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Effects on *in vitro* digestibility and rumen fermentation of maize straw silage as a partial dietary replacement for Napier grass

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ABSTRACT. Maize straw is an agricultural by-product that has the potential to be used as an alternative feed for ruminants. In order to utilise this crop residue rationally, their nutritional value should be improved to promote their use as silage. This study aimed to evaluate *in vitro* fermentability and digestibility of maize by-product silage in ruminant feed rations based on *Indigofera zollingeriana* as a substitute for Napier grass (NG). A completely randomised design with 3 treatments and 8 replications was applied. The proportion of forage and concentrate in rations was 45:55% on a dry matter (DM) basis. The experimental treatments included a ration containing 35% NG (R0), a ration containing 17.5% NG and 17.5% maize straw silage (MSS) (R1), and lastly a ration containing 35% MSS (R2). The results showed that increasing the levels of maize straw silage in rations reduced *in vitro* digestibility including DM and organic digestibility, ammonia (NH_3), total and proportional volatile fatty acid (VFA) contents, gas production, and the total protozoan and bacterial populations ($P < 0.01$). However, the numerical rumen pH values (6.45–6.71), VFA levels (69.08–89.23 mM), NH_3 concentrations (10.50–12.88 mM), and gas production (185.46–193.33 ml/g DM) were within the normal range for ruminant requirements in terms of gas production and *in vitro* feed fermentation in the rumen. There was no significant difference found in methane production ($P > 0.05$). Considering rumen fermentation products and pH ruminal condition, it can be concluded that maize straw silage up to 50% can be used as a feed substitute in ruminant rations.

Introduction

As the global population continues to increase, there is a growing need for sustainable food production systems that can provide sufficient nutrition for both humans and animals. The concept of a circular food system has emerged as an approach to minimise waste, improve resource efficiency, and reduce the environmental impact of food production. Livestock in particular can play a vital role in this system by recycling by-products and biomass from grasslands into valuable food and nutrient-rich manure. In a circular food system, livestock contribute significantly to the global food supply by converting low-opportunity-cost feeds and agricultural by-products into nutritious food (van Zanten et al., 2019). These feed sources, which would otherwise go to waste, are recycled by farm animals, thus minimising resource loss. However, one major challenge in utilising these by-products is their high content

of crude fibre, particularly derived from the lignin cellulose component. This complex carbohydrate can be difficult for livestock to digest.

Maize straw is a fibrous material that often remains in the field after maize harvest. However, it has the potential to be used as a feed source for ruminant animals such as cows, goats, and sheep. Cellulose and haemicellulose are the primary cell wall constituents of maize straw. According to Preston (2016), maize straw contains 29% acid detergent fibre (ADF), 48% neutral detergent fibre (NDF), 9% crude protein (CP), 7% ash, 0.5% calcium and 0.25% phosphorus. The high crude fibre content and low protein content of agricultural waste are limiting factors for their use as animal feed. To make agricultural wastes, including maize straw, more suitable for animal feed, various treatments can be employed, encompassing physical, chemical, and biological approaches (Rabemanolontsoa and Saka, 2016). Ensiling is the most frequently applied technology for forage preservation. Silage is a type of animal feed that is preserved in a silo under anaerobic conditions and retains 40–70% water content. It enhances the nutritional value and digestibility of forages and can be stored for an extended period (up to one year) without significant loss of nutrients. Consequently, silage serves as a valuable reserve of forage during dry seasons (Kung et al., 2018; Trisnadewi and Cakra, 2020).

