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 OMDS, 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 OMDS and ME GP 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: Cellulolytic bacteria, Juncus acutus, Maize silage, Metabolizable energy, Organic matter digestibility

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

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

Nurcan ÇETİNKAYA 1 Funda ERDEM 2

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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 rumen mikroorganizmalarının etkisi konusunda araştırmaların hacimini artırmaya yönelik olarak Juncus acutus ve mısır silajı üç farklı oranda (100:0 (A), 50:50 (B), 0:100 (C)) karıştırılarak kaba yem örnekleri hazırlanmıştır. A, B ve C örneklerinin % organik madde sindirilebilirliği (OMS), (metabolik enerji) ME OMDS, 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 arasında önemli farklılıklar tespit edildi (P<0.05). Bakteri miktarlarındaki artış Fibrobacter succinogenes>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.

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estimated from chemical composition and in-vitro organic matter digestibility (OMD) [4]. 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 digestibility and metabolisable values in feed evaluation for ruminants [3]. 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 production 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 [9]. 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 [10]. Ruminal degradation of cellulose is mediated primarily by cell-associated enzymes produced by a few predominant cellulytic bacteria [10]. The rate and extent of fiber digestion in the rumen in large measure are dependent on the population size of these cellulytic bacteria. Fibrobacter succinogenes, Ruminococcus flavefaciens and Ruminococcus albus are presently recognized as the major cellulytic 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 applied research [10]. Real-time PCR has been successfully used for quantifying protozoa, cellulyotic fungi and cellulyotic 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 Department of Animal Nutrition and Animal Diseases, OMU Faculty of Veterinary Medicine. Real-time PCR analyses were conducted in Samsun Public Health Laboratories, Ministry 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 mainenance level with a diet containing 65% alfalfa 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 overnight [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® 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 [9], 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
RESULTS

Chemical composition of different ratio of *Juncus acutus*: maize silage samples A, B and C collected from Kızılirmak Delta is presented in Table 1. There was significant differences 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\textsubscript{ADF} values, the highest was found in roughage C, but the lowest was in roughage A.

Cumulative GP\textsubscript{100}/200 mg DM, OMD\%, ME\textsubscript{OMD} (MJ/kg DM), ME\textsubscript{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\textsubscript{100}/200 mg DM, OMD\%, ME\textsubscript{OMD}, ME\textsubscript{GP}, and ME\textsubscript{ADF} 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 were quantified using the relative quantification Δ C\textsubscript{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.

### Table 1. Chemical composition and ME\textsubscript{ADF} (MJ/kg DM) values of roughages A, B and C

| Roughage Sample | A (n=20) | B (n=20) | C (n=20) |
|-----------------|----------|----------|----------|
| DM (105°C)      | X±5x     | X±5x     | X±5x     |
| 97.36±0.21\textsuperscript{a} | 95.43±0.18\textsuperscript{a} | 94.51±0.06\textsuperscript{a} |
| ASH             | 4.11±0.02\textsuperscript{a} | 5.15±0.04\textsuperscript{a} | 6.30±0.08\textsuperscript{a} |
| OM              | 93.25±0.05\textsuperscript{a} | 90.28±0.03\textsuperscript{a} | 88.21±0.09\textsuperscript{a} |
| CP              | 10.13±0.06\textsuperscript{b} | 8.41±0.05\textsuperscript{b} | 6.55±0.06\textsuperscript{b} |
| EE              | 1.53±0.05\textsuperscript{a} | 1.69±0.06\textsuperscript{a} | 1.94±0.05\textsuperscript{a} |
| NDF             | 73.14±0.08\textsuperscript{a} | 60.65±0.06\textsuperscript{a} | 47.62±0.03\textsuperscript{a} |
| ADF             | 45.84±0.04\textsuperscript{b} | 37.95±0.03\textsuperscript{b} | 31.45±0.04\textsuperscript{b} |
| ADL             | 12.43±0.04\textsuperscript{a} | 9.23±0.04\textsuperscript{a} | 6.19±0.06\textsuperscript{a} |
| ME\textsubscript{ADF} (MJ/kg DM) | 8.65±0.01\textsuperscript{a} | 9.67±0.02\textsuperscript{a} | 10.52±0.01\textsuperscript{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)

