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Research Article

Effects of Different *Juncus acutus*: Maize Silage Ratios on Digestibility and Rumen Cellulolytic Bacteria [1]

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Abstract

The objectives of this study were to estimate the digestibility of different ratios of *Juncus acutus* and maize silage and to investigate the effects of them on rumen bacteria. Three different ratios of *Juncus acutus* and maize silage 100:0 (A), 50:50 (B) and 0:100 (C) were prepared and their gas productions were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times by ANKOM [RF] gas production system. OMD%, ME_{OMD}, ME_{GP}, and b values of A, B, C were 42.06, 51.06 and 60.21%; 6.72, 8.16 and 9.63 MJ/kg DM; 5.15, 6.28 and 7.55 MJ/kg DM; 20.85, 35.24 and 48.11 mL respectively. There were significant variations between the chemical composition, gas production, OMD%, ME_{GP} and ME_{OMD} values of A, B and C ($P<0.05$). Abundance of ruminal bacteria were as following *Fibrobacter succinogenes*>*Ruminococcus flavefaciens*>*Ruminococcus albus* values at all incubation times. In conclusion, mixing of *Juncus acutus* with maize silage in 50:50 ratio increased the amount of rumen cellulolytic bacteria and 22% of both OMD and ME of *Juncus acutus*. Supplementation of maize silage to *Juncus acutus* in ruminant diet may improve the utilization of *Juncus acutus* through providing of nitrogen and fermentable carbohydrates to rumen bacteria.

Keywords: *Cellulolitic bacteria, Juncus acutus, Maize silage, Metabolizable energy, Organic matter digestibility*

Juncus acutus ve Mısır Silajının Farklı Oranlarının Sindirilebilirlik ve Rumen Selülolitik Bakterileri Üzerine Etkisi

Özet

Bu çalışma ile farklı oranlarda karıştırılan *Juncus acutus* ve mısır silajının sindirilebilirliğinin ve rumendeki selülolitik bakteriler üzerine etkisinin belirlenmesi amaçlandı. *Juncus acutus* ve mısır silajı üç farklı oranda (100:0 (A), 50:50 (B), 0:100 (C)) karıştırılarak kaba yem örnekleri hazırlandı ve 0, 3, 6, 12, 24, 48, 72 ve 96 saatlik inkübasyonlarda gaz üretim (GÜ) değerleri belirlendi. A, B ve C örneklerinin % organik madde sindirilebilirliği (OMS), (metabolik enerji) ME_{OMS} ve $ME_{GÜ}$, potansiyel gaz üretimi (b) değerleri sırasıyla %42.06, 51.06 ve 60.21; 6.72, 8.16 ve 9.63 MJ/kg KM; 5.15, 6.28 ve 7.55 MJ/kg KM; 20.85, 35.24 ve 48.11 mL bulundu. A, B ve C örneklerinin kimyasal kompozisyonları, gaz üretimi, %OMS, $ME_{GÜ}$ ve ME_{OMS} değerleri arasında önemli farklılıklar tespit edildi ($P<0.05$). Bakteri miktarlarındaki artış *Fibrobacter succinogenes*>*Ruminococcus flavefaciens*>*Ruminococcus albus* şeklinde tespit edildi. Sonuç olarak *Juncus acutus* ile mısır silajının 50:50 oranında karıştırılması rumen bakterilerinin oranını ve *Juncus acutus*'un OMS ve ME değerlerini %22 oranında artırdı.

