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Optimization of Madura Cattle Performance Fed Ammoniated Rice Straw and Concentrate Containing *Hibiscus tiliaceus* Leaf

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ABSTRACT

The leaves of *Hibiscus tiliaceus*, known for their flavonoid and fumaric acid contents, may support more stable rumen fermentation by promoting propionic acid production and helping maintain a healthy pH in high-concentrate diets. When paired with ammoniated rice straw, this supplement could improve how cattle utilize nutrients while lowering the risk of subacute ruminal acidosis in feedlot settings. This study evaluates the effectiveness of *H. tiliaceus* leaf flour as a dietary supplement in feedlot Madura cattle. Fifteen cattle (initial weight 264.43 ± 22.68 kg) were assigned to three diet treatments: rice straw plus concentrate (RSC), ammoniated rice straw plus concentrate (ARSC), and ammoniated rice straw plus concentrate supplemented with HTLF (ARSC+H) and statistically analyzed using a completely randomized design. Concentrates were fed at 2.5% of body weight, while rice straw and ARS were provided *ad libitum*. Ammoniation involved treating rice straw with 5% urea and 2.5% cassava pulp. The treatments significantly (p<0.01) increased digestibility parameters (dry and organic matter digestibility (DMD, DMO), crude fat digestibility (CFD), crude protein digestibility (CPD), crude fiber digestibility (CFD), and nitrogen retention (NR)), microbial protein synthesis (MPS) and production (MPP), energy utilization (energy digestibility (ED), metabolizable energy output (MEO), energy retention (ER)), volatile fatty acid (VFA) production, average daily gain (ADG), and feed efficiency (FE). RSC showed lower values compared to ARSC and ARSC+H (p<0.01), while differences between ARSC and ARSC+H were not significant (p>0.05). The highest MPS, MPP, and ADG were observed in ARSC+H, with the best FE also in ARSC+H. In conclusion, ARSC+H enhances nutrient digestibility, MPS, and fattening performance in Madura cattle, indicating its potential as an effective feed strategy.

Keywords: *flavonoid; Hibiscus tiliaceus; metabolism; nitrogen; rumen*

INTRODUCTION

High-concentrate feeding has become a standard practice in feedlot systems to accelerate growth and improve production efficiency. These energy-rich diets are primarily formulated to supply glucogenic precursors, such as propionic acid, which are essential for rapid tissue accretion. However, these formulations also have some disadvantages. Excessive fermentation of starch in the rumen can cause a surge in short-chain fatty acids (SCFAs), leading to a significant drop in rumen pH. This condition, known as subacute ruminal acidosis (SARA), has emerged as a prevalent metabolic disorder in intensively managed ruminants (Lee *et al.*, 2017).

SARA negatively impacts animal welfare and productivity by reducing feed intake, impairing fiber digestibility, and disrupting the balance of rumen microbiota. It also contributes to secondary complications, including laminitis, diarrhea, and liver abscesses, which ultimately compromise performance and economic returns (DeClerck *et al.*, 2020; Meissner *et al.*, 2017). To address this, antibiotics such as monensin were historically used to stabilize ruminal fermentation. Yet, increasing concerns over antimicrobial resistance have prompted regulatory bans, including the European Union's prohibition in 2006 (Seradj *et al.*, 2018) and Indonesia's enforcement of a similar policy under UU 41/2014 and Permentan 14/2017, which effectively banned Antibiotic Growth Promoters (AGPs) from 2018 onwards.

In light of these restrictions, attention has turned to natural feed additives, particularly those derived from plants, as safer alternatives. These compounds, rich in bioactive secondary metabolites, have shown the potential to enhance rumen health, reduce pathogenic load, and improve fermentation efficiency (Hassan *et al.*, 2020). Among these, Hibiscus tiliaceus, a locally available tropical plant, has gained interest due to its content of flavonoids, saponins, tannins, and essential oils, which have demonstrated antimicrobial, antiprotozoal, and fermentation-modulating effects

(Arowolo & He, 2018; Flachowsky & Lebzien, 2012). Furthermore, the presence of fumaric acid, an intermediate in propionic acid synthesis, adds to its potential to mitigate acidotic conditions commonly associated with high-concentrate diets (Bata & Rahayu, 2016, 2017).

While concentrate manipulation is crucial in preventing SARA, the choice of forage is equally significant. In many tropical production systems, rice straw remains the primary basal roughage due to its abundance and low cost. However, its high lignin and silica content and low fermentable carbohydrate and nitrogen levels limit its nutritive value (Van Soest, 2006). Urea-ammoniation has been widely adopted as a chemical treatment to improve its digestibility. This method increases nitrogen content and modifies fiber structure, promoting better rumen degradability. Despite its benefits, the effectiveness of this process is limited. Only a fraction of the applied urea is converted into usable ammonia, while the remainder is lost or poorly synchronized with energy release, especially in the absence of readily fermentable carbohydrates (Saadullah *et al*., 1981; Sarwar *et al*., 2004).

To address these shortcomings, several studies have explored the co-supplementation of ammoniated straw with fermentable carbohydrate sources such as corn-steep liquor, acidic molasses, or cassava pulp (Bata & Hidayat, 2010; Bata *et al*., 2020). These combinations have been shown to enhance nitrogen retention and overall feed quality. Yet, the challenge remains to find an additive that not only improves low-quality roughage utilization but also helps stabilize rumen fermentation under high-concentrate feeding regimes.

