Effect of Concentrate Mixture on Rumen Microbial Activity in Male Goats across Different Breeds

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Abstract

Twenty seven males of three goat breeds namely Toggenburg, German Alpine and Small East Africa were used in a factorial experimental design to determine the effect of supplementation on microbial performance. The concentrate mixture(CM) used for supplementing a basal diet of Napier grass(NG) was compounded from ground maize grain, sunflower seed cake, Mineral Salt and dairy premix. The corresponding basal dietary treatments were designated at three levels of concentrate mixture (gms/day/goat) as follows; i) Control (C); (NG+0), ii) Low Plane (L); (NG+ 200) and (iii) High Plane (H); (NG+400). The experimental animals were randomly selected from a population of thirty six male goats of three breeds that were initially used during a digestibility trials. The pH and the ammonia nitrogen (N) of the ruminal liquor were inversely related to each other in control and pH being highest ($P \leq 0.05$) and N the lowest ($P \leq 0.05$). The pH and the Ammonia nitrogen of the rumen liqour was investigated at four set of hours namely 0, 3, 6 and 12 hours. The highest level of ruminal pH was observed at 0 hours before the concentrate mixture was offered. However the highest levels of Ammonia nitrogen in the rumen was observed after the concentrate diet was offered three hours. The pH level decreased three hours later after the concentrate mixture diet was offered. The Ammonia Nitrogen level decreased on a linear scale as time progressed except after three hours. The lowest Ammonia Nitrogen level was recorded after 12 hours of rumen liqour collection. There was significance difference in the pH among the three breeds and the three different levels of concentrate mixture. Low ammonia nitrogen production among the low plane dieters is attributed to low availability of soluble carbohydrates resulting to low digestible energy production in the fermentation chambers (rumen) of ruminant. Increase in level of concentrate mixture supplementation ($P<0.05$) affected the levels of microbial production of Ammonia and pH.

Key words: Goat breeds, Concentrate mixture, digestibility trials, pH, Ammonia Nitrogen, Rumen liquor.

INTRODUCTION

Ruminants have a unique characteristics associated with fibrous fodder material utilization through anaerobic fermentation (Kibria *et al*., 1991). The rumen located at the beginning of the gastro intestinal tract plays a major role because over 50% of the digestion takes place at the rumen. The rumen of a ruminant is a complex, open and self-contained ecosystem where nutrients consumed by the microorganisms such as bacteria, protozoa and fungi are digested anaerobically. Among the rumen microorganisms, bacteria are the most abundant and important microorganisms in the rumens and play an important role in all aspects of rumen fermentation process. Rumen anaerobic fungi have a potential plant cell wall degrading ability they play an important role in the degradation of forage (Han *et al*., 2019).

The feed consumed by ruminant animals is fermented by rumen microbes to produce volatile fatty acids, mainly butyric, propionic and acetic acid. The three volatile fatty acids diffuse through the ruminal wall to undergo several metabolic processes in the body of the ruminant animals to serve as source of energy and protein for the host animal (Weimer, 1998). Microbial activities in the rumen differ depending on the type of roughages consumed by the animals. Short- chain volatile fatty acids such as acetic, propionic, butyric acids, carbon dioxide, and methane and ammonia nitrogen are the products of fermentation in the rumen (McSweeney *et al*., 2012). The interactions between the microorganisms and the host animals results in a symbiotic relationship that allows ruminants to digest diets rich in fiber and low in protein (Mingyung *et al.,* 2019). Goats adapt to their feeding behaviour depending on the composition of the diet offered, especially in terms of the quality and quantity of feed consumed during the day. Small ruminants like goats adapt to the diet offered depending on the percentage of concentrate in the diet (Peyraud *et al*., 2006). Feeding a concentrate mixture to goat meat (chevon) could lead to a major negative consequence through the occurrence of subacute ruminal acidosis. Acidosis is defined as a decrease in rumen pH below a threshold value of six (Peyraud *et al*., 2006).

