Kafkas Univ Vet Fak Derg
21 (4): 499-505, 2015
DOI: 10.9775/kvfd.2014.12767

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

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

Nurcan ÇETİNKAYA [1] Funda ERDEM [2]

This study was supported by Ondokuz Mayis University Research Fund (Project number: PYOVET.1904.14.003, 2014)

[1] Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ondokuz Mayis University, TR-55139 Samsun - TURKEY
[2] Department of Moleculer Microbiology, Public Health Laboratory, Ministry of Health, TR-55060 Samsun - TURKEY

Article Code: KVFD-2014-12767 Received: 07.12.2014 Accepted: 17.02.2015 Published Online: 17.03.2015

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

İletişim (Correspondence)
+90 541 4543732
fundaerdemtr@gmail.com

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*.

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 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].

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 değişi*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.

REFERENCE

1. Moore KJ, Jung HG: Lignin and fiber digestion. *J Range Manage*, 54, 420-430, 2001.

2. De Boever JL, Aerts JM, Vanacker JM, De Brabander DL: Evaluation of the nutritive value of maize silages using a gas production technique. *Anim Feed Sci Tech,* 123-124, 255-265, 2005. DOI: 10.1016/j.anifeedsci.2005.04.019

3. Ayan AK: Natural Resources of Kizilirmak Delta. Report of Kizilirmak Delta, OMU Faculty of Agriculture. Samsun, Turkey, 2007.

4. Erdem F: Determination the digestibility of *Juncus acutus* by *in-vıtro* gas production and its effect on ruminal cellulolytic bacteria by real-time pcr methods. *PhD Thesis,* Ondokuz Mayıs University, 2014.

5. Menke KH, Steingass H: Estimation of the energetic feed value obtained from chemical analysis and *in-vitro* gas production using rumen fluid. *Anim Res Dev*, 28, 7-55, 1988.

6. Getachew G, Blummel M, Makkar HPS, Becker K: *In-vitro* gas measuring techniques for assesment of nutritional quality of feeds: A review. *Anim Feed Sci Tech,*72, 261-281,1998. DOI: 10.1016/S0377-8401(97)00189-2

7. Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W: The estimation of the digestibility and metabolisable energy content of ruminant feding stuffs from the gas production when they are incubated with rumen liquor. *J Agric Sci,* 93, 217-222, 1979. DOI: 10.1017/S0021859600086305

8. Makkar HPS: Recent advances in the *in-vitro* gas method for evaluation of nutritional quality of feed resources. Food and Agricultural Organisation, Assessing Quality and Safety of Animal Feeds. ISSN 0254-6019, ISBN 92-5-105046-5. Publishing Management Service, Information Division, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy, 2004.

9. Sing B, Tomar SK, Kundu SS: *In-vitro* Gas Production Technique for Feed Evaluation. 1-115, Karnal - 132 001, Haryana, India Intech Printers & Publishers #.353, Mughal Canal Market, 2010.

10. Klieve AV, Yokoyama MT, Forster RJ, Ouwerkerk D, Bain PA, Mawhinney EL: Naturally occuring DNA transfer system associated with membrane vesicles in cellulolytic *Ruminococcus spp*. of ruminal origin. *Appl Environ Microbiol,* 71, 4248-4253, 2005. DOI: 10.1128/AEM.71.8.4248-4253.2005

11. Van Soest PJ: Nutritional Ecology of the Ruminant. 2nd ed., 140-155, Cornell University Press, Ithaca, N.Y, 1994.

12. Weimer PJ: Cellulose degradation by ruminal microorganisms. *Crit Rev Biotechnol*, 12, 189-223, 1992. DOI: 10.3109/07388559209069192

13. Koike S, Kobayyashi Y: Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens*. *FEMS Microbiol Lett,* 204, 361-366, 2001. DOI: 10.1111/j.1574-6968.2001.tb10911.x

14. Koike S, Pan J, Kobayashi Y, Tanaka K: Kinetics of in sacco fiber-attachment of representative ruminal cellulolytic bacteria monitored by competitive PCR. *J Dairy Sci,* 86, 1429-1435, 2003. DOI: 10.3168/jds.S0022-0302(03)73726-6

