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In vitro Study on the Effect of Forage Type and Chemical Nature on Vigor of Rumen Microbial Community

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Abstract: Effect of forage type and chemical composition on vigor of rumen microbial community was investigated *in vitro*. Dry herbage samples used as culture media substrates were obtained from *Sorghum almum*, *Vicia villosa* and *Commelina benghalensis* established and harvested at 6, 10 and 14 weeks. Chemical analyses and constitution of incubation buffer solutions were done following standard procedures. Rumen liquor was obtained from 3 mature male goats (LW: 23±2 kg) fitted with rumen cannulae, housed in a well-ventilated group pen and fed a basal diet of fresh grass and supplemented with Lucerne hay. Bottles (225 mL capacity) containing 120 mL of the liquor-buffer mixture (1:2 v/v) and 1.2 g of substrate (30:70 soluble starch:dry forage samples milled to pass 1mm sieve) were incubated (39°C) under anaerobic condition and microbial culture harvested after 24 h and determined. Data was analyzed using SAS. As expected, DM and fibre content increased whereas CP and EE content decreased with advancing forage maturity. It was noted that, culture media based on herbage harvested young had higher bacteria and protozoal biomass compared to those based on older herbage. The overall mean for bacterial and protozoal biomass in *Sorghum almum*, *Vicia villosa* and *Commelina benghalensis* based media were 0.325, 0.3782 and 0.3712 mg mL^{-1} and 0.2221, 0.2344 and 0.2539 mg mL^{-1}, respectively. Results therefore, indicate that forage type and chemical composition had significant effect on the vigor of rumen microbial community.

Key words: Rumen, bateria, protozoa, chemical composition, forage maturity

INTRODUCTION

Forages roughages represent a major component of diets for ruminants. Their nutritive value can be gauged on the basis of their dry matter digestibility and voluntary intake, which in turn related to their chemical components (Seven and Çerçi, 2006). Ruminants utilize these forages through a symbiotic relationship with enzyme producing microorganisms (Russell and Rychlik, 2001). These microbes have the ability to break down ligno-cellulosic fibre to the benefit of the host animal. Efficient functioning of these organisms, however, requires a stable rumen environment (Cheng *et al*., 1991) and sufficient supply of essential nutrients (Ørskov, 1995). Sufficient supply of essential nutrients (ammonia, branched-chain fatty acids, fermentable carbohydrates) promotes high microbial population and therefore, high microbial biomass (Ørskov and Ryle, 1990). Bacteria (cellulolytic, amylolytic and proteolytic), protozoa (*Entodinium caudatum*, *Epidinium caudatum*, *Ophryoscolex caudatum* and the small *Entodinium* sp.) and fungi have been shown to be the microorganisms involved in the cell wall digestion in the rumen. Comparatively, bacteria are believed to play a major role because of their numerical predominance and metabolic diversity (Sylvester *et al*., 2004). Opinions are wide and divergent on the role of protozoa in the digestion process. Many reports are however in agreement that the protozoa predation on bacteria, compounded by their own autolysis is counter productive in that, it deprives the host animal of microbial protein and increases excess ruminal ammonia (Williams and Coleman, 1992). Available literature indicates that, although rumen fungi possess superior ability such as penetration of plant cell wall, thereby physically disrupting the lignified stem tissue and solubilization of lignin (Joblin *et al*., 1989) their contribution to fibre digestion might be low due to small biomass (8% of total microbial biomass) (Orpin and Joblin, 1997). The complex interrelationships between the different species of microorganisms and between the organisms and the nature of the substrate, is still less understood.

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Knowledge of the variation of ruminal microbial density with changing dietary nutrients is important for understanding the implication of chemical and physical variability of feedstuffs on rumen ecosystem. We report findings of an *in vitro* study to evaluate effects of chemical and physiological changes associated with advancing maturity of *Sorghum almum*, *Vicia villosa* and *Commelina benghalensis* on microbial density and population.

