International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; 8(5): 423-427

www.veterinarypaper.com
Received: 28-06-2023
Accepted: 01-08-2023

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Shea nut meal effect on rumen fermentation parameters of sheep

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DOI: https://dx.doi.org/10.22271/veterinary.2023.v8.i5g.780

Abstract
Although ruminants have a greater capacity to utilize various feed sources with lesser risks, the impact of non-conventional feed on rumen function should be understood. This study investigated the effects of Shea nut meal (SNM) different inclusion levels on rumen parameters of sheep fed 70% Rhodes grass hay and 30% maize bran basal diets. Five diets (D) containing SNM at different levels of D1=0% SNM, D2=5% SNM, D3=10% SNM, D4=15% SNM and D5=20% SNM were fed to fifteen ewes clustered into three, in a completely randomized design (CRD). Rumen samples were collected in the 10th week of the feeding trial at hour 0, 3, 6, 9 and 12 post feeding and analyzed for pH, NH_3-N, volatile fatty acids & protozoa count. SNM increased mean pH, and was in a range of (6.58-6.8) in SNM diets compared D1 (6.2). Mean NH_3-N (mg/100ml) in SNM groups decreased with SNM increasing inclusion. Propionate (mmol/L) production was favored for SNM diets; 5% (1.379), 10% (1.375), 15% (1.377), 20% (1.365) compared to D1 (1.682) but total volatile fatty acids (mmol/L) (p<0.05) reduced with SNM increased inclusion. Mean protozoa counts were similarly reduced with SNM increased inclusion; 5% (7.40), 10% (6.80), 15% (6.40), 20% (5.07) compared to D1 (8.53). It was concluded that SNM had no adverse effects on the rumen parameters but rather enhanced the rumen function through improved propionate production and exertion of ant protozoan properties, making it a safe supplement and/or additive source of essential oils and perhaps tannins when needed in formulation of ruminant feeds.

Keywords: NH_3-N concentration, pH, protozoa count and volatile fatty acid concentration

Introduction
The intensification of livestock farming to meet a sustainable global demand for proteins of animal origin has birthed exploration for new possible feed ingredients for the livestock feed industry. Ruminants have a greater capacity to utilize various feed sources with lesser risks due to the unique well developed digestive system they possess, and the presence of microorganisms that not only aid fiber digestion but also detoxify toxins [35]. The impact of new feed ingredients on rumen function must, however, be understood to inform tolerable limits of such new feed for better formulations and animal performance. This is because the rumen function influences the ruminants' digestive heath, and will have a general impact on the human wellbeing through the quality of products produced and the enteric methane emitted [35, 18]. Not only does enteric methane impact the environment, but also causes a feed energy loss [9] which also disturbs the volatile fatty acid profile, affecting post absorptive metabolism [24]. Protozoa are key to facilitating CH_4 formation. This is due to their ability to produce H2 and harboring methanogens, protecting them from oxygen toxicity [26]. Redirecting hydrogen flow away from CH_4 to VFAs increases ruminant productivity [23]. Because volatile fatty acids are the precursors of almost 70% of the energy requirement in ruminants [3]. Feeding poor forages alone favors production of acetate and butyrate and little of propionate [22, 23, 20, 19]. When comparing propionate, acetate and butyrate are not hydrogen sinks and their production results in the build-up of hydrogen which is then used in the formation of methane [6, 28, 23]. Supplementing diets of ruminants with concentrates at 30-40% improves rumen efficiency through maintaining high pH, optimum NH_3-N concentration levels and increasing microbial protein synthesis and VFAs production, [21, 32, 35], which results in improvement in diet digestibility and reduction in methane production [17, 20].

Plant compounds such as fat of unsaturated fatty acids included at levels of 5%-8% are reported to even yield better results as they act as hydrogen sinks, reducing methane production by about 33% [7] and tannins and saponin when added at 4% inhibit protozoa growth and cause a beneficial ruminal response that improves performance [28]. Shea nut meal (*Vitellaria paradoxa*) of recent has gained attention because of its increased availability [7, 1] and surprisingly, the meal has all the mentioned metabolites in adequate quantities [8] [1]. This study seeks therefore, to investigate SNM impact on rumen fermentation parameters of sheep *in vivo* since no study has yet been done.

