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Effect of local potash inclusion in mineral-salt-blocks on *in vitro* gas production and post-incubation fermentative parameters using sheep and goat inoculum

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Abstract

Mineral deficiencies arise from the long-term feeding of tropical grasses to ruminants thereby necessitating supplementation. However, commercial mineral supplements are expensive to buy for small holder farmers thereby necessitating cheap and readily available alternative. This study was designed to evaluate the effect of industrial salt only and mineral-salt-blocks formulated to contain graded levels of local potash (0, 6, 8 and 10% w/w replacement of industrial salt) on in vitro gas production, fermentation characteristics and nutrient degradability using sheep and goat rumen inoculum in a 2 × 5 factorial arrangement. Megathyrsus maximus was used as substrate and it was supplemented with four modified mineral-salt-blocks (MSB1–MSB4) or industrial salt (19:1 substrate-to-supplement ratio) and incubated for 48 h at 39°C following the Menke and Steingass technique. Data collected were subjected to two-way analysis of variance. Mineral supplement type affected (p<0.05) cumulative gas production, fermentation kinetics, metabolizable energy, organic matter digestibility, short-chain fatty acids, volatile fatty acids, ammonia-nitrogen and in vitro crude protein degradability. The 8% local potash inclusion (MSB3) recorded the highest total volatile fatty acids (90.00 mol/100 mL) and in vitro crude protein degradability (66.72%), while industrial salt supplementation yielded the highest in vitro dry matter degradability (50.00%). Sheep inoculum produced higher (p<0.05) cumulative gas volume, asymptotic gas production, metabolizable energy and total volatile fatty acids than goat inoculum. Methane production was unaffected (p>0.05) by mineral supplement type or inoculum source. In conclusion, local potash at 8% inclusion to MSB enhanced in vitro crude protein degradability and elevated total volatile fatty acid concentration, indicating improved rumen fermentation efficiency. Mineral-salt-blocks enriched with 8% local potash may serve as effective and affordable mineral supplements for small ruminants fed tropical grasses.

Keywords: Kaun; inoculum source; mineral supplementation; salt licks; small ruminants; rumen fermentation

Effet de l'incorporation de potasse locale dans des blocs de sels minéraux sur la production de gaz in vitro et les paramètres de fermentation post-incubation à partir d'inoculums provenant de moutons et de chèvres

Résumé

Les carences minérales résultent d'une alimentation à long terme des ruminants à base d'herbes tropicales, ce qui rend nécessaire une supplémentation. Cependant, les compléments minéraux commerciaux sont coûteux pour les petits exploitants agricoles, d'où la nécessité de trouver une alternative peu coûteuse et facilement accessible. Cette étude a été conçue pour évaluer l'effet du sel industriel seul et de blocs de sels minéraux formulés pour contenir des teneurs graduelles en potasse locale (remplacement du sel industriel à raison de 0, 6, 8 et 10 % p/p) sur la production de gaz in vitro, les caractéristiques de fermentation et la dégradabilité des nutriments, à l'aide d'inoculums de rumen de moutons et de chèvres, dans un plan factoriel 2 × 5. Megathyrsus maximus a été utilisé comme substrat ; celui-ci a été complété par quatre blocs de sel minéral modifiés (MSB1–MSB4) ou par du sel industriel (rapport substrat/complément de 19:1), puis incubé pendant 48 h à 39 °C selon la technique de Menke et Steingass. Les données recueillies ont fait l'objet d'une analyse de variance à deux facteurs. Le type de complément minéral a influencé (p < 0,05) la production cumulative de gaz, la cinétique de fermentation, l'énergie métabolisable, la digestibilité de la matière organique, les acides gras à chaîne courte, les acides gras volatils, l'azote ammoniacal et la dégradabilité in vitro des protéines brutes. L'inclusion de potasse locale à 8 % (MSB3) a enregistré les valeurs les plus élevées en acides gras volatils totaux (90,00 mol/100 ml) et en dégradabilité in vitro des protéines brutes (66,72 %), tandis que l'ajout de sel industriel a donné la dégradabilité in vitro de la matière sèche la plus élevée (50,00 %). L'inoculum provenant de moutons a produit un volume cumulé de gaz, une production asymptotique de gaz, une énergie métabolisable et une teneur totale en acides gras volatils supérieurs (p < 0,05) à ceux de l'inoculum provenant de chèvres. La production de méthane n'a pas été influencée (p > 0,05) par le type de complément minéral ni par la source de l'inoculum. En conclusion, l'ajout de 8 % de potasse locale au MSB a amélioré la dégradabilité in vitro des protéines brutes et augmenté la concentration totale en acides gras volatils, ce qui

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indique une meilleure efficacité de la fermentation ruminale. Les blocs de sel minéral enrichis à 8 % de potasse locale peuvent constituer des compléments minéraux efficaces et abordables pour les petits ruminants nourris avec des graminées tropicales.