MATERIALS AND METHODS

Animals, diets and rumen liquor: The study was conducted at the college of animal science Yangzhou University in Jiangsu, P. R. China. The rumen fluid used to seed rumen microbes into the *in vitro* culture media was collected from 3 mature male goats (LW: 23±2 kg) fitted with rumen cannula and housed in a well ventilated group pen with a slatted wooden floor. The animals were fed *adlib* with a basal diet comprising of fresh grass fodder and supplemented with Lucerne hay. The ration was offered in 2 equal portions at 08:00 and 16:00 h daily starting 15 days before commencement of the study. The animals had free access to clean drinking water and balanced mineral salt. A total of 500 mL of rumen liquor (strained through 2 layers of cheesecloth) was collected by suction tubing through the cannula 4 h after the morning feed into pre-heated glass bottles cushioned with woolen rag to minimize heat loss and pre-flashed with Carbon dioxide (CO_2). At the laboratory, the liquor bottled was placed into a water bath maintained at 39°C.

Test forages and chemical analyses: The test forages were Columbus grass (*Sorghum almum*), Vetch (*Vicia villosa* Roth) and *Commelina benghalensis* (the giant Naivasha type). The forages were each established in 3 randomly demarcated blocks of 12 plots each (2×2 sq. m; N = 36). Plots and blocks were separated by weed free guard rows of 30 and 60 cm, respectively. The forages were later harvested sequentially (block 1, 2 to 3) at 6, 10 and 14 weeks post-emergence. Composite samples of freshly harvested herbage were taken for dry matter determination, chemical analysis and *in vitro* studies. Feed samples were dried in a forced-air oven at 65°C for 24 h, their Dry Matter (DM) content calculated and then grounded to pass 1 mm screen. Ash was determined (AOAC, 1990, ID 942.05) and micro-Kjeldahl N in feed (AOAC, 1990, ID 954.01). Crude protein was calculated as Kjeldahl N×6.25. Neutral-Detergent Fibre (NDF), Acid-Detergent Fibre (ADF) and Acid-Detergent Lignin (ADL) were determined by the procedures of Van Soest and Robertson (1985). Ether Extract (EE) was determined by extracting the sample with petroleum ether using a Gerhart Soxtherm 2000 Automated (AOAC, 1990, ID 920.39).

Incubation medium: The *in vitro* incubation media were constituted following the procedure of Menke *et al.* (1979). In this procedure, a mixture comprising of 477 mL of buffer (7.9 g NH_4HCO_3, 70.6 g $NaHCO_3$, 2000 mL distilled water); 237 mL macro-mineral solution (11.4 g Na_2HPO_4, 12.4 g KH_2PO_4, 1.2 g $MgSO_4.6H_2O$, 2000 mL distilled water), 0.237 mL of micro-mineral solution (13.2 g $CaCl_2.2H_2O$, 10 g $MnCl_24H_2O$, 1 g $CoCl_26H_2O$, 8 g $FeCl_3.6H_2O$, 100 mL distilled water), 0.297 mL of resazurine, 0.297 g Na_2S, 0.318 mL of 6 M NaOH and 529 mL distilled water was constituted. The flask containing the solution was placed in he water bath (39°C) and gently bubbled with CO_2 until the blue color turned to pink and then clear. The rumen liquor and medium solution were then mixed at the ratio of 1:2 (v/v mL), respectively in a water bath maintained at 39±0.2°C.

Treatment diets, culture and separation of microbes: Treatment diets were formulated to comprise 30% soluble starch and 70% test forages (dry milled samples from each of the 3 test forages harvested at 6, 10 and 14 weeks growth stage). Exactly 1.2 g of each diet was weighed accurately in triplicate into 225 mL conical flasks in a water bath maintained at 39°C. Immediately after, 120 mL of incubation medium (1:2 v/v mL rumen liquor-buffer mixture), maintained under anaerobic condition and 39°C, was injected into each flask and closed with a stopper fitted with 2 fine syringe needles to allow continuous fluxing with CO_2. The water bath temperature was maintained at 39±0.2°C and automatic shaker was set at medium speed and allowed to run throughout the study (Menke *et al.*, 1979). Microbial cultures were harvested after 24 h incubation period. Rumen liquor-buffers were strained through a 100-μm-nylon filter to separate a Liquid Phase (LP) and Feed Residues (FR). The LP and FR associated bacterial and protozoal separated according to the methods described by Martin *et al.* (1994). Fungal zoospores were removed from bacterial and protozoal biomass through the use of anti-fungal agents (cychloheximide [0.05 mg mL^{-1}] and nystatin [200 U mL^{-1}]). For protozoa counting, 4 mL of the rumen liquor-buffer fluid was preserved at 4°C with 4 mL of 4% formaldehyde and Protozoal (Pr) numbers were determined by counting protozoa present in 4 known volumes using a standard counting chamber and light microscope (Koenig *et al.*, 2000). The equation used was: Pr mL^{-1} = [0.25*A] * 16*10^4*B; where A is the total number of protozoa in the 4 volumes and B is the dilution factor for the sample used.