Materials and Methods

Experimental animals, diet and study design

Rumen liquor samples were collected from Fifteen (15), five months old growing Corriedale ewes that were set in a completely randomized design (CRD) and were being fed diets of Rhodes grass hay (70%) and maize bran (30%) as a basal diet with shea nut meal offered as a supplement at five levels of i.e., D1 = basal diet + 0% SNM, D2 = basal diet + 5% SNM, D3 = Basal diet + 10% SNM, D4 = basal diet + 15% SNM and D5 = basal diet + 20% SNM. The detailed inclusion levels of different ingredients are as in [4].

Sample collection and preparation

Rumen fluid samples were collected on the 10th week of the feeding period using a flexible oral stomach tube as described in [37]. The samples were collected from 2 donor animals of individual groups and pooled, The were collected at 0, 3, 6, 9 and 12 hr post feeding into thermal flasks and taken to the laboratory within 15 minutes time, but pH measured immediately. Individual group liquors were divided into three portions. The first portions (about 50 ml each) rumen liquor was centrifuged at 16000 xg for 15 minutes and the supernatant was added 5 ml of 25% metaphosphoric solution and then stored at -20 °C, which was later used for VFAs analysis. The second portion of the liquor (about 50ml) was centrifuged at 10000 xg for 15 minutes and the supernatant was acidified with 5ml of 25% metaphosphoric acid solution and stored at -40 °C and was used for NH_3-N analysis. The third portion (50 ml) of the rumen fluid was fixed with 2ml of 10% formalin solution and was later strained under a two layered phyllo cloth of about 2000 µm pore size as in [31] and was stored at -20 °C awaiting protozoa count.

Rumen pH, NH_3-N, VFAs Analysis and Protozoa count

Rumen pH was measured using a mobile pH meter (6.6; tecnal, SP, Brazil). Ammonia nitrogen concentration (NH_3-N) for individual groups sampled at different times was analyzed using Kjeldahl method described in [21], without acid digestion and NH_3-N concentration was estimated using the formula:

$NH_3\text{-}N = V*0.005*f*14*100/A$

Where NH_3-N = ammonia nitrogen concentration (mg/100ml), V= volume of hydrochloric acid (ml), f= the factor for the correction of hydrochloric acid concentration obtained with Na_2CO_3 solution (0.005N), 14= atomic weight of nitrogen and A=aliquot volume (ml).

The VFAs were analyzed using Gas chromatograph (trace GC ultra-thermos scientific) with Crotonic acid from Shangai kefeng chemical reagent Co., ltd China as an internal standard and individual VFAs were identified by comparing relative retention time with the known standards of acetic, propanoic and butyric obtained from Sigma-Aldrich Co. LLC. USA. The GC temperature conditions were set at injection port temperatures of 220 °C, injection volume 1 uL, at a temperature program of 100 °C for 1 min, 100 °C to 190 °C increasing by 20 °C/min 190 °C for 3 min and total analysis time was 7.5 min as in [15] in procedure.

Rumen protozoa density was microscopically identified and counted using Sedgewick-Rafter counting chamber [11].

Statistical Analysis

A general linear model of the statistical analysis systems computer package (SAS 2009) was used to analyze data. Means were separated using least significance difference, and the difference between means was considered at (p<0.05). Diets, sampling time and errors were taken as sources of variation.

