.

The treatments given in this study were:

T1= Pakchong grass 50% + *Tithonia diversifolia* 35% + Concentrate 14% + mineral 1%

T2= Pakchong grass 50% + *Tithonia diversifolia* 40% + Concentrate 9% + mineral 1%

T3= Pakchong grass 50% + *Tithonia diversifolia* 45% + Concentrate 4% + mineral 1%

T4= Pakchong grass 50% + *Tithonia diversifolia* 49% + mineral 1%

The parameters measured in this study were the digestibility of crude fat, crude fiber, and nitrogen-free extract(NFE).

Table1. Chemical composition of experimental ration ingredients (%)

Chemical Composition	Feed Ingredients				
	Pakchong Grass	Tithonia	Corn	Bran	Cocoa Husk
Dry matter	93.39	87.88	85.49	91.33	89.39
Organic matter	89.46	84.01	96.23	87.44	95.37
Crude matter	11.69	19.56	13.58	11.09	13.10
Crude fat	1.34	1.09	3.99	6.28	11.39
Crude fiber	21.76	17.44	10.29	9.23	31.21
Ash	16.92	13.88	3.77	12.56	11.01
NFE	48.29	48.03	68.38	60.83	33.57
TDN	62.84	64.72	77.96	73.25	64.88
NDF	66.57	61.12	58.62	48.11	44.60
ADF	41.71	40.15	34.66	23.08	27.32
Cellulose	34.18	34.57	19.70	11.05	25.67
Hemicellulose	24.86	20.97	23.96	25.03	17.28
Lignin	6.29	4.57	7.50	3.78	5.95
Silica	1.24	4.92	0.70	2.28	3.38

Notes: Total Digestible Nutrient (TDN), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Nitrogen Free Extract (NFE).

Table 2. Formulation of Research Ration

Feed Ingredients	Treatment(%)			
	T1	T2	T3	T4
Pakchong grass	50	50	50	50
Tithonia	35	40	45	49

Concentrate	14	9	4	0
Mineral	1	1	1	1
Total	100	100	100	100

Table 3. Chemical Composition (%) of Ration Treatments

Chemical Treatment (%)	Treatment			
	T1	T2	T3	T4
Dry matter	89.96	89.89	89.81	89.75
Organic matter	87.06	86.64	86.22	85.89
Crude matter	14.42	14.78	15.14	15.42
Crude fat	2.15	1.81	1.47	1.20
Crude fiber	19.53	19.49	19.45	19.42
Ash	14.74	14.92	15.11	15.26
NFE	48.14	47.97	47.81	47.68
TDN	63.98	63.68	63.37	63.13
NDF	61.51	62.12	62.74	63.23
ADF	38.70	39.35	40.00	40.52
Cellulose	31.79	32.59	33.39	34.02
Hemicellulose	22.81	22.77	22.73	22.70
Lignin	5.49	5.45	5.41	5.38
Silica	2.67	2.80	2.93	3.03

Notes: Total Digestible Nutrients (TDN), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Nitrogen-Free Extract (NFE).

2.4 Research Procedures

The raw materials in this study are *Tithonia diversifolia* plants and pakchong grass taken in the district of Lima Puluh Kota. The tithonia plants were dried with sunlight, placed in the oven at 60°C, and then mashed using a grinding machine. Tithonia and pakchong grass were finely weighed along with other feed ingredients according to the composition of the ration. Add 200 ml of McDougall solution and 50 ml of rumen fluid to each Erlenmeyer flask, ensuring a steady flow of CO₂ to keep the conditions aerobic. Seal the flask with a vented rubber cover for gas release. Then, incubate the flasks in a shaker water bath at 39°C for 48 hours. After incubation, remove the flask and place it in a basin with ice to cease microbial activity, and promptly measure the pH of the rumen fluid. Then, centrifuge for 15 minutes to separate the supernatant with residue using filter paper. The residue attached to the filter paper was then oven to 60°C for 12 hours. After the smooth residue was tested for crude fiber and fat content, the residue was then mashed using a porcelain mortar and pestle.

2.5 Data Analysis

Data analysis was conducted using analysis of variance (ANOVA) with IBM SPSS Statistics version 26.0 (IBM Corp, NY, USA). Additionally, Duncan's Multiple Range Test (DMRT) was employed as a subsequent analysis.