Statistical analysis and calculations: The chemical constituents in the 3 test forages were analysed as a randomised complete block design using SAS (2002). The statistical model used was: $Y_i = \mu+A_i+e_i$; where

Y_i = The concentration of the chemical constituents measured; μ = overall mean, A_i = Age at harvest (i = 6, 10, 14 weeks) and e_i = random experimental error. The model used for rumen microbes was: $Y_{ij} = \mu+A_i+B_j+A_i*B_j+e_{ij}$; where Y_{ij} = the microbial biomass or population, μ = overall mean, A_i = forage age at harvest (i = 6, 10 and 14 weeks), B_j = forage type (j = 1, 2 and 3), A_i*B_j = forage age-type interaction and e_{ij} = random experimental error. The Proc GLM was used to perform the Analysis of Variance (ANOVA) and means were contrasted with significance assumed when p = 0.05 (SAS, 2002). Linear and quadratic regression functions were fitted using SPSS (2003) to describe the exhibited change patterns.

RESULTS AND DISCUSSION

Mean chemical values recorded for *Sorghum almum* (Table 1) were consistent with those reported for *Sorghum almum* (Kallah *et al*., 1999), *Pennisetum purpureum* (Orodho, 2006) and *Chloris gayana* (Keftasa, 1990). The *Vicia villosa* chemical values compared well with those reported for *Vicia sativa* (Lanyasunya *et al*., 2006a), *Vicia villosa* (Caballero *et al*., 2001) and *Lablab purpureus* (Murphy *et al*., 1999), whereas those of *Commelina benghalensis* compared well with those reported for *Commelina dffusa* (Lanyasunya *et al*., 2006b), *Commelina benghalensis* (CGIAR, 2006), *Ipomoea batatas* (Snijders *et al*., 1992) and *Ipomoea aquatica* (Chat *et al*., 2005). As expected the Dry Matter (DM) and fibre concentration increased with advancing maturity of the forage (Table 1). Crude Protein (CP) and Ether Extract (EE) of both *Sorghum almum* and *Commelina benghalensis* decreased with age ($p<0.0001$ and $p<0.001$, respectively). *Vicia villosa* EE also decreased with advancing maturity ($p<0.0001$). Contrary to the expected however, CP in *Vicia villosa*, registered a marginal increase of 9.4%. From the results, it was also noted that mean Relative Feed Value (RFV) also varied with advancing maturity of the forages. The mean RFV for *Sorghum almum* decreased by 38.65% between 6 and 14 weeks. Evidently however, the mean RFV for *Vicia villosa* and *Commelina benghalensis* registered marginal decline ($p>0.05$). Increase of DM and fibre concentration and decrease of CP and EE with advancing maturity of tropical forages in well documented (Arthington and Brown, 2005). The observed increase in fibre content in the 3 forages was associated with accumulation of structural components, whereas the decrease of CP content with advancing maturity was attributed to the decrease in the actual concentration of CP in the leaf swards, stem and leaf to stem ratio. The observed increase in CP content with advancing maturity of *Vicia villosa* was attributed to the accumulation of reproductive parts (floral, rapid pod filling and fully formed seed grains) (Hadjipanayiotou and Economides, 2001) which concurred with Murphy *et al*. (1999) and Caballero *et al*. (2001). The recorded RFV values for *Vicia villosa* and *Commelina benghalensis* (Table 1) were above the 151 suggested as the lower bound for high quality forage (Canbolat *et al*., 2006) which suggests that, these two forages are of high quality. Invariably however, the RFV value for *Soghum almum* was much lower suggesting the reverse.