Mots-clés : Kaun ; source d'inoculum ; supplémentation minérale ; blocs de sel ; petits ruminants ; fermentation ruminale

Introduction

Tropical grasses which are the principal feed resources for small ruminants across West Africa are typically known to be low in crude protein, excessive in fibre and deficient in minerals like phosphorus, sodium, sulphur, zinc and copper (Suttle 2010). These minerals serve as essential cofactors for cellulolytic and proteolytic enzymes in the rumen. Phosphorus drives ATP-dependent microbial attachment to plant fibre, sulphur participates in microbial amino acid synthesis, and trace minerals like zinc and copper are structural components of metalloenzymes involved in fibre degradation (Durand and Komisarczuk 1988; Genther and Hansen 2015). Modest deficit in mineral supply suppresses fibre digestion and widens the gap between the potential and realized nutritive values of tropical forages because cellulolytic bacteria in the rumen are sensitive to mineral shortfalls (Smith *et al.,* 2005; Huws *et al.,* 2018; Adegbeye *et al.,* 2020). Correcting forage mineral deficiencies through affordable and accessible mineral supplementation is a direct pathway to improving feed utilization.

Commercial mineral supplements address these deficits effectively but their cost, erratic supply and large-unit packaging place them beyond the reach of smallholder farmers who keep the bulk of Nigeria's small ruminant population (Godde *et al.,* 2021). This drives farmers to rely on indigenous mineral sources, amongst which is local potash. Local potash also known locally as "Kaun" or as "Trona" in the geological sector is a dried lake salt comprising largely of sodium bicarbonate (Davidson *et al.,* 1974). It is occasionally encountered as a saline lake deposit or evaporation product, and as an efflorescence on arid soil. It is a product of the salt industry in many parts of Nigeria and North Africa (RMRDC, 1996). It is also notable for its concentration of phosphorus, iron, copper and zinc (Uzogara *et al.,* 1990). Despite its widespread use in ethnoveterinary practices across southern Nigeria, the effect of local potash on rumen fermentation dynamics has been minimally evaluated.

Mineral-salt-blocks (MSB) offer a practical delivery mechanism for local potash because their hard physical form regulates intake through licking thereby reducing the risk of mineral toxicity. The *in vitro* gas production technique provides a rapid and reproducible method of estimating fermentation potential and predicting nutritive value of feedstuffs and supplements (Menke and Steingass, 1988; Getachew *et al.,* 1998). Gas volume during incubation reflects the extent of substrate degradation by rumen microorganisms, and correlates positively with *in vivo* digestibility (Yáñez-Ruiz *et al*., 2016). A fundamental requirement of the technique however, is a source of rumen inoculum, and the choice of donor species introduces a recognised source of variation into fermentation estimates (Cone *et al.,*1996; Bueno *et al.,* 2005). Several studies have compared rumen fluid from different ruminant species as inoculum for *in vitro* systems. Bueno *et al.* (2005) reported that donor species influences gas production kinetics of tropical feedstuff, Molina-Alcaide *et al.* (2017) found goat inoculum to be superior for tanniferous browse, and Aderinboye *et al.* (2016) observed significant species versus substrate interactions using inoculum from cattle, sheep and goat. Calabrò *et al.* (2005) reported that inter-species microbial community differences significantly impact *in vitro* fermentation outcomes. These studies have however all manipulated the incubation substrate while keeping the mineral environment constant with very limited study carried out to evaluate if fermentation response to mineral supplements differs between ruminant species.

This study was therefore designed to evaluate the effect of MSB containing graded levels of local potash on *in vitro* gas production, fermentation kinetics, methane gas production and nutrient degradability of *Megathyrsus maximus,* using rumen inoculum harvested from slaughtered West African dwarf (WAD) sheep and goats. It was hypothesized that local potash inclusion would enhance fermentation parameters and the magnitude of its effect would differ between the source of inoculum owing to inter-species variation in rumen microbial composition and mineral sensitivity.

Material and methods

The study was carried out at the laboratory of the Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (7°15′N, 3°26′E; altitude 76 m above sea level).

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Formulation and determination of mineral composition of mineral-salt-block

Modified-salt-block was formulated using the ingredient composition on Table 1 with industrial salt being partially replaced with local potash at four levels (0, 6, 8 and 10% - w/w) to produce four mineral-salt-blocks designated MSB1, MSB2, MSB3 and MSB4, respectively. Industrial salt served as the primary sodium source and intake regulator, bone meal provided calcium and phosphorus, limestone powder functioned as calcium source and binding agent, termitarium soil collected from a termite mound served as mineral source and secondary binder, while local potash purchased from a local market in Abeokuta served as mineral source. The ingredients were mixed together and made into a paste using water (5% - w/v), the paste was then poured into cellophane-lined plastic moulds and compressed using flat wooden slabs (Omoniyi *et al.,* 2013). The blocks were thereafter sundried on raised platform for 7 days. Mineral concentrations were determined by wet digestion using nitric-perchloric acid mixture (AOAC, 2012). The mineral elements calcium, magnesium, iron, copper, zinc, and manganese were determined using atomic absorption spectrophotometer using the Buck Scientific model 200 (East Norwalk, USA), while flame photometer (FP 410 Corning) was used to estimate sodium and potassium contents. Phosphorus concentration was determined calorimetrically using Vanadomolybdate method (Parkinson and Allen, 1975).

