p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, March 2024, 47(1):97-103
DOI: https://doi.org/10.5398/tasj.2024.47.1.97
Available online at https://journal.ipb.ac.id/index.php/tasj

Improving the Rumen Molar Proportion of Glucogenic Volatile Fatty Acids with the Inclusion of Siam Weed (*Chromolaena odorata*) Meal in Pelleted Diet of Fattened Cattle

M. L. Mullik[a,*], T. O. D. Dato[a], Y. M. Mulik[b], & G. Oematan[a]
[a]Study Program of Animal Husbandry, Faculty of Animal Husbandry, Marine and Fisheries, Universitas Nusa Cendana
Jalan Adisucipto Penfui, Kupang, NTT, Indonesia
[b]Department of Animal Husbandry, Politeknik Pertanian Negeri Kupang
Jalan Yohanes, Penfui, Kupang, NTT, Indonesia
*Corresponding author: marthenmullik@staf.undana.ac.id
(Received 01-08-2023; Revised 05-12-2023; Accepted 14-12-2023)

ABSTRACT

The objective of this metabolism study was to evaluate the efficacy of Siam weed (*Chromolaena odorata*) meal in pelleted diets for fattened cattle. Four 2-year-old Bali steers were assigned to four dietary treatments using a 4x4 Latin square experimental design. The treatments were pellets containing chromolaena meal at 10% (10COM), 20% (20COM), 30% (30COM), or 40% (40COM). The pellet was given at 2% liveweight (LW), and kume (*Sorghum plumosum* var. Timorense) grass hay was offered *ad libitum*. The diets were isonitrogenous (20%) and energy (11.5 MJ ME/kg DM). Dietary intake, digestibility, and rumen fermentation were the variables measured. The results showed that increasing chromolaena meal to 40% substantially decreased the nutrient intake. Dry matter intake decreased from 2.5% LW in the 10COM to 2.19% LW in the 40COM. Likewise, crude protein intake decreased from 749 g/d (10COM) to 661 g/d (40COM). On the contrary, digestibility, rumen pH, ammonia concentration (116–125 mg/dL), and volatile fatty acids were not affected. It might be concluded that chromolaena can be used as a protein source for ruminants, but at high levels of inclusion (40%) tends to reduce intake.

Keywords: Bali cattle; digestibility; kume grass; rumen fermentation; VFA

INTRODUCTION

Apart from being labeled as an evil weed (OISC, 2018) for its negative impacts on the agricultural systems (Ganguly *et al.*, 2022), Siam weed (*Chromolaena odorata*) could serve as a potential alternative feed source for both ruminants and non-ruminants (Jiwuba *et al.*, 2018; Tiamiyu & Okunlade, 2020) due to its high biomass production and high protein content (Oematan *et al.*, 2020), and well-balanced amino acid profile (Fasuyi *et al.*, 2005), and anti-oxidant properties (Lartey *et al.*, 2020). However, its utilization as livestock feed remains challenging due to its inherent anti-nutrient properties (Olawale *et al.*, 2022). Akintunde *et al.* (2021) reported that *C. odorata* leaf meal contains 3.15 mg/g oxalate, 2.09% phytate, 0.60% saponin, 6.30% flavonoids, 0.60 mg/g total phenolics, 0.002% tannins, and 1.66% alkaloids. With such a variety of anti-nutrients, treatments to eliminate them are required if a higher proportion in the ration is the target. Various pre-treatments have been tested yielding variable results. Mulik *et al.* (2016) evaluated the impact of five processing methods on chromolaena leaf meal found that anaerobic fermentation, boiling or soaking in plain water, and sun-drying reduced tannin concentrations by 43%-62% compared to the untreated materials. These researchers observed the greatest reduction with the anaerobic fermentation technique. The inclusion of cattle rumen liquor during the ensilage process of chromolaena leaf resulted in a 20.77% decrease in total tannin concentration (Ridla *et al.*, 2016). Whereas in an *in vivo* trial on fattened cattle, Bira (2015) found that increasing the proportion of sun-dried chromolaena leaf meal from 10% to 40% in complete diets provided in mash form led to reduced intake, digestibility, and rumen fermentation products.

Based on these findings, the current study aimed to examine the effectiveness of including chromolaena meal in pelleted complete diets on intake, digestibility, and rumen fermentation products of fattened Bali cattle. The rationale for this experiment is that the addition of heat and physical pressure during the pelleting process might enhance the biological value of chromolaena included in the concentrate for fattened cattle.

MATERIALS AND METHODS

The Livestock, Marine, and Fishery Ethics Committee of the Faculty of Animal, Marine, and Fishery Sciences of Nusa Cendana University approved

the protocols for cattle housing, handling, measuring of intake and digestibility, and rumen fluid collection techniques through Ethical Research Clearance Reference Number 020/3.SK/KEPPKP/II/2022 dated 10 February 2022.

