Digestibility of *Juncus acutus* and its effects on ruminal cellulolytic bacteria

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**ABSTRACT**

The objectives of this study were to estimate the digestibility of *Juncus acutus* and to investigate its effects on rumen cellulolytic bacteria to consider *J. acutus* as an alternative roughage source in ruminant nutrition. *Juncus acutus* samples were collected from three different stations in Kızılırmak Delta and their proximate analysis was carried out. Organic matter digestibility (OMD) and metabolizable energy (ME) values of *J. acutus* were estimated from gas measured by the *in vitro* gas production method. The effects of *J. acutus* on the abundance of rumen cellulolytic bacteria *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* were determined by real-time PCR method. Crude nutrient values of *J. acutus* collected from three stations were analysed. Mean OMD %, ME<sub>OMD</sub> and ME<sub>GP</sub> values of *J. acutus* from three stations were ranged from 42.64 to 42.48%, 6.78 to 6.82 MJ/kg DM and 5.05 to 5.26 MJ/kg DM respectively. Ruminal *F. succinogenes*, *R. flavefaciens* and *R. albus* abundance calculated from threshold (C<sub>t</sub>) values in rumen fluids obtained from 0 to 96 h incubations showed an increases in following order *F. succinogenes* > *R. flavefaciens* > *R. albus*. The CP % of *J. acutus* was found higher from cereal straw and close to low-quality alfalfa hay and dry meadow grass. *Juncus acutus* increased the amount of rumen cellulolytic bacteria. In the light of obtained results, it can be concluded that *J. acutus* may be considered as a new alternative roughage source in ruminant nutrition.

**Introduction**

Changing environmental conditions such as rising temperatures because of global warming seriously affect agricultural productivity (FAO 2007; El-Hage Scialabba & Muller-Lindenlauf 2010). Forages are the most important feed source for ruminants worldwide. They are suited to utilisation by herbivores that have a capacity for microbial digestion of cell wall constituents (Wilkins 2000). Ruminant nutritionists have been increasingly searching alternative forages for ruminant animals.

The nutritive value of ruminant feedstuffs is determined by the amount of its chemical components, as well as degree of digestion. There are a few *in vitro* techniques useful to evaluate the nutritive value of feedstuffs at relatively practical and low cost. Mathematical descriptions of gas production profiles permit analysis of data, evaluation of substrates and media related differences and fermentability of soluble and slowly fermentable components of feedstuffs (Sallam et al. 2007). Gas production can be regarded as an indicator of carbohydrates degradation. Sallam (2005) suggested that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microorganisms in the *in vitro* system. The *in vitro* gas production method has been widely used to estimate organic matter digestibility and metabolisable energy values in feed evaluation for ruminants (Sharifi et al. 2013; Polyorach et al. 2014; Hernandez et al. 2015).

Rumen microbial ecosystem consists of bacteria, archaea, protozoa, fungi and bacteriophages (Klieve et al. 2005). The digestion of plant material is performed through a complex symbiotic relationship of microbiota within the rumen (Mackie 1997). Cellulolytic bacteria are considered to be the most important for the biological degradation of dietary fibres because of their fibrolytic activity and biomass in the rumen. The main three predominant cellulolytic bacteria *Fibrobacter succinogenes* (*F. succinogenes*), *Ruminococcus flavefaciens* (*R. flavefaciens*) and *Ruminococcus albus* (*R. albus*) take an active role in ruminal digestion of cellulose.

*Juncus acutus* is the most abundant plant in wetlands worldwide. *Juncus acutus* is prevailing plant of...
the natural grassland in Kizilirmak Delta that has been considerably consumed by water buffaloes, Samsun, Turkey. Potential production capacity of *J. acutus* is around 8650 tons only in Kizilirmak Delta.

It is the first time *J. acutus* has been studied as an alternative roughage source. Therefore, the objectives of this study were to estimate the digestibility of *J. acutus* by *in vitro* gas production method and to investigate its effects on rumen cellulolytic bacteria by the real-time PCR method to consider *J. acutus* as a new alternative roughage source in ruminant nutrition.