Table 1: Chemical composition of the herbage samples used to constitute culture treatment diets

		Sorghum almum				*Vicia villosa* Roth				Commelina benghalensis			
Component	N	6	10	4	SEM	6	10	14	SEM	6	10	14	SEM
DM (g kg^{-1})	12	125.5^{a}	153.9^{b}	217.1^{c}	5.87	121.7^{a}	148.7^{a}	196.9^{a}	3.48	74.3^{a}	90.8^{b}	122.3^{c}	3.19
OM (g kg^{-1} DM)	12	753.1^{a}	781.7^{b}	803.5^{c}	5.48	811.3^{c}	717.6^{a}	765.9^{b}	8.16	709.4^{b}	625.8^{a}	627.3^{a}	10.21
CP (g kg^{-1} DM)	12	156.3^{c}	97.8^{b}	52.1^{a}	2.19	199.3^{a}	205.8^{a}	218.1^{b}	2.64	175.9^{c}	121.8^{b}	92.5^{a}	2.07
CF (g kg^{-1} DM)	12	195.8^{a}	346.3^{b}	356.6^{b}	6.72	173.3^{a}	225.3^{b}	241.8^{b}	6.28	125.1^{a}	189.6^{b}	211.1^{c}	7.08
NDF (g kg^{-1} DM)	12	519.9^{a}	664.3^{b}	691.7^{c}	3.96	347.7^{a}	352.3^{a}	354.9^{a}	6.27	326.4^{a}	371.1^{b}	398.6^{c}	14.17
ADF (g kg^{-1} DM)	12	259.6^{a}	377.6^{b}	422.2^{c}	5.89	260.7^{a}	312.9^{b}	315.8^{b}	6.69	215.5^{a}	374.3^{b}	309.2^{c}	7.50
ADL (g kg^{-1} DM)	12	35.4^{a}	36.8^{a}	55.4^{b}	2.74	48.3^{a}	51.5^{b}	55.3^{b}	1.76	37.8^{a}	51.5^{b}	49.7^{b}	1.92
EE (g kg^{-1} DM)	12	39.5^{c}	16.5^{b}	10.5^{a}	1.79	62.1^{c}	24.6^{b}	19.2^{a}	1.53	22.1^{c}	18.2^{b}	11.4^{a}	1.17
HC (g kg^{-1} DM)	12	260.4^{a}	286.5^{b}	269.5^{a}	4.91	86.9^{b}	41.9^{a}	36.4^{a}	6.42	110.9^{b}	96.9^{a}	89.6^{a}	15.38
Cellul. (g kg^{-1} DM)	12	224.2^{a}	340.9^{b}	366.8^{c}	5.43	212.5^{a}	261.5^{b}	260.6^{b}	5.71	177.7^{a}	222.8^{b}	259.3^{c}	8.43
NFE (g kg^{-1} DM)[1]	12	361.5^{b}	321.1^{a}	384.3^{b}	7.11	376.5^{c}	302.8^{b}	245.9^{a}	7.41	286.3^{a}	296.2^{ab}	313.3^{b}	5.36
NSC (g kg^{-1} DM)[2]	12	132.5^{b}	72.9^{a}	122.4^{b}	3.79	260.1^{b}	261.1^{b}	215.9^{a}	9.81	149.1^{a}	192.7^{b}	199.6^{b}	16.20
CHO (g kg^{-1} DM)[3]	12	557.4^{c}	667.3^{b}	740.9^{a}	7.31	549.8^{b}	487.2^{a}	528.6^{b}	7.24	511.4^{ab}	485.8^{a}	524.4^{b}	8.49
RFV [4]	12	122.9^{c}	83.3^{b}	75.4^{a}	1.03	183.6^{b}	169.8^{a}	169.7^{a}	4.02	158.6^{ab}	169.6^{b}	151.3^{a}	5.47

[1]NFE (g kg^{-1} DM) = DM-(EE+CP+Ash+CF) (Van Soest, 1994); [2]NSC was calculated as 100% - (CP%+NDF%+EE% +Ash%); [3]CHO was determined as as described by Arieli *et al*. (1999), where CHO (g kg^{-1} DM) = OM content-(CP+EE); [4]RFV = [(88.9-(0.78×ADF%))×(120/NDF%)]/1.29 (Agric-Facts, 2006); N-Number of samples; DM-Dry Matter; OM-Organic Matter; CP-Crude Protein; CF-Crude Fibre; NDF-Neutral Detergent Fibre; ADF and ADL-Acid Detergent fibre and Lignin; HC and Cellul.-Hemicellulose and Cellulose; NSC and CHO-Non-structural and Structural Carbohydrates; RFV-Relative Feed Value

Table 2: Least square means for rumen microbial parameters in culture media based on herbage harvested at different maturity stages