Maize straw or maize stover have already been used as ruminant feed in animal husbandry. A study by Arief et al. (2020) demonstrated that a ration based on maize waste (as substitution of *Tithonia diversifolia*), at a proportion of up to 30%, supplemented for 30 days, maintained the production quality of Etawa crossbred dairy goat. Similarly, research conducted on lactating dairy cows showed that partial replacement of mixed forage diet (37% dry matter (DM)) affected the rumen fermentation environment (pH, temperature, oxidation reduction) (Qin et al., 2017). Dong et al. (2014) suggested that feeding a high-concentrate maize straw diet (35% DM) induced epigenetic changes that may have altered the methylation of specific genes involved in increasing fat and decreasing protein synthesis, possibly by altering the amounts of lipopolysaccharides (LPS) released into the mammary blood. In order to enhance the utilisation of maize straw, several studies have focused on improving its nutritional value through ensiling. Fermentation of maize stover for 21 days has been found to elevate the CP content, resulting in an increase from 8.30% to 8.47–16.04% (Kurniawan et al., 2019). Moreover, Zhang et al. (2023) reported that ensiling maize straw for 60 days increased the protein content from 9.81% to 9.99%, whereas the NDF content decreased from 58.89% to 50.03%, and the ADF content decreased from 32.47% to 28.96%. Gao et al. (2019) have pointed out that selecting the appropriate time for ensilaging following maize harvest is an important condition in obtaining high-quality maize stover silage.

Napier grass (NG) is commonly used feed source for ruminants in many tropical countries. We hypothesised that maize straw silage, when incorporated into rations at levels up to 35%, would not significantly alter the digestibility and rumen fermentation characteristics compared to NG. The combination of straw silage with Indigofera is expected to be an ideal complete feed for ruminants. *Indigofera zollingeriana* serves as a feed ingredient providing protein, with an average crude protein content of 28–34%, depending significantly on the plant part (Antari et al., 2022). The current study aimed to evaluate the *in vitro* fermentability and digestibility of maize by-product silage as a substitute for NG in diets. By investigating nutritional value and fermentability of maize straw silage in the rumen, our objective was to promote its use as a sustainable and efficient feed source for ruminants in the circular food system.

Material and methods

Diet preparation

Maize straw was harvested at the mature stage of about 115 days, and then chopped into 35 cm pieces. Silage was prepared from chopped maize straw with 1% molasses (w/w), thoroughly mixed and fermented in anaerobic conditions for 21 days. The diet was composed of 45% forage and 55% concentrate (DM basis), with minimum crude protein and total digestible nutrient (TDN) contents of 12% and 65%, respectively. The treatments consisted of different proportions of NG and maize straw silage (MSS): R1 (50% NG and 50% MSS), and R2 (100% MSS). The control treatment (R0) consisted of 100% NG. The nutrient content and composition of the treatments are presented in Table 1.

Chemical analyses

The dry matter and crude fibre contents of the samples were determined in duplicate using the procedures outlined in the Indonesian National Standard (1992). Similarly, the ash, ether extract, CP, calcium, and phosphorus contents of the samples were also determined in duplicate following the procedures specified by AOAC International (2005). A bomb calorimeter was used to determine the gross

Table 1. Composition and nutrient content of treatment rations

Item	Treatments		
	R0	R1	R2
Feedstuff, % DM			
Forage	45	45	45
napier grass	35	17.5	0
maize straw silage	0	17.5	35
pellet of *Indigofera*	10	10	10
Cassava pulp	15	15	15
Concentrate	40	40	40
maize bran	10	10	10
rice bran	11.25	11.25	11.25
coconut cake	17.81	17.81	17.81
premix	0.38	0.38	0.38
dicalcium phosphate	0.38	0.38	0.38
$CaCO_3$	0.19	0.19	0.19
Total	100	100	100
Nutrient contents, %			
DM	49.67	52.36	55.06
ash	9.71	8.77	7.84
crude protein	15.88	14.28	12.68
crude fibre	17.29	18.07	18.84
ether extract	3.21	2.89	2.57
nitrogen free extract [a]	54.40	56.48	58.56
Ca	0.43	0.44	0.44
P	0.67	0.63	0.59
neutral detergent fibre	61.42	61.78	62.13
acid detergent fibre	30.68	31.79	32.90
haemicellulose [b]	30.74	29.99	29.23
total digestibility nutrient [c]	61.41	59.94	58.46
gross energy, kcal/kg	4 547.83	4 563.07	4 578.30