Substrate preparation and chemical analysis

Megathyrsus maximus (Guinea grass) was harvested from naturally established grassland, chopped, oven-dried at 105°C to constant weight for dry matter determination, milled through 1mm screen and stored in airtight containers. Proximate composition was determined according to AOAC (2012), while fibre fractions were analyzed according to Van Soest *et al.* (1991).

Table 1: Ingredient Composition (%) of Mineral-Salt-Blocks

Ingredients	MSB1	MSB2	MSB3	MSB4
Industrial salt	58	52	50	48
Local potash	0	6	8	10
Bone meal	15	15	15	15
Limestone powder	10	10	10	10
Termitarium soil	17	17	17	17
Total	**100**	**100**	**100**	**100**

Note. Water to mix (5% - w/v). MSB = Mineral-salt-block.

In vitro gas production procedure

The *in vitro* gas production followed the technique of Menke and Steingass (1988). Rumen fluid was collected from three West African dwarf goats and three West African dwarf sheep maintained at the Small Ruminant Unit. The donor animals had been fed Guinea grass, cassava peels and wheat offal (75:15:10 ratio) for at least 14 days prior to the collection. Rumen fluid was collected before morning feeding via oesophageal suction tube (Babayemi and Bamikole, 2006) into two separate pre-warmed (39°C) thermos flasks and transported immediately to the laboratory. The fluid was strained through four layers of cheesecloth under continuous flushing with CO_2 to maintain anaerobic conditions. Approximately 200mg of substrate (comprising of oven-dried 190 mg *Megathyrsus maximus* and 10mg respective mineral supplement) were weighed into fibre bags placed into 100mL plastic syringes. Each syringe was then filled with 40mL of inoculum containing strained rumen fluid mixed with buffer solution (Menke and Steingass, 1988). The experimental design was a 2 x 5 factorial arrangement in a

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Completely randomized design, with two inoculum sources (sheep and goat), and five mineral supplements (MSB1, MSB2, MSB3, MSB4 and industrial salt), giving 10 treatments combinations. The various treatments were replicated 10 times, and six blanks (three per inoculum source) containing inoculum medium alone were included. The syringes were incubated at 39°C with gas production recorded at 3, 6, 9, 12, 18, 24, 36 and 48h. Blank-corrected gas volume was used for all calculations. At the end of the 48-h incubation, 4mL of 10M NaOH was introduced into each syringe (n=4 per treatment) to absorb CO_2, and the residual gas volume was recorded as methane (Fievez *et al.,* 2005). Net methane production was expressed as a proportion (%) of total gas volume.

Post-incubation analyses and calculations

After 48 h, residues were recovered by filtration into pre-weighed crucibles, oven-dried at 105°C for 24 h and weighed. The crude protein content of residues was determined following standard procedure of AOAC (2012). *In vitro* dry matter degradability (IVDMD) and *in vitro* crude protein degradability (IVCPD) were calculated as the proportional loss of initial substrate dry matter or crude protein, respectively. The pH of post-incubation fluid was measured immediately using a portable pH meter. Ammonia-nitrogen (NH_3-N) was determined colorimetrically. Total volatile fatty acids (TVFA) were measured by steam distillation (Markham, 1942), and individual VFAs (acetic, propionic and butyric acids) were quantified by gas chromatography. Gas production data were fitted to the non-linear model of France *et al.* (2000): $A = b(1 - e^{-c(t-L)})$, where A is the volume of gas produced at time t, b is the asymptotic gas production (mL/g DM) from the fermentable fraction, c is the fractional rate of gas production (/h) and L is the discrete lag time (h). Metabolizable energy (ME, MJ/kg DM) was predicted as 2.20 + 0.136GV + 0.057CP + 0.0029CF (Menke and Steingass, 1988), Organic matter digestibility (OMD, %) was calculated as 14.88 + 0.889GV + 0.45CP + 0.651XA (Menke and Steingass, 1988), while Short-chain fatty acid (SCFA, µmol/g DM) was estimated as 0.0239GV − 0.0601 (Getachew *et al.,* 1998). Where GV is total 24-h gas volume, CP is crude protein (% DM) and CF is crude fibre (% DM) and XA is ash (% DM).

Statistical analysis

Data were analysed as a 2 × 5 factorial using the General Linear Model procedure of SAS (SAS Institute, 2002). The model included the fixed effects of mineral supplement type (5 levels), inoculum source (2 levels) and their interaction. Where the F-test was significant ($p<0.05$), means were separated using Duncan's multiple range test. When a significant interaction was detected, simple-effect means are discussed.