**Materials 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 analysis 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**

Three fistulated Karayaka rams (2 years old, BW = 50 ± 5 kg) were used for rumen fluid collection for the gas production method. Animals were fed twice daily at the maintenance level with a diet containing 65% alfalfa hay and 35% concentrate (Samsun Feed Processing Factory; 13% CP, 10% CS, 4% EE, 9% Ash) after 3 weeks adaptation period. Twenty *J. acutus* samples were randomly collected from different plants of each station: Station I (36°19′53.81″, E, 41°16′30.29″N), Station II (36°6′51.52″E, 41°36′24.24″N) and Station III (36°5′33.18″E, 41°38′16.85″N), Kizilirmak Delta, Samsun, Turkey. Stations were chosen to represent geographical differences of Kizilirmak Delta considering with riverside, abundance of plant, soil type, etc. Cut *J. acutus* samples were weighed, dried and ground in a mill to pass a 1-mm mesh screen, and kept in labelled plastic containers for later laboratory analysis.

**Chemical analysis**

Collected samples were milled through a 1-mm sieve for chemical analysis and gas production methods. Dry matter (DM), ash, ether extract (EE) contents and nitrogen (N) contents were determined according to AOAC (2006) procedure. Crude protein (CP) was calculated as N × 6.25 AOAC (2006). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by Van Soest et al. (1991) using ANKOM fibre analyzer (Ankom Technology, Macedon, NY).

**In vitro gas production method**

Rumen fluid was obtained from three fistulated rams fed twice daily at the maintenance level with a diet containing alfalfa hay (65%) and concentrate (35%). Rumen fluid was collected from the ruminen with manually operated vacuum pump and transferred into pre-warmed thermos flasks, transported to the laboratory, filtered through eight layers of cheesecloth and flushed with CO₂. The ANKOM RF gas production method was used for the incubation. Each experimental unit consisted of 250 mL glass jar with attached module top. The module tops used contained the communication system. Gas accumulating in the head-space 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 *J. acutus* was weight into 250 mL glass jar and incubated at 39 °C overnight. The fluid was collected from animals which were fed at least 3 h before sampling into pre-heated thermos flask. The buffer was prepared according to Menke and Steingass (1988), and buffer mixed with rumen fluid 4:1. A mixture of 100 mL of this media was added to preheated units containing *J. acutus* samples. The glass jar was then closed and put into an incubator. Media and incubation preparation were done under anaerobic conditions by constantly flushing CO₂, at a temperature 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 and 96 h. The average cumulative pressure was measured for each sample. Pressure was converted to mL of gas at standard temperature and pressure and gas produced per gram DM substrate incubated used was there after calculated. Cumulative gas production data at 24 h were fitted to the model of Ørskov and McDonald (1979). Gas \( (Y) = b \left(1 - e^{-ct}\right) \), where \( b \) is the gas production from the insoluble fraction (mL), \( c \) is the gas production rate constant for the insoluble fraction (mL/h), \( t \) is the incubation time (h), \( T_{1/2} \) is the time taken to produce the half of the gas volume was calculated using equations of \( T_{1/2} = \frac{\ln 2}{c} \), \( T_{1/2} = 0.693/c \) (Menke et al. 1979). OMD %, ME₆₃ and ME₂₀₀ (MJ/kg DM) values of *J. acutus* were estimated from gas measured by *in vitro* method at 24 h by...
using the following equations (Menke & Steingass 1988):

\[
MEGP (MJ/kg DM) = 2.2 + 0.136 GP + 0.057 CP + 0.0029 EE
\]
\[
OMD (\%) = 57.2 + 0.365 GP + 0.304 CP - 1.98 ADL
\]
\[
GP (mL/200 mg DM)
\]
\[
ME_{OMD} (MJ/kg DM) = 0.16 OMD
\]
\[
OMD: \text{organic matter digestibility}
\]
\[
GP: \text{gas production}
\]
\[
ME_{GP}: \text{metabolisable energy calculated from gas production}
\]
\[
ME_{OMD}: \text{metabolisable energy calculated from organic matter digestibility}
\]

**Real-time PCR analysis**

**Pretreatment of samples with Chelex-100 for DNA extraction**

DNA isolation from rumen fluids obtained from 0, 3, 6, 12, 24, 48, 72 and 96 h incubations was carried out by applying general bacterial DNA isolation procedure (Aldrich & Cullis 1993; Yang et al. 2008). The DNA containing sample (1 mL) was centrifuged, and the supernatant was removed and discarded. The pellet was resuspended in the remaining supernatant to obtain a concentrated sample. Then, sample was mixed 200 µL Chelex-100 extraction solution. The mixture was boiled for half an hour. The samples were placed immediately on ice for 2 min. Then they were centrifuged at 13 000 rpm for 5 min. Since Chelex-100 inactivates the PCR, only the supernatant was transferred to a new Eppendorf tube and 5 µL were used for the PCR reaction.