R0 – 0% maize straw silage (control), R1 – 50% maize straw silage (50% substitute Napier grass), R2 – 100% maize straw silage (100% substitute Napier grass); [a] based on the following formula = 100 – dry matter – crude protein – crude fat – crude fibre, [b] based on the formula of Van Soest (1991); [c] based on the formula of Hartadi et al. (1980)

energy of the samples, whereas NDF and ADF contents were determined using procedures described by van Soest et al. (1991). The TDN content was calculated based on the method provided by Hartadi et al. (1980).

In vitro procedure

In vitro fermentation was carried out according to the procedure of Theodorou and Brooks (1990). In a 100 ml *in vitro* tube, 0.75 g of sample plus 50 ml of McDougall buffer and 25 ml of rumen fluid was treated with CO_2 for 30 s. Subsequently, the tube was sealed with a vented rubber stopper and incubated in a shaker water bath at 39 °C for 24 to 72 h. After 24 h, approx. 2–3 drops of $HgCl_2$ were added to fix the microorganisms. The tube content was filtered through a predetermined weight of Whatman paper No. 41 using a vacuum pump. Whatman paper was folded and dried for 24 h at 105 °C to determine DM, then ashed at 450–600 °C in an electrical furnace for 8 h to determine organic matter (OM) digestibility. *In vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) were calculated by determining the difference between the initial DM and OM weights of the sample and the respective DM and OM of the residues. These values were expressed as percentages. The supernatant obtained from the fermentation process was used to determine the pH value, total volatile fatty acid (VFA) content using gas chromatography, ammonia (NH_3) concentration (Conway and O'Malley, 1942), as well as the counts of protozoa and bacteria (Oginomoto and Imai, 1981).

Gas production was recorded after incubation for 0, 2, 4, 6, 8, 12, 24, 36, 48, and 72 h using a glass syringe. Gas production kinetics was calculated according to the following equation: $p = a + b (1 – e^{–ct})$ (Ørskov and McDonald, 1979), where: p – gas produced at the time (t), a – gas production from the immediately soluble fraction (ml); b – gas production from the insoluble fraction (ml); c – gas production rate constant; (a+b) – potential gas production (ml); t – incubation time (h). The gas sample was stored in vacuum tubes (Venoject) to analyse methane gas concentrations using gas chromatography.

Statistical analysis

The experiment was based on a completely randomised design using three treatments, as described above, with eight replications for each treatment. Data were analysed using analysis of variance (Steele and Torrie, 1990). Any significant differences ($P < 0.05$) between the means were further tested using Duncan's multiple range test. All statistical analyses were performed using SAS software version 9.0.

Results

In vitro digestibility, pH value and NH_3 concentration

Changes in the fermentation properties of rations containing maize straw silage during 21 days of ensiling are presented in Table 2. IVDMD and IVOMD significantly decreased ($P < 0.01$) as the proportion of maize straw silage in the ration increased. A higher percentage of maize straw silage also significantly elevated ($P < 0.01$) ruminal pH, which ranged from 6.45 to 6.71 in this study. Additionally, the concentration of NH_3 decreased ($P < 0.01$) with increasing proportion of maize straw

Table 2. Digestibility, pH value, and NH_3 concentration of *in vitro* rations containing maize straw silage

Variables	Treatments			*P*-value
	R0	R1	R2	
Dry matter digestibility, %	56.38 ± 0.85[a]	52.53 ± 1.32[b]	51.18 ± 0.87[c]	**
Organic matter digestibility, %	54.45 ± 0.89[a]	50.74 ± 0.16[b]	49.12 ± 0.86[c]	**
pH value	6.45 ± 0.08[c]	6.55 ± 0.09[b]	6.71 ± 0.10[a]	**
NH_3, mM	12.88 ± 0.60[a]	12.04 ± 0.74[b]	10.50 ± 0.97[c]	**

R0 – 0% maize straw silage (control), R1 – 50% maize straw silage (50% substitute Napier grass), R2 – 100% maize straw silage (100% substitute Napier grass). Data are presented as mean value ± SEM; [abc] – means within a column with different superscripts are significantly different; ** – $P < 0.01$

silage in the ration. The study found a significant decrease of up to 0.84 mM and 2.38 mM, when maize straw silage was used to substitute 50 and 100% of the forage, respectively (NG).