Results and discussion

Mineral composition of ingredients and mineral-salt-blocks

Local potash (LP) contained the highest concentrations of phosphorus (28.77%), copper (6150 ppm) and zinc (7250 ppm) among all ingredients, while termitarium soil was the richest source of iron (24700 ppm) and manganese (550 ppm) (Table 2). Graded inclusion of local potash in the blocks produced a significant ($p<0.05$) linear increase in calcium, phosphorus, potassium, iron, copper and zinc concentrations, accompanied by a corresponding linear decrease in sodium (Table 3). Phosphorus rose nearly three-fold from 1.55% in MSB1 to 4.43% in MSB4, while copper and zinc increased by more than 250-fold and 9-fold, respectively. Magnesium and manganese remained unaffected ($p>0.05$). The progressive mineral enrichment with local potash inclusion is consistent with earlier reports that local potash is a repository of various essential mineral elements required by grazing animals (Adeleye, 2004). The magnitude of phosphorus increase carries particular relevance for rumen function, Komisarczuk *et al.* (1987) demonstrated in a semi-continuous fermenter that phosphorus depletion severely reduces cellulose digestion and microbial protein synthesis, and Durand and Komisarczuk (1988) established that phosphorus is required for cellulolysis at a higher critical concentration than for hemicellulolysis or amylolysis. Recent evidence confirms that phosphorus availability remains a rate-limiting factor for microbial colonization of plant fibre under conditions typical of tropical ruminant diets (Zebeli *et al.,* 2012). The concurrent increase in copper and zinc is nutritionally significant because these trace elements serve as cofactors for microbial metalloenzymes (Genther and Hansen, 2015), the authors confirmed that relatively small changes in trace mineral concentration can modulate fibre digestion in the rumen. The reciprocal decrease in sodium with potash inclusion, though statistically significant, was practically minor (21.58 to 20.34%) and unlikely to limit microbial sodium requirements (Suttle, 2010).

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Table 2: Mineral composition (% and ppm) of ingredients

Minerals	Industrial salt	Bone meal	Limestone powder	Termitarium soil	Local potash
Major minerals (%)					
Calcium	0.03	27.22	25.80	0.82	3.12
Phosphorus	0.00	10.35	0.00	0.00	28.77
Sodium	35.8	5.41	0.04	0.00	23.41
Potassium	0.09	0.34	0.63	1.46	4.68
Magnesium	0.01	0.52	0.17	0.00	0.48
Trace (Minor) minerals (ppm)					
Iron	40	440	3000	24700	9450
Copper	0.0	16.3	0.0	0.0	6150
Zinc	5.0	312	350	0.0	7250
Manganese	0.0	20	180	550	5.0

Table 3: Mineral composition (% and ppm) of mineral-salt-blocks

Minerals	**MSB1**	**MSB2**	**MSB3**	**MSB4**	**SEM**	***p***
Major minerals (%)						
Calcium	6.82^{b}	7.01^{a}	7.07^{a}	7.13^{a}	0.04	0.021
Phosphorus	1.55^{d}	3.28^{c}	3.85^{b}	4.43^{a}	0.33	<0.001
Sodium	21.58^{a}	20.84^{b}	20.59^{c}	20.34^{d}	0.14	0.033
Potassium	0.41^{d}	0.69^{c}	0.78^{b}	0.87^{a}	0.05	0.004
Magnesium	0.10	0.13	0.14	0.15	0.01	0.102
Trace (Minor) minerals (ppm)						
Iron	4588^{b}	5152^{a}	5341^{a}	5529^{a}	127	0.019
Copper	2.45^{d}	371.45^{c}	494.45^{b}	617.45^{a}	70.18	<0.001
Zinc	84.76^{d}	519.46^{c}	664.36^{b}	809.26^{a}	82.87	0.003
Manganese	114.5	114.8	114.9	115.0	0.3	0.229

^{abcd} - Means on the same row with different superscripts are significantly different (P<0.05). MSB 1 – Salt block with 0% local potash, MSB 2 – Salt block with 6% local potash, MSB 3 – Salt block with 8% local potash, MSB 4 – Salt block with 10% local potash, SEM – Standard Error of Mean

In vitro gas production

Mineral supplement type significantly (p<0.05) influenced cumulative gas production at all incubation time points except the 3rd hour (Table 4). After 48 h, MSB1 (0% LP) produced the highest gas volume (13.43 mL/200 mg DM), followed by MSB3 (8% LP -12.00 mL), industrial salt (11.10 mL), MSB2 (6% LP - 11.02 mL) and MSB4 (10% LP - 10.71 mL). Sheep inoculum generated higher (p<0.05) cumulative gas volumes than goat inoculum at most time points, with final 48-h values of 11.97 and 11.23 mL/200 mg DM for sheep and goat, respectively. Significant (p<0.05) interactions between mineral supplement type and inoculum source were detected at all time points except the 6th hour. The highest 48-h gas volume (13.75 mL/200 mg DM) was recorded for MSB1 incubated with sheep inoculum, while the lowest (9.67 mL) was observed for MSB4 with goat inoculum (Table 4). The higher gas volume for MSB1-supplemented substrate relative to industrial salt suggests that even the basal mineral-salt-block formulation (without potash) provided a more favourable mineral profile for rumen microbial fermentation than industrial salt alone, because MSB1 still contains bone meal, limestone and termitarium soil, which collectively supply calcium, phosphorus, iron and manganese. The depression in gas volume at 10% potash inclusion (MSB4) may reflect an inhibitory effect of excessive alkalinity or elevated osmotic pressure on certain rumen microbial populations, consistent with the established principle that mineral supplementation follows an optimum-response curve beyond which returns diminish (Suttle, 2010; Kholif and Olafadehan, 2021). The premise that ruminant's species differ in their digestibility of different forage plants has been the been the basis for several *in vitro* studies carried out comparing inoculum from different ruminant species (Szumacher-Strabel *et al*., 2004; Selmi *et al.,* 2015;

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Aderinboye *et al.,* 2016). Selmi *et al.* (2015) observed that substrate incubated with goat inoculum recorded higher cumulative gas volume than substrate incubated with sheep inoculum. This however contradicts observation of this study where 48h gas volume was similar for sheep and goat inoculum with sheep inoculum recording higher gas volume than goat inoculum at lower time points. This variation is attributable to substrate incubated (fodder shrubs in their study vs grass in this study), Goats are browsers while sheep are grazers.