		Age at harvest (weeks)						
Parameter	N	6	10	14	SEM	CV	R^2	P
Sorghum almum								
Bacterial mass (mg mL^{-1})	4	0.4008^{c}	0.3224^{b}	0.2518^{a}	0.0030	1.8586	0.9927	****
Protozoal mass (mg mL^{-1})	4	0.2665^{c}	0.2219^{b}	0.1777^{a}	0.0016	1.4441	0.9942	****
Protozoal Pop. ($\times 10^4$ mL^{-1})	4	0.8323^{c}	0.6534^{b}	0.4717^{a}	0.0057	1.7065	0.9957	****
B:P ratio	4	1.5038^{c}	1.4531^{b}	1.4168^{a}	0.0040	0.5445	0.9642	****
Vicia villosa Roth								
Bacterial mass (mg mL^{-1})	4	0.4030^{c}	0.3721^{b}	0.3597^{a}	0.0027	1.4092	0.9396	****
Protozoal mass (mg mL^{-1})	4	0.2385^{c}	0.2344^{b}	0.2302^{a}	0.0019	1.5855	0.5303	****
Protozoal Pop. ($\times 10^4$ mL^{-1})	4	0.9991^{c}	0.8987^{b}	0.7799^{a}	0.0068	1.5259	0.9829	****
B:P ratio	4	1.6896^{c}	1.5874^{b}	1.5629^{a}	0.0034	0.4217	0.9886	****
Commelina benghalensis								
Bacterial mass (mg mL^{-1})	4	0.4011^{c}	0.3873^{b}	0.3254^{a}	0.0018	0.9512	0.9914	****
Protozoal mass (mg mL^{-1})	4	0.2548^{c}	0.2708^{b}	0.2362^{a}	0.0010	0.7894	0.9852	****
Protozoal Pop. ($\times 10^4$ mL^{-1})	4	1.0067^{c}	0.9415^{b}	0.7393^{a}	0.0048	1.0759	0.9947	****
B:P ratio	4	1.5741^{c}	1.4303^{b}	1.3778^{a}	0.0027	0.3735	0.9968	****

N-Number of samples; SEM-Standard Error Mean; CV-Coefficient of Variation; Means with the same superscript ($^{a, b, c}$) within the same row are not significantly different (p>0.05); B:P ratio-Bacterial biomass to Protozoal biomass ratio; **** p<0.0001

Table 3: Quadratic regression function fitted to describe the change pattern of microbial biomass and population with herbage age at harvest

Parameter	Quadratic function	R^2	P
Sorghum almum			
Bacterial mass (mg mL^{-1})	$Y = 0.4872-0.0903X+0.0039X^2$	0.993	****
Protozoal mass (mg mL^{-1})	$Y = 0.3117-0.0455X+0.0003X^2$	0.994	****
Protozoal Pop. ($\times 10^4$ mL^{-1})	$Y = 1.0083-0.1747X-0.0014X^2$	0.996	****
B:P ratio	$Y = 1.5689-0.0723X+0.0072X^2$	0.964	****
Vicia villosa Roth			
Bacterial mass (mg mL^{-1})	$Y = 0.4523-0.0586X+0.0092X^2$	0.991	****
Protozoal mass (mg mL^{-1})	$Y = 0.2424-0.0038X-0.0001X^2$	0.985	****
Protozoal Pop. ($\times 10^4$ mL^{-1})	$Y = 1.0810-0.0728X-0.0092X^2$	0.995	****
B:P ratio	$Y = 1.8693-0.2186X+0.0388X^2$	0.989	****
Commelina benghalensis			
Bacterial mass (mg mL^{-1})	$Y = 0.3668+0.0583X-0.0240X^2$	0.940	****
Protozoal mass (mg mL^{-1})	$Y = 0.1882+0.0918X-0.0253X^2$	0.530	****
Protozoal Pop. ($\times 10^4$ mL^{-1})	$Y = 0.9349+0.1402X-0.0685X^2$	0.983	****
B:P ratio	$Y = 1.8093-0.2809X+0.0457X^2$	0.997	****

Y = Dependent variable (Bacterial or Protozoal biomass, Protozoa population, B:P ratio); X-herbage age at harvest (Weeks), **** p<0.0001