The benefit of supplementation of goats with a concentrate diet rich in minerals and vitamin results in enhanced animal performance which includes improved rumen function, increased energy and protein intake and improved feed efficiency (Mahgoub *et al*., 2005, Reverdin *et al*., 2020). Small ruminant animals like sheep and goats have the ability not only to survive in different management systems but also can utilize poor quality roughages (Abedo *et al.*, 2013). In small holder ruminant production system, feeding of goats occupies a prominent place (Venereo and Hermosillo, 2014). Feed resources meant for ruminant animals are natural pastures available in many tropical regions and are normally of poor quality with limited supply of required nutrients (Yulistiani *et al*., 2003).

Therefore, feeding small ruminant animals with these roughages requires supplementation with a proper concentrate mixture to improve their intake and digestibility (Wildeus *et al*., 2007).

A few studies have evaluated the effects of the inclusion of sunflower cake to a basal diet of Napier grass. This feeding practices of supplementing Napier grass with sunflower cake to the ruminants animals has attracted a lot of interest particularly to small ruminants like growing goats. This type of feeding has resulted to improved efficiency of nutrient utilization and to obtain optimum body weight. Therefore, the objective of this study was to determine feed intake, growth performance and nutrient digestibility in goats fed different inclusion levels of sunflower cake to Napier grass based diets (Rashid *et al*., 2016). A few studies have evaluated the effects of the inclusion of sunflower cake to a basal diet of Napier grass. This feeding practices of supplementing Napier grass with sunflower cake to the ruminants animals has attracted a lot of interest particularly to small ruminants like growing goats. This type of feeding has resulted to improved efficiency of nutrient utilization and to obtain optimum body weight. Therefore, the objective of this study was to determine feed intake, growth performance and nutrient digestibility in goats fed different inclusion levels of sunflower cake to Napier grass based diets (Rashid *et al*., 2016).

The plane of nutrition is considered critical to high quality carcass production from local goats and especially the Small East Africa goats which have shown fast and positive response towards supplementation (Shija *et al*., 2013). It is desirable feeding strategy to increase dietary levels of concentrates when low quality roughages are fed to meat goats for improved meat quality and quantity. .However when concentrate proportion is beyond 50-60% in the diet, rumen pH decreases (Morand-Fehr, 2005) leading to off- feed problem (Wanngsness & Muller 1981) which impares digestibility. In small ruminants such as goats if the lowering of rumen pH last several hours symptoms of acidosis can appear as diarrhea and fall feed in take occurs (Morand- Fehr, 2005). Modification of rumen microbial ecosystem is a main target for studies that aims to improve animal efficiency and decreases methane emission. Dietary intervention is the main driver of change in the rumen microbiome. Consequently understanding the modification of rumen microbiome under different diets or feed additives opens the door to designing suitable strategies to improve small ruminant animal productivity.

Ruminants need a suitable level and length of fiber to maintain the normal rumen pH (Xue, *et al*., 2022). Gastro Intestinal microbes are affected by many factors, including diet living environment and age (Jim *et al*., 2016). Goat fed roughages in combination with more concentrate were better in growth performance. Increased concentrate supplementation increased carcass performance. The rumen contains a highly complex microbial ecosystem that plays an important role in converting solar energy in plants into nutrients for ruminants and generates animal food products, such as meat and milk for humans. Therefore understanding the effect of the dietary concentrates to forage(C:F) ratio on ruminal microbiota is of great significance for the growth and development of ruminants.

Rumen adaptation to their environment conditions was closely linked to their rumen microbial communities. Native breeds, Dhofari goats represents well adapted genetic resources for their traditional production systems and unique local conditions. Understanding the rumen ecosystem and microbiome essential for achieving more efficient feed conversion, thereby ensuring a sustainable supply of livestock products. The syombiotic relationship between the rumen microbiome and the host is crucial in providing dietary energy and essential nutrients which ultimately influences animal health and productive performance. Furthermore, feeding patterns, fed additives, ration formulation, animal age and health conditions and species as well as their geographical origin have been reported as factors affecting rumen microbiota composition and structure. Hence introducing certain feed additives modulates rumen fermentation responses and microbiome diversity which ultimately affects the host animal health and hospitality. The Inclusion of concentrates in the diet has shown microbial potential, altering the rumen bacteria community structure and fermentation pattern to increase volatile fatty acids (Zaiat et al., 2024).