Anahtar sözcükler: *Juncus acutus, Metabolik enerji, Mısır silajı, Organik madde sindirilebilirliği, Selülolitik bakteri*

INTRODUCTION

Nowadays, one of the most important problems of the livestock sector is finding roughage without considering quality in Turkey. Mainly crop residues like wheat, barley and rice straw have been used to meet roughage requirement. A large proportion of crop residues consists of indigestible lignin [1]. Therefore, the use of straw as roughage in ruminant feeding should be used in conjunction with other easily digestible high quality roughages which will have a positive effect on the digestive system. Maize silage is a high energy roughage with high dry matter yield relative to the other roughage crops. Maize silage has low concentrations of protein and some minerals, but high concentrations of fermentable carbohydrates. Energy value of maize silage is mostly

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estimated from chemical composition and *in-vitro* organic matter digestibility (OMD) [2]. Therefore, maize silage are often preferred together with straw and hay in rations. In Kizilirmak Delta in Turkey, farmers mix maize silage with straw for cattle and buffalo nutrition.

Juncus acutus is the most abundant plant in wetlands. There are about 2549.22 ha of natural grassland in the Kizilirmak Delta [3]. *Juncus acutus* presents mainly in Yorukler, Doganca and Sarikoy districts having 519.843 ha land and its *Juncus acutus* production capacity is 8.650 tons. This amount corresponds to 3.719 tons on dry matter basis. Total *Juncus acutus* production capacity of 23 wetlands in Turkey is approximately 85.537 tons. *Juncus acutus* are consumed by water buffaloes which is part of the natural habitat of Kizilirmak Delta. *Juncus acutus* has been proposed as an alternative roughage to cereal straw and also in term of CP % to medium-quality roughage [4].

The *in-vitro* gas production method have been widely used to estimate organic matter digetibility and metabolisable energy values in feed evaluation for ruminants [5]. Advantages and disadvantages of *in-vitro* gas methods are discussed by Gatechew *et al.*[6]. A simple *in vitro* approach is described by Menke *et al.*[7] which is convenient and fast, and allows a large number of samples to be handled at a time. Makkar [8] highlights the potential of a novel approach using an *in-vitro* gas prodution methods for evaluation of nutritional quality of feed resources. Recently, *in-vitro* gas production technique for feed evaluation well reviewed by Singh *et al.*[9].

Rumen microbial ecosystem consist of bacteria, archaea, protozoa, fungi, and bacteriophages [10]. Bacteria are the most numerous of these microorganisms and play major role in the biological degradation of dietary fiber. Cellulose is the major component of forages, and its digestion and subsequent fermentation by ruminal microbes provide much of the energy for forage-fed ruminants [11]. Ruminal degradation of cellulose is mediated primarily by cell-associated enzymes produced by a few predominant cellulolytic bacteria [12]. The rate and extent of fiber digestion in the rumen in large measure are dependent on the population size of these cellulolytic bacteria. *Fibrobacter succinogenes, Ruminococcus flavefaciens* and *Ruminococcus albus* are presently recognized as the major cellulolytic bacterial species found in the rumen [13-15].

Recent advances in molecular biology techniques allow the analysis of such bacteria without cultivation, there by many functional but uncultured, bacteria as new targets for basic and aplied research [16]. Real-time PCR has been successfully used for quantifying protozoa, cellulolytic fungi and cellulolytic bacterial species [4,17-19].

The objectives of this study were to estimate the digestibility of different ratios of *Juncus acutus* and maize silage and to investigate the effects of them on rumen bacteria.

MATERIAL and METHODS

The study was approved by the Local Ethics Committee on Animal Experiments of Ondokuz Mayis University, Turkey (OMU, 18.12.2012, HADYEK 2012/70). Chemical analyses and *in-vitro* gas production experiments were carried out in the Ruminant Feed Evaluation Laboratory of Departmant of Animal Nutrition and Animal Diseases, OMU Faculty of Veterinary Medicine. Real-time PCR analyses were conduted in Samsun Public Health Laboratories, Ministery of Health.

Animals and Feeds

Rumen fluid was obtained from three fistulated Karayaka rams (2 years old, BW = 50±5 kg) fed twice daily at the maintanence level with a diet containing 65% alfaalfa hay and 35% concentrate (Samsun feed processing factory; 1 3% CP, 10% CS, 4% EE, 9% Ash) after three weeks adaptation period. Twenty *Juncus acutus* samples were collected from Kizilirmak Delta. Twenty maize silage samples were taken from dairy cattle enterprise in Doganca Bafra, Turkey. Cut roughage samples were dried in oven at 105°C over-night [20], ground in a mill to pass a 1 mm mesh screen, and kept at room temperature till laboratory analysis.

