Estimation of organic matter digestibility, metabolizable energy, phenolic compounds and antioxidant activity of stems and seeds of the *Juncus acutus* plant in ruminant nutrition

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Abstract

The objectives of the present study were to determine the organic matter digestibility (OMD), metabolizable energy (ME), antioxidant activity and total phenolic and flavonoid concentrations of stems and seeds of *Juncus acutus*. Stem and seed samples were collected from Hamsiloz Bay in Sinop, Turkey, and the proximate analysis was carried on them. The OMD percentage and ME values of the samples were estimated from gas measured by the *in vitro* gas production method. Phenolic and flavonoid concentrations and total antioxidant activity were determined spectrophotometrically. Mean OMD, MEOMD and MEGP levels and gas production kinetic parameters A, c and T 1/2 of *J. acutus* stem and seed samples were 40.3% and 47.7%; 6.44 and 7.63 MJ/kg DM, 5.96 and 7.07 MJ/kg DM, 35.12 and 47.19 mL, 0.055% and 0.092%, and 12.60 and 7.53 h, respectively. Mean OMD percentage, ME and gas production kinetic parameters A, c and T1/2 of the stems were significantly different than that of the seed. The antioxidant activity, total phenolic and flavonoid concentrations of stems and seeds of *J. acutus* were 88.45 and 88.48 IC50 mg/mL, 19.70 and 40.99 mg GAE/100 g and 0.63 and 1.20 mg Qe/100 g, respectively. Mean values of total phenolic and flavonoid concentrations of stems were significantly different from that of the seeds. In conclusion, both stems and seeds of *J. acutus* may be considered alternative feed sources for ruminants. Furthermore, when *J. acutus* stems and seeds are included in ruminant diet, the phenolic compounds may contribute to the intake of natural antioxidants.

Keywords: *In vitro* gas production, *Juncaceae*, flavonoid compounds, spiny rush, sharp rush

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Introduction

*Juncus acutus* (*Juncaceae*) is a perennial plant that belongs to the genus *Juncus* and is commonly known as spiny rush or sharp rush. There are approximately 300 species of *J. acutus* and they are widely distributed throughout the world, occurring naturally in Africa, Europe and North America. It is a natural salt-tolerant plant, most abundant in wetlands, and grows under natural range conditions. Total *J. acutus* production potential of 23 wetlands of Turkey is estimated at 85 537 tons (Erdem, 2014), but there are no reports on the worldwide potential of *J. acutus*. The nutritive value of *J. acutus* has been studied in terms of chemical composition, organic matter digestibility (OMD) and metabolizable energy (ME) (Erdem, 2014), using the *in vitro* gas production method (Menke & Steingass, 1988; Blummel & Ørskov, 1993; Blummel et al., 2003). Consequently, it has been proposed as an alternative roughage source for ruminants (Erdem, 2014).

*Juncaceae* has been identified as a new source of natural antioxidants in feed (Meot-Duros et al., 2008). Because of increased concern about synthetic antioxidants that can be toxic to animal DNA, there is great interest in finding new and safe antioxidants from natural sources (Muraina, 2009). Plant phenolics include phenolics acids, flavonoids, tannins and the less common stilbenes and lignans, which are known to have antiviral, anti-inflammatory, anti-allergic and anti-carcinogenic properties (Carr et al., 2000; Dai et al., 2010). Phenolics in plants can vary from simple phenolic acids to highly polymerized substances such as tannins. They may also be associated with other plant components such as carbohydrates and proteins. There is consequently no universal extraction procedure or single method for evaluating the total antioxidant...
activity of plants (Dai et al., 2010; Swapana et al., 2013). Among the familiar methods, the 2,2 diphenyl-1-
picrylhydrazyl radical (DPPH) method is widely used owing to its stability, simplicity and simple reaction system, which involves only direct reaction between free radicals and antioxidants (Noipa et al., 2011).

Erdem (2014) had previously studied the medium part of *J. acutus* stems and reported its ME and OMD values. However, studies have not been done on antioxidant activity, and total phenolic and flavonoid concentrations of the stems and seeds of the *J. acutus* plant. The objectives of this study were to estimate and compare the nutritive value, antioxidant activity, total phenolic and flavonoid concentrations of its stems and seeds in terms of ruminant nutrition.