It is important to understand how goats respond to different concentrate supplemental regimes (Luginbuhl, 2015) and their possible integration into various production systems. The plane of nutrition is considered critical to high quality carcass production from local goats and especially the Small East Africa goats which have shown fast and positive response towards supplementation (Shija *et al*., 2013). Therefore, the objective of this present study was to investigate how microbial fermentation of a basal diet is affected when three male breeds of goats are fed with a concentrate mixture compounded from sunflower seed cake and maize flour.

Materials and Methods

Study site

The experiment was carried out at the Goat Unit of Chuka University Main Campus farm, in Tharaka Nithi County, Kenya. The global positioning system (GPS) location of the experimental site was Latitude -0.322401 and Longitude 37.658465. The site is situated between Latitude 00 07’23” and 00 26’19”South and Longitudes 37 18’37” and 37 28’33”East, 5200 meters above sea level at the Eastern slopes of Mount Kenya and about 190 Km North East of Nairobi.

The study site experiences a bi-modal rainfall pattern with long and short rains falling during the months of March to May and October to December respectively. Within the study site, short rains are more reliable and the total rainfall amounts to more than 700mm with annual temperature averages between 21 to 25 ^{o}C (Makanga *et al*., 2015).

Experimental diets
The basal diet used in this experiment was wilted Napier grass (NG). The Napier grass was harvested and delivered into the study site a day before the actual day of feeding. In the site, it was wilted in open sunlight before being chopped into small particles of about 2 cm using a mechanical chaff cutter and put into a king size gunny bag for day's ration. A concentrate mixture (CM) constituting of ground maize flour (MF) (70%) and sunflower seed cake (SFC) (30%) was offered before the basal diet was consumed by the goats. The concentrate mixture was offered as low (L) and high (H) per day while the control (C) were offered only chopped NG. Clean and fresh water was provided in plastic containers firmly fixed in the metabolic crates. A mineral block was also provided in the feed trough.

Experimental animals' acquisition
Twenty seven (27) male goats, nine (9) from every breed namely Toggenburg (T), Alpine (A) and Small East Africa Goat (S) were used in the study. The Alpine goats were sourced from Manyatta in Embu County while Toggenburg and Small East Africa were sourced from Karimba village and Kandigi village in Tharaka Nithi County respectively. The animals were housed individually in well ventilated open timber pens, and sprayed with an acaricide for ectoparasites control on a monthly basis and drenched with an anthelmintic on quarterly basis for the control of endo-parasites (Praharee, 2022). The animals were randomly assigned into three (3) treatment groups with four (4) goats per breed consuming each treatment. The experimental animals were raised in individual shed with free access to water and mineral salts.

Sampling of experimental Animals
The experimental animals were randomly selected from a population of thirty six goats that had been kept at the experimental site for eleven months. Twenty seven male goats of the three breeds Toggenburg, Alpine and Small East Africa aged 16 months with an average body weight of 20 kgs and tagged were used in the experiment.

Prior to the selection of the animals the live weight of all the experimental animals was determined and recorded. The body condition and the live weight of the experimental animals were key factors considered during the selection criteria. The animals were properly managed against endo-parasites and ecto-parasites through routine spraying, deworming and vaccinated against Contagious Caprine Pre-Pneumonia (CCPP). All the experimental animals were sprayed with ectomin acaricide after every 3-4 weeks as a preventive measure against ecto-parasites during the period of the experiment.

Experimental Housing and Management
The experimental goats were housed individually in separate well ventilated pens measuring 4 x3 feets^{2}and 5 feet's in height. All the goats were housed in individual metabolic crates with free access to mineral salt block and fresh water provided in plastic containers. Four male goats from each breed were randomly distributed to the three experimental treatment levels comprising control, low and high.

Feeding of the basal diet
Napier grass (*Pennisetum purpureum*) (NG) which was offered as the basal diet was harvested from three areas which are within the vicinity of Chuka University main Campus farm namely; Chogoria, Njaina and Kibumbu. The Napier grass was harvested (Bayble *et al*., 2007) and transported to the study sites on a daily basis. The Napier grass was later wilted in open sunlight for a day before being chopped into small particles of about 2 cm using a mechanical chaff cutter and put into a king size gunny bag of about 30 kilogrammes for a day's ration. The collected Napier grass material was analysed for its chemical composition at Nutrition Laboratory of University of Nairobi, Kabete Campus. The chemical composition for NG was determined using both proximate and Van Soest analysis (Syahirah *et al*., 2022).