### Table 2. Cumulative gas production data at 24 h

| Roughage Sample | A (n=20) | B (n=20) | C (n=20) |
|-----------------|----------|----------|----------|
| GP (mL/200 mg DM) | X±5x     | X±5x     | X±5x     |
| 5.15±0.04\textsuperscript{a} | 5.16±0.04\textsuperscript{a} | 5.17±0.04\textsuperscript{a} |
| GP (mL/200 mg DM) | X±5x     | X±5x     | X±5x     |
| 5.15±0.04\textsuperscript{a} | 5.16±0.04\textsuperscript{a} | 5.17±0.04\textsuperscript{a} |
| GP (mL/200 mg DM) | X±5x     | X±5x     | X±5x     |
| 5.15±0.04\textsuperscript{a} | 5.16±0.04\textsuperscript{a} | 5.17±0.04\textsuperscript{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 (Ct) 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.

| Parameter          | Roughage Sample |
|--------------------|-----------------|
|                    | A (n=20)        | B (n=20)        | C (n=20)        |
| GP(b) (GP/mL/200mg DM) | 17.56±0.41a    | 26.57±0.35b    | 36.63±0.39c    |
| OM (%):            | 42.06±0.07a    | 51.06±0.15b    | 60.21±0.16c    |
| ME(MJ/kg DM):      | 6.72±0.03a     | 8.16±0.02b     | 9.63±0.02c     |
| ME(MJ/kg DM):      | 5.15±0.07a     | 6.28±0.05b     | 7.55±0.05c     |
| b (mL):            | 20.85±0.26a    | 35.24±0.25b    | 48.11±0.45c    |

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 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-Aghsaghal 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 Convolvulus 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.[38]; for Eucalyptus camaldulensis leaves by Akcil and Denek [39].

The ADL contents of roughages A, B and C samples

| t(h) | Fibrobacter succinogenes (mean fold *) | Ruminococcus flavefaciens (mean fold *) | Ruminococcus albus (mean fold *) |
|------|--------------------------------------|----------------------------------------|---------------------------------|
|      | Roughage Sample                      | Roughage Sample                        | Roughage Sample                 |
| 0    | A 1 1 1 SEM                           | A 1 1 1 SEM                             | A 1 1 1 SEM                     |
| 3    | 1.08a 1.10a 1.17a 0.01                | 1.05a 1.07a 1.11a 0.02                 | 1.01a 1.03a 1.06a 0.01           |
| 6    | 1.20a 1.19a 1.29a 0.05                | 1.11a 1.12a 1.19a 0.03                 | 1.05a 1.08a 1.09a 0.03           |
| 12   | 1.32a 1.36b 1.56b 0.05                | 1.21a 1.28a 1.30a 0.04                 | 1.12a 1.21a 1.22a 0.02           |
| 24   | 1.99a 2.63a 2.92a 0.04                | 1.55a 2.39a 2.43a 0.04                 | 1.47a 1.68a 1.95a 0.04           |
| 48   | 2.32a 3.48b 3.87b 0.05                | 1.92a 2.65a 2.72a 0.03                 | 1.73a 2.01a 2.23a 0.05           |
| 72   | 2.49a 3.51a 3.90a 0.05                | 2.21a 2.87a 2.96a 0.05                 | 1.92a 2.20a 2.28a 0.04           |
| 96   | 2.53a 3.56a 3.92a 0.06                | 2.27a 3.05a 3.20a 0.05                 | 2.00a 2.24a 2.31a 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 error. 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 [19]. 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. [43] reported that high ADL level of feedstuffs adversely 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\(_{\text{GFP}}\), ME\(_{\text{OMD}}\) and OMD% are significantly affected by nutrient content of roughages A, B and C (Table 2).

ME\(_{\text{GFP}}\) and ME\(_{\text{OMD}}\) 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. [46]. The OMD% value of roughage B was similar to that reported for Convolvulus arvensis by Canbolat [56].

There were significant differences between roughages in terms of estimated ME\(_{\text{GFP}}\), ME\(_{\text{OMD}}\) 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\(_{\text{T}}\)) 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 [46]; Wanapat and Cherdthong [48]; Koike and Kobayashi [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\(_{\text{T}}\)) 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 [43]. 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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