**Real-time PCR assays**

Real-time PCR assays of isolated DNA samples were performed on C 1000 Bio-rad real-time PCR device (Bio-Rad Laboratories, Inc., Berkeley, CA). Assays were set up using the EVA Green PCR Master Mix (2X) (Seegene Technologies, Seoul, Korea).

The targeted bacteria were three predominant cellulolytic bacteria *F. succinogenes*, *R. flavefaciens* and *R. albus*. Primer for *F. succinogenes* Forward (Fs219f): 5’-GGTATGGGATGAGCTTGC-3’ Reverse (Fs654r): 5’-GCCTGC CCGTGAACCTAC-3’. *R. albus* Forward (Ralph1f): 5’-CCCTA AAAGCAGTGATTTG-3’, Reverse (Ralph3r): 5’-CCCTCCTT GCGTTAGAAA-3’ and *Fibrobacter flavefaciens* Forward (Rf154f): 5’-TCTGGAACCGATGGA-3’, Reverse (Rf425r): 5’-CCTTAAGACAGAGTTTACAA-3’. Those primers were chosen from previously published sequences that demonstrates species-specific amplification (Koike & Kobayashi 2001).

PCR conditions for *F. succinogenes* were as follows: 30 s at 94 °C for denaturing, 30 s at 60 °C for annealing and 30 s at 72 °C for extension (48 cycles), except for 9 min of denaturation in the first cycle and 10 min of extension in the last cycle. Amplification of 16s rDNA for *R. flavefaciens* and *R. 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 *J. acutus* samples which were collected from three different stations was quantified using the relative quantification ΔC*_T* (Livak & Schmittgen 2001). The mean values of each bacterium at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation time of *J. acutus* were collected from three different stations.

**Statistical analysis**

One-way analysis of variance and multiple comparisons among treatment means were performed by Duncan’s new multiple range (SAS 2007). Means differences were considered significant at *p* < 0.05.

**Results and discussion**

**Chemical analysis**

Chemical composition of *J. acutus* collected from Stations I, II and III is presented in Table 1.

There were no significant variations except CP % and ADL % (*p* > 0.05) between the chemical compositions of *J. acutus* samples collected from three different stations in Kızılirmak Delta (Table 1).

The CP contents of *J. acutus* collected from Stations I, II and III ranged from 9.77 to 10.03%.

| Constituents | Station I | Station II | Station III |
|--------------|-----------|------------|-------------|
| DM | 97.68 ± 0.23 | 97.99 ± 0.24 | 97.78 ± 0.26 |
| ASH | 4.02 ± 0.03 | 4.10 ± 0.02 | 4.21 ± 0.01 |
| OM | 93.66 ± 0.09 | 93.89 ± 0.13 | 93.57 ± 0.08 |
| CP | 10.03 ± 0.06 | 9.79 ± 0.07 | 9.77 ± 0.07 |
| EE | 1.47 ± 0.06 | 1.48 ± 0.03 | 1.46 ± 0.02 |
| NDF | 73.66 ± 0.10 | 73.70 ± 0.08 | 73.70 ± 0.10 |
| ADF | 46.98 ± 0.04 | 46.58 ± 0.11 | 46.17 ± 0.10 |
| ADL | 12.27 ± 0.06 | 12.17 ± 0.09 | 12.01 ± 0.12 |
| ME_{ADF}, MJ/kg DM | 8.61 ± 0.01 | 8.55 ± 0.02 | 8.60 ± 0.01 |

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; ME_{ADF}, Metabolisable energy calculated from ADF.