Chemical Analysis

All roughage samples were milled through a 1 mm sieve then three different ratios of *Juncus acutus* and maize silage 100:0 (A), 50:50 (B) and 0:100 (C) were prepared. Prepared roughage samples A, B and C were used for chemical analysis, gas production and real-time PCR methods. Dry mater (DM), ash, ether extract (EE) and nitrogen (N) contents of samples were analysed according to AOAC methods [20]. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by Van Soest *et al.*[21].

In-Vitro Gas Production

The ANKOM^{RF} gas production method was used for the incubation [22]. Each experimental unit consisted of 250 mL glass jar with attached module top. The module tops having the communication system were used. Gas accumulating in the headspace of bottle was automatically released when the pressure inside the units reached 1.5 kPa above ambient pressure. Pressure was measured every 10 min. Approximately 1 g of the milled feed samples was weight into 250 mL glass jar and incubated at 39°C overnight.

They were fed at least 3 h before the rumen fluid was collected. The fluid was collected into pre-heated thermos-flask. The buffer was prepared according to Menke and Steingass [5], and buffer mixed with rumen fluid 4:1. A mixture of 100 mL of this media was added to preheated units containing feed samples. The glass jar were then closed and put into an incubator. Media and incubation preparation were done under anaerobic conditions by

constantly flushing CO_2, at a temprature of about 39°C and pH of about 6.5-6.8. The incubation procedure was repeated three times. The samples were incubated for 0, 3, 6,12, 24, 48, 72, 96 h. The average cumulative pressure measured for each sample. Pressure was converted to mL of gas at standard temprature and pressure. Then after gas produced per gram DM incubated substrat was calculated. Cumulative gas production data at 24 h was fitted to the model of Ørskov and McDonald [23]. Gas (Y) = b (1-e^{-ct}), where; b = the gas production from the insoluble fraction (mL), c = the gas production rate constant for the insoluble fraction (mL/h), t = incubation time (h). OMD%, ME_{GP}(MJ/ kg DM), and ME_{OMD} (MJ/ kg DM) values of roughage samples A, B and C were estimated from measured pressure by *in-vitro* method at 24 h by using below equations [5].

ME (MJ/kg DM) = 2.2 + 0.136 GP + 0.057 CP + 0.0029 EE

OMD (%) = 57.2 + 0.365 GP + 0.304 CP-1.98 ADL

GP (mL/200 mg DM)

ME (MJ/kg DM) = 0.16 OMD

Real -Time PCR Analysis

The effects of roughages A, B and C on rumen cellulolytic rumen bacteria *Fibrobacter succinogenes* > *Ruminococcus flavefaciens* > *Ruminococcus albus* were determined by real-time PCR method. DNA isolation of rumen fluids obtained from 0, 3, 6, 9, 12, 24, 48, 72 and 96 h incubations were carried out by applying bacterial DNA isolation procedure by using Chelex-resin [24]. Real-time PCR assays of isolated DNA samples were performed on C 1000 Bio-rad real-time PCR device. Assays were set up using the EVA Green PCR Master Mix (2X) (Seegene Technologies; Taewon Bldg., 91, Ogeum-ro, Songpa-gu, Seoul, 138-828, Korea).

The targeted bacteria were 3 predominant cellulolytic bacteria *Fibrobacter succinogenes, Ruminococcus flavefaciens* and *Ruminococcus albus.* Primer for *Fibrobacter succinogenes* Forward(Fs219f):5´-GGTATGGGATGAGCTTGC-3´,

Reverse(Fs654r):5´-GCCTGCCCCTGAACTATC-3´,

Ruminococcus albus Forward(Ral281f):5´-CCCTAAAA GCAGTCTTAGTTCG-3´,

Reverse(Ral439r):5´-CCTCCTTGCGGTTAGAACA-3´and

Fibrobacter flavefaciens Forward (Rf154f):5´TCTGGAAA CGGATGGTA-3´,

Reverse(Rf425r): 5´-CCTTTAAGACAGGAGTTTACAA-3´. Those primers were chosen from previously published sequences that demostrates species-specific amplication [13].