In this context, *Hibiscus tiliaceus* leaf flour offers a unique solution. Its flavonoid and organic acid content may not only support more efficient fermentation patterns, by favoring propionic acid production over lactic acid, but also buffer rumen pH and suppress pathogens that contribute to SARA. *In vitro* research by (Bata *et al*., 2021) revealed that extracts from this plant increased propionic acid concentration, enhanced microbial protein synthesis, and reduced methane output, suggesting a multi-faceted benefit. However, its application in feedlot cattle, particularly in high-concentrate feeds and ammoniated rice straw diets, remains underexplored.

Therefore, this study aims to evaluate the effectiveness of *H. tiliaceus* leaf flour as a dietary supplement in feedlot Madura cattle. By integrating it into a feeding system based on high-concentrate rations and ammoniated rice straws, this research seeks to optimize nutrient utilization, support rumen stability, and offer a viable alternative to synthetic additives under intensive production conditions.

MATERIALS AND METHODS

Animal Ethics

All procedures involving animals in this study were conducted following ethical guidelines and were approved by the Ethical Clearance Committee of the Veterinary Faculty, Gajah Mada University, with number 130/EC-FKH/int. 2023.

Animal, Diets, and Experimental Design

Fifteen healthy Madura cattle with an initial body weight of 264.43 ± 22.68 kg and a variance coefficient of 9.25% were vaccinated and dewormed, and they were allocated to receive randomly three treatments diet with 5 replicates using a single-factor completely randomized design. The three dietary treatments were rice straw plus concentrate diet (RSC), ammoniated rice straw plus concentrate (ARSC), and ammoniated rice straw plus concentrate supplemented with *H. tiliaceus* leaf flour (ARSC+H).

The treatment involved feeding rice straw (RS), ammoniated rice straw (ARS), and concentrate (C) diets, with the nutrient content shown in Table 1. A proximate analysis was conducted to evaluate the nutritional composition of feed ingredients, including dry matter (DM), ash, crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen-free extract (NFE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and gross energy (GE). This analysis provides a comprehensive evaluation of the nutrient profile, particularly assessing the impact of ammoniation on rice straw quality. Concentrate feed ingredients were composed of cassava pulp, pollard bran, rice bran, coconut meal, palm kernel meal, salt, urea, proaminosin, dolomite, minerals, and molasses. *H. tiliaceus* leaf flour (HTLF) from the small leaf type was then ground and filtered with a size of 0.5 mm. We mixed the HTLF flour with the concentrated ingredients using a mixer. The amount of HTLF used in this research was 0.48% of concentrated dry matter, according to (Bata & Rahayu, 2016). This study consists of three treatments, namely RSC, ARSC, and ARSCH. The feed composition and nutrient content are presented in Table 2. The feed composition ratio was determined according to cattle consumption during the feeding trial.

The ammonization of rice straw used 5% urea and an additional 2.5% (DM rice straw) of tapioca waste (cassava pulp). The urea and cassava pulp were mixed well and added to water until the concentration reached 10%, as per the guide (Bata & Rahayu, 2016). Rice straw ammonization was done using a thick plastic container and two silos with a volume of 500 kg and 250 kg. The cassava pulp-urea solution was sprayed evenly to the rice straw with a thickness of 5 cm. The ammonization process was conducted for a minimum of 14 days. This research was undertaken for 84 days, including 14 days of adaptation period and 5 days of digestion and balance trial.

Feeding Management

The animals were fed on concentrates at 2.5% of body weight, while RS and ARS were offered ad libitum for 84 days (14 days of adaptation, 65 days of feeding trial, and 5 days of digesta collection and balance trial). The concentrates were offered at 07.00 a.m. and 2.00 p.m. while RS and AR were at 09.00 a.m., 5.00 p.m.,

Table 1. Nutrient content of ingredients of experimental diets for Madura cattle

Nutrients	Ingredients (DM Basis)			
	RS	ARS	Concentrate	Concentrate + HTLF
Dry matter (%)	90.93	89.63	91.78	91.46
Ash (%)	23.40	22.72	13.13	12.58
Crude protein (%)	4.87	8.76	14.32	13.59
Crude fiber (%)	39.03	34.23	24.76	23.53
Ether extract (%)	1.41	1.02	3.79	4.25
NFE %	31.29	33.27	44.00	46.05
NDF%	78.66	78.75	69.85	58.31
ADF%	49.26	46.09	27.29	20.80
GE (Mcal/kg)	2.730	2.673	3.152	3.277

Note: RS, Rice straw; ARS, Ammoniated rice straw; HTLF, *Hibiscus tiliaceus* leaf flour; NFE, nitrogen free extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; GE, gross energy.

Table 2. Feed composition of experimental diets for Madura cattle

Feed	Diets		
	RSC	ARSC	ARSC+H
Rice straw (%)	27		
Ammoniated rice straw (%)		30	29
Concentrate (%)	73	70	71
Hibiscus tiliaceus leaf extract (%)			0.48
Nutrient composition			
Dry matter (%)	91.55	91.14	90.93
Ash (%)	15.90	16.01	15.52
Crude protein (%)	11.77	12.65	12.19
Crude fiber (%)	28.61	27.60	26.63
Ether extract (%)	3.15	2.96	3.31
NFE %	40.57	40.78	42.34
NDF%	72.23	72.52	64.24
ADF%	33.22	32.93	28.13
GE (Mcal/kg)	3.04	3.01	3.10

Note: RS, rice straw; ARS, ammoniated rice straw; HTLF, *Hibiscus tiliaceus* leaf flour; NFE, nitrogen free extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; GE, gross energy.

and 8.00 p.m. Drinking water was supplied *ad libitum*. During the feeding trial, we weighed the offered and refused concentrates, RS, and ARS, and took samples for analyses of dry matter every week to count the feed efficiency (FE).