Study Design
The design of the study was a 3×3 factorial experiment. The experimental animals were blocked into group of three animals per breed each based on their initial body weight determined before the experiment commenced. Each treatment diet was randomly assigned three animals in the group within the three blocks.

Experimental Procedure and Collection of Samples
Rumen liquor was collected for a period of three (3) days post-feeding 27 selected male goats using a stomach tube connected to a vacuum pump (figure 1). Approximately 50ml of rumen liquor was taken from the middle part of the rumen of each male goat (Morsy *et al.,* 2015).

Figure 1: Extraction of rumen liquor for pH and ammonia nitrogen analysis

Rumen liquor samples were collected at zero, three, six and twelve hours post feeding time. The rumen liquor was immediately recorded for pH using a pH meter. The rumen liquor removed from each male goat was stored in plastic bottles and was preserved by adding 2.5mls of Sulfuric acid diluted to 10% using distilled water. The rumen liquor was stored in labeled plastic bottles that had a capacity of 100 milliliters for later analysis of ammonia nitrogen. The rumen liquor samples collected were pooled together and recorded during the experimental period.

Analysis of Samples

pH

The pH of rumen liquor was analyzed immediately after its collection from the rumen of the twenty seven male goats using a general purpose pH Meter.

Ammonia Nitrogen

Fifty milliliters of rumen liquor well labeled and stored in a deep freezer was used to analyse for ammonia nitrogen concentration (Mingung *et al.,* 2019). The samples were filtered through a three-fold cheese cloth firmly fixed into a glass tube. Determination of ammonia nitrogen concentration from every sample was evaluated according to the Kjeldahl method.

Statistical Analysis

Data collected from each experimental animal for both ammonia nitrogen and pH was later input in the Excel data sheet statistical analysis. The data was analyzed using GLM procedures in a two-way analysis of variance (ANOVA) with the aid of the standard error test for the comparison of means (SPSS, 2008).

Results

Effect of supplementation on ruminal pH levels

The mean of the ruminal pH and Ammonia Nitrogen (mg^{-1}/l) for the breeds Toggenburg, Alpine and Small East Africa is summarized in Table 1 respectively. The mean of ruminal pH and Ammonia Nitrogen (mg^{-1}/l) at different level of concentrate mixture is summarized in Table 1 respectively.

Table 1: Rumen fermentation parameters (Mean±SE) of goats fed diet at different levels of Concentrate mixture (Low, Medium, High grammes per day).

Breed	pH	Ammonia Nitrogen(mg^{-1}/l)
T	6.8^{bac}±0.0	5.8^{abc}±0.5
A	6.8^{ab}±0.0	5.7^{bac}±0.5
S	6.7^{cb}±0.0	5.3^{cab}±0.6
Levels	pH	Ammonia
L	6.7^{c}±0.0	5.2^{cba}±0.4
M	6.8^{ba}±0.1	5.8^{bca}±0.5
H	6.9^{ab}±0.0	5.9^{acb}±0.6
Hours	pH	Ammonia
0	6.9^{a}±0.0	5.4^{b}±0.3
3	6.8^{cd}±0.0	9.1^{a}±0.6
6	6.6^{d}±0.1	5.1^{cb}±0.5
12	6.8^{bd}±0.0	2.7^{d}±0.2

Value are expressed as mean +_ SE $^{a, b, c, d,:}$ values in the same row between the breeds having different super-script are significantly different (P<0.05). T (Toggenburg), A (Alpine), S (Small East Africa). B (breed), L (control), M (low plane), H (high plane).

Breeds ruminal ammonia nitrogen levels

The ruminal ammonia nitrogen levels for three breeds of goat, Toggenburg, Alpine and Small East Africa are summarized in Table 1. The mean of ruminal ammonia nitrogen at different concentrate mixture degree is summarized in Table 1. The ammonia nitrogen level for the experimental animals supplemented at high grammes per day was higher than the other two levels of concentrate diet mixture with the low group having the lowest level. The ammonia nitrogen level for Toggenburg was the highest followed by the one for Alpine breed and lowest level being that of Small East Africa. The concentration of the rumen volatile fatty acids and the ammonia nitrogen increased from before feeding to three hours after feeding. This showed a decrease trend at six hours after feeding the concentrate mixture. The addition of extra fat in the male goats' diet of Napier grass (basal diet) in the form of a concentrate mixture constituted from sunflower seed cake and maize grain could have caused some effect on the rumen fermentation and micro nutrient digestibility.