Results and Discussions

Effect of increasing levels of SNM in diets of sheep on ruminal pH

The pH range post sampling hours of 0, 3, 6, 9 and 12 was variable across diets but was much affected for diet 0% at hour 3. The results agree with [36] who reported that pH is affected by sampling time. On the other hand, Shea nut meal had a general impacted on the mean pH. The mean ruminal pH from 0, 3, 6, 9 and 12-hour sampling post feeding was increased with SNM increasing inclusion in diets of sheep and was within optimum safe range in SNM and control diet : D2 (6.58), D3 (6.62), D4 (6.65) D5 (6.8) and D1 (6.2) respectively. pH results for sampling hours post feeding are presented in figure 1. The type of ration fed to ruminants is believed to influence the rumen pH that in turn affects the rumen microbes and thus the metabolic process. Acute ruminal acidosis could result especially if rumen pH is decreased to and maintained at a low range of 5.5-5.0 for quite long [19]. The low pH would eventually disrupt the microflora population, feed intake, digestibility, ruminal NH_3-N concentration and the concentration of the different volatile fatty acids [12, 14, 7]. In this study, it was observed that SNM diets had high and slightly steady pH post feeding compared to the control diet 0%. This could be attributed to the presence of ether extracts. The outcome agrees with [34] who reported that pH was not affected when essential oils such as olive oil were included in diets of sheep and [13] who also observed a pH range of 6.2-6.51 when diets of sheep were supplemented with palm oil, linseed oil, whole soybean and protected. It was therefore concluded that; just like other essential oils, SNM exerts neutralizing properties and does not cause lowered pH that is observed with grain concentrate feeding [17, 14], making it a potential additive in prevention of ruminal acidosis

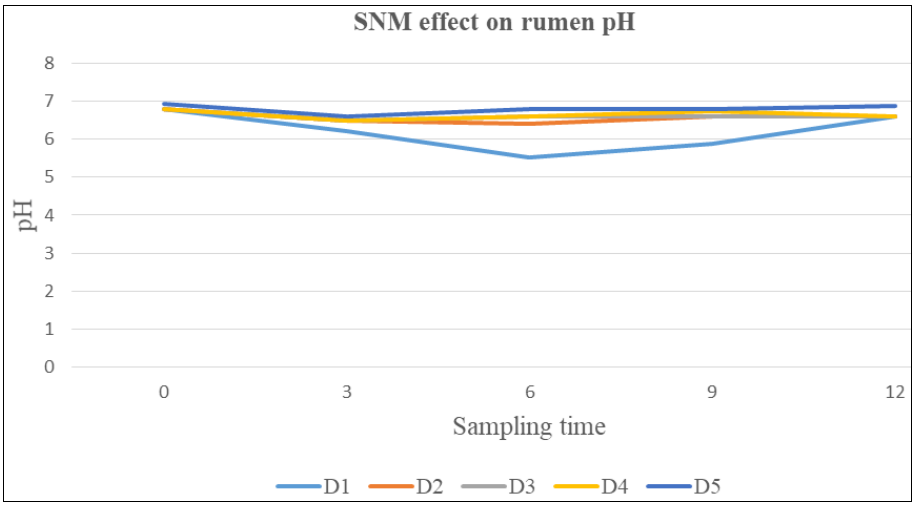

Fig 1: Effect of SNM increasing inclusion on rumen pH of sheep fed Rhodes grass hay and maize bran basal diets

Effect of increasing levels of SNM in sheep diets on ruminal NH_3-N concentration (mg/100 ml)

The ruminal NH_3-N concentration post feeding was monitored at i.e., 0, 3, 6, 9 and 12 hours post feeding which was observed variable. The NH_3-N concentration in the control diet D0 in the initial hours of 0 and 3 were (p<0.05) higher from the rest of the diets. However, Diets D2 and D3 at 9hour recorded (p<0.05) higher NH_3-N and D4 at 6hour. Nevertheless, the mean NH_3-N concentrations were observed to decrease (p<0.05) with increasing SNM inclusion in the diets, with diet D0 recording the higher mean NH_3-N. Still yet, the NH_3-N concentrations were within optimum levels in all diets as ruminal NH_3-N concentration of about 5mg is thought to be optimum for ruminal microbial activities [29], though higher concentrations are believed to improve total bacterial count, feed intake and digestibility [31]. The reduction in the mean NH_3-N with increasing SNM in the diets can be attributed to the presence of tannins in SNM that must have protected the rapid degradation of proteins and the findings agree with [37, 8] who similarly reported decrease in NH_3-N with increasing SNM inclusion. Dietary fat with different fatty acid profile is also reported to improve rumen fermentation through reducing ruminal NH_3-N concentration [5, 17, 14]. It can thus be concluded that SNM could become a potential source of tannins for protection of ruminal protein degradation hence enhancing the flow of proteins to the intestines. The results of NH_3-N are showed in table 1.