PCR conditions for *Fibrobacter succinogenes* was as follows: 30 sec. at 94°C for denaturing, 30 sec. at 60°C for anneling and 30 sec. at 72°C for extension (48 cycles), except for 9 min of denaturation in the first cycle and 10 min of extention in the last cycle. Amplification of 16 sec. rDNA for *Ruminococcus flavefaciens* and *Ruminococcus albus* was carried out similarly except an annealing temperature of 55°C.

The relative abundance of three predominant bacteria in rumen fluids obtained from 0, 3, 6, 12, 24, 48, 72 and 96 h incubations of *Juncus acutus* samples which were collected from three different stations was quantified using the relative quantification Δ C_T[25]. The mean values of each bacteria at 0, 3, 6, 12, 24, 48, 72 ve 96 h incubation time of *Juncus acutus* which were collected from three different station.

Statical Analysis

One-Way analysis of variance and multiple comparisons among treatment means were performed by Duncan's new multiple range [26]. Means differences were considered significant at P<0.05.

RESULTS

Chemical composition of different ratio of *Juncus acutus*: maize silage samples A, B and C collected from Kizilirmak Delta is presented in *Table 1*. There was significant differencies between roughages in terms of chemical composition (P<0.05). Roughage A was very rich in DM, OM, CP, NDF, ADF and ADL contents and higher than that of the others roughages B and C, however roughage C was the lowest. Besides, ash, EE and ME_{ADF} values, the highest was found in roughage C, but the lowest was in roughage A.

Cumulative GP_{mL}/200 mg DM, OMD%, ME_{OMD} (MJ/ kg DM), ME_{GP} (MJ/kg DM) and potential gas production (b) mL of roughages A, B and C at 24 h are presented in *Table 2.* Cumulative GP_{mL}/200 mg DM, OMD%, $ME_{OMD,}$ $ME_{GP,}$ and

Table 1. *Chemical composition and ME_{ADF} (MJ/kg DM) values of roughages A, B and C*

Tablo 1. *A, B ve C kaba yemlerinin kimyasal kompozisyonu ve ME_{ADF} (MJ/ kg KM)* değerleri

%	Roughage Sample		
	A (n=20) X±Sx	B (n=20) X±Sx	C (n=20) X±Sx
DM (105°C)	97.36±0.21^a	95.43±0.18^b	94.51±0.06^c
ASH	4.11±0.02^c	5.15±0.04^b	6.30±0.08^a
OM	93.25±0.05^a	90.28±0.03^b	88.21±0.09^c
CP	10.13±0.06^a	8.41±0.05^b	6.55±0.06^c
EE	1.53±0.05^c	1.69±0.06^b	1.94±0.05^a
NDF	73.14±0.08^a	60.66±0.06^b	47.62±0.03^c
ADF	45.84±0.04^a	37.95±0.03^b	31.45±0.04^c
ADL	12.43±0.04^a	9.23±0.04^b	6.19±0.06^c
ME_{ADF} (MJ/kg DM)	8.65±0.01^c	9.67±0.02^b	10.52±0.01^a

A: *100% Juncus acutus,* ***B:*** *50% Juncus acutus + 50% maize silage,* ***C:*** *100% maize silage. n: number of samples; Means with in a row with different superscripts differ (P<0.05)*

b values of roughages A, B, C were 17.56, 26.57 and 36.63 mL; 42.06, 51.06 and 60.21%; 6.72, 8.16 and 9.63 MJ/kg DM; 5.15, 6.28 and 7.55 MJ/kg DM; 20.85, 35.24 and 48.11 mL respectively.