Table 2 presents the mean microbial biomass in the different culture media. Results showed that *in vitro* culture media based on herbage harvested young contained high microbial biomass than those based on mature herbage. Bacterial biomass in culture media based on *Sorghum almum*, *Vicia villosa* and *Commelina benghalensis* herbage harvested at 6 weeks were 37.18, 10.75 and 18.87% higher than in those based on forage harvested at 14 weeks. Protozoal biomass and population also varied with age of the herbage. In *Vicia villosa* 3.48 and 21.94% higher protozoal biomass and population respectively, were recorded in the media based on the young herbage. The same was observed in *Commelina benghalensis* based media, with those based on herbage harvested at 6 weeks recording, respectively 7.3 and 26.56% higher protozoal biomass and population than those based on the more mature herbage. Bacterial to protozoal biomass ratio (B:P) also varied between media and forages (Table 2). The B:P ratios in the *Sorghum almum*, *Vicia villosa* and *Commelina benghalensis* declined by 5.79, 7.49 and 12.47%, respectively with advancing maturity of the herbage used to constitute culture media. Quadratic functions were also fitted to describe the microbial biomass, protozoal population and B:P ratio change with substrate in the culture media (Table 3). Results showed that, the equations achieved high fit as evidenced by high coefficients of determination and p-values. Microbial biomass and population comparisons were also made between the 3 forages. Results indicated that forage type had strong influence on microbial biomass and population. *Vicia villosa* and *Commelina benghalensis* based culture media recorded high bacterial ($p<0.001$ and $p<0.01$, respectively) and protozoal ($p<0.05$ and $p<0.01$, respectively) biomass than those based on *Sorghum almum*. Mean bacterial biomass values for *Vicia villosa* and *Commelina benghalensis* were also different ($p<0.05$). The same was observed for protozoal population. *Vicia villosa* and *Commelina benghalensis* recorded respectively 36.79 ($p<0.0001$) and 37.29% ($p<0.0001$) higher protozoal population than in *Sorghum almum* based media.

The findings of this study, concurred with earlier reports (Williams and Coleman, 1992). Ørskov and Ryle (1990) reported increased bacterial activity with increased urea supplementation of low quality feeds. Navas-Camacho *et al*. (1993) also recorded changes of protozoal population with incremental level of dry leaves of a tree legume (*Enterolobium ciclocarpum*) in the diet of ruminants. Since the incubation medium solutions were the same, contained the same amount of soluble starch and hosted in the same temperature, the observed variations in bacterial biomass, protozoal biomass and protozoal populated were directly attributed to the effect of the inherent differences in chemical composition and physical structure of the herbage materials used as a substrate. This assertion is consistent with that of Varga and Kolver (1997) who stated that structure and chemical composition are key plant factors that regulate fibre digestion. High fibre can present a barrier to their complete digestion in the rumen, especially the association of lignin with polysaccharide constituents, whereas low CP contents can prevent multiplication of microbes (Ushida and Jouany, 1990). Orpin (1984) also stated that the type of substrate entering the rumen determines the microbial population. Ørskov (1995) also stated that, feedstuffs low in Nitrogen (N) and high in lingo-cellulosic compounds and therefore, low in fermentable energy severely limits the rumen microbial growth and biomass. These findings supported our results, which showed progressive decline in microbial biomass and population with advancing forage maturity. The rapid decrease in microbial biomass in *Sorghum almum* based culture media, was attributed to the increase in NDF content above 600 g kg^{-1} DM suggested as the critical limit for efficient utilization of fibrous diets (Van Soest, 1994) and the decrease in CP content to below 70 kg^{-1} DM also suggested as the level below which efficient ruminal microbial functions will be impaired (Larbi *et al*., 1992). The observed differences in microbial densities between the forages, was attributed to their differences in chemical composition. *Vicia villosa* and *Commelina benghalensis* are comparatively low fibre and high protein forages (Table 1), which might have spurred higher colonization, hence higher microbial biomass (Cheng *et al*., 1990). Forage legumes are relatively good source of degradable N (Klopfenstein *et al*., 2001) and fermentable energy, so their diets are likely to attract higher microbial colonization compared to low quality roughages. The observed variation of B:P ratio with diets was attributed to the different rates of bacteria and protozoa generation, different rates of engulfment of bacteria by protozoa and protozoal autolysis (Williams and Coleman, 1992). These complex interrelationships were however not elucidated in this study.

CONCLUSION

Results demonstrated that age at harvest effected concentration of chemical constituents. It was also evident that bacterial biomass, protozoal biomass and protozoal population, varied with age and type of herbage used as substrate. From these observations it was therefore concluded that forage dietary nature and chemical composition has significant effect on the vigor of rumen microbial community and therefore, feed digestion. Further research is however need to elucidate the complex interrelationships between the individual microbial species and the various feed chemical nutrients.

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