Table 1: Effect of increasing SNM inclusion in sheep diets on ruminal NH_3-N concentration (mg/100 ml) over sampling time

Sampling time	Diets					Sig level	SEM
	D1	D2	D3	D4	D5		
0	15.27^{a}	13.73^{b}	13.37^{b}	13.60^{b}	13.47^{b}	0.05	0.321
3	18.87^{a}	17.07^{b}	17.20^{b}	16.90^{b}	18.33^{a}	0.05	0.329
6	17.17^{ab}	16.93^{b}	17.87^{ab}	18.33^{a}	15.70^{c}	0.05	0.388
9	16.33^{b}	18.27^{a}	16.93^{a}	16.40^{b}	14.40^{c}	0.05	0.318
12	17.13^{ab}	17.90^{ab}	17.80^{a}	16.40^{b}	16.23^{b}	0.05	0.701
Mean	16.95	16.78	16.63	16.33	15.63		

SEM= Standard error of the means, ^{ab}Means within the row with different letter superscripts are different at (p<0.05).

Effect of increasing levels of SNM on sheep diets on the concentration of selected Ruminal VFAs, total VFAs and acetate propionate ratio (mmol/L)

The concentration of the selected volatile fatty acids (butyrate, propionate & acetate) post feeding was considerably variable. Mean butyrate and acetate (p<0.05) diminished with SNM inclusion but propionate increased though the increase was not (p>0.05) different among the diets.

Total volatile fatty acids (tVFAs) were higher in control diet 0% compared to the SNM diets whose tVFAs decreased (p<0.05) with increased SNM inclusion. The declining tVFAs concentration with increased SNM can be attributed to increase in ether extract and tannins in SNM diets. Tannins bound nutrients, hence forming complexes and reducing digestibility and thus VFAs production. Also, fats increase VFAs ruminal absorption thus must have contributed to the low concentrations detected [17, 16]. These findings agree with [34] who reported a reduction in VFAs in an *In vitro* experiment, and [8] who observed an increase in tVFAs when shea nut cakes were incubated together with polyethylene glycol (PEG). The acetate propionate ratio, however, improved with increasing SNM inclusion. This can be credited to the presence of fats. Fats with essential oil properties are believed to lower ruminal acetate yet increase propionate production and thus improving the molar acetate: propionate ratio composition [30, 33]. The low acetate concentration could have been as a result of depression in the activity and growth of the fibrolytic bacterial species and thus reduced degradation of fibrous material. Oily products such as rubber seed oil, flaxseed oil and olive, sunflower, linseed oil, coconut oil and fish oil respectively were reported to change the rumen VFAs profile by increasing propionate proportion versus acetate proportion [7, 5, 13, 30, 33] a pathway with diminished methanogenesis [6, 26].

The results of selected VFAs, tVFAs and acetate propionate ratio are presented in table 2.

Table 2: Effect of increasing SNM inclusion on the concentration of the selected VFAs, tVFAs and acetate propionate ratio

Sampling time	Diets					Sig level	SEM
	D1	D2	D3	D4	D5		
Acetate							
0	43.10^{a}	33.87^{b}	32.53^{b}	32.33^{b}	32.23^{b}	0.05	0.6389
3	51.37^{a}	40.93^{b}	40.87^{b}	41.47^{b}	41.40^{b}	0.05	0.3429
6	55.40^{a}	52.97^{b}	54.10^{ab}	54.10^{ab}	53.37^{b}	0.05	0.5698
9	65.50^{a}	56.07^{b}	56.27^{b}	56.20^{b}	56.53^{b}	0.05	0.7920
12	74.07^{a}	59.27^{b}	59.27^{b}	58.63^{b}	58.23^{b}	0.05	1.3954
Mean	57.88	48.42	48.54	48.46	48.37		
Butyrate							
0	19.80^{a}	17.97^{b}	17.60^{b}	17.57^{b}	17.77^{b}	0.05	0.1461
3	25.00^{a}	18.80^{b}	18.73^{b}	18.73^{b}	18.73^{b}	0.05	0.2595
6	24.37^{a}	20.27^{b}	20.03^{b}	20.47^{b}	20.20^{b}	0.05	0.2211
9	23.50^{a}	22.10^{b}	22.17^{b}	22.53^{ab}	22.27^{b}	0.05	0.3474
12	20.60^{a}	18.83^{b}	18.70^{b}	18.67^{b}	18.70^{b}	0.05	0.1687
mean	22.65	19.59	19.45	19.59	19.53		
Propionate							
0	32.20^{a}	33.87^{a}	34.07^{a}	34.10^{a}	34.27^{a}	0.05	0.7294
3	35.80^{a}	36.97^{a}	36.47^{a}	36.20^{a}	36.90^{a}	0.05	0.6731
6	35.73^{a}	35.93^{a}	36.23^{a}	36.03^{a}	36.13^{a}	0.05	0.9563
9	34.87^{a}	35.43^{a}	35.63^{a}	35.37^{a}	35.77^{a}	0.05	0.7486
12	33.50^{a}	34.10^{a}	34.33^{a}	34.70^{a}	34.07^{a}	0.05	1.4435
Mean	34.42	35.26	35.35	35.28	35.43		
Total VFAs	114.9^{a}	103.5^{b}	103.5^{b}	103.4^{b}	103.3	0.5	0.6033
Ace: prop ratio	1.682^{a}	1.379^{b}	1.375^{b}	1.377^{b}	1.365^{b}	0.5	0.0177