In vitro volatile fatty acids

VFAs are the primary source of energy for ruminants. As shown in Table 3, a higher proportion of maize straw significantly reduced ($P < 0.01$) the concentration of total VFAs. When compared to forage (R0), VFA levels were 14.8–22% higher in maize straw silage, while the fermentable fraction of 50% maize straw silage (R1) was higher than 100% maize straw silage (R2). The results showed that replacing 50% forage in ration R1 did not alter the proportions of acetate and iso-butyrate compared to the control (R0). However, it increased the percentage of propionate, N-butyrate, while decreasing the concentration of N-valerate and the acetate to propionate ratio (C2:C3).

Table 3. Volatile fatty acid (VFA) concentration of *in vitro* rations containing maize straw silage

Variables	Treatments			*P*-value
	R0	R1	R2	
Total VFA, mM	89.23 ± 0.69[a]	75.95 ± 0.49[b]	69.08 ± 0.49[c]	**
Partial VFA, %				
acetate (C_2)	55.02 ± 0.38[b]	54.47 ± 0.40[b]	56.81 ± 0.43[a]	**
propionate (C_3)	22.41 ± 0.17[b]	24.07 ± 0.18[a]	22.44 ± 0.29[b]	**
iso-butyrate ($_iC_4$)	2.16 ± 0.18	2.05 ± 0.13	1.98 ± 0.20	NS
N-butyrate ($_nC_4$)	12.41 ± 0.23[b]	13.49 ± 0.38[a]	13.47 ± 0.38[a]	*
iso-valerate ($_iC_5$)	4.03 ± 0.68	3.77 ± 0.06	3.38 ± 0.29	NS
N-valerate ($_nC_5$)	3.97 ± 0.06[a]	2.15 ± 0.23[b]	1.92 ± 0.20[b]	**
C2:C3	2.45 ± 0.0[b]	2.26 ± 0.03[c]	2.53 ± 0.05[a]	**

R0 = 0% maize straw silage (control), R1 = 50% maize straw silage (50% substitute Napier grass), R2 = 100% maize straw silage (100% substitute Napier grass). Data are presented as mean value ± SEM; [abc] – means within a column with different superscripts are significantly different; **= $P < 0.01$; *= $P < 0.05$; NS= not significant ($P > 0.05$)

In vitro gas production parameters and CH_4 generation

As presented in Table 4, rations containing maize straw silage showed a significant decrease ($P < 0.01$) in total *in vitro* gas production, but no difference in CH_4 production was observed. Higher gas production was detected in silage R0 compared to R1 and R2 during 72 h of *in vitro* fermentation.

Table 4. Kinetic parameters of *in vitro* gas production and CH_4 production of rations containing maize straw silage

Variables	Treatments			*P*-value
	R0	R1	R2	
Total gas production, ml/g DM	193.33 ± 1.87[a]	189.61 ± 1.48[b]	185.46 ± 2.27[c]	**
CH_4 production, ml/l	99.68 ± 5.02	97.84 ± 9.36	100.63 ± 19.18	NS

R0 – 0% maize straw silage (control), R1 – 50% maize straw silage (50% substitute Napier grass), R2 – 100% maize straw silage (100% substitute Napier grass); DM – dry matter. Data are presented as mean value ± SEM; [abc] – means within a column with different superscripts are significantly different. ** – $P < 0.01$; NS – non-significant ($P > 0.05$)

Based on the curve-fitting model shown in Figure 1, the gas production rate increased significantly from 0 to 24 h of incubation. Afterward, there was a slight increase up to 60 h of incubation, followed by a plateau in gas production up to 96 h. Regarding the specific data presented for R0 and R1, it appears that the volume of gas produced at the start of incubation (0 h) until 12 h increased in R0 but was lower compared to R1. However, at 24 h of incubation, there was a higher increase in gas volume in R0 compared to R1.