Fibrobacter succinogenes, Ruminococcus flavefaciens and *Ruminococcus albus* values calculated from threshold (C_T) values in rumen fluids obtained from 0, 3, 6, 12, 24, 48, 72 and 96 h incubations of roughages A, B, C collected from Kizilirmak Delta by real-time PCR method are shown in *Table 3*.

DISCUSSION

Chemical Analysis

Chemical composition of roughages A, B and C collected from Kizilirmak Delta are presented in *Table 1*. There was considerable variation between roughages in terms of chemical composition (P<0.05). The crude protein content of roughages changed from 6.55 to 10.13%. Roughage A was very rich in crude protein and higher than that of the other silages. Roughage C was very poor in crude protein. The crude protein content of roughage A was similar to that reported for *Juncus acutus* by Erdem [4]. The crude protein content of roughage B was similar to that reported for maize silage by Nkosi *et al*.[27]; for orange pulp by Akinfemi *et al*.[28]. The crude protein content of roughage C was similar to that reported for maize silage by Ozturk *et al*.[29], Karakozak and Ayasan [30] and Podkowka and Podkowka [31].

Table 2. *Cumulative gas production volume at 24 h (GP), potantial gas production volume (b), organic matter digestibility (OMD), metabolic energy (ME_{OMD} and ME_{GP}) of roughages A, B, and C*

Tablo 2. *A, B ve C kaba yemlerinin 24 saatlik kümülatif gaz üretim hacmi (GÜ), potansiyel gaz üretim hacmi (b), organik madde sindirilebilirliği (OMS) ve metabolik enerji (ME_{OMS} ve $ME_{GÜ}$)*

Parameter	Roughage Sample		
	A (n=20) X±Sx	B (n=20) X±Sx	C (n=20) X±Sx
GP_{mL}(GP_{mL}/ 200mg DM)	17.56±0.41^c	26.57±0.35^b	36.63±0.39^a
OMD (%)	42.06±0.07^c	51.06±0.15^b	60.21±0.16^a
ME_{OMD} (MJ/kg DM)	6.72±0.03^c	8.16±0.02^b	9.63±0.02^a
ME_{GP} (MJ/kg DM)	5.15±0.07^c	6.28±0.05^b	7.55±0.05^a
b (mL)	20.85±0.26^c	35.24±0.25^b	48.11±0.45^a

***A:** 100% Juncus acutus, **B:** 50% Juncus acutus + 50% maize silage, **C:** 100% maize silage. n:number of samples; Means with in a row with different superscripts differ (P<0.05)*

There were statistically significant differences between of roughages A, B and C in terms of NDF, ADF and ADL (P<0.05). The NDF contents of roughage A, B and C was found 73.14%, 60.66% and 47.62% respectively. The NDF content of roughage A was similar to that reported for *Juncus acutus* by Erdem [4]; for rice straw by Rahman *et al*.[32]. The NDF content of roughage B was similar to that reported for bromegrass by Doane *et al*.[33]. The NDF content of roughage C was similar to that reported for pea hay by Canbolat *et al*.[34]; for tomato pomace by Mirzaei-Aghsaghali *et al*.[35].

The ADF contents of roughages A, B and C was found 45.84%, 37.95% and 31.45% respectively. The ADF content of roughage A was similar to that reported for *Juncus acutus* by Erdem [4]. The ADF content of roughage B was similar to that reported for *Convoivuius arvensis* by Canbolat [36]. The ADF content of roughage C was similar to that reported for *Onobrychis sativa* hay by Canbolat [37]; for tomato pomace by Mirzaei-Aghsaghali *et al*.[35]; for *Eucalyptus camaldulensis* leaves by Akcil and Denek [38].