Variable Measured, Sample Collection, and Chemical Analysis

Nutrient utilization and digestibility. Five days following the feeding trial, daily records of feed offered and refusals were maintained to determine nutrient composition. Total digestibility of nutrients, nitrogen balance, and energy utilization were assessed using the total collection method (Krause *et al*., 1998). Rice straw (RS) and ammoniated rice straw (ARS) were core-sampled, while concentrate feed was grab-sampled and composited for each treatment prior to chemical analysis (Lewis *et al*., 1996). RS, ARS, concentrates, and fecal samples were processed by grinding to analyze DM, OM/ash, CF, EE, and CP, following the methods of AOAC (2019).

During the collection period, total fecal output was recorded daily. The animals were equipped with harnesses to ensure effective separation of feces. A 5% representative sample was dried and stored for further analysis. The DM content of each sample was determined, while ash, CF, EE, and CP or nitrogen (N) analyses were performed after pooling fecal samples per treatment per animal.

Nitrogen utilization. Urine samples were collected from individual animals and filtered through multiple layers of cheesecloth according to the method described by Balcells *et al*. (1992a). The urine was directed into containers containing 10% sulfuric acid (H_2SO_4) to reduce pH and prevent ammonia volatilization. Urine samples were stored at -20 °C until further analysis.

Feed, feed refusals, and fecal samples were analyzed for DM, ash, CF, EE, and digestible energy (DE), according to AOAC (2019). Nitrogen content was calculated as CP to analyze intake, composition, urine, and subsequent digestion. Nitrogen retention was determined as dietary nitrogen intake (NI) minus total nitrogen excreted (fecal N and urinary N), following the approach of Katsande *et al*. (2016). Net nitrogen utilization (NNU) is determined using nitrogen intake (NI) and nitrogen retention (NR), while protein biological value (PBV) is calculated based on nitrogen absorption (NA) and nitrogen retention (NR). Purine derivative concentrations, including allantoin, uric acid, xanthine, and hypoxanthine, in urine samples were measured according to Balcells *et al*. (1992b). High-performance liquid chromatography (HPLC) was conducted using two 4.6 mm × 250 mm C-18 reverse-phase columns (Spherisorb), with effluent detection at 205 nm. The HPLC system was manufactured by Agilent Technologies, USA.

Protein synthesis (MPS) was estimated via the colorimetric procedure as described by Chen & Gomes (1992). Microbial protein production (MPP) was determined using the equation MPP= MPS × 6.25 (g/d). The efficiency of MPS (EMPS) was expressed in g N/kg digestible organic matter in the rumen (DOMR) and calculated as DOMR= organic matter (OM) intake × OM digestibility × 0.65, with EMPS= MPS/DOMR.

Energy balance, digestibility, methane production, volatile fatty acid, and growth efficiency. At the end of the experiment, the cattle were weighed using a digital cattle scale. The average daily gain (ADG) was determined by the difference between the final and initial weights over the length of the experiment period. The energy utilization was determined by measuring energy intake (EI), digestible energy intake (DEI), metabolizable energy intake (MEI), energy retention (RE), RE to EI ratio, and RE to DEI ratio using the total collection method (Cole & Ronning, 1974). RE was determined from the difference between digestible energy intake and the total urine energy output. MEI was determined by the difference between energy retention and methane energy output. Methane energy output and E1 were calculated using estimation by (Ryle & Ørskov, 1990), specifically methane energy output calculated with the formula (((2pa+2pb)-pp)/4) x 210.8, pa is the proportion of acetate, pb is the proportion of butyrate, and pp is the proportion of propionate. Concentrations of VFA partial was measured using gas chromatography techniques (Guan *et al*., 2008). The efficiency of conversion of hexose to VFA (E_1) was calculated using estimates by (Ryle & Ørskov, 1990), i.e., percentage of (0.622 pa+ 1.092 pp+ 1.560 pb)/(pa+pp+2pb), where pa is the proportion of acetate, pb is the proportion of butyrate, and pp is the proportion of propionate.

Performance of cattle. Metabolic body weight ($BW^{0.75}$) was derived by elevating body weight (kg) to the exponent of 0.75. Initial and final $BW^{0.75}$ were recorded using a digital cattle scale at the beginning and conclusion of the experiment. Average daily gain ($ADG^{0.75}$) (kg/day) was computed as the difference between the final and initial $BW^{0.75}$, divided by the study duration. Feed efficiency (%) was determined by calculating the ratio of ADG to dry matter intake (DMI), reflecting the efficiency of feed conversion.

Statistical Analysis

All data were expressed as means and were statistically analyzed using SPSS software with a one-way ANOVA under a completely randomized design. Mean differences were evaluated using the Tukey post hoc test, with significance established at p<0.05.

RESULTS

Dry Matter Intake and Nutrient Digestion

Data on nutrient intake and digestibility of every treatment are presented in Table 3. Supplementation with *Hibiscus tiliaceus* leaf flour (ARSC+H) significantly increased DMD and OMD compared to the RSC diet (p<0.01). Similarly, CFD and CPD were significantly lower in cattle fed the RSC diet compared to ARSC and ARSC+H (p<0.01). The ARSC diet increased CFD by 49.79% and CPD by 8.46% compared to RSC, while the ARSC+H diet resulted in similar CPD but slightly lower CFD compared to ARSC (p>0.05). Despite the improvements in digestibility, feed intake remained unchanged (p>0.05) across treatments. There was no difference between ARSC and ARSC+H (p>0.05) on both parameters.