200 mg DM,

Means within a row with different superscripts differ (*p* < 0.05).
Statistically significant differences were obtained among Stations I, II and III (p<0.05). These differences may be originated from geographical status such as soil humidity, soil type and nutrient contents of soil of selected stations in delta. Station I was rich in crude protein and higher than those of the other stations. Crude protein of station III is not significantly different from station II (p>0.05). Station III was poor in crude protein and lower than those of the other stations. The CP content of J. acutus was found similar to Astragalus (Avci et al. 2012) and to apple tree leaves (Nahand et al. 2010). Juncus acutus can be used as a roughage source due to the CP % of J. acutus close to low-quality alfalfa hay and dry meadow grass. The EE contents of J. acutus ranged from 1.46 to 1.48%. The differences between EE content were not statistically significant among Stations I, II and III (p>0.05). The EE contents of J. acutus obtained in the current study were similar to found apple tree leaves (Nahand et al. 2010). It is well known that the lower ether extract content of forages refers to a decrease in digestibility of feeds (Menke & Steingass 1988). The ash contents of J. acutus ranged from 4.02 to 4.21%. The differences between ash content were not statistically significant among Stations I, II and III (p>0.05). The ash content of J. acutus was found similar to rapeseed straw (Canbolat 2013). The NDF and ADF contents of J. acutus ranged from 73.66 to 73.70% and 46.08 to 46.58% respectively. The differences between NDF and ADF contents were not statistically significant among Stations I, II and III (p>0.05). The NDF and ADF contents of J. acutus were found similar to rice straw (Rahman et al. 2010). It can be considered as good-quality roughage sources since fibrous feeds with NDF content of less than 45% of DM were classified as high-quality roughage feeds (Singh & Oosting 1992). The ADL contents of J. acutus were ranged from 12.01 to 12.27%. A statistically significant difference was observed between stations (p<0.05). ADL content of J. acutus collected from Station I was the higher than those of other stations. Station III was the lowest ADL contents in three different stations. The ADL content of J. acutus was found similar to wheat straw (Kalkan & Filia 2011). It is known that if cell-wall components in feed decreases, its digestibility increases. This reason is that it is desirable to have low NDF, ADF and ADL contents in feeds. The differences between MEADF values based on chemical analysis were not statistically significant among Stations I, II and III (p>0.05).

It is obvious that any variation in chemical composition and cell-wall components can be resulted in different nutritive values; because chemical composition is one of the most important indices of nutritive value of feedstuffs (Aghjanzadeh-Golshani et al. 2010). It could supply that geographic variations, climatic conditions, soil characteristics and extent of foreign materials (Iqbal et al. 2006; Maheri-Sis et al. 2007).

### In vitro gas production method

Cumulative $P_{psi}$/g DM, cumulative GP$_{ml}$/200 mg DM, OMD %, ME$_{OMD}$ (MJ/kg DM), ME$_{GP}$ (MJ/kg DM) and gas production parameters ($b$, $c$, $T_{1/2}$) of J. acutus collected from Stations I, II and III at 24 h incubation are presented in Table 2. Cumulative $P_{psi}$/g DM, cumulative GP$_{ml}$/200 mg DM, OMD %, ME$_{OMD}$, ME$_{GP}$, $b$, $c$ and $T_{1/2}$ were found as 7.39, 7.15 and 6.79 mL; 18.31, 17.73 and 16.87 mL; 42.64, 42.48 and 42.54%; 6.82, 6.79 and 6.81 MJ/kg DM; 5.26, 5.17 and 5.05 MJ/kg DM; 21.10, 20.45 and 21.94 mL; 0.09, 0.08 and 0.05 mL/h; 7.29, 8.33 and 12.65 h, respectively.

The cumulative volume of gas production increased with increasing incubation time (Table 2). A statistically significant difference was observed among Stations I, II and III at 24 h and 48 h of incubations (p<0.05). It may be due to different ADL contents of J. acutus collected from three stations. The decline in gas production and estimated parameters is possibly associated with an increase in cell-wall contents (NDF, ADF and ADL) of J. acutus with maturity. It is well known that cell-wall contents are more indigestible fractions of plant. The ADL contents of J. acutus were between 12.01 and 12.27% and the cumulative volume of gas production was observed between 16.87 and 18.31 mL at 24 h of incubation. Mertens et al. (1997) reported that high ADL level of feedstuffs adversely affects gas production; however, NDF content increases gas production. The cumulative volume of gas production value of J. acutus was obtained in the current study.

| Parameters | Station I | Station II | Station III |
|------------|-----------|------------|-------------|
| ME$_{OMD}$ MJ/kg DM | 6.82 ± 0.02$^a$ | 6.79 ± 0.02$^b$ | 6.81 ± 0.02$^b$ |
| ME$_{GP}$ MJ/kg DM | 5.26 ± 0.05$^a$ | 5.17 ± 0.06$^b$ | 5.05 ± 0.05$^b$ |
| b, mL | 21.1078 ± 0.2606$^{ab}$ | 20.4587 ± 0.2700$^{ab}$ | 21.9427 ± 0.4543$^{ab}$ |
| c, mL/h | 0.0950 ± 0.0034$^a$ | 0.0832 ± 0.0038$^b$ | 0.0548 ± 0.0031$^b$ |
| $T_{1/2}$ h | 7.29 ± 0.13$^c$ | 8.33 ± 0.16$^b$ | 12.65 ± 0.18$^b$ |

b, potential gas production (mL); c, the gas production rate constant for the insoluble fraction (mL/h); $T_{1/2}$, the time taken to produce the half of the total gas pool (h).