SEM= Standard error of the means, ^{ab}Means within the row with different letter superscripts are different at (p<0.05).

Effect of SNM increasing inclusion on ruminal Protozoa count for subsequent hours post feeding (mg)

The results of protozoa count from different SNM treated diets are presented in Table 3. Shea nut meal addition to sheep diets had a negative impact on protozoa counts. The mean total protozoa count reduced with increasing SNM inclusion with the lower count observed at 20% (5.07), which was (p<0.05) lower compared to the control 0% (8.53). The reduction in protozoa count in SNM diets can be attributed to the tannins and ether extract present in SNM. This finding agrees with [8, 34] who reported that tannins in shea nut by products negatively affected protozoa populations. Phytochemicals of some plants such as C-12:0 & C-14:0 medium chain fatty acids, and moderate tannins have been reported to be toxic to the protozoans [16, 26]. Other studies using different oils at different levels of inclusion have also reported reductions in protozoa [27, 25, 7, 30, 33, 13]. Rumen protozoa are important in methanogenesis, and it is believed that a reduction of their numbers below 7 $\log_{10}$ cells/ml could potentially reduce methane production and improve the flow of bacterial protein to the intestines [26, 28, 10].

Table 3: Mean ruminal protozoan count/ mg rumen fluid for subsequent hours post feeding

Hours post feeding	Diets					Sig Level	SEM
	D1	D2	D3	D4	D5		
0	12.33^{a}	8.33^{b}	8.33^{b}	7.00^{b}	6.67^{b}	0.05	0.596
3	6.33^{a}	5.67^{a}	5.67^{a}	5.00^{ab}	3.00^{b}	0.05	0.683
6	6.00^{a}	6.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	0.05	0.699
9	8.33^{a}	8.00^{a}	6.67^{ab}	6.33^{ab}	4.67^{c}	0.05	0.745
12	9.67^{a}	9.00^{a}	8.33^{ab}	8.67^{a}	6.67^{b}	0.05	0.537
Mean	8.53^{a}	7.4^{ab}	6.80^{b}	6.40^{c}	5.07^{c}	0.05	0.469

^{abc}Means within the row with the same letter superscripts are not significantly different. D1= (0%), D2 = (5%), D3 = (10%), D4 = (15%), D5 = (20%), SEM = Standard error mean

Conclusion

It is established that shea nut meal has no adverse effects on the rumen fermentation parameters of sheep as optimum pH levels and NH_3-N were maintained within acceptable range. The acetate propionate ratio was improved with ant protozoan properties realized. SNM could serve as a potential additive source for essential oils and perhaps tannins when required in ruminant feed for rumen modification. Further studies should however be done to investigate SNM effect on rate of absorption, passage and/or potential alteration of VFAs metabolism at tissue metabolism as SNM diets lowered mean NH_3-N and VFAs concentrations

Acknowledgement

The authors thank the Center of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM) for the financial support.

Conflict of interest

The authors declare that there is no conflict of interest in publishing this manuscript.

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