The research findings of Hristov and Jouany, (2024) found that the volatile fatty acids concentration increased and the ammonia nitrogen decreased a few hours after supplementation causing the decrease of pH in the rumen with sunflower seed cake supplementation as it was in the present study.

Effect of time on ruminal pH and ruminal ammonia Nitrogen levels

The pH of the ruminal liquor and the ruminal ammonia nitrogen at four set of hours namely 0, 3, 6 and 12 hours is summarized in Table 1. The highest level of ruminal pH was at 0 hours before the concentrate mixture diet was offered. The pH levels decreased three hours later after the concentrate mixture diet was offered and the trend continued but later stabilized after 12 hours.

The highest levels of ammonia nitrogen amount in the rumen was after the concentrate diet was offered 3 hours. The ammonia nitrogen level decreased on a linear scale as time progressed except after three hours. The lowest ammonia nitrogen level was recorded after 12 hours of rumen liquor collection.

Table 2: Effect of breeds and plane of nutrition on level of significance

Parameters	Level of significance(pH)	
B		P<0.05
L		P<0.05
B x L		P>0.05
Parameter	Level of significance (Ammonia nitrogen)	
B		P<0.05
L		P<0.05
H		P<0.05

B (breeds), L (level of concentrate mixture diet), H (hours of sample collection)

The pH and ammonia nitrogen in this present study for the three breeds of goats showed significant difference. There was significance difference in the pH among the three breeds of goats and the three different levels of concentrate mixture (CM) as shown on Table 2. The ruminal pH value for the treatment in the present study varied from 6.7 to 6.9. However the range of 5.8 to 6.2 is considered the most acceptable for fibre digestion (Sung *et al.*, 2007). The decreased ruminal pH values with the supplementation of the concentrate mixture may be associated with increased energy density resulting in increased concentration of volatile fatty acids in both treatments of low and high plane compared to the control group (Morsy *et al*., 2015). Rumen ammonia nitrogen level depends on many factors including the dietary crude solubility. Reduction of ammonia nitrogen concentration with supplementation is paralleled with increased ruminal volatile fatty acids which is an indicator of higher fermentation rate and increased microbial protein synthesis (Khalif *et al*., 2014).

DISCUSSIONS

Effect of supplementation on breed ruminal pH levels

The changes in the levels of concentrates in the goats' diets affected the protein digestibility, rumen fermentation and the relative abundances of some rumen bacteria. This agrees with some previous and recent studies that reported changes in rumen fermentation and microbiota due to changing diet composition especially the proportion of dietary fiber and starch in the concentrate. The goats in this study were fed with a basal diet of Napier grass a roughage whose nutritive value can vary depending on the age and the location of harvesting. The basal diet was offered adlib but the concentrate supplement was quite restricted particularly for the low level group. Askari, *et al*., 2023 reported that increasing the level of concentrate supplement in the diet of sheep and goats did not affect the nutrients digestibility except for the crude protein digestibility that was increased by increasing the concentrate level. Increasing the dietary concentrate level and subsequent increase in protein intake increased the rumen ammonia which is used in microbial protein synthesis and increase in protein utilization. Askari, *et al*., 2023 indicated that rumen pH lower than 6 negatively affects rumen fermentation and animal performance which suggests that higher concentrates supplementation in growing goats. The results of pH in this present study are comparable with those obtained by Dependra KC, (2015), when the growth performance of Khari goats in Nepal on effect of by-pass protein was studied. The ruminal pH can vary depending on the diet and the time after feeding (Silveira *et al*., 2007). The high pH observed before feeding is related to the low amount of nutrients available to the rumen microbial metabolism and rumination activity of the animal. According to Orskov (1986) values above 6.2 ruminal fluid is considered as a minimum limit for adequate fermentation of the fiber (Krause *et al*., 2005). The ruminal pH levels in this present study were within the normal range of 6.0-7.0 for effective microbial rumen digestion. The ruminal pH range from 5.8 to 7.0 depends on the amount and extent of protein degradation and nature of carbohydrate utilized in the compounded supplement (Phillipsson, 2000). The level of pH in the rumen digester depend mostly on the type of the basal diet offered to ruminant animals amidst other factors such as supplementation of low quality forages with a concentrate mixture as it was in the present study. Rumen digester with a pH below 5.7 is reported to drastically reduce dry matter intake in ruminant animals (Nocek, 1997).