$^{ab}$Significant differences within a row with different superscripts differ (p<0.05).

Table 2. Cumulative gas production volume at 24 h (GP), potential gas production volume (b), organic matter digestibility (OMD) and metabolisable energy (ME$_{OMD}$ and ME$_{GP}$) of Juncus acutus.
Juncus acutus was found similar to wheat straw (Kalkan & Filya 2011). It is well documented that the nutrient contents of feeds affect their potential gas production and level of gas produced tends to decrease or increase with changing chemical content of feeds (Doane et al. 1997; Getachew et al. 2004; Boga et al. 2014). When the amount of crude protein is lower than 10% crude protein, the gas production levels of feeds reduce significantly.

In vitro gas production volume, kinetic parameters, metabolic energy values and OMD % are significantly affected by differences in nutrient content of J. acutus collected from three stations. The energy level and the digestibility of organic matter of J. acutus samples were determined by multivariate regression equations using gas production together with crude nutrient contents. Calculated ME<sub>GP</sub>, ME<sub>OMD</sub> and OMD % levels are presented in Table 2.

While the ME<sub>GP</sub> value of J. acutus collected from Station II was not statistically significantly different from Stations I and III in terms of ME<sub>GP</sub> (p>0.05); the differences between ME<sub>GP</sub> were statistically significant between Stations I and III (p<0.05). The reason for energy content differences between stations can be due to the difference in chemical composition (especially soluble carbohydrates, CP and NDF, and ADL) and volume of gas production (Menke & Steingass 1988; Getachew et al. 2004). There was a positive correlation between metabolic energy calculated from in vitro gas production.

The OMD % of J. acutus ranged from 42.48 to 42.64%. The OMD % value of J. acutus collected from Station I was statistically significantly different from Stations II and III in terms of OMD % (p<0.05). The OMD % of J. acutus was found similar to rice straw (Rahman et al. 2010). OMD % differences seen between stations may be associated with CP and gas production volumes. OMD % increases due to increasing gas production and CP content. In contrast, OMD % decreases due to increasing NDF, ADF and ADL contents.

The mean values of in vitro gas production volumes and kinetic parameters (b, c, and T<sub>1/2</sub>) of J. acutus from Stations I, II and III were statistically significant (p<0.05). This may be due to the major differences in the kinetic parameters of gas production, which is the difference in the amount of CP and ADL. Moreover, potential gas production, b and c values may be affected in the presence of secondary metabolites in J. acutus. Fibrous feedstuffs will tend to show lower fermentation rates and the period of fermentation of the non-soluble fraction will be more extensive due to the higher concentration of cell wall components.

Potential gas production of J. acutus was lower than that of the leaves of some exotic tree grown in Turkey and bindweed hay but c value was similar to each other (Canbolat 2012a,b). T<sub>1/2</sub> values of J. acutus calculated from Ørskov and McDonald (1979) model was similar to wheat straw, oat straw and berseem (Sing et al. 2010).

Real-time PCR analysis

Fibrobacter succinogenes, R. flavefaciens and R. albus values calculated from threshold (C<sub>T</sub>) values in rumen fluids obtained from 0, 3, 6, 12, 24, 48, 72 and 96 h incubations of J. acutus samples by real-time PCR method at three different Stations I, II and III showed an increase in the order of F. succinogenes > R. flavefaciens > R. albus (Table 3). This ranking is in agreement with reported values by Hung and Wanapat (2013) and Polyorach et al. (2014). The population of F. succinogenes compared with R. flavefaciens and R. albus was highest at three different Stations I, II and III. Furthermore, R. albus was the lowest compared with F. succinogenes and R. flavefaciens in all stations. Apparently, because F. succinogenes and R. flavefaciens can colonize the cellulose more rapidly than R. albus (Hung & Wanapat 2013), R. albus always was less abundant than that of F. succinogenes and R. flavefaciens because it was less effective in colonizing cellulose and was probably reduced to growing on soluble products released by the other species during cellulose hydrolysis (Shi et al. 1997). It is possible that dietary conditions might have influenced on reduced numbers of cellulolytic bacteria. Moreover, the nature of substrates and environmental factors such as temperature and the existence of cations and soluble carbohydrates have been suggested as factors governing bacterial attachment (Miron et al. 2001). And also the quantification of cellulolytic bacteria was affected by diet and sampling