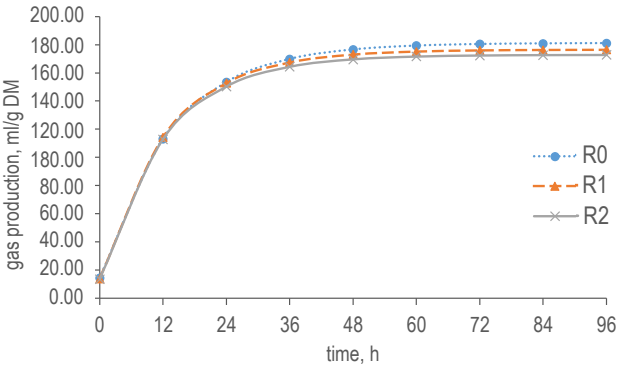

Figure 1. Cumulative total gas production of experimental diet during 96 h of incubation (fitted value)

R0 – 0% maize straw silage (control), R1 – 50% maize straw silage (50% substitute Napier grass), R2 – 100% maize straw silage (100% substitute Napier grass), DM – dry matter

Table 5. Total population of bacteria and protozoa in rations containing maize straw silage in the rumen

Variables	Treatments			*P*-value
	R0	R1	R2	
Total population of bacteria, log CFU/ml	9.79 ± 0.02[a]	9.71 ± 0.03[b]	9.52 ± 0.02[c]	**
Total population of protozoa, log CFU/ml	5.57 ± 0.01[a]	5.46 ± 0.01[b]	5.42 ± 0.02[c]	**

R0 – 0% maize straw silage (control), R1 – 50% maize straw silage (50% substitute Napier grass), R2 – 100% maize straw silage (100% substitute Napier grass); CFU – colony forming units. Data are presented as mean value ± SEM; [abc] – means within a column with different superscripts are significantly different; ** – $P < 0.01$

Population of bacteria and protozoa

Table 5 demonstrates significant variations ($P < 0.01$) in the total populations of bacteria and protozoa in diets containing forage and maize straw silage. In the present study, the bacterial population decreased as the proportion of maize straw silage in the ration increased. A significant decrease of 0.08 log CFU/ml was observed when forage was substituted with 50% maize straw silage (R1), and a decrease of 0.27 log CFU/ml was recorded when all forage was replaced. The mean counts of bacterial and protozoan populations in the current study ranged from 9.52–9.79 and 5.42–5.57 log CFU/ml, respectively.