This metabolism study employed a 4 x 4 Latin Square Experimental Design to examine four levels of chromolaena leaf meal in pelleted complete diets. The tested pellets contain chromolaena meal of 10% (10COM), 20% (20COM), 30% (30COM), or 40% (40COM). Feedstuffs used in the formulation of the complete diets comprised chromolaena leaf meal, *Leucaena leucocephala* leaf meal, maize flour, rice bran, fish meal, urea (to balance the nitrogen in the diet), and a mineral mix. The pellet composition was formulated according to SCA (2007) to achieve a daily weight gain of at least 0.6 kg/day. The energy and protein contents of the pellets were maintained as isoenergetic (11.4 MJ ME/kg DM) and isoproteinous (20% CP) for all treatments. The level of chromolaena in the pellets replaced leucaena leaf meal in equal amounts (10%-40%), as both feedstuffs contain similar crude protein and energy density. The nutrient composition of the feedstuffs is presented in Table 1.

In this metabolism experiment, four 2-year-old male Bali cattle with an average weight of 175 kg (±9.23 kg) were used. The pellets were provided at 2% of the animals' live weight, and kume grass (*Sorghum plumosum* var. Timorense) hay and drinking water were supplied *ad libitum*. The animals were randomly assigned to the treatments in each treatment period. Each treatment period consisted of a 10-day adaptation to diets and pens and a 7-day data collection.

The parameters measured were feed intake and digestibility of feed and nutrients, as well as rumen fermentation products. Feed intake was known by deducting the amount of feed offered with refusal over a 24 hour period. Feed intake was measured daily for five days in each collection period. Feed was provided twice a day, at 8 A.M. and 4 P.M. Daily feces production of individual cattle was collected and weighed during the intake measurement. Feces samples were collected daily for each animal (kept separate) and analyzed for nutrient content at the end of each period, allowing for the calculation of digestibility coefficients.

The rumen fluid samples from each animal were collected twice on the last day of each treatment period. The first sample was taken 0 hours before feeding, and the second was taken 3-4 hours after feeding. Rumen fluid was collected using a tube inserted into the rumen through the mouth and connected to a vacuum pump. Approximately 250 mL of rumen fluid was drawn and filtered through four layers of socking, and about 100 mL was transferred to a plastic container with a few drops of concentrated sulfuric acid to lower the pH below 3. Subsequently, about 100 mL was transferred to two plastic containers (50 mL each), properly capped, and stored in a freezer while awaiting VFA and ammonia analysis.

The nutrient content of the feed samples was analyzed according to the procedures outlined in AOAC (2005). Dry matter was determined by oven drying the samples at 65 °C for 48 hours. Organic matter content in the samples was quantified by furnace burning at 550 °C for four hours. The Micro-Kjeldahl technique was applied for crude protein analysis. Crude lipid content was measured using the ether extract technique, while crude fiber was determined by the boiling technique.

The pH of the rumen liquor was measured using a digital pH meter immediately after the rumen fluid was collected. Partial volatile fatty acids (VFA) concentration in the rumen fluid was determined by gas chromatography-mass spectrometry using a dimethyl carbonate extraction technique, as described by Foote (2022). Methane production based on partial VFA concentration was estimated using formula of Moss *et al.* (2000).

The obtained data were analyzed using the General Linear Model (GLM) for univariate data following the principles of the Latin Square Design. The treatments were considered significantly different at a p-value of ≤0.05. The Duncan multiple range test was applied for between-treatment comparisons. Data processing was performed using SPSS 25.0 (IBM, 2017).

RESULTS

The mean daily dry matter (DM) intake ranged from 2.19% live weight (LW) to 2.57% LW. The animals fed pellets containing 40% chromolaena (40COM) showed the lowest intake (2.19% LW), whereas the highest intake (2.57% LW) was recorded when animals were fed pellets containing 20% chromolaena (20COM). Increasing the levels of chromolaena in the pellets from 10% to 40% significantly suppressed ($p<0.05$)

Table 1. Nutrient composition of the feedstuff used in the experiment

Feedstuff	Dry matter (g/kg)	Organic matter (g/kg DM)	Crude protein (g/kg DM)	Crude lipid (g/kg DM)	Total carbohydrate (g/kg DM)	Crude fiber (g/kg DM)	Metabolizable energy (MJ/kg DM)
Kume grass hay	850	845	62	56	850	713	9.7
Pellet	870	812	200	63	604	551	11.4
Chromolaena meal	831	890	210	61	619	682	10.4
Leucaena meal	889	899	246	59	594	663	11.7
Maize meal	860	925	102	65	758	512	12.6
Rica bran	886	880	142	87	651	671	12.2
Fish meal	930	900	694	82	124	0	12.0
Urea	950	0	2,600	0	0	0	0.0
Mineral mix	960	0	0	0	0	0	0.0

all consumption variables. The total intake trend was observed in both grass hay and pellet consumption (Table 2). The reduction trend for all nutrient intakes was similar to that of DM intake.

Contrary to the intakes, all digestibility variables were not significantly affected by the levels of chromolaena in the pellets (Table 2). The digestibility values were 59%-63%, 65%-68%, and 82%-83% for DM, OM, and CP, respectively. Nitrogen retention showed a highly significant decline (p= 0.008) from 100 g/d in cattle-fed pellets containing 10% chromolaena to 87 g/d when the chromolaena meal reached 40% in the pellet (40COM).