Table 3. Fibrobacter succinogenes, Ruminococcus flavefaciens and Ruminococcus albus values 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.

| T     | Sta I | Sta II | Sta III | SEM  | Sta I | Sta II | Sta III | SEM  | Sta I | Sta II | Sta III | SEM  |
|-------|-------|--------|---------|------|-------|--------|---------|------|-------|--------|---------|------|
| 0     | 1     | 1      | 1       | 1    | 1     | 1      | 1       | 1    | 1     | 1      | 1       | 1    |
| 3     | 1.13<sup>a</sup> | 1.04<sup>b</sup> | 1.23<sup>b</sup> | 0.03 | 1.05 | 1.03 | 1.07 | 0.02 | 1.03 | 1.04 | 1.03 | 0.01 |
| 6     | 1.13<sup>a</sup> | 1.30<sup>a</sup> | 1.48<sup>a</sup> | 0.05 | 1.23 | 1.10 | 1.19 | 0.03 | 1.05 | 1.07 | 1.04 | 0.03 |
| 12    | 1.63<sup>a</sup> | 1.85<sup>a</sup> | 1.89<sup>a</sup> | 0.03 | 1.32 | 1.37 | 1.37 | 0.04 | 1.14 | 1.23 | 1.23 | 0.02 |
| 24    | 2.96<sup>a</sup> | 2.73<sup>a</sup> | 2.75<sup>a</sup> | 0.06 | 2.29<sup>a</sup> | 2.41<sup>a</sup> | 1.94<sup>a</sup> | 0.05 | 1.90<sup>a</sup> | 1.64<sup>a</sup> | 1.59<sup>a</sup> | 0.04 |
| 48    | 3.90<sup>a</sup> | 3.58<sup>a</sup> | 3.70<sup>a</sup> | 0.05 | 2.69<sup>a</sup> | 2.67<sup>a</sup> | 2.53<sup>a</sup> | 0.03 | 2.29<sup>a</sup> | 2.23<sup>a</sup> | 2.32<sup>a</sup> | 0.05 |
| 72    | 3.90<sup>a</sup> | 3.68<sup>a</sup> | 3.70<sup>a</sup> | 0.08 | 2.71<sup>a</sup> | 2.69<sup>a</sup> | 2.58<sup>a</sup> | 0.04 | 2.31 | 2.28 | 2.37 | 0.04 |
| 96    | 3.94<sup>a</sup> | 3.68<sup>a</sup> | 2.68<sup>a</sup> | 0.06 | 2.71<sup>a</sup> | 2.73<sup>a</sup> | 2.60<sup>a</sup> | 0.04 | 2.29 | 2.29 | 2.39 | 0.03 |

SEM, mean of standard error; Sta, Station; T, incubation time (h).<sup>abc</sup>Means within a row with different superscripts differ (p<0.05).
time. *Fibrobacter succinogenes* quantity in Station I was the higher than that of other stations. These differences could be based on the CP content in Station I that was the highest in the other stations. It is known that feeds must contain a minimum of 10% crude protein for the optimal activity of microbial flora (Norton 2003). Goel et al. (2007) reported that *F. succinogenes* population increased by 21% due to the use of protein source of high-level.

Gas production values of *J. acutus* at 0, 3, 6, 12, 24, 48, 72 and 96 h of incubations were compatible with *F. succinogenes*, *R. flavefaciens* and *R. albus* values calculated from threshold (C_T) values in rumen fluids obtained from 0 to 96 h.

Conclusions
As a result, the CP % of *J. acutus* was obtained higher than cereal straw and close to low-quality alfalfa hay and dry meadow grass. *Juncus acutus* increased the amount of three predominant rumen cellulolytic bacteria. In terms of OMD %, ME, MEOMD and in some amount of three predominant rumen cellulolytic bacteria. In terms of OMD %, ME, MEOMD and in some degree of CP % values of *J. acutus* may be proposed as medium-quality roughage source for ruminant nutrition.

Disclosure statement
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding information
The authors would like to extend their sincere appreciation to the Ondokuz Mayis University for Research Fund Project Q2 no. PYOVT.1904.14.003, 2014.

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