3. Result and Discussion

Table 4. Crude Fiber, Crude Fat, and NFE Digestibility Results

Treatment	Parameter		
	DCF	DCFat	DNFE
T1	64.79 ^c ±0.18	63.03 ^c ±0.18	66.22 ^c ±0.20

T2	66.64 ^d ±0.20	64.56 ^d ±0.23	68.06 ^d ±0.18
T3	63.65 ^b ±0.14	61.87 ^b ±0.19	65.41 ^b ±0.22
T4	60.98 ^a ±0.22	60.09 ^a ±0.20	63.62 ^a ±0.16
SE	0.26	0.28	0.24

Note: Standard Error (SE); Digestibility of Crude Fiber (DCF); Digestibility of Crude Fat (DCFat); Digestibility of Nitrogen-Free Extract (DNFE).

3.1 Crude Fiber Digestibility

In the T2 treatment, crude fiber digestibility was higher compared to the T1, T3, and T4 treatments. However, increasing the level of tithonia caused a decrease in crude fiber digestibility. Tithonia in the ratio is optimal in treatment T2, which uses 40% tithonia, 9% concentrate, and 1% minerals. At this level, the protein content remains beneficial, and the effects of anti-nutrients such as tannins are still tolerable, so fiber digestibility is not significantly reduced.

In the T3 and T4 treatments, there was a decrease in crude fiber digestibility. Tithonia is due to anti-nutrient compounds such as phytate and tannin. These compounds can affect nutrient digestibility, especially fiber because they bind minerals or proteins that microbes need in the rumen. [11] confirmed that *Thitonia diversifolia* contains anti-nutrient compounds such as tannins and phytates that can reduce nutrient digestibility, especially fiber and minerals. Tannins have a role in decreasing protein degradation within the rumen, but at higher levels, these compounds can reduce the efficiency of fiber fermentation by rumen microbes. Phytates, conversely, bind to minerals such as calcium and magnesium, inhibiting their availability for microbial digestion.

3.2 Crude Fat Digestibility

This study shows that the feed composition in T2, which has a digestibility of 64.56%, provides an optimal balance between fiber and concentrates that support rumen microbial activity in digesting fat. It shows that the feed composition in T2 provides an optimal balance between fiber and fat. This condition will support the activity of rumen microbes in digesting fat more efficiently. Rumen microbes, especially lipolytic bacteria, play an essential role in the hydrolysis of fat into volatile fatty acids, which can then be absorbed and utilized as an energy source. [12] stated that crude fat digestibility is affected by the composition of the feed ration, especially the content of crude fat content and fiber fraction contained in the feed ingredients. High fat content, but balanced with fiber, can increase the efficiency of rumen microbes in digesting fat and fiber. The increasing percentage of tithonia digestibility decreases due to the content of anti-nutritional compounds in tithonia.

Anti-nutritional compounds such as tannins, saponins, and alkaloids in tithonia interfere with fat digestion [13] [14]. Tannins can bind to proteins and lipolytic enzymes, which reduces the availability of essential nutrients for the hydrolysis of fat in the rumen. This condition can explain the decreased crude fat digestibility in the T3 and T4 treatments. In addition, these anti-nutritional compounds can also inhibit overall rumen microbial activity, thus impacting the efficiency of fat fermentation.

3.3 NFE Digestibility

Based on the study results presented in Table 4, using tithonia as a substitute for concentrates at varying levels significantly affects nitrogen-free extract *in vitro* ($P < 0.01$). The DMRT further test showed that treatment T1 significantly differed ($P < 0.01$) from treatments T2, T3, and T4. Additionally, treatment T2 showed a significant difference ($P < 0.01$) compared to treatment T3 and T4, while treatment T3 also differed significantly ($P < 0.01$) from treatment T4.

Table 4 shows that tithonia up to 40% in the ration can reduce the use of concentrates, still able to maintain the value of protein, TDN, and NFE in the ration. NFE is a food substance easily digested by rumen microorganisms. However, along with the increase in tithonia, NFE

digestibility decreased due to anti-nutrient content in tithonia. Some anti-nutritional substances often found in *Tithonia diversifolia* include tannins, saponins, and alkaloids. Tannins can reduce the efficiency of NFE digestion by inhibiting the activity of enzymes responsible for breaking down starch and simple carbohydrates, thus reducing the availability of energy produced from NFE [15]. Tannins reduce the efficiency of rumen fermentation by inhibiting amylolytic microbes that convert starch into VFA, which is the final product of NFE digestion.

4. Conclusion

This study showed that the administration of *Tithonia diversifolia* as an alternative concentrate in the formulation of 50% patching grass, 40% tithonia, 9% concentrate, and 1% minerals could significantly increase the content of crude fat, crude fiber, and nitrogen-free extract (NFE). The best results were shown by the digestibility of crude fiber (66.64%), crude fat (65.56%), and NFE (68.06%), so this formulation has the potential to increase feed efficiency in livestock.

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