15. Koike S, Kobayashi Y: Fibrolytic rumen bacteria: their ecology and functions. *Asian- Austr J Anim Sci*, 22, 131-138, 2009. DOI: 10.5713/ajas.2009.r.01

16. Russell JB, Muck RE, Weimer PJ: Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. *FEMS Microbiol Ecol*, 67, 183-197, 2009. DOI: 10.1111/j.1574-6941.2008.00633.x

17. Cherdthong A, Wanapat M, Kongnum P, Pilajun R, Khejornsart P: Rumen fermentation, microbial protein synthesis and cellulolytic bacterial population of swamp buffaloes as affected by roughage to concentrate ratio. *J Anim Vet Adv,* 9, 1667-1675, 2010. DOI: 10.3923/javaa.2010.1667.1675

18. Wanapat M, Cherdthong A: Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in Swamp buffalo. *Curr Microbiol*, 58, 294-299, 2009. DOI: 10.1007/s00284-008-9322-6

19. Kongmun P, Wanapat M, Pakdee P, Navanukraw C: Effect of coconut oil and garlic powder on *in-vitro* fermentation using gas production technique. *Livest Sci*, 127, 38-44, 2010. DOI: 10.1016/j.livsci.2009.08.008

20. AOAC: Official Methods of Analysis, 18th ed., Association of Official Analytical Chemists, Inc., Arlington, VA, 2006.

21. Van Soest PJ, Robertson JD, Lewis BA: Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci,* 74, 3583-3597, 1991. DOI: 10.3168/jds.S0022-0302(91)78551-2

22. ANKOM, 2011: RF Gas Info. pdf. http://www.ankom.com, *Accessed:* 27 February 2012.

23. Ørskov ER, McDonald I: The estimation of protein degradability in the rumen from incubation measurement weighed according to rate of passage. *J Agric Sci*, 92, 499-503, 1979. DOI: 10.1017/S0021859600063048

24. Kwon HB, Su HS, Jong SL, Sung RM, Suk MK, Jang RL, Dongsu C, Won JJ: Rapid and simple method for DNA extraction from plant and algal species suitable for PCR amplification using a chelating resin Chelex 100. *Plant Biotech Reports,* 4, 49-52, 2010. DOI: 10.1007/s11816-009-0117-4

25. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2ΔΔC(T)Method. *Methods*, 25, 402-408, 2001. DOI: 10.1006/meth.2001.1262

26. SAS: SAS STATİSTİC SOFTWARE, SAS Campus DRİVE. Cary NC, USA, 2007.

27. Nkosi BD, Meeske R, Langa T, Thomas RS: Effects of bacterial silage inoculants on whole-crop maize silage fermentation and silage digestibility in rams. *S Afr J Anim Sci,* 41, 350-359, 2011.

28. Akinfemi A, Adesenya AO, Aya VE: Use of an *in-vitro* gas production technique to evaluate some nigerian feedstuffs. *American-Eurasian J Sci Res*, 4, 240-245, 2009.

29. Ozturk D, Kizilsimsek M, Kamalak A, Canbolat O, Ozkan CO: Effects of ensiling alfalfa with whole cropmaize on chemical composition and nutritive value of silage mixtures. *Asian-Aust J Anim Sci,* 19, 526-532, 2006. DOI: 10.5713/ajas.2006.526

30. Karakozak E, Ayaşan T: Değişik yem bitkileri karışımından hazırlanan silajlarda inokulant kullanımının flieg puanı ve ham besin maddeleri üzerine etkileri. *Kafkas Univ Vet Fak Derg,* 16, 987-994, 2010. DOI: 10.9775/kvfd.2010.2197

31. Podkowka Z, Podkowka L: Chemical composition and quality of sweet sorghum and maize silages. *JCEA*, 12, 294-303, 2011. DOI: 10.5513/JCEA01/12.2.915

32. RahmanMM, Alam MR, Amin MR, Das NG: Comparative study of the nutritive values of the different varieties of rice straw. *Bang J Anim Sci*, 39, 75-82, 2010.