The mean pH value of the rumen liquor collected at 0 h before and 3-4 h after feeding was in the range of 6.70–6.90 and was not affected by the levels of chromolaena in the pellets (Table 3). The rumen ammonia (NH_3) concentration was also not affected by the treatments. The lowest NH_3 value was 109 mg/L (30COM), and the highest was 125 mg/L (20COM). Both total and partial VFA concentrations in the rumen fluid were not affected by the treatments. The total VFA concentration ranged from 130.1 mM (40COM) to 134.7 mM (30COM). The difference in VFA values between treatments failed to be detected in the Duncan Multiple Range Test. Estimated methane production was in a range of 47.26–49.36 mM, and was not significantly different between treatment groups.

When the partial VFAs were expressed as molar proportions to the total, all measured VFAs (acetate, propionate, and butyrate) showed a significant decline in the C_2 equivalent VFAs (acetate and butyrate), while the C_3 (propionate) showed an increasing trend as the level of chromolaena in the pellets increased (Table

Table 2. Nutrient intake and digestibility of fattened cattle offered a pelleted diet containing *Chromolaena odorata* meal at different levels

Variables	Treatments				SEM	p-value
	10COM	20COM	30COM	40COM		
Intake:						
Grass DM (kg^{-d})	1.61^{b}	1.68^{b}	1.63^{b}	1.41^{a}	0.01	0.004
Grass DM (% LW)	0.83^{b}	0.86^{b}	0.84^{b}	0.72^{a}	0.01	0.015
Pellet DM (kg^{-d})	3.25^{b}	3.33^{b}	3.14^{ab}	2.87^{a}	0.01	0.019
Pellet DM (% LW)	1.68^{b}	1.71^{b}	1.62^{b}	1.47^{a}	0.01	0.010
Total DM (kg^{-d})	4.86^{b}	5.02^{b}	4.78^{b}	4.28^{a}	0.01	0.002
Total DM (% LW)	2.51^{b}	2.57^{b}	2.46^{b}	2.19^{a}	0.01	0.001
Total OM (kg^{-d})	4.00^{b}	4.14^{b}	3.93^{b}	3.52^{a}	0.06	0.015
Total crude protein (g^{-d})	749^{b}	771^{b}	730^{b}	661^{a}	13.13	0.005
Total crude lipid (g^{-d})	295^{b}	305^{b}	290^{b}	260^{a}	4.41	0.002
Digestible OM (kg^{-d})	2.75^{b}	2.81^{b}	2.60^{b}	2.32^{a}	0.074	0.013
Protein:DOM ratio	273	275	281	288	8.962	0.582
Digestibility:						
Dry matter (%)	63	62	60	59	1.43	0.362
Organic matter (%)	68	67	66	65	1.21	0.341
Crude protein (%)	83	83	82	82	0.64	0.317
Nitrogen retained (g^{-d})	100^{b}	103^{b}	96^{b}	87^{a}	2.12	0.008

Note: The amount of chromolaena meal in the pellets were 10% (10COM), 20% (20COM), 30% (30COM), and 40% (40COM). Means in the same row with different superscripts differ significantly (p<0.05). DM= dry matter; OM= organic matter; SEM=standard error of the treatment means.

Table 3. Rumen fermentation products of fattened cattle offered a pelleted diet containing *Chromolaena odorata* meal at different levels

Variables	Treatments				SEM	p-value
	10COM	20COM	30COM	40COM		
pH of rumen fluid	6.90	6.70	6.73	6.71	0.34	0.542
Rumen NH_3-N (mg/L)	116.0	125.0	109.0	119.0	12.10	0.808
Total VFA (mM)	128.4	132.3	134.7	130.1	2.58	0.725
Acetate (mM)	88.4	86.3	88.3	85.5	4.63	0.961
Butyrate (mM)	12.3	12.9	16.4	10.6	1.98	0.564
Propionate (mM)	27.6	30.5	30.1	34.5	2.78	0.562
VFA proportion:						
Acetate (%)	68.9^{b}	66.5^{ab}	65.5^{a}	65.5^{a}	1.13	0.059
Butyrate (%)	9.6^{b}	9.9^{b}	12.2^{c}	8.1^{a}	0.50	0.001
Propionate (%)	21.5^{a}	22.3^{ab}	23.5^{b}	26.4^{c}	0.44	0.001
C_2 eq : propionate ratio	3.65^{bc}	3.48^{b}	3.25^{b}	2.79^{a}	0.09	0.001
Methane production (mM)#	47.44	47.49	47.26	49.36	3.24	0.842

Note: The amount of chromolaena meal in the pellets were 10% (10COM), 20% (20COM), 30% (30COM), and 40% (40COM). Means in the same row with different superscripts differ significantly (p<0.05). #methane production (mM)= (0.45*acetate) - (0.275*propionate) + (0.4*butyrate) (Moss *et al*., 2000). SEM=standard error of the treatment means.

3). The C_2:C_3 ratios in the rumen fluid significantly (p = 0.001) declined from 3.65 in the 10COM treatment to 2.75 in the 40COM treatment.

DISCUSSION

Intake and Digestibility

The findings of the current experiment, presented in Table 2, suggest that the decrease in consumption at the 40% chromolaena was not due to feed nutrient content, as all treatments had similar protein and energy content. The decrease is likely associated with an increased intake of secondary metabolites as the proportion of chromolaena flour in the diet increases. The review of Kato-Noguchi & Kato (2023) showed that *C. odorata* contains secondary metabolites in many chemical classes, such as flavonoids, phenolic acids, saponins, terpenoids, and tannins. Other anti-nutritional properties, such as phytic acid and cyanogenic glycosides, are also present in this plant (Ngozi *et al.*, 2009). Likely, the processing of chromolaena to produce pellets in the present study did not totally eliminate these anti-nutritional properties.