In vitro fermentation parameters, methane production and volatile fatty acid profile

Mineral supplement type significantly ($p<0.05$) affected NH3-N, TVFA and individual VFA concentrations but (Table 5). MSB3 (8% potash) recorded the highest TVFA (90.00 mol/100 mL), acetic acid (60.00 mol/100 mL), propionic acid (20.00 mol/100 mL) and butyric acid (6.00 mol/100 mL), all significantly higher than values for MSB2 and MSB4. Industrial salt supplementation produced the highest NH3-N (6.94%), similar to MSB3 and MSB1 (6.87 and 6.71%), but higher than MSB2 and MSB4 (6.19 and 6.20%). Neither mineral supplement type and inoculum source nor their interaction significantly ($P > 0.05$) affected total methane gas volume or net methane production (Table 5). Sheep inoculum yielded higher ($p<0.05$) TVFA (85.80 vs. 72.84 mol/100 mL), NH3-N (6.76 vs. 6.41%) and individual VFA concentrations but lower pH (6.43 vs. 6.76) than goat inoculum.

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Table 4: Main and interaction effect of mineral supplement type and inoculum source on *in vitro* gas production (ml/200mg DM)

Inoculum source	Mineral supplement	Hours of incubation (hrs)							
		3	**6**	**9**	**12**	**18**	**24**	**36**	**48**
Mineral supplement									
	Industrial salt	1.24	2.57^{ab}	4.57^{a}	4.71^{bc}	6.23^{ab}	8.43^{b}	9.95^{b}	11.10^{b}
	MSB 1	1.43	3.00^{a}	4.64^{a}	5.50^{a}	6.93^{a}	10.07^{a}	11.64^{a}	13.43^{a}
	MSB 2	1.00	2.60^{ab}	3.81^{b}	4.81^{bc}	5.62^{b}	8.00^{b}	9.60^{b}	11.02^{b}
	MSB 3	1.43	2.57^{ab}	4.43^{a}	5.00^{ab}	6.14^{ab}	8.71^{b}	10.29^{b}	12.00^{ab}
	MSB 4	1.14	2.29^{b}	3.71^{b}	4.42^{c}	5.42^{b}	8.00^{b}	9.57^{b}	10.71^{b}
	SEM	0.09	0.10	0.12	0.10	0.15	0.22	0.24	0.27
	p	0.372	0.011	0.009	<0.001	0.004	0.007	0.025	0.017
Inoculum source									
Goat		0.93^{b}	2.37^{b}	3.97^{b}	4.77	5.83	8.03^{b}	9.33^{b}	11.23
Sheep		1.48^{a}	2.78^{a}	4.43^{a}	4.98	6.25	9.10^{a}	10.87^{a}	11.97
SEM		0.09	0.10	0.12	0.10	0.15	0.22	0.24	0.27
p		<0.001	0.003	<0.001	0.097	0.072	0.041	0.035	0.108
Interaction effect									
Goat	Salt*	0.67^{b}	2.00	4.00^{bcd}	4.33^{bc}	5.67^{bcd}	7.67^{cd}	9.00^{cd}	10.33^{bc}
	MSB 1	1.00^{ab}	3.00	4.33^{ab}	5.50^{a}	6.50^{abc}	9.50^{ab}	10.50^{bc}	13.00^{a}
	MSB 2	1.00^{ab}	2.50	4.00^{bcd}	5.00^{ab}	6.00^{abcd}	8.00^{bcd}	9.50^{bcd}	11.50^{abc}
	MSB 3	1.00^{ab}	2.33	4.00^{bcd}	5.00^{ab}	6.00^{abcd}	8.00^{bcd}	9.33^{bcd}	11.67^{abc}
	MSB 4	1.00^{ab}	2.00	3.33^{d}	4.00^{d}	5.00^{d}	7.00^{d}	8.33^{d}	9.67^{c}
Sheep	Salt*	1.67^{a}	3.00	4.00^{bcd}	5.00^{ab}	6.67^{ab}	9.00^{abc}	10.67^{bc}	11.67^{abc}
	MSB 1	1.75^{a}	3.00	4.75^{a}	5.50^{a}	7.25^{a}	10.50^{a}	12.50^{a}	13.75^{a}
	MSB 2	1.00^{ab}	2.67	3.67^{cd}	4.67^{abc}	5.33^{cd}	8.00^{bcd}	9.67^{bcd}	10.67^{bc}
	MSB 3	1.75^{a}	2.75	4.33^{ab}	5.00^{ab}	6.25^{abcd}	9.25^{abc}	11.00^{ab}	12.25^{ab}
	MSB 4	1.25^{ab}	2.50	4.00^{bcd}	4.75^{abc}	5.75^{bcd}	8.75^{abcd}	10.50^{bc}	11.50^{abc}
SEM		0.09	0.10	0.12	0.10	0.15	0.22	0.24	0.27
p		0.004	0.305	0.029	0.021	0.027	0.004	0.012	0.004