33. Doane PH, Schofield P, Pell AN: Neutral detergent fiber disappearance and gas and volatile fatty acid production during the *in-vitro* fermentation of six forages. *J Anim Sci,* 75, 3342-3352, 1997.

34. Canbolat O, Kara H, Filya I: Comparison *of in-vitro* gas production, metabolizable energy, organic matter digestibility and microbial protein production of some legume hays. *Uludag Univ Zirrat Fak Derg*, 27, 71-81, 2013.

35. Mirzaei-Aghsaghali A, Maheri-sis N, Mansouri H, Razeghi ME, Safaei AR, Aghajanzadeh Golshani A, Alipoor K: Estimation of the nutritive value of tomato pomace for ruminant using *in-vitro* gas production technique. *African J Biotech,* 10, 6251-6256, 2011.

36. Canbolat O: Potential nutritive value of field binweed *(Convoivuius arvensis L.)* hay harvested at three different maturity stages. *Kafkas Univ Vet Fak Derg,* 18, 331-335, 2012. DOI: 10.9775/kvfd.2011.5533

37. Canbolat O: The Effect of some essential oils on *in-vitro* digestibility, rumen fermentation characteristics and methane gas production. *Igdır Univ J Inst Sci & Tech*, 2, 91-98, 2012.

38. Akcil E, Denek N: Investigation of different levels eucalyptus (*Eucalyptus camaldulensis*) leaves effect on *in-vitro* methane production of some roughages. *Harran Univ Vet Fak Derg,* 2, 75-81, 2013.

39. Kalkan H, Filya I: Effects of cellulase enzyme on nutritive value,

in-vitro digestion characteristics and microbial biomass production of wheat straw. *Kafkas Univ Vet Fak Derg,* 17, 585-594, 2011. DOI: 10.9775/kvfd.2010.4246

40. Gungor T, Basalan M, Aydogan I: The determination of nutrient contents and metabolizable energy levels of some roughages produced in Kirikkale region. *Ankara Univ Vet Fak Derg,* 55, 111-115, 2008.

41. Canbolat O: Comparison of *in-vitro* gas production, organic matter digestibility, relative feed value and metabolizable energy contents of some cereal forages. *Kafkas Univ Vet Fak Derg,* 18, 571-577, 2012. DOI: 10.9775/kvfd.2011.5833

42. Mertens DR, Weimer PJ, Waghorn GC: Inocula differences affect *in-vitro* gas production kinetics. USA Dairy Forage Research Center, *Research Summaries*, 53-54, 1997.

43. Polyorach S, Wanapat M, Cherdthong A: Influence of yeast fermented cassava chip protein (yefecap) and roughage to concentrate ratio on ruminal fermentation and microorganisms using *in-vitro* gas production technique. *Asian Australas J Anim Sci,* 27, 36-45, 2014. DOI: 10.5713/ajas.2013.13298

44. Hung LV, Wanapat M: Effects of Leucaena leaf pellet on bacterial diversity and microbial protein synthesis in swamp buffalo fed on rice straw. *Livest Sci*, 151, 188-197, 2013. DOI: 10.1016/j.livsci.2012.11.011

45. Shi Y, Odt CL, Weimer PJ: Competition for cellulose among three predominant ruminal cellulolytic bacteria under substrate-excess and substrate-limited conditions. *Appl Environ Microbiol*, 63, 734-742, 1997.

46. Shi Y, Weimer PJ: Utilization of individual cellodextrins by three predominant ruminal cellulolytic bacteria. *Applied Environ Microbiol*, 62, 1084-1088, 1996.

47. Chenost M, Aufrere J, Macheboeuf D: The gas-test technique as tool for predicting the energetic value of forage plants. *Anim Res*, 50, 349-364, 2001. DOI: 10.1051/animres:2001137

48. Norton BW: The nutritive value of tree legumes. from http://www.fao.org/ag/AGP/AGPC/doc/Pub/licat/Guttshel/x5556e0j.htm, 1-10, 2003.