Discussion

In vitro digestibility, pH value and NH_3 concentration

In vitro digestibility reflects the degree of substrate degradation by microorganisms in fermentation systems. DM disappearance of maize straw silage at 50% (R1) and 100% (R2) was 3.85% and 5.2%, respectively, while IVODM decreased by 3.71–5.33%. The relatively lower digestibility of the ration based on maize straw silage could be attributed to the fact that the straw was harvested at 115 days of maturity, when the process of cell wall synthesis was ongoing, which led to an increase in the lignin cellulose fraction (complex carbohydrates). The increase in lignin content is associated with the development of secondary wall in the stem, sheath and leaf tissue, which ultimately reduces forage digestibility. These findings are consistent with reduced IVDMD and IVODM in silage, associated with higher NDF and lower CP content in the ration, leading to lower total degradable DM content. Furthermore, it is worth noting that the proportion of alkali-soluble lignin also appears to be relatively high in diets R1 and R2, despite having a higher NFE content in the formulation. NFE consists of sugars, organic acids, pectin, haemicellulose, and alkali-soluble lignin (Cherney, 2000). Several studies reported that increasing NDFom or total NDF content in the diet reduced the effective DM and OM degradability (Ali et al. 2014; De Boever et al., 2017). These results were confirmed in the present study as NDF in R0 was numerically the lowest and CP in R0 was the highest among the treatments. The study by Wang et al. (2021) further supported the observed decrease in IVDMD and IVOMD. The latter authors recorded significant decreases in IVDMD in the range of 3.3–5.49% (from 510 g/kg DM to 493 and 482 g/kg DM), and IVOMD in the range of 4.7–8.5% (from 503 g/kg DM to 460 and 479 g/kg DM, re spectively), with increasing proportion of maize silage in the ration from 0 to 20 and 40%. Additionally, they showed that the NDF content in the diets containing 0, 20, and 40% maize silage were 395, 434, and 550 g/kg DM, respectively, and the protein content was 207, 201, and 105 g/kg DM, respectively (Wang et al., 2021). Our findings were also consistent with other study focused on IVDMD and IVOMD in mixtures of sweet sorghum and alfalfa silage, and reported values in the range of 457.89–666.56 g/kg DM and 498.01–749.03 g/kg DM, respectively (Zhang et al., 2015).

Also, the results of our study are in line with previous study by Chen et al. (2017) who recorded IVDMD in maize stover silage at 51.04%. Moreover, Ribeiro et al. (2015) studied the effect of five tropical roughage sources and showed that DM and OM apparent digestibility and ruminal digestibility did not differ between treatments, but numerically maize silage exhibited better digestibility, which aligned with its higher CP, ether extract, and non-fibrous carbohydrate contents.

As pH is the main indicator of internal homeostasis of the rumen environment, a relatively stable ruminal pH measurements are indicative of efficient rumen fermentation (Chen et al., 2017). The range of rumen pH in the present study was 6.45–6.71, which was within the normal range of 5.5–7.0 for ruminal fermentation (Chen et al., 2019). Our findings were consistent with other studies showing that higher proportion of non-fibre carbohydrates with lower NDF content in ruminant diets resulted in low rumen pH (Zhang et al., 2015; Granja-Salcedo et al. 2016; De Boever et al., 2017; Wang et al., 2021).

In the present study, NH_3 concentration in R0 reflected enhanced protein degradation during *in vitro* fermentation compared to the MSS-based diets (R1, R2). This finding was consistent with previous studies by Zhang et al. (2015) and Wang et al. (2021), who observed decreasing NH_3 levels with increasing maize silage percentage. Specifically, NH_3 concentration decreased by 7.2 mg/dl and 11.7 mg/dl when the percentage of maize silage was increased to 40 and 100%, respectively (Wang et al., 2021). The average NH_3 concentration in our study was 10.50–12.88 mM, which was within the optimum concentration range for the growth of rumen microbes. Schwab and Broderick (2017) conducted a review on ruminal ammonia concentrations and their impact on microbial nitrogen flow in the rumen. Their findings suggested that ruminal ammonia concentrations ranging from 5 to 11 mM were necessary to maximise microbial N flow from the rumen, depending on the diet and fermentation conditions. These authors also reported that the optimum concentration of ruminal ammonia appeared to be dependent on the diet as well as factors such as the type of N supplements, carbohydrate fermentability, and possibly other factors affecting passage rates (e.g., DM intake).