The ADL contents of roughages A, B and C samples

Table 3. *The mean fold changes of Fibrobacter succinogenes, Ruminococcus flavefaciens and Ruminococcus albus in rumen fluids obtained from 0, 3, 6, 12, 24, 48, 72 and 96 h incubations of roughages A, B and C*

Tablo 3. *A, B ve C kaba yemlerinin 0, 3, 6, 12, 24, 48, 72 ve 96 saatlik inkübasyonlarından elde edilen rumen sıvısındaki Fibrobacter succinogenes, Ruminococcus flavefaciens ve Ruminococcus albus bakterilerinin ortalama kat artışlarının değiş*mi

t(h)	***Fibrobacter succinogenes*** **(mean fold *)**				***Ruminococcus flavefaciens*** **(mean fold *)**				***Ruminococcus albus*** **(mean fold *)**			
	Roughage Sample				Roughage Sample				Roughage Sample			
	A	B	C	SEM	A	B	C	SEM	A	B	C	SEM
0	1	1	1		1	1	1		1	1	1	
3	1.08^b	1.10^b	1.17^a	0.01	1.05^b	1.07^b	1.11^a	0.02	1.01^b	1.03^b	1.06^a	0.01
6	1.20^b	1.19^b	1.29^a	0.05	1.11^b	1.12^b	1.19^a	0.03	1.05^b	1.08^a	1.09^a	0.03
12	1.32^b	1.36^b	1.56^a	0.05	1.21^b	1.28^a	1.30^a	0.04	1.12^b	1.21^a	1.22^a	0.02
24	1.99^c	2.63^b	2.92^a	0.04	1.55^c	2.39^b	2.43^a	0.04	1.47^c	1.68^b	1.95^a	0.04
48	2.32^c	3.48^b	3.87^a	0.05	1.92^c	2.65^b	2.72^a	0.03	1.73^c	2.01^b	2.23^a	0.05
72	2.49^b	3.51^b	3.90^a	0.05	2.21^c	2.87^b	2.96^a	0.05	1.92^c	2.20^b	2.28^a	0.04
96	2.53^b	3.56^b	3.92^a	0.06	2.27^c	3.05^b	3.20^a	0.05	2.00^c	2.24^b	2.31^a	0.03

***A:** 100% Juncus acutus, **B:** 50% Juncus acutus + 50% maize silage, **C:** 100% maize silage; t: incubation times (h); **SEM:** Mean of Standard eror. Means within a row with different superscripts differ (P< 0.05); *** fold:** amount of microbial population at each incubation time over 0 h (control) which was taken as 1*

was found 12.43%, 9.23% and 6.19% respectively. The ADL content of A roughage was similar to that reported for *Juncus acutus* by Erdem [4]; for wheat straw by Kalkan and Filya [39]. The ADL content of roughage B was similar to that reported for good quality alfalfa hay by Gungor *et al*.[40]. The ADL content of roughage C was similar to that reported for maize silage by Gungor *et al*.[40]; for cereal roughages from corn and wheat by Canbolat [41].

In-Vitro Gas Production

The cumulative volume of gas production increased with increasing incubation time. A statistically significant difference was observed between roughages A, B and C samples of gas production at all incubation times (P<0.05). It may be due to different ADL content of roughages A, B and C. Mertens *et al*.[42] repoted that high ADL level of feedstuffs adversly affect gas production however NDF content increase gas production. The ADL contents and cumulative volume of gas production of roughages A, B and C were 12.43, 9.23 and 6.19%; 17.56, 26.57 and 36.63 mL at 24 h of incubation respectively. At all incubation time, gas production of roughage C was significantly higher than the others (P<0.05) and gas production of roughage A was significantly lower than the others (P<0.05).

In-vitro gas production, kinetic parameters, ME_{GP}, ME_{OMD} and OMD% are significantly affected by nutrient content of roughages A, B and C *(Table 2)*.