Similar trends have been reported in sheep and rabbit studies when chromolaena levels reached 40% of the total diet (Apori *et al.*, 2001; Aro *et al.*, 2009). Empirically, the data in Table 2 show an increase in consumption when the chromolaena level reached 20%. Research on sheep in Ghana (Yakubu, 2012) also demonstrated an increased consumption and digestibility when the inclusion level of chromolaena increased to 20%. This suggests that the inclusion of up to 20% chromolaena in the pellet improved intake due to the stimulative effects of the extra nutrients from the pellet, which outweigh the suppressive effects of the anti-nutritional compounds in the chromolaena. However, when the proportion of chromolaena in the pellets reached 30%, the animals started to show a significant decline in the intake of grass hay and pellet. The solid argument might be that a higher concentration of anti-nutritional compounds in the ingested pellet triggers negative feedback on the animal's appetite. The low palatability of chromolaena was also observed by Oematan (2020) in fattened cattle supplemented with chromolaena silage.

The intake of other nutrients (OM, CP, EE) also decreased, following the trend of dry matter intake. This is logical since the nutrient density of all diets was the same, making the level of dry matter intake the determinant of the consumption level of other nutrients. The decrease in consumption indicates that although chromolaena has been made into flour and processed into pellets, its secondary metabolites have not been eliminated entirely, thus still exerting a negative effect. Yakubu (2012) also found a negative impact on the reproductive hormone profile, testicular health, and sperm in experimental rats when using chromolaena leaf meal in diets.

Unlike consumption, digestibility parameters were not significantly affected by the increased level of chromolaena in the pellets (Table 2). The dry matter digestibility shown in this study is relatively low, considering the high crude protein content (20%) and dense metabolizable energy (11.4 MJ ME/kg DM) in the pellets, which were given at 2% of live weight. However, this can be explained by the physical form of the feed, which has been processed into flour and then pelleted, resulting in a relatively high proportion of feed bypassing rumen fermentation, as is commonly reported for processed feed (Miller *et al.*, 2020).

In the present study, the lack of significant differences in digestibility variables suggests that although empirical data (Table 2) indicated a declining trend in digestibility variables, the relatively large variability (as indicated by the SEM values) may have diluted the treatment effects. Additionally, the pelleting process, which involved heat and pressure, may have contributed to a small improvement in the biological value of the diets. Oematan *et al.* (2020) have also reported that the inclusion of bio-fermented chromolaena in fattening diets exhibits no negative effects on intake, digestibility, and the growth of fattened cattle. This finding is in line with other studies (Mulik *et al.*, 2016; Ridla *et al.*, 2016; Oematan *et al.*, 2020) that anaerobic microbial fermentation significantly reduces anti-nutrient compounds and improves nutrient profiles of chromolaena.

The ratio of crude protein (CP) intake to digestible organic matter intake (DOMI) reflects the amount and balance of animal protein and energy consumed. In Table 2, it can be seen that the CP-DOMI ratio ranged between 273-288 g CP/kg DOMI. This value is well above the minimum standard (170 g of rumen-degradable protein per kg DOM) recommended for optimal microbial protein production (SCA, 2007; NRC, 2016). Such a favorable ratio in the consumed diets in this study aims to provide a daily weight gain of over 0.6 kg.

Nitrogen retention values can be used as a rough indicator to predict the possible average daily gain (ADG) that can be achieved by the animals. The lowest significant nitrogen retention was found in 40COM (87 g N/day) compared to the other three treatments (96-103 g N/day). If we use the assumption (SCA, 2007; NRC, 2016) that all retained CP in the body of experimental cattle occupies 20% of each empty body weight, it can be roughly estimated that the ADG of the experimental animals would range between 435-515 g/day. The predicted ADG indicates that the formulated feed in this study can support an ADG of over 0.6 kg/day. However, the ADG achieved by the experimental animals was still below 0.6 kg/day due to the dry matter intake ranging from 2.19%-2.57% of BW, which is still below the ideal level of 3% of LW. The low nitrogen retention in the 40COM treatment was due to the significantly lowest total CP intake compared to the other three treatments (Table 2). On the other hand, the digestibility among all treatments was not significantly different. Therefore, retention is determined more by the level of CP intake rather than digestibility.

Rumen Fermentation

Ruminal pH is a critical factor in the normal and stable function of the rumen (Kitkas *et al.*, 2022) because

of its profound effect on microbial populations and fermentation products and physiological functions of the rumen, mainly motility and absorptive function (Arowolo *et al.,* 2022). Ruminal pH is the product of coordinated acid-base management efforts in the rumen. A high protein diet increases the rumen's buffer capacity, elevating ruminal pH (Lopes *et al.,* 2023), and a high intake of easily fermentable carbohydrates lowers the pH (Humer *et al*., 2017). This happens when the removal rate of lactic acid and VFAs from the rumen and rumen buffering capacity cannot keep up with their production rate (Bach *et al.,* 2023). The normal ruminal digestion of dairy cattle occurs when the rumen pH is between 6.0 and 6.5 (Lam *et al.,* 2015), whereas Haerr & Drackley (2012) proposed a range value of 6.0–6.9 for cattle feed total mixed ration. This implies that the values of the ruminal pH in the present experiment (7.71-6.90) were normal, yet Umar *et al.* (2011) found that the normal ruminal pH for two indigenous cattle in Indonesia (Madura dan Brahman cross) was around 8.0–8.2.