^{abcd}*- Means on the same column with different superscripts are significantly different (P<0.05). MSB 1 – Salt block with 0% local potash, MSB 2 – Salt block with 6% local potash, MSB 3 – Salt block with 8% local potash, MSB 4 – Salt block with 10% local potash, SEM – Standard Error of Mean.*

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The interaction between mineral supplement type and inoculum source was significant ($p<0.05$) for all fermentation parameters. The highest TVFA (98.40 mol/100 mL) was recorded for MSB3 with goat inoculum, while the lowest (50.40 mol/100 mL) was recorded for MSB4 with goat inoculum. The observed variation in response of sheep and goat inoculum to MSB3 supplementation relative to industrial salt in this study indicates that the fermentation response to potash-enriched salt blocks is modulated by the microbial composition of the donor species, confirming the inter-species sensitivity hypothesised at the outset of this study. The 48-unit range for TVFA within goat inoculum treatments (50.40–98.40 mol/100 mL), compared with a narrower range (81.60–91.20) within sheep inoculum treatments, suggests that goat rumen microbiota is more sensitive to mineral supplement composition than sheep rumen microbiota under these conditions. This differential sensitivity may reflect differences in microbial community diversity and structure between the two species (Bueno *et al*., 2005; Hartinger *et al*., 2018). Molina-Alcaide *et al.* (2017) similarly reported divergent fermentation responses between sheep and goat inocula, although their study examined tanniferous substrates rather than mineral supplements. The absence of a significant methane response to mineral supplementation indicates that the salt blocks did not shift the balance between hydrogenotrophic and acetoclastic methanogenesis (Suttle, 2010), an important finding for climate-smart livestock management, as it confirms that the supplementation strategy does not compound enteric methane emissions (Adegbeye *et al.*, 2020).

Fermentation kinetics, predicted nutritive value and degradability

Mineral supplement type significantly ($p<0.05$) affected the asymptotic gas production (b_g) and fractional rate of gas production (c_g) but not lag time (Table 6). All MSB-supplemented substrates recorded higher asymptotic gas production (35.10–41.62 mL/g DM) than industrial salt-supplemented substrate (21.92 mL/g). Conversely, the fractional rate of gas production was higher for industrial salt (0.017/h) than for the MSBs (0.008–0.010/h), indicating faster but less extensive fermentation with industrial salt alone. Sheep inoculum produced a higher asymptotic gas production (42.50 vs. 24.96 mL/g; $p<0.05$) and longer lag time (3.08 vs. 2.13 h; $p<0.05$) than goat inoculum. The interaction effect was significant ($p<0.05$) for all kinetic parameters; MSB3 and MSB4 with sheep inoculum yielded the highest asymptotic gas values (48.93 and 53.03 mL/g), while MSB4 with goat inoculum recorded only 20.40 mL/g. ME values ranged from 4.13 MJ/kg DM for MSB2 and MSB4 to 4.41 MJ/kg DM for MSB1, with MSB1 and MSB3 recording significantly higher ME, SCFA and OMD than industrial salt, MSB2 or MSB4. Industrial salt supplementation produced the highest IVDMD (50.00%), while MSB4 recorded the lowest (36.67%). The most pronounced treatment effect was observed for IVCPD: MSB3 (8% potash) yielded 66.72%, a value 6.19 percentage units above industrial salt (60.53%) and 15.12 units above MSB4 (51.60%). MSB3 with sheep inoculum produced the highest IVCPD across all treatment combinations (75.47%), while MSB4 with goat inoculum recorded the lowest (46.23%). The higher asymptotic gas production for MSB-supplemented substrates relative to industrial salt, coupled with their lower fractional rate, indicates that the mineral-salt-blocks promoted more extensive but slower degradation of the fermentable fraction of *M. maximus*. Extensive fibre degradation is nutritionally desirable because it increases total nutrient extraction from the forage (Yáñez-Ruiz *et al*., 2016; Moorby and Fraser, 2021). The higher asymptotic gas production for sheep inoculum across all MSB treatments is consistent with reports of significant species differences in *in vitro* gas production kinetics by Bueno *et al.* (2005) and Aderinboye *et al*. (2016). Such inter-species differences likely reflect variations in microbial community composition, enzyme activities and protozoa-to-bacteria ratios between donor animals (Cone *et al.,* 1996; Calabrò *et al.,* 2005; Hartinger *et al.,* 2018). The significantly higher IVCPD for MSB3 (66.72%) relative to all other supplements represents the most prominent finding of this study. The mineral profile of MSB3 appears to have been particularly adequate for proteolytic bacterial growth. Recent evidence indicates that dietary mineral supply can selectively favour proteolytic microbial taxa in the rumen (Detmann *et al.,* 2019).