ME_{GP} and ME_{OMD} values of roughages A, B and C were 6.72, 8.16 and 9.63 MJ/kg DM; 5.15, 6.28 and 7.55 MJ/kg DM respectively. The OMD% value of roughages A, B and C was found 42.06%, 51.06% and 60.21% respectively. There were statistically significant differences between of roughages in terms of OMD% (P<0.05). Obtained differences among OMD% of roughages A, B and C were associated with gas production. The OMD% value of roughage A was similar to that reported for *Juncus acutus* by Erdem [4]; for rice straw by Rahman *et al*.[32]. ME, OMD and gas production values of *Juncus acutus* were the significantly improved by treatment maize silage due to maize has low concentrations of protein and some minerals, but high concentrations of fermentable carbohydrates. The OMD% value of roughage B was similar to that reported for corn cobs and guinea corn threshed tops by Akinfemi *et al*.[28]. The OMD% value of roughage B was similar to that reported for *Convoivuius arvensis* by Canbolat [36].

There were significant differences between roughages in terms of estimated ME_{GP}, ME_{OMD} and OMD% levels (P<0.05). It may be due to the major causes of the differences in the amount of CP and ADL. The lag time for all roughages was very low and very close to zero. Therefore, lag time was ignored. However, potential gas production (b) value may be affected in the presence of secondary metabolites in *Juncus acutus*. Potential gas production of roughage C was higer than the other roughages. Potential gas production value of roughage A was similar to that reported for *Juncus acutus* by Erdem [4]. Potential gas production value of roughage C was similar to that reported for Mirzaei-Aghsaghali *et al*.[35].

Positive associative effects occured when *Juncus acutus* was mixed with maize silage in 50:50 ratio which increased the OMD and ME values of *Juncus acutus*. This observed effect maybe due to providing energy and protein for rumen microorganisms in required ratio from a mixture of *Juncus acutus* and maize silage.

Real-Time PCR Analysis

Fibrobacter succinogenes, Ruminococcus flavefaciens and *Ruminococcus albus* values calculated from threshold (C_T) values in rumen fluids obtained from 0, 3, 6, 12, 24, 48, 72 and 96 h incubations of roughages A, B and C by real-time PCR method showed an increases as FS > RF > RA *(Table 3)*. This ranking is in agreement with reported values by Polyorach *et al*.[43]; Hung and Wanapat [44]; Erdem [4]; Wanapat and Cherdthong [18]; Koike and Kobayyashi [13]. The population of *Fibrobacter succinogenes* compared to *Ruminococcus flavefaciens* and *Ruminococcus albus* was highest in all roughages A, B and C. Furthermore *Ruminococcus albus* was the lowest compared with *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* in all roughages. Our obtained results showed that supplementation of maize silage to *Juncus acutus* provides nitrogen and fermentable carbohydrates to rumen cellulolitic bacteria and this caused to increase in the following order of *Fibrobacter succinogenes, Ruminococcus flavefaciens* and *Ruminococcus albus* growth. Apparently because *F. succinogenes* and *R. flavefaciens* can colonize the cellulose more rapidly than *R. Albus* [44,45]. *R. albus,* always was less abundant than was *F. succinogenes* and *R. flavefaciens* because it was less effective in colonizing cellulose and was probable reduced to growing on soluble products released by the other species during cellulose hydrolysis [46].

Gas production values of roughage samples A, B and C at 3, 6, 12, 24, 48, 72, 96 h of incubations were compatible with *Fibrobacter succinogenes, Ruminococcus flavefaciens* and *Ruminococcus albus* values calculated from threshold (C_T) values in rumen fluids obtained from 0, 3, 6, 12, 24, 48, 72, 96 h of incubations. There is a strong relationship between the OMD of feedstuffs and the rate of gas production [47]. Feedstuffs should contain at least 10% CP for optimum microbial activity in the rumen [48]. Mixing of *Juncus acutus* with maize silage is being a good combination for rumen bacteria because of high protein content of *Juncus acutus* (10% CP).

Mixed *Juncus acutus* with maize silage in 50:50 ratio may be used as medium quality roughage source in ruminant nutrition. It may be suggested to do further study on *in-vivo* condition to explore more about *Juncus acutus* and its potential effects on animal performance.

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