The concentration of ruminal NH_3 has a positive correlation with ingested protein, indicating that higher protein intake leads to higher ruminal NH_3 levels. The range of ruminal NH_3 concentration reported in this study (109–125 mg/L) falls within the normal range reported by Arowolo *et al.* (2022) for dairy cattle. This suggests that the protein intake in the current study is adequate, considering that the concentrate used has a protein content of 20%. The non-significant effects of the levels of chromolaena in the concentrate can be predicted since all treatments in the study were iso-proteonous.

Microbial fermentation plays a significant role in fulfilling 75% of the energy requirements of ruminant animals through VFA production (Newbold & Ramos-Morales, 2020). In this context, efficient cattle display elevated molar concentrations of VFAs when considering the total VFA concentration. However, due to the wide range in rumen digesta liquid volume, the concentrations of VFAs in the rumen are not appropriate indicators of the status of ruminal fermentation. Therefore, molar proportion of the individual VFAs would be a more reliable estimate (Hall *et al.,* 2015).

The molar proportion of the partial VFAs in the present study was significantly affected (p= 0.01) by the incremental levels of chromolaena in the concentrate. The C_2 equivalent VFAs (acetate and butyrate) decreased from 78.5% in treatment with 10% chromolaena to 73.6% in treatment with 40% chromolaena. In contrast, the proportion of propionate (C_3) significantly increased from 21.5% (10COM) to 26.4% (40COM). This change in the molar proportions of the individual VFAs resulted in a significant (p= 0.01) change in the C_2:C_3 ratio.

Although the composition of rumen microbes was not measured in this study, the reduction in the molar proportion of C_2 equivalent VFAs suggested that the increasing level of chromolaena induced the growth and fermentation process of propionate-producing rumen microbes. A more realistic proposition is that various secondary metabolite/s in the chromolaena (Kato-Noguchi & Kato, 2023) meal might be the cause since Abubakar *et al.* (2020) reported a significant anti-bacterial activity of chromolaena extract. Up to now, there have been no reports on the effects of *C. odorata* on rumen microbes, creating a knowledge gap regarding how the inclusion of chromolaena alters the rumen microbial ecosystem. However, studies on the same anti-nutritional compounds found in Chromolaena, such as tannins, saponins, phytic acid, could be used to explain the reduction in acetate observed in our study. *In vitro* studies (Vasta & Bessa, 2012) demonstrated that the inclusion of tannins in the fermenters led to a reduction in the acetate:propionate ratio. Considering that acetate is primarily produced by cellulolytic bacteria, the lower acetate:propionate ratio could suggest an impairment of cellulolytic bacteria activity. *In vivo* study (Guerreiro *et al.,* 2022) also reported that condensed tannin extracted from *Cistus ladanifer* L. influenced the ruminal microbial composition.

Methane gas represents wasted energy resulting from the ruminant fermentation process (Rivera-Ferre *et al.,* 2016). A byproduct of this microbial degradation is hydrogen, which, along with carbon dioxide, is subsequently reduced by methanogenic archaea to produce methane gas (Lakamp *et al.,* 2022). Efforts exist to mitigate this ruminant energy inefficiency (Morgavi *et al.,* 2023), one of which involves the utilization of dietary additives and rumen modifiers to alter enteric methane production (Alemu *et al.,* 2019). One approach to manipulating rumen conditions involves the application of phytochemicals to control protozoal populations, thereby reducing enteric methane production (Almeida *et al.,* 2021).

The methane values in the present study were computed from individual VFAs using the formula of Moss *et al*. (2000), which adopted that acetate acid and butyrate acids (C_2) are involved in H2 production, whereas propionate (C_3) is involved in H2 utilization. This implies that diets giving rise to propionate production will reduce methane production, and vice versa for C_2 VFAs (Olijhoek *et al*., 2022). Data presented in Table 3 suggests that increasing the level of chromolaena in the diet by up to 40% had no significant effect (p= 0.824) on methane molar concentrations (mM) in the rumen fluid. The insignificant effects on the CH_4 variable are anticipated, as the molar concentration of partial VFAs (Table 3) did not differ between treatment groups (p=0.84). This might be attributed to two factors. Firstly, although all intake variables were significantly affected by the level of chromolaena meal in the pellets, the nutrient density in the pellets and organic matter digestibility were not significantly different between treatments. The methane values presented in Table 3 were mmol/L, not mmol or L/day, as the volume of the rumen fluid was not measured; hence methane concentration is rather dictated by digestibility coefficients than intake parameters. Jayanegara et al. (2012), in their meta-analysis, found that some of the CH_4 decrease was due to the concomitant decline in *in vivo* organic matter digestibility. Secondly, exposure of chromolaena to heat during sun drying, milling, and pelleting might have reduced potential negative methane suppressive properties in chromolaena, such

as tannic acids (Yang *et al*., 2017), condensed tannins (Thompson *et al*., 2023; Berça *et al*., 2023), mimosine (Min *et al*., 2020), oxalate, and phytic acid (Akintunde *et al*., 2021), saponins (Martin *et al*., 2024), and nitrate (Patra & Yu, 2016).