Nitrogen Utilization and Microbial Protein Production

Nitrogen utilization and microbial protein production are presented in Table 4. The analysis of variance showed that the treatments significantly

Table 3. Nutrient intake and digestion of Madura cattle fed ammoniated rice straw and concentrate containing *Hibiscus tiliaceus* leaf

Variables	Diets			Sig.
	RSC	ARSC	ARSC+H	
Feed intake (DM/kg)	7.79 ± 0.78	7.46 ± 0.79	6.84 ± 1.38	0.491
RS/ARS (DM/kg)	2.07 ± 0.20	2.24 ± 0.16	1.92 ± 0.38	0.357
Concentrate (DM/kg)	5.27 ± 0.64	5.22 ± 0.90	4.92 ± 1.16	0.546
RS or ARS/C DM ratio	27 : 73	30 : 70	29 : 71	-
DMD (%)	58.29 ± 4.05^{a}	63.88 ± 3.26^{b}	65.68 ± 2.16^{c}	0.001
OMD (%)	62.34 ± 3.74^{a}	66.96 ± 3.13^{b}	69.21 ± 1.93^{c}	0.001
CFD (%)	40.54 ± 5.41^{a}	60.70 ± 3.23^{b}	57.60 ± 2.77^{b}	0.001
CPD (%)	72.37 ± 3.02^{a}	78.49 ± 2.27^{b}	78.42 ± 1.48^{b}	0.001
EPD (%)	76.65 ± 4.94	71.59 ± 2.95	72.71 ± 2.95	0.348

Note: [abc]Means in the same row not having at least one common superscript differ significantly (p<0.05); RSC, rice straw + concentrate; ARSC, ammoniated rice straw + concentrate; ARSC+H, ARS + concentrate supplemented with *Hibiscus tiliaceus* leaf flour; DMD, dry matter digestibility; OMD, organic matter digestibility; CFD, crude fiber digestibility; CPD, crude protein digestibility; EPD, effective protein digestibility.

Table 4. Nitrogen utilization of Madura cattle fed ammoniated rice straw and concentrate containing *Hibiscus tiliaceus* leaf

Variables	Diets			Sig.
	RSC	ARSC	ARSC+H	
Nitrogen intake (g/day)	0.71 ± 0.05	0.71 ± 0.05	0.74 ± 0.04	0.546
Fecal nitrogen (g/day)	0.24 ± 0.01^{b}	0.16 ± 0.01^{a}	0.14 ± 0.03^{a}	0.001
Nitrogen digestion (%)	65.6 ± 3.40^{a}	76.6 ± 0.70^{b}	81.04 ± 3.28^{b}	0.001
Urine nitrogen (mL/day)	0.12 ± 0.02	0.12 ± 0.02	0.10 ± 0.03	0.741
Nitrogen retention (g/day)	0.34 ± 0.05^{a}	0.42 ± 0.04^{b}	0.49 ± 0.05^{b}	0.001
Net nitrogen utilization (%)	0.48 ± 0.04^{a}	0.59 ± 0.03^{b}	0.66 ± 0.05^{b}	0.001
Protein biological value (%)	0.74 ± 0.03	0.77 ± 0.04	0.81 ± 0.05	0.063
MPS (g/N day)	445 ± 3.07^{a}	459 ± 3.40^{b}	504.57 ± 2.10^{c}	0.001
EMPS (g N/ kg OMD/ day)	22.0 ± 2.80	22.2 ± 3.25	26.25 ± 6.34	0.344
MPP (g/day)	2786 ± 19.18^{a}	2874 ± 21.27^{b}	3153.60 ± 13.13^{c}	0.001
OMD (kg/day)	20.5 ± 2.45	21.1 ± 3.11	20.05 ± 4.37	0.951

Note: [abc]Means in the same row not having at least one common superscript differ significantly (p<0.05). RSC, rice straw + concentrate; ARSC, ammoniated rice straw + concentrate; ARSC+H, ARS + concentrate supplemented with *Hibiscus tiliaceus* leaf flour; MPS, microbial protein synthesis; EMPS, efficiency of MPS; MPP, microbial protein production; OMD, organic matter digestibility.

affected ($p<0.01$) fecal nitrogen (FN), nitrogen digestibility (ND), nitrogen retention, net nitrogen utilization (NNU), microbial protein synthesis (MPS), and microbial protein production (MPP), but did not affect ($p>0.05$) nitrogen intake (NI), urine nitrogen (UN), protein biological value (PBV), microbial protein synthesis efficiency (ESPM) and organic matter digestibility (OMD). The lowest fecal nitrogen (FN) ($p<0.01$) was in cattle fed ARSC+H and ARSC followed by RSC. The highest ND, NR, NNU, MPS, and MPP in cattle fed on ARSC+H ($p<0.01$), followed by RSC and ARSC, respectively. The protein biological value (PBV) of cattle in ARSC+H tended to be higher ($p=0.063$) compared to ARSC and RSC.