Slow and continuous supply of fermentable nitrogen and carbohydrates in the rumen of a ruminant animal increases the digestion rate of low quality roughages (Mirza *et al*., 2004). The influence of a concentrate diet on the ruminal pH and ruminal ammonia nitrogen and how the two parameters are affected by different hours of rumen liquor collection is paramount. The effect of supplementing a poor quality basal diet with a concentrate mixture diet significantly affected the ruminal microbial environment. Supplementation with a concentrate mixture diet provides proper utilization of low quality forage and consequently contributes to better diet digestibility and increased body live weight. The performance of the experimental animals supplemented at a concentrate mixture of high grammes per day was better than the other two experimental groups due to readily available carbohydrates that facilitated the growth of cellulolytic microbes. The increased level of microbes in the rumen contributed to better utilization of the roughages through provision of amino acids at the intestine and tissue level (Martin *et al*., 2006).

Breed ruminal ammonia nitrogen levels

Goats are similar to other ruminants for the basic functions in the rumen environment. The biomass consumed by ruminants is partly fermented in the rumen by the microbes and is generally converted to microbial protein, volatile fatty acids and fermentation gases.(Sauvant *et al.*, 2006). The transit fractional rate responses to feeding level and proportion of concentrates are generally similar for cattle and small ruminants (Sauvant *et al*., 2006). On the other, there are no publications where the efficiency of microbial growth in the rumen of goat has been compared for the same diet to that of other ruminants. . Nevertheless the global similarly between goats and cows has been used in the feeding systems of ruminants (INRA, 2018), even if goats present some specific digestive features. Ammonia nitrogen is regarded as the most important nitrogen source for microbial protein synthesis in the rumen (Puwadon *et al*., 2018) and the level in the rumen is usually high when feeds are more digestible (Erdman *et al.,*1986).

Kinyua, (2019), found that there was a significance difference ($p < 0.05$) in the rumen pH of Alpine and Toggenburg for the three trial diets fed at 6^{th} and 9^{th} hours post feeding. It was concluded that goats poses a unique characteristic of being able to maintain larger rumen ammonia nitrogen levels without noticeable rumen distension compared to sheep. Low ammonia nitrogen production among the Low dieters is attributed to low availability of soluble carbohydrates resulting to low digestible energy production in the fermentation chambers of ruminant animals called rumen (Oetzel, 2000). Maloney and Flynn (1992) reported that high ammonia nitrogen concentration may be needed to maximize forage digestion and consequently allow greater hay intake. Krebs and Leng (1984) also reported that slow and continuous supply of fermentable nitrogen and carbohydrates in rumen increases the digestion rate of roughages such as wheat straw. Leng 1999 reported that availability of fermentable nitrogen and readily available carbohydrates supplied through a concentrate mixture can facilitate the growth of cellulolytic bacterias which might result in better utilization of a basal diet like wheat bran.

Effect of time on pH and ammonia nitrogen

Goats utilizes most of their time ruminating than eating, regardless of the diet offered. This behaviour of goats is in accordance with study results obtained in lactating goats (Kawas *et al*., 1991) and cows (Maekawa *et al*., 2002). There was a significant difference observed by the interactions of treatments with the days of sampling. This suggests that ammonia nitrogen levels in the rumen might be associated with the shift of the microbial population with time due to the addition of the concentrate mixture. Fibre degrading bacteria populations were affected by interaction between treatment and the day of sampling. A mean rumen pH of 6.25 corresponds to around 4 hours of rumen digesta, but rumen digesta of pH below 6.0 could be used as a threshold to define the occurance of subcute acidosis (Rapetti *et al*., 2005).

Conclusion

This study demonstrates that supplementation of a basal diet such as Napier grass (NG) with a concentrates mixture diet improves rumen fermentation. Incorporation of a concentrates mixture diet positively increased the pH of in the rumen male goats of different breeds.

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