CONCLUSION

Chromolaena has great potential as a local feed ingredient and source of protein. Including chromolaena up to 40% in concentrate and feed at 2% LW decreases all intake parameters, yet it does not affect digestibility indexes. However, increasing levels of chromolaena meal in the concentrate increases glucogenic VFA, resulting in a better molar ratio of glucogenic:ketogenic fatty acids, yet does not affect methane production.

CONFLICT OF INTERESTS

The authors declare that there was no conflict of interest in undertaking this experiment.

ACKNOWLEDGEMENT

The authors acknowledge the financial support provided by the Ministry of Research and Technology/ Research and Innovation Agency through Research Grant No. 2987/E4/AK.04/2021.

REFERENCES

Abubakar, M. A., A. C. Etonihu, P. E. Kigbu, J. E. Owuna, & S. I. Audu. 2020. Phytochemical and antimicrobial analyses of leaf extracts of cerathoteca sesamoides and *Chromolaena odorata*. International Journal Research GRANTHAALAYAH 8:65-74. https://doi.org/10.29121/granthaalayah.v8.i8.2020.435

Akintunde, A. O., L. C. Ndubuisi-Ogbona, O. A. Ajayi, C. Chioma, W. A. Jimoh, & O. J. Afodu. 2021. Utilization of *Chromolaena odorata* leaf meal as a supplement in broiler chickens' diet. Nigerian Journal Animal Science 23:189-198.

Alemu, A. W., A. Romero-Perez, R. C. Araujo, & K. A. Beauchemin. 2019. Effect of encapsulated nitrate and microencapsulated blend of essential oils on growth performance and methane emissions from beef steers fed backgrounding diets. Animals 9:21. https://doi.org/10.3390/ani9010021

Almeida, A. K., R. S. Hegarty, & A. Cowie. 2021. Meta-analysis quantifying the potential of dietary additives and rumen modifiers for methane mitigation in ruminant production systems. Anim. Nutr. 7:1219-1230. https://doi.org/10.1016/j.aninu.2021.09.005

AOAC. 2005. Official Methods of Analysis.17th ed. Assoc. of Official Analytical Chemist, Washington DC.

Apori, S. O., F. N. Odoi, & E. Ganyo. 2001. Intake and digestibility of organic matter and nitrogen in *Chromolaena odorata* leaf meal-based diets by sheep. Ghana Journal Agricultural Science 34:11-14. https://doi.org/10.4314/gjas.v34i1.1823

Aro, S. O., I. B. Osho, V. A. Aletor, & O. O. Tewe. 2009. *Chromolaena odorata* in livestock nutrition. J. Med. Plant Res. 3:1253-1257.

Arowolo, M. A., X. M. Zhang, M. Wang, R. Wang, J. N. Wen, L. Z. Hao, J. H. He, W. J. Shen, Z. Y. Ma, & Z. L. Tan. 2022. Proper motility enhances rumen fermentation and microbial protein synthesis with decreased saturation of dissolved gases in rumen simulation technique. J. Dairy Sci. 105:231–241. https://doi.org/10.3168/jds.2021-20165

Bach, A., M. Baudon, G. Elcoso, J. Viejo, & A. Courillon. 2023. Effects on rumen pH and feed intake of a dietary concentrate challenge in cows fed rations containing pH modulators with different neutralizing capacity. J. Dairy Sci. 106:4580-4598. https://doi.org/10.3168/jds.2022-22734

Berça, A. S., L. O. Tedeschi, A. da Silva Cardoso, & R. A. Reis. 2023. Meta-analysis of the relationship between dietary condensed tannins and methane emissions by cattle. Anim. Feed Sci. Technol. 298:115564. https://doi.org/10.1016/j.anifeedsci.2022.115564

Bira, G. A. 2015. Determination of the ideal level of *Chromolaena odorata* in concentrate for fattening cattle based on consumption rate, digestibility, rumen dynamics, and blood profiles. Thesis. Department of Animal Science, Universitas Nusa Cendana, Indonesia.

Fasuyi, A. O., K. S. O. Fajemilehin, & S. O. Oro. 2005. Nutritional potentials of Siam weed *(Chromolaena odorata)* leaf meal on laying hens: Biochemical and haematological implications. Pak. J. Nutr. 4:336-341. https://doi.org/10.3923/pjn.2005.336.341

Foote, A. P. 2022. Technical Note: Analysis of volatile fatty acids in rumen fluid by gas chromatography mass spectrometry using a dimethyl carbonate extraction. J. Anim. Sci. 100:skac207. https://doi.org/10.1093/jas/skac207

Ganguly, R. K., Md. A. Al-Helal, & S. K. Chakraborty. 2022. Management of invasive weed *Chromolaena odorata* (Siam weed) through vermicomposting: An eco-approach utilizing organic biomass valorization. Environ. Technol. Innov. 28:102952. https://doi.org/10.1016/j.eti.2022.102952

Guerreiro, O., A. E. Francisco, S. P. Alves, D. Soldado, L. Cachucho, A. U. Chimenos, F. Duarte, J. Santos-Silva, R. J. B. Bessa, & E. Jerónimo. 2022. Inclusion of the aerial part and condensed tannin extract from *Cistus ladanifer* L. in lamb diets – Effects on rumen microbial community and fatty acid profile. Anim. Feed Sci. Technol. 291:115398. https://doi.org/10.1016/j.anifeedsci.2022.115398