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Table 5: Main and interaction effect of mineral supplement type and inoculum source on *in vitro* fermentation kinetics, calculated parameters and degradability

Inoculum source	Mineral supplement	Parameters							
		TMGV (ml)	NMP (%)	pH	NH_3-N (%)	TVFA (mol/100mL)	Acetic (mol/100mL)	Propionic (mol/100mL)	Butyric (mol/100mL)
Mineral supplement									
	Salt*	4.52	39.95	6.56	6.94^{a}	83.40^{ab}	55.60^{b}	18.53^{ab}	5.56^{ab}
	MSB 1	5.57	40.54	6.64	6.71^{ab}	80.40^{b}	53.60^{b}	17.87^{b}	5.36^{b}
	MSB 2	5.10	45.47	6.58	6.19^{b}	72.60^{c}	48.40^{c}	16.13^{c}	4.84^{c}
	MSB 3	5.14	42.73	6.50	6.87^{a}	90.00^{a}	60.00^{a}	20.00^{a}	6.00^{a}
	MSB 4	5.00	46.61	6.71	6.20^{b}	70.20^{c}	46.80^{c}	15.60^{c}	4.68^{c}
	SEM	0.26	1.64	0.05	0.14	2.69	1.79	0.60	0.18
	p	0.526	0.449	0.094	0.023	0.037	0.044	0.008	0.001
Inoculum source									
Goat		4.73	42.00	6.76^{a}	6.41^{b}	72.84^{b}	48.56^{b}	16.19^{b}	4.86^{b}
Sheep		5.32	43.85	6.43^{b}	6.76^{a}	85.80^{a}	57.20^{a}	19.07^{a}	5.72^{a}
SEM		0.26	1.64	0.05	0.14	2.69	1.79	0.60	0.18
p		0.482	0.317	0.047	0.041	0.036	0.021	0.011	0.007
Interaction effect									
Goat	Salt*	4.00	37.03	6.73^{bc}	6.52^{bcd}	75.60^{d}	50.40^{d}	16.80^{d}	5.04^{d}
	MSB 1	4.33	33.10	6.83^{ab}	7.09^{abc}	78.60^{cd}	52.40^{cd}	17.47^{cd}	5.24^{cd}
	MSB 2	6.00	51.86	6.72^{bc}	5.70^{de}	61.20^{e}	40.80^{e}	13.60^{e}	4.08^{e}
	MSB 3	4.67	39.90	6.45^{cd}	7.43^{a}	98.40^{a}	65.60^{a}	21.87^{a}	6.56^{a}
	MSB 4	4.67	48.15	7.08^{a}	5.30^{e}	50.40^{f}	33.60^{f}	11.20^{f}	3.36^{f}
Sheep	Salt*	4.92	42.14	6.39^{cd}	7.37^{ab}	91.20^{ab}	60.80^{ab}	20.27^{ab}	6.08^{ab}
	MSB 1	6.50	46.15	6.44^{cd}	6.33^{cd}	82.20^{bcd}	54.80^{bcd}	18.27^{bcd}	5.48^{bcd}
	MSB 2	4.42	40.68	6.43^{cd}	6.69^{abc}	84.00^{bcd}	56.00^{bcd}	18.67^{bcd}	5.60^{bcd}
	MSB 3	5.50	44.86	6.55^{cd}	6.32^{cd}	81.60^{bcd}	54.40^{bcd}	18.13^{bcd}	5.44^{bcd}
	MSB 4	5.25	45.46	6.35^{d}	7.10^{abc}	90.00^{abc}	60.00^{abc}	20.00^{abc}	6.00^{abc}
SEM		0.26	1.64	0.05	0.14	2.69	1.79	0.60	0.18
p		0.721	0.208	0.039	0.032	0.004	<0.001	0.007	<0.001

*^{abcd}- Means on the same column with different superscripts are significantly different (P<0.05). * - Industrial salt, MSB 1 – Salt block with 0% local potash, MSB 2 – Salt block with 6% local potash, MSB 3 – Salt block with 8% local potash, MSB 4 – Salt block with 10% local potash, SEM – Standard Error of Mean, TMGV – Total methane gas volume, NMP – Net methane production, NH_3-N – Ammonia nitrogen, TVFA – Total volatile fatty acid.*

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Table 6: Main and interaction effect of mineral supplement type and inoculum source on *in vitro* fermentation kinetics, calculated parameters and degradability