Energy Utilization

The treatments significantly decreased ($p<0.05$) fecal energy output (FEO) and increased ($p<0.05$) energy digestibility (ED) (Table 5). The lowest FEO was observed in cattle fed ARSC+H, followed by ARSC and RSC ($p<0.05$). Energy digestibility was significantly higher in ARSC and ARSC+H compared to RSC ($p<0.05$). The efficiency ratio of energy retention to energy intake (ER to EI) was significantly lower ($p<0.05$) in cattle-fed RSC compared to ARSC and ARSC+H. However, no significant difference ($p>0.05$) was observed between ARSC and ARSC+H. The efficiency ratio of energy retention to digested energy intake (ER to DEI) was significantly lower ($p<0.05$) in ARSC+H compared to RSC and ARSC. Total volatile fatty acid (VFA) production and individual VFA concentrations (C_2, C_3, and C_4) were significantly higher ($p<0.05$) in cattle fed ARSC+H compared to ARSC and RSC. However, there were no significant differences ($p>0.05$) between ARSC and RSC. Methane energy output (MEO) was significantly higher ($p<0.01$) in ARSC+H compared to RSC and ARSC, while no significant differences ($p>0.05$) were observed between RSC and ARSC. The C_2/C_3 ratio and E1 were not significantly affected ($p>0.05$) by the treatments.

Performances of Madura Cattle

The treatments significantly increased ($p<0.05$) the average daily gain (ADG) and feed efficiency (FE) (Table 6). The highest ADG ($p<0.05$) was observed in cattle fed ARSC+H, followed by ARSC and RSC. Feed efficiency was significantly improved ($p<0.01$) in ARSC+H compared to ARSC and RSC, with ARSC+H showing the best (highest) feed efficiency, followed by ARSC and RSC.

Table 5. Energy balance, digestibility, methane production, volatile fatty acid, and growth efficiency of Madura cattle fed ammoniated rice straw and concentrate containing *Hibiscus tiliaceus* leaf

Variables	Treatments			Sig.
	RSC	ARSC	ARSC+H	
Energy intake (MJ/d)	99.39 ± 2.41	93.57 ± 2.46	89.03 ± 4.28	
Fecal energy output (MJ/d)	37.20 ± 1.26^{b}	29.82 ± 0.57	28.45 ± 1.29^{a}	
Digested energy intake (MJ/d)	62.17 ± 1.794	63.75 ± 2.203	60.58 ± 3.08 0	0.865
Energy digestibility (%)	62.94 ± 3.744^{a}	68.13 ± 3.013^{b}	68.04 ± 1.971^{b}	0.017*
Urine energy output (MJ/d)	0.305 ± 0.015^{ab}	0.146 ± 0.007^{a}	0.502 ± 0.039^{bc}	0.000**
Methane energy output (MJ/d)	11.38 ± 0.668^{a}	13.54 ± 1.517^{a}	25.48 ± 0.612^{b}	0.016*
Metabolizable energy intake (MJ/d)	51.55 ± 1.616^{a}	50.21 ± 2.007^{a}	35.09 ± 2.730^{b}	0.856
Energy retention (MJ/d)	61.86 ± 1.779	63.60 ± 2.198	60.08 ± 3.068	0.869
ER to EI Ratio (%)	62.24 ± 3.718^{a}	67.97 ± 3.007^{b}	67.48 ± 2.124^{b}	0.021*
ER to DEI Ratio (%)	99.50 ± 0.047^{b}	99.77 ± 0.033^{b}	99.17 ± 0.263^{a}	0.000**
Acetic acid (C_2) (mMol)	22.74 ± 4.833^{a}	24.28 ± 8.295^{a}	43.05 ± 3.415^{b}	0.011*
Propionic acid (C_3) (mMol)	4.520 ± 0.591^{a}	4.903 ± 1.262^{a}	8.620 ± 0.947^{b}	0.004**
Butyric acid (C_4) (mMol)	2.880 ± 1.031^{a}	4.370 ± 2.932^{a}	9.060 ± 1.301^{b}	0.025*
Total VFA (mM)	30.14± 5.844^{a}	33.55 ± 12.461^{a}	60.73 ± 5.663^{b}	0.010*
Ratio of $C_2 : C_3$	5.013 ± 0.807	4.890 ± 0.442	5.003 ± 0.153	0.208
E_1 (%)	71.50 ± 0.837	71.91 ± 0.395	72.09 ± 0.239	0.090

Note: [abc]Means in the same row not having at least one common superscript differ significantly ($p<0.05$). RSC, Rice straw + concentrate; ARSC, ammoniated rice straw + concentrate; ARSC+H, ammoniated rice straw + (concentrate + 0.48% *Hibiscus tiliaceus* leaf flour; ER, energy retention; EI, energy intake; DEI, digestible energy intake; VFA, volatile fatty acid; E1, efficiency of conversion of fermented hexose energy to VFA energy.

Table 6. Performances of Madura cattle fed ammoniated rice straw and concentrate containing *Hibiscus tiliaceus* leaf

Variables	Diets		
	RSC	ARSC	ARSC +H
Initial body weight$^{0.75}$ (kg)	71.66±6.58	67.20±5.51	67.59±3.66
Final body weight$^{0.75}$ (kg)	85.22±7.25	77.32±5.51	75.77±4.64
ADG$^{0.75}$ (kg/day)	0.57±0.05^{a}	0.62±0.75^{a}	0.82±0.09^{b}
Feed efficiency (%)	8.18±0.30^{a}	8.88±0.37^{b}	12.02±0.44^{c}

Note: [abc]Means in the same row not having at least one common superscript differ significantly ($p<0.05$). RSC, rice straw plus concentrate; ARSC, ammoniated rice straw + concentrate; ARSC+H, ARS+Concentrate supplemented with *Hibiscus tiliaceus* leaf flour; ADG, average daily gain.