**Material and Methods**

Seed of *J. acutus* samples was collected randomly by hand, and stem samples were collected from 20 plants at Hamsiloz Bay, Sinop, Turkey. The stems were chopped into small pieces with garden scissors. Duplicate samples were prepared for each analysis. Samples were weighed and dried in an oven at 65 °C. After drying, all stems and seeds were ground in a mill to pass through a 1 mm screen, and kept in plastic boxes pending laboratory analysis.

Dry matter (DM) (105 °C overnight), ash (525 °C for 8 h), nitrogen (N) (Kjeldahl method), crude protein (CP = N x 6.25), ether extract (EE) and crude fibre (CF) levels of all parts were determined according to the AOAC (2006). The chemical analyses and *in vitro* gas production experiments were carried out in the Ruminant Feed Evaluation Laboratory of Department of Animal Nutrition and Nutritional Diseases and total antioxidant activity, total phenolic and flavonoid concentrations were determined at the Department of Biochemistry, Faculty of Veterinary Medicine, OMU, Samsun, Turkey.

At Florya Farm, beef cattle were fed grass hay (100 g CP/kg; 6 MJ ME/kg DM) ad libitum, as well as 10 kg of a compound feed in the morning and evening. The compound feed contained 140 g CP, 11 MJ ME, 10 - 20 g Ca, 5 g P, 10 000 IU retinol, 2000 IU vitamin D3 and 30 mg α-tocopherol per kg DM. Rumen fluid was collected in preheated thermos flasks from three cattle that had been freshly slaughtered before morning feeding at the Florya Meat Joint-Stock Company, Samsun, Turkey. The collected rumen fluid was transferred to the laboratory within 10 minutes, and used the *in vitro* gas production (ANKOM, 2011), as follows: Approximately 1 g sample was transferred to a 250 mL glass jar (module) and incubated at 39 °C overnight. The module tops contained a communication system with a computer, and data were recorded automatically. A rumen-buffer mixture was prepared according to the Menke & Steingass (1988) method: It was done under anaerobic conditions by continuously flushing CO2 at 39 °C and keeping the pH between 6.4 and 6.7. The buffer was mixed with rumen fluid at a ratio of 4 : 1, and 100 mL of this fluid were added to a preheated glass jar containing the feed samples. It was then closed and put into a shaking waterbath. The incubation per sample was done in triplicate. The average cumulative pressure was recorded for each sample at 10 min intervals. Recorded pressure values as psi were converted to mL gas production. Total mL gas produced from 1 g sample was adjusted to 200 mg stems and seeds of *J. acutus*, to estimate OMD and ME using the equations of Menke & Steingass (1988):

\[
\text{Gas} \ (Y) = A \ (1-e^{-ct}),
\]

Where: \( A \) = the total gas production (mL),
\( c \) = the gas production rate constant (%),
\( t \) = incubation time (h).

\( T_{1/2} \) = the time taken to produce 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). The OMD %, MEGP, and ME\textsubscript{OMD} (MJ/ kg DM) values of *J. acutus* were estimated from gas measured by the *in vitro* method at 24 h using the equations of Menke & Steingass (1988):

\[
\text{MEGP (MJ/kg DM)} = 2.2 + 0.136 \ \text{GP} + 0.057 \ \text{CP} + 0.0029 \ \text{EE}
\]
\[
\text{OMD} \ (%) = 14.88 + 0.889 \ \text{GP} + 0.45 \ \text{CP} + 0.0651 \ \text{CF}
\]
\[
\text{GP (mL/200 mg DM)}
\]
\[
\text{MEOMD (MJ/kg DM)} = 0.16 \ \text{OMD}
\]

Total antioxidant activity and free radical scavenging activity of the stem and seed samples were determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Meda et al., 2005; Dimins et al., 2010). The absorbance was measured at 520 nm. Quercetin (0 - 50 mg/L) and ascorbic acid (0 - 40 mg/L) were used as positive controls. The radical scavenging activity was calculated as follows:
Inhibition % = [(blank absorbance - sample absorbance)/blank absorbance] x 100

The mean concentrations for stem and seed samples were calculated from three readings using 50% inhibition values (IC50).

The Folin-Ciocalteu method was used to determine total phenolic concentration. The absorbance of the reaction mixture was measured at 750 nm against a methanol blank. Gallic acid (0 - 200 mg/L) was used as standard to produce the calibration curve. The mean of three readings was used to estimate the total phenolic concentration and the results were expressed as mg of gallic acid equivalents (GAE)/100 g of stem or seed samples (Meda et al., 2005; Lin et al., 2007).