Inoculum source	Mineral supplement	Parameters							
		bg (mL/g)	cg (ml/hr)	Lag time (hr)	ME (MJ/kg DM)	SCFA (mmol)	OMD (%)	IVDMD (%)	IVCPD (%)
Mineral supplement									
	Salt*	21.92^{b}	0.017^{a}	2.52	4.18^{b}	0.217^{b}	33.39^{b}	50.00^{a}	60.53^{b}
	MSB 1	37.25^{a}	0.010^{b}	2.35	4.41^{a}	0.254^{a}	34.89^{a}	44.17^{ab}	57.12^{bc}
	MSB 2	35.10^{a}	0.009^{b}	2.26	4.13^{b}	0.209^{b}	33.07^{b}	46.67^{a}	57.89^{b}
	MSB 3	41.62^{a}	0.008^{b}	3.33	4.22^{ab}	0.224^{ab}	33.68^{ab}	47.50^{a}	66.72^{a}
	MSB 4	39.05^{a}	0.008^{b}	2.89	4.13^{b}	0.208^{b}	33.05^{b}	36.67^{b}	51.60^{c}
	SEM	2.46	0.001	0.21	0.03	0.005	0.22	1.40	1.57
	p	<0.001	<0.001	0.186	0.038	0.007	0.046	0.039	0.032
Inoculum source									
Goat		24.96^{b}	0.013^{a}	2.13^{b}	4.10^{b}	0.20^{b}	32.83^{b}	43.00	53.41
Sheep		42.50^{a}	0.008^{b}	3.08^{a}	4.30^{a}	0.24^{a}	34.20^{a}	47.00	64.14
SEM		2.46	0.001	0.21	0.03	0.005	0.22	1.40	1.57
		<0.001	<0.001	0.020	0.022	0.013	0.030	0.084	0.520
Interaction effect									
Goat	Salt*	20.06^{d}	0.021^{a}	1.70^{b}	4.05^{cd}	0.196^{cd}	32.54^{cd}	48.33^{abc}	56.62^{bc}
	MSB 1	26.60^{cd}	0.013^{bc}	2.16^{ab}	4.25^{bc}	0.229^{bc}	33.87^{bc}	45.00^{abc}	54.34^{cd}
	MSB 2	25.88^{d}	0.012^{bc}	2.15^{ab}	4.12^{bcd}	0.207^{bcd}	32.98^{bcd}	40.00^{bc}	51.89^{cd}
	MSB 3	31.87^{bcd}	0.010^{bc}	2.11^{ab}	4.10^{bcd}	0.203^{bcd}	32.83^{bcd}	45.00^{abc}	57.97^{bc}
	MSB 4	20.40^{d}	0.013^{bc}	2.51^{ab}	3.96^{d}	0.181^{d}	31.95^{d}	36.67^{c}	46.23^{d}
Sheep	Salt*	23.31^{d}	0.014^{ab}	3.13^{ab}	4.28^{bc}	0.233^{bc}	34.02^{bc}	51.67^{ab}	64.43^{b}
	MSB 1	45.23^{ab}	0.008^{bc}	2.50^{ab}	4.53^{a}	0.273^{a}	35.65^{a}	43.33^{abc}	59.91^{bc}
	MSB 2	42.02^{abc}	0.007^{bc}	2.34^{ab}	4.14^{bcd}	0.210^{bcd}	33.13^{bcd}	53.33^{a}	63.90^{b}
	MSB 3	48.93^{a}	0.006^{c}	4.25^{a}	4.32^{ab}	0.240^{ab}	34.32^{ab}	50.00^{ab}	75.47^{a}
	MSB 4	53.03^{a}	0.005^{c}	3.18^{ab}	4.25^{bc}	0.232^{bc}	33.87^{bc}	36.67^{c}	56.98^{bc}
SEM		2.46	0.001	0.21	0.03	0.005	0.22	1.40	1.57
		<0.001	<0.001	0.043	0.045	0.037	0.047	0.026	0.006

*^{abcd}- Means on the same column with different superscripts are significantly different (P<0.05). * - Industrial salt, MSB 1 – Salt block with 0% local potash, MSB 2 – Salt block with 6% local potash, MSB 3 – Salt block with 8% local potash, MSB 4 – Salt block with 10% local potash, SEM – Standard Error of Mean, b_g – the potential rate of gas production from the fermentable fraction, c_g – fractional rate of gas production, ME – Metabolizable energy, SCFA – Short chain fatty acid, OMD – Organic matter digestibility, IVDMD – In vitro dry matter digestibility, IVCPD – In vitro crude protein digestibility.*

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The depression in IVCPD at 10% potash (MSB4, 51.60%) parallels the decline in gas volume and TVFA, reinforcing the interpretation that this level exceeds the optimal dose. The divergence between IVDMD and IVCPD responses underscores the distinct mineral requirements of cellulolytic versus proteolytic microbial communities (Durand and Komisarczuk, 1988; Genther and Hansen, 2015). The consistent superiority of sheep over goat inoculum for most parameters contrasts with Molina-Alcaide *et al.* (2017), who found goat inoculum superior for tanniferous browse. This discrepancy likely reflects substrate-dependent interactions, *M. maximus* is non-tanniferous, and sheep rumen microbiota may be more efficient at degrading this substrate, the feeding habit of the two species (bowsers vs grazers) further explains why sheep inoculum performed better on the grass in this study, while goat inoculum performed better on tanniferous browse in the earlier study (Aderinboye *et al.,* 2016). Rumen microbial profiling studies have demonstrated substantial inter-species differences in bacterial community structure between sheep and goats even on identical diets (Hartinger *et al.,* 2018).

Conclusion

In conclusion, the inclusion of local potash at 8% (w/w replacement of industrial salt) enhanced *in vitro* crude protein degradability and elevated VFA production of *Megathyrsus maximus*, while 10% inclusion depressed gas production, degradability and VFA concentration, confirming 8% as an optimal dose. Sheep inoculum produced higher asymptotic gas production, metabolizable energy and total volatile fatty acids than goat inoculum for all mineral supplement types, and goat rumen microbiota showed greater sensitivity to mineral supplement composition than sheep microbiota. Methane production was unaffected by mineral supplementation, confirming that the salt blocks do not compound enteric methane emissions from small ruminants. Further *in vivo* studies is recommended to verify the results from this study before recommendation for practical adoption by small holder farmers.

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