DISCUSSION

We observed similar results (p>0.05) for energy, nitrogen/protein, and DM consumption across all treatments. However, total DM and concentrate consumption in cattle fed ARSC+H tended to decrease compared to cattle fed ARSC and RSC. This result was similar to what Paniagua *et al*. (2019) found when they added citrus aurantium flavonoid extract to high-concentrate feed for cows. They found that it didn't change how much concentrate the cattle ate but instead made it more efficient at using concentrate. Yu *et al*. (2023) reported that the addition of citrus flavonoid extract did not affect DM consumption. Aguiar *et al*. (2012) found that different levels of total flavonoid propolis consumed by dairy cows did not affect the consumption of DM, OM, CP, and NDF compared to control. These results contrasted with those reported by (Orzuna-Orzuna *et al*., 2023), who reported an increase in dry matter intake (DMI) in response to flavonoid supplementation. Hao *et al*. (2023) also reported an increase in DMI in finishing lambs that consumed sea-buckthorn flavonoid-supplemented feed. These differences are likely due to the feed and the types, dosages, and sources of flavonoids offered to the ruminant cattle, and they align with the research of Ampapon and Wanapat (2021).

Although DM consumption was not different across treatments, cattle that consumed feed supplemented with *H. tiliaceus* leaf flour (ARSC+H) exhibited the highest levels of DMD and OMD compared to cows fed on ARSC and RSC. In a similar study, Aguiar *et al*. (2012) found that DMD levels were significantly higher in cattle that were fed 2.81 mg/kg DM of flavonoid total propolis compared to cattle that were fed 1.22 mg/kg of the same supplement. Protein digestibility CPD, ND, CFD, and ED of cattle fed on ARSC+H were higher than those feeding on RSC (Table 3). This is because using a lot of concentrate (73.36% total DMI) in RSC with non-ammoniated straw and no *H. tiliaceus* leaf flour can make the rumen pH too acidic, which can make it hard for microorganisms to do their activities. Cattle-fed ARSCH+H had a higher rumen pH than cattle-fed RSC due to the high concentration of easily degradable non-protein nitrogen (NPN) in the ammoniated rice straw in ARSCH. The ammoniation of rice straw results in the release of ammonia, which serves as a crucial buffering agent in the rumen by neutralizing excess acids generated during the fermentation process. This buffering capacity is fundamental in maintaining ruminal pH homeostasis, thereby fostering an optimal microbial environment essential for efficient fiber degradation and nutrient assimilation (Nagaraja & Titgemeyer, 2007). By mitigating excessive declines in pH, ammonia helps preserve rumen functionality, reducing the incidence of acidosis and enhancing overall ruminant health and productivity (Matthews *et al*., 2019). Ammoniated rice straw helps maintain rumen pH within the optimal range (usually 6.0–7.0) for fiber-digesting bacteria. This is beneficial because too low a pH (below 5.5) can inhibit fiber-digesting microbes and lead to acidosis (Zhang *et al*., 2025). In addition to ammoniated rice straw, increased nutrient digestibility (CPD, ND, CFD, and ED) in the ARSCH cattle group was also due to the addition of flavonoids through *H. tiliaceus* leaf flour. Flavonoids stimulate *Megasphaera elsdenii* growth, converting lactic acid from high-concentrate diets into propionic acid, maintaining ruminal pH and microbial activity, which enhances nutrient digestibility. Lack of certain types and amounts of flavonoids can inhibit the growth of lactic acid bacteria like *Streptococcus bovis* and *Lactobacillus* spp. and help the growth of lactic acid bacteria like *M. elsdenii* (Balcells *et al*., 2012; Li *et al*., 2022). This selective inhibition can lower the amount of lactic acid that builds up and helps lactic acid bacteria like *M. elsdenii* and *Selenomonas ruminantium* do their activities (Balcells *et al*., 2012). Lactic acid bacteria help prevent acidosis by regulating rumen pH, ensuring stable microbial activity. Li *et al*. (2017) demonstrated that flavonoids significantly increase the cellulolytic bacteria (*R. albus*) in the rumen of fattening steers fed with diets containing ensiled mulberry leaves. Zhan *et al.* (2017) demonstrated that alfalfa flavonoid extract (AFE) in lactating cows tended to increase protein and crude fiber digestibility. Flavonoids have selective antimicrobial properties. They may inhibit the growth of some harmful ruminal bacteria, like *E. coli* and *Clostridium*, but they help the growth of good bacteria, like Fibrobacter and *Ruminococcus species*, which are needed for fiber digestion (Seradj *et al*., 2018). These results showed that flavonoids can affect nutrient digestibility and utilization; however, flavonoids derived from different plants and given at different levels will have different effects (Paniagua *et al*., 2022).

When flavonoids were added to cattle-fed ARSC+H, it did not enhance (p>0.05) CPD, ND, CFD, and ED compared to cattle-fed ARSC. Chen *et al*. (2015) reported that the supplementation of flavonoid mulberry leaf did not impact the DM, OM, NDF, and ADF digestibility. The presence of high NPN in the ammoniated rice straw in ARSC, which degrades easily, likely led to a more alkaline ruminal pH in the cows fed on ARSC+H, as explained in the previous section. Therefore, the addition of flavonoids did not significantly enhance the nutrient digestibility of cattle ARSC compared to those fed RSC.