In vitro volatile fatty acids

The molarity of ruminal VFAs in the current study was positively correlated with ruminal digestibility, indicating that IVDMD and IVOMD were associated with increased VFA production. This finding was consistent with the study by Wang et al. (2021), who found that increasing proportion of sweet maize stalk in the ration (0 to 100%) resulted in a gradual decrease in IVDMD (1.7–9.7%) and IVOMD (2.4–9.5%), accompanied by a decrease in total VFA levels (1.6–6.9 mM). The latter report was in line with a meta-analysis by Brandao and Faciola (2019), who indicated that total VFAs linearly increased with DMI and decreased exponentially with increasing dietary NDF content. In addition, it has been reported that the quadratic response of total VFAs to dietary CP, maximized at 18% dietary CP, which is related to nitrogen availability for favouring microbial growth. However, the average total VFA content in the current study was in the range of 69.08–89.23 mM, as determined by gas chromatography analysis. These values were within an optimum range for rumen physiology and acidity, as reported by Allen (1997) and McDonald et al. (2011). The latter authors considered VFA concentration at the level of 70–150 mM as normal rumen fluid VFA production that supported microbial growth.

The increased concentration of propionate (1.66%) and N-butyrate (1.09%) in fermented rumen fluid with MSS (R1) could be attributed to the ruminal microbial activity. However, when forage was completely substituted with 100% MSS (R2), it increased the proportion of acetate, even though the percentage of propionate was similar. Acetic acid is produced in large quantities, ranging from 28 to 88 mM, while propionic acid is usually produced at approximately one-third of the amount of acetic acid (McDonald et al., 2011). Acetate is the major VFA produced during NDF fermentation, while propionate is generated during the fermentation of non-structural carbohydrates such as starch. However, the relationship between NDF content and VFA production is not always straightforward, as other factors like the type and availability of other nutrients can also affect VFA production. In the present study, the higher percentage of acetate was due to a higher proportion of fibre, including crude fibre, NDF and ADF. This result was supported by the meta-analysis of Brandao and Faciola (2019), who observed an increase in the molar percentage of acetate with an increase in the proportion of degraded NDF in digested organic matter. The relationship between the NDF content and acetate production was found to be curvilinear.

The molar ratios of propionate and butyrate were shown to exhibit opposite trends in response to dietary NDF (Brandao and Faciola, 2019). However, the present data demonstrated an increased propionate response in R1 which had a higher NDF content than R0. Inconsistent results in relation to propionate and butyrate percentage were also observed in the study by Tian et al. (2022), where the proportion of propionate and butyrate in rations containing maize straw silage was higher compared to rations containing ramie silage, despite the NDF content of the rations being 554 and 508 g/kg, respectively. Likewise, N-valerate concentration in our study was significantly affected by the treatments, with the highest concentration observed in the control (R0). Branched-chain VFAs, such as iso-butyrate and iso-valerate, could arise from the fermentation of branched-chain amino acids (Saro et al., 2014). Therefore, the tendency for higher iso-butyrate and iso-valerate concentrations in the ration based on NG compared to MSS (R1 and R2) in the present work could be attributed to the higher CP concentration and its extensive degradation. Our findings were also consistent with the results of Wang et al. (2021), who described a correlation between iso-butyrate, iso-valerate, and CP content in the diet. Additionally, Liu et al. (2014) suggested

that a diet consisting of 60% maize stover with low protein content could be combined with the addition of isovalerate (16.8 g/day), which could stimulate microbial or digestive enzyme activity. These authors reported that the inclusion of isovaleric acids enhanced the degradation of cellulose and haemicellulose in the rumen by increasing the population of cellulolytic bacteria.

In vitro gas production parameters and CH_4 production

The current study demonstrated that gas production in rations containing different fibrous feedstuffs (NG and MSS) ranged from 3.72 to 7.88%. This variability could be related to the composition of structural carbohydrates, soluble or digestible carbohydrates, and fermentative processes. A possible explanation is that NG (R0) had a lower NDF content compared to MSS. A negative relationship between NDF and gas production has been previously reported by Chen et al. (2019). In the present study, the high IVDMD and IVOMD values of forage corresponded with elevated *in vitro* gas production.