Hall, M. B., T. D. Nennich, P. H. Doane, & G. E. Brink. 2015. Total volatile fatty acid concentrations are unreliable estimators of treatment effects on ruminal fermentation *in vivo*. J. Dairy Sci. 98:3988-3999. https://doi.org/10.3168/jds.2014-8854

Humer, E., J. R. Aschenbach, V. Neubauer, I. Kröger, R. Khiasord, W. Baumgartner, & Q. Zebeli. 2017. Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. J. Anim. Physiol. Anim. Nutr. 102:380–392. https://doi.org/10.1111/jpn.12850

IBM. 2017. SPSS Statistics Version 25. International Business Machines Corporation. Armonk NY, USA.

Jayanegara A, F. Leiber, & M. Kreuzer. 2012. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from *in vivo* and *in vitro* experiments. Anim. Physiol. Anim. Nut. 96:365e75. https://doi.org/10.1111/j.1439-0396.2011.01172.x

Jiwuba, P. C., I. P. Ogbuewu, & K. Nwachukwuguru. 2018. Performance and economy of production of broilers fed Siam weed (*Chromolaena odorata*) leaf meal (SWLM). Trop. Anim. Health Prod. 50:1305–1311. https://doi.org/10.1007/s11250-018-1559-x

Kato-Noguchi, H. & M. Kato. 2023. Evolution of the secondary metabolites in invasive plant species *Chromolaena odorata* for the defense and allelopathic functions. Plants 12:521. https://doi.org/10.3390/plants12030521

Kitkas, G. C, G. E. Valergakis, M. Kritsepi-Konstantinou, A. I. Gelasakis, P. D. Katsoulos, E. Kalaitzakis, & N. K. Panousis. 2022. Association between ruminal pH and

rumen fatty acids concentrations of Holstein cows during the first half of lactation. Ruminants 2:382–389. https://doi.org/10.3390/ruminants2040026

Lakamp, A. D., R. L. Weaber, J. M. Bormann, & M. M. Rolf. 2022. Relationships between enteric methane production and economically important traits in beef cattle. Livest. Sci. 265:105102. https://doi.org/10.1016/j.livsci.2022.105102

Lam, S., S. Miller, J. Munro, T. Caldwell, J. Cant, M. Steele, & M. Montanholi. 2015. Ruminal pH and Temperature. The Elora Beef Gaezette, 2015 May 1st, Factsheet 4 Issu1. https://doi.org/10.13140/RG.2.1.1130.8400

Lartey, K. A., Dan-Ju, Qiao-Hui, Zhang, Chao-Qun, S., F. Yang, L. Hong-Ying, & Ch. Jin-Jun. 2020. *Chromolaena odorata* as a potential feed additive bioresource to alleviate heat stress in chickens in the humid tropics. Worlds Poult. Sci. J. 76:119-133. https://doi.org/10.1080/00439339.2020.1729673

Lopes, A. S. M., J. S. de Oliveira, G. F. L. Cruz, D.S. Vieira, F. N. S. Santos, M. L. P. Lemos, J. K. Pinheiro, L. S. de Sousa, C. J. B. de Oliveira, & E. M. Santos. 2023. Effects of non-protein nitrogen on buffel grass fiber and ruminal bacterial composition in sheep. Livest. Sci. 272:105237. https://doi.org/10.1016/j.livsci.2023.105237

Martins, L. F., S. F. Cueva, C. F. A. Lage, M. Ramin, T. Silvestre, J. Tricarico, & A. N. Hristov. 2024. A meta-analysis of methane-mitigation potential of feed additives evaluated *in vitro*. J. Dairy Sci. 107:288-300. https://doi.org/10.1016/S0022-0302(23)00819-6

Miller, M. D., J. S. Lanier, S. K. Kvidera, H. M. Dann, C. S. Ballard, & R. J. Grant. 2020. Evaluation of source of corn silage and trace minerals on rumen characteristics and passage rate of Holstein cows. J. Dairy Sci. 103:8864–8879. https://doi.org/10.3168/jds.2020-18490

Min, B. R., S. Solaiman, H. M. Waldrip, D. Parker, R. W. Todd, & D. Brauer. 2020. Dietary mitigation of enteric methane emissions from ruminants: A review of plant tannin mitigation options. Anim. Nutrition 6:231-246. https://doi.org/10.1016/j.aninu.2020.05.002

Morgavi, D. P., G. Cantalapiedra-Hijar, E. C. Martin, P. Noziere, M. Popova, I. Ortigues-Marty, R. Muñoz-Tamayo, & E. M. Ungerfeld. 2023. Review: Reducing enteric methane emissions improves energy metabolism in livestock: Is the tenet right? Animal 17:100830. https://doi.org/10.1016/j.animal.2023.100830

Moss, A. R., J-P. Jouany, & J. Noewbold. 2000. Methane production by ruminants: Its contribution to global warming. Annales De Zootechnie 49:231–253. https://doi.org/10.1051/animres:2000119