Cattle fed on ARSC+H had the lowest (p<0.01) levels of N in their feces (FN) and tended to decrease (p=0.741) urine nitrogen (UN) when compared to cattle fed on RSC or ARSC (Table 4). As a result, the cattle that received a diet of ARSC+H treatment had the highest levels of ND, NR, and NNU and tended to increase (p=0.063) the protein biological value (PBV). Hao *et al*., 2023) reported that the addition of sea-buckthorn flavonoids for finishing lambs diet reduced the UN and increased the N retention (NR). A variety of factors, including diet composition, microbial activity, and rumen dynamics, influence nitrogen utilization in the rumen of cattle. Flavonoids may reduce the activity of proteolytic bacteria that break down proteins into ammonia (Donadio *et al*., 2021; Xie *et al*., 2014). This can result in lower ammonia concentrations in the rumen, leading to better N utilization and less N waste.

Flavonoids can improve the efficiency of N utilization by changing the rumen environment and microbial populations. This can improve animal performance and lower N excretion. Therefore, cattle fed ARSC+H produced higher ADG and FE compared to those that received RSC and ARSC feed.

Adding flavonoids can change the main signs of N use in the rumen, such as ND, NR, and NNU, as well as the process of microbial protein synthesis. The results showed that the cattle fed on ARSC+H showed the highest MPS and PPM, followed by ARSC and RSC, while EMPS tended to be high (p=0.344) in cattle fed on ARSC+H compared to RSC and ARSC. The addition of flavonoids to cattle diets can significantly influence factors affecting microbial protein synthesis in the rumen (Kim *et al*., 2015). When added to high-concentrated feed for beef cattle, flavonoids would help more protein microbes get into the duodenum, which would make metabolic amino acids and ADG more available (Balcells *et al*., 2012). Flavonoids can improve microbial protein synthesis by changing the pH of the rumen, the structure of the microbial population, and the availability of nutrients and energy. They can also protect against oxidative stress. However, the specific type of flavonoid and its interaction with other dietary components influence their dose-dependent effects (Min & Solaiman, 2018). Hao *et al*. (2023) reported that the addition of sea-buckthorn flavonoids for finisher lambs can increase the SPM. Li *et al*. (2022) also reported that the use of mulberry flavonoids at a dose of 45 g/day increases SPM in water buffalo. When bioflavex was given to steers in feedlots, it stopped the pH from dropping, improved rumen fermentation by changing the activity of bacteria that break down lactate, raised the molar proportion of propionate, and lowered the molar proportion of acetate. This shows that it plays a positive role in changing the activity of rumen microbiota (Seradj *et al*., 2018).

The ruminal microbe and ruminants require energy from the volatile fatty acids (VFA) derived from carbohydrate fermentation for protein synthesis and growth. A sufficient supply of energy from the fermentation can increase the production of microbial protein and cattle performance. The supplementation of flavonoids, such as in cattle fed on ARSC+H, increased the energy production of VFAT, C_2, C_3, and C_4 compared to RSC and ARSC (Table 5). This volatile fatty acid is a source of energy for microorganisms and their hosts. This result aligns with the elevated levels of SPM, PPM, ADG, and FE in the cattle belonging to the ARSC+H group. The high level of VFA in ARSC+H is because flavonoid stimulates the activities of fiber-degrading bacteria, which are positively correlated with the production of acetic acid and lactic acid bacteria, which convert lactic into propionic in the rumen. The improved activities of these bacteria will increase both total and partial VFA. In 2012, Bodas *et al*. found that flavonoids are important feed additives that make fiber-digesting bacteria, like *Fibrobacter succinogenes* and *Ruminococcus spp*., work better in cattle that are fed high concentrates. Flavonoids have also, directly and indirectly, made lactic acid bacteria like *M. elsdenii* work better by increasing the activity of enzymes involved in lactic metabolism. Enhancing the conversion of lactic into propionic, the primary energy source for cattle fattening, can decrease or prevent acidosis (Yesudhas *et al*., 2023) and improve energy efficiency. This mechanism optimizes ruminal fermentation and fermentation products due to the higher level of flavonoid.

The types and amount of produced VFA affect energy availability and energy retention. Cows fed on ARSC+H showed the highest total and partial VFA compared to those feeding on RSC and ARSC. However, the efficient conversion from glucose to VFA (E_1) and the C_2/C_3 ratio of ARSC+H were relatively the same. This resulted in the ER levels in RSC, ARSC, and ARSC+H remaining relatively unchanged. One of the causes was the high production of methane (MEO) in ARSC+H, which is a byproduct of unused energy. Therefore, reducing methane emissions can enhance energy retention by reducing the amount of energy wasted during fermentation. Strategies like altering the diet (e.g., increasing starch or using feed additives that promote propionate production over acetate) can reduce methane emissions while improving energy efficiency. Enhanced energy retention improves performance outcomes such as weight gain and feed efficiency.

In this study, feeding feedlot cattle on RSC feed with a high concentrate that contains easily fermentable carbohydrates increases the risk of acidosis. This is due to the rapid production of short-chain fatty acids (C_2, C_3, and C_4), which are the primary energy source for cattle fattening. The rumen's supporting capacity will be reduced by the fast-accumulating fatty acid, leading to acidosis or subacute ruminal acidosis. Meanwhile, changes in the structure and diversity of the ruminal bacteria community (Mao *et al*., 2016) lead to the progressive release of lipopolysaccharide (LPS), a component of gram-negative bacteria cell walls (Eckel & Ametaj, 2016; Plaizier *et al*., 2017). It has been shown that lipopolysaccharides can move through the digestive system's epithelial tissue and cause inflammation (Huo *et al*., 2022). Liu *et al*. (2020) reported that feeding the high-concentrate diet (HCD) promoted the pH reduction and the LPS release in the rumen. Furthermore, feeding the HCD increased inflammatory response and disturbed the ruminal bacterial stability. Therefore, cattle fed RSC in this study produced low ADG and FE.