CH_4 is an unavoidable product generated from the anaerobic fermentation of dietary carbohydrates in the rumen, and methanogenesis is regulated by specific biological regulatory mechanisms. The mean production of CH_4 in the current study were in the range of 97.84–100.63 ml/l. There was a tendency for decreased methane production in R1 (1.83%), which was accompanied by a decrease in acetate molar percentage and the acetate to propionate ratio. Conversely, methane production increased in R2 (0.9%), where 100% of the forage was substituted with MSS. Several studies have found correlations between CH_4 production and NDF content. For instance, Salinas-Chavira et al. (2013) reported a strong relationship between CH_4 production and NDF content, while Singh et al. (2012) found positive correlations between CH_4 production and the ADF and cellulose content in forage. In addition, Kara (2019) observed positive correlations between CH_4 production and NDF content, IVDMD, IVOMD, and gas production.

Population of bacteria and protozoa

In the present study, the abundance of the bacterial population decreased linearly with increasing MSS percentage in the ration. A significant decrease of 0.08 log CFU/ml was observed when 50% of the forage was substituted with MSS (R1), and a decrease of 0.27 log CFU/ml when all forage was replaced with MSS (R2). Interestingly, our findings differed from previous reports that suggested an increase in the number of amylolytic bacteria and lactobacilli with increasing NDF content in the diet, along with a decrease in protozoa populations (Liu et al., 2013). In our study, the protozoan population declined by 1.97–2.69% (0.11–0.15 log CFU/ml) as the dietary NDF content increased. This suggests that non-structural carbohydrates in the ration containing MSS can be readily digested and utilised by microbes; however, the fibre content of MSS cannot be degraded as effectively as NG, resulting in a lower population of bacteria and protozoa.

The increasing utilisation of MSS in the rations resulted in a reduced population of bacteria and protozoa. Such inconsistent results were previously observed by Tian et al. (2022), who demonstrated that rami silage contained higher CP and lower NDF contents compared to MSS, but did not show significant differences in the population of bacteria (11.3–11.4 $\log_{10}$ copies/g DM), protozoa (7.50–7.66 $\log_{10}$ copies/g DM), fungi (8.09–8.27 $\log_{10}$ copies/g DM), and methanogens (9.33–9.51 $\log_{10}$ copies/g DM) in the rumen of goats. However, it is important to note that rumen microbes can be affected by various factors such as pH, feed materials, and nutrient composition (Sato, 2016; Kim et al., 2016). The main nutrients required by rumen microbes are carbohydrates and proteins. Thus, sufficient availability of these nutrients can increase rumen microbial biomass and subsequently impact feed digestibility. Hackmann and Firkins (2015) pointed out that rumen microbes do not allocate all adenosine triphosphate for growth, but also direct some of it towards activities such as maintenance (as has long been known), reserve carbohydrate synthesis or energy spilling (futile cycles that dissipate heat). In addition, the pH value obtained in this study was sufficient for proper rumen microbial activity and growth. This indicated that rumen microbial fermentation processes were productive, as evidenced by the concentration of VFA and NH_3 within ranges meeting the requirements of rumen microorganisms.

Conclusions

The increasing proportion of maize straw silage in the rations resulted in reduced *in vitro* digestibility and fermentation, i.e. lower dry matter and organic digestibility, reduced levels of ammonia (NH_3), total volatile fatty acids (VFA), gas production, and total protozoan and bacterial populations. However, the numerical values of rumen pH (6.45–6.71), VFA (69.08–89.23 mM), NH_3 concentration (10.50–12.88 mM), and gas production (185.46–193.33 ml/g dry

matter) remained within the normal range for ruminant requirements in terms of rumen *in vitro* feed fermentation. It should be noted, however, that the digestibility rate was still lower (<60%), which is an undesirable result and a more appropriate strategy is needed to overcome this problem. Nevertheless, considering the rumen fermentation products and pH ruminal conditions, it can be concluded that the addition of maize straw silage, up to 50% of the ration, can potentially serve as a forage substitute in ruminant rations.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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