Mulik, Y. M., M. Ridla, I. Prihantoro, & M. L. Mullik. 2016. Anaerobic fermentation effectively reduces concentration of total tannins in Siam Weed (*Chromolaenan odorata*). Jurnal Ilmu Ternak Veteriner 21:19-21. https://doi.org/10.14334/jitv.v21i1.1301

Newbold, C. J. & E. Ramos-Morales. 2020. Review: Ruminal microbiome and microbial metabolome: Effects of diet and ruminant host. Animal 14:s78-s86. https://doi.org/10.1017/S1751731119003252

Ngozi, I. M., I. C. Jude, & I. C. Catherine. 2009. Chemical profile of *Chromolaena odorata* L. (King and Robinson) leaves. Pak. J. Nutr. 8:521-524. https://doi.org/10.3923/pjn.2009.521.524

NRC. 2016. Nutrient Requirement of Beef Cattle. 8th Revised Edition. National Academy Press.

Oematan, G., E. Hartati, M. L. Mullik, & N. Taratiba. 2020. Bio-the nutritional values of *Chromolena odorata* utilization as Bali cattle feed source. Int. J. Sci. Res. 9:1524-1533.

Oematan, G. 2020. Optimation of the Rumen Biofermentation and Growth of Bali Cattle using *Chromolaena odorata* Supplemented with Hydroxy Metheonine Analogue and Unsaturated Fatty Acid. PhD Thesis. Universitas Nusa Cendana, Indonesia.

OISC. 2018. Survey and Control of *Chromolaena odorata* in the Kahuku Training Area, O'ahu, Hawai'i Annual Progress Report October 1, 2017—March 31.

Olawale, F., K. Olofinsan, & O. Iwaloye. 2022. Biological activities of *Chromolaena odorata*: A mechanistic review. S. Afr. J. Bot. 144:44-57. https://doi.org/10.1016/j.sajb.2021.09.001

Olijhoek, D. W., **A. L. F. Hellwing, S. J. Noel, P. Lund, M. Larsen, M. R. Weisbjerg, & C. F. Børsting.** 2022. Feeding up to 91% concentrate to Holstein and Jersey dairy cows: Effects on enteric methane emission, rumen fermentation and bacterial community, digestibility, production, and feeding behavior. J. Dairy Sci. 105:9523–9541. https://doi.org/10.3168/jds.2021-21676

Patra, A. K. & Z. Yu. 2016. Effective reduction of enteric methane production by a combination of nitrate and saponin without adverse effect on feed degradability, fermentation, or bacterial and archaeal communities of the rumen. Bioresource Technol. 148:352-360. https://doi.org/10.1016/j.biortech.2013.08.140

Ridla, M., Y. M. Mulik, I. Prihantoro, & M. L. Mullik. 2016. Penurunan total tanin silase semak bunga putih (*Chromolaena odorata*) dengan aditif tepung putak (*Corypha elata* Robx) dan isi rumen sapi. Buletin Peternakan 40:165-169. https://doi.org/10.21059/buletinpeternak.v40i3.12838

Rivera-Ferre, M. G., F. López-i-Gelats, M. Howden, P. Smith, J. F. Morton, & M. Herrero. 2016. Re-framing the climate change debate in the livestock sector: mitigation and adaptation options. Wiley Interdiscip. Rev. Clim Change 7:869-892. https://doi.org/10.1002/wcc.421

SCA. 2007. Feeding Standards for Australian Livestock. Standing Committee on Ruminants. CSIRO, Australia.

Smith, F. E. & T. A. Murphy. 1993. Analysis of Rumen Ammonia & Blood Urea Nitrogen. Univ. Nebraska, Lincoln, NE. [March 10, 2023].

Tiamiyu, A. M. & O. A. Okunlade. 2020. Benefits and detriments of Siam weed (*Chromolaena odorata*): A review. Biochem. Biotechnol. Res. 8:21-28.

Thompson, J. P., S. Stergiadis, O. C. Carballo, T. Yan, F. Lively, S. Huws, K. Theodorido, & J. Gilliand. 2023. O88 Effect of grazing cattle on willow silvopastoral systems on animal performance and methane production. Animal Science Proceedings 14:599-600. https://doi.org/10.1016/j.anscip.2023.04.089

Umar, M., M. Arifin, & A. Purnomoadi. 2011. Ruminal condition between madura cattle and ongole crossbred cattle raised under intensive feeding. J. Indones. Trop. Anim. Agric. 36:213-218. https://doi.org/10.14710/jitaa.36.3.213-218

Vasta, V. & R. J. B. Bessa. 2012. Manipulating Ruminal Biohydrogenation by the Use of Plants Bioactive Compounds. In: Patra, A. (eds) Dietary Phytochemicals and Microbes. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-3926-0_9

Yakubu, M. T. 2012. Effect of a 60-day oral gavage of a crude alkaloid extract from *Chromolaena odorata* leaves on hormonal and spermatogenic indices of male rats. J. Adrol. 33:1199-1207. https://doi.org/10.2164/jandrol.111.016287

Yang, H., C. Wei, G. Y. Zhao, Z. W. Xu, & S. X. Lin. 2017. Effects of dietary supplementing tannic acid in the ration of beef cattle on rumen fermentation, methane emission, microbial flora and nutrient digestibility. J. Anim. Physiol. Anim. Nutr. 101:302-310. https://doi.org/10.1111/jpn.12531