In this study, cattle that were fed ARSC+H had higher levels of CPD, CFD, OMD, DMD, NR, NNU, BV, SPM, PPM, ED, VFA, and tended to have higher levels of E_1 and EMPS. This is why cattle in this group perform better than cattle in the RSC and ARSC. Cattle fed on ARSC+H had a higher level of ADG than the RSC, which tended to be higher than ARSC. Meanwhile, a higher level of FE was evident in ARSC+H than in RSC and ARSC control (Table 6). Rahayu *et al*. (2021) reported that adding *H. tiliaceus* leaf flour to high-concentrated feed for Bali cattle (70% of total DM) resulted in better ADG levels than the control. Zhan *et al*. (2017), among others, reported that supplementing dairy cattle feed with flavonoids enhanced the relative abundance of ruminal microbes *Tenericutes* and *Mollicutes*, a finding that positively correlated with the ADG of finisher sheep (Du *et al*., 2019). Flavonoid supplementation for growing sheep reduces the relative abundance of ruminal bacteria from the Veillonellaceae family (Zhao *et al*., 2022), a finding that negatively correlates with the ADG of sheep (Zhang *et al*., 2020). Du *et al*. (2019) found that adding plants with flavonoids to beef cattle's food increases the amount of the ruminal microbe *Rikenellaceae* in the fluid around their rumen, which is linked to ADG and FE in a positive and negative correlation, respectively (Yi *et al*., 2023).

Cows fed on ARSC+H showed a higher ADG and FE than cows fed on ARSC or RSC. This is due to the role of flavonoid in *H. tiliaceus*, which improves ruminal metabolism by increasing the digestibility of energy, fermentation products of total VFA and partial VFA (such as acetic and propionic acid), and by enhancing the efficiency of glucose conversion to VFA (E_1), as discussed in the previous section. Additionally, flavonoids prevent acidosis, which hinders growth and feed efficiency in cows fed on RSC and ARSC. Based on the type and amount of flavonoid, it can stop the growth of lactic acid bacteria like S*treptococcus bovis* and *Lactobacillus spp*. and help the growth of lactic-using bacteria like *M. elsdenii* (Balcells *et al*., 2012; Li *et al*., 2022). This selective inhibition can lower the amount of lactic acid that builds up and help lactic acid bacteria like *M. elsdenii* and *Selenomonas ruminantium* do their job. This mechanism plays a crucial role in preventing the accumulation of lactic acid, which can lower the ruminal pH and lead to acidosis. This condition can hinder the growth and efficiency of feed utilization, just as it did for the cows fed on RSC and ARSC in our study.

Bacteria can convert lactic into different types of VFA, such as propionic, which can be assimilated and harnessed as a source of energy. Additionally, fumaric acid serves as the primary ingredient in *H. tileaceus*, leading to a higher propionic acid production in ARSC+H compared to other treatments (Bata *et al*., 2021). During the feedlot process, propionic acid is used for glucose synthesis, which will synthesize glycerol and fatty acids. Both compounds are the key components of triglycerides stored in the adipose tissue that increase body fat deposition (Nagaraja & Titgemeyer, 2007). Propionic acid generates a higher energy efficiency than other volatile acids, such as acetic acid. In addition, the formation of propionic acid (C_3) in the rumen requires hydrogen, which is also the main component of methane. Therefore, an increase in C_3 will decrease the amount of methane the rumen excretes. This results in a greater improvement in feed efficiency for cows that consume feed with flavonoid (ARSC+H) compared to those that only consume ARSC or RSC. Nozière *et al*. (2011) stated that the improved feed efficiency indicates a higher proportion of energy conversion into body mass (Brunes *et al*., 2021), thus accelerating an effective fattening process.

Increased ADG in cows fed ARSC+H results from improved health, enhanced fermentation (VFA, PPM, SPM), and better nutrient utilization. Flavonoid supplementation (200–400 mg/kg DM) increases IGF-1 levels, which correlate positively (r = 0.61–0.67) with ADG in ruminants (Balcells *et al*., 2012). The current meta-analysis (Orzuna-Orzuna *et al*., 2023) also discovered that adding flavonoids to the body raised levels of antioxidant enzymes (SOD, CAT, and GPx) and immunoglobulins (IgA, IgG, and IgM). These actions may minimize oxidative stress and improve animal health, potentially improving animal performance. Previous research (Paniagua *et al*., 2019) showed that giving beef cattle that eat high-concentrate diets 400 mg/kg DM of flavonoids improves the health of their rumen epithelial cells. The presence of papillae in the rumen epithelium may increase the absorption of volatile fatty acids, leading to an increase in ADG (Kern *et al*., 2016).

CONCLUSION

Supplementing *Hibiscus tiliaceus* leaf flour (ARSC+H) at 0.48% of DM enhanced nutrient digestibility, nitrogen utilization, and energy efficiency in Madura cattle. Despite unchanged feed intake, ARSC+H improved crude protein and fiber digestibility, nitrogen retention, and microbial protein synthesis. It also increased energy digestibility and volatile fatty acid production, though methane output was higher. Additionally, ARSC+H improved feed conversion efficiency and weight gain, supporting its potential as a sustainable feed additive.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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