The total flavonoid concentration was determined using a standard curve with quercetin (0 - 50 mg/L). The absorbance of the reaction mixture was determined at 415 nm against a methanol blank. The mean of three readings was used to estimate the total flavonoid concentration, and the results were expressed as mg of quercetin equivalents (QE)/100 g of the stem or seed samples (Meda et al., 2005; Lin et al., 2007).

The data obtained from the chemical analysis and in vitro gas production were analysed with the software package SAS (2007). Differences between mean values of seed and stem samples were obtained using the t-test.

Results and Discussion

The chemical composition of the stems and seeds of *J. acutus* is presented in Table 1. The CP and EE levels of the seeds were higher than that of the stems (P<0.01). The CP level of the seeds was similar to levels reported for safflower seed by Koyama et al. (2009), for apple pomace by Mirzaei-Aghsaghali et al. (2011) and pomegranate seed by Taher-Maddah et al. (2012). The chemical composition of *J. acutus* seeds showed that these seeds could supply moderate concentrations of protein. The EE level of the *J. acutus* seeds was similar to that reported for bambara groundnut (Ajayi et al., 2010). The ash and crude fibre levels of the stems were higher (P<0.01) than that of the seeds, but the ash content of the seeds was similar to those reported for apple pomace by Mirzaei-Aghsaghali et al. (2011) and Muiumba seeds by Rodrigues et al. (2014). The crude fibre level of the *J. acutus* stems was similar to that reported for low-quality lucerne hay (Gungor, 2008) and maize cobs (Akinfemi et al., 2009).

| Juncus acutus | Stem (n = 20) (x ± S_ε) | Seed (n = 20) (x ± S_ε) |
|---------------|------------------------|------------------------|
| Dry matter    | 438.9 ± 0.5            | 427.5 ± 0.6            |
| Crude protein | 51.0^b ± 2.7           | 93.1^a ± 1.4           |
| Ether extract | 17.2^b ± 2.4^b         | 27.8^a ± 1.2           |
| Ash           | 47.9^a ± 1.0           | 26.4^b ± 0.7           |
| Crude fibre   | 295.7^a ± 2.7^a        | 184.5^b ± 2.1          |

Table 1: Chemical composition (g/kg DM) of stems and seeds of *Juncus acutus*

Mean cumulative gas production volume at 24 h (GP_{ml}/200 mg DM), OMD %, ME_{OMD} (MJ/kg DM), ME_{GP} (MJ/kg DM) and gas production parameter (A, c and T_{1/2}) of the stems and seeds are presented in Table 2. The gas volume at 24 h incubation, OMD, ME_{OMD}, ME_{GP} and gas production kinetics (A and c) of the seeds were higher than those of the stems (P<0.05). The cumulative GP_{ml}/200 mg DM of the stems was similar to that reported for ensiled pomegranate seeds by Taher-Maddah et al. (2012). However, the cumulative GP_{ml}/200 mg DM of *J. acutus* seeds was similar to those reported for cottonseeds (Nezarati et al., 2014). ME_{GP} of *J. acutus* seeds was similar to those reported for guinea corn threshed top (Akinfemi et al., 2009), *Moringa stenopetala* seeds (Melesse, 2012) and *Trifolium alexandrinum* (Boga et al., 2014). It was proven that gas volume after 24 h incubation was correlated with the ME of feedstuffs (Menke & Steingass, 1988; Chen et al., 2011). Gas production volume is considered an indication of carbohydrate degradation. In addition, gas production is a good parameter from which to estimate OMD, fermentation product and microbial protein synthesis of the substrate in the rumen (Sallam, 2005). The OMD % of *J. acutus* seeds was
similar to those reported for maize cobs (Akinfemi et al., 2009), Lima bean (Ajayi et al., 2010) and grass pea seeds (Riasi et al., 2014). The OMD might be affected by plant variety and proportion of cell wall concentration. Furthermore, Kilic & Garipoglu (2009) reported that in vitro OMD has a high relationship with gas volume and gas production rate. MEOMD of the J. acutus seeds was similar to that reported for J. acutus stems by Erdem (2014). Potential gas production (A) of the J. acutus stems was similar to that reported for chickling vetch (Seifdavati & Taghizadeh, 2012) and Moringa stenopetala seeds (Melesse, 2012). The rate constant of gas production (c) of the J. acutus stems was similar to that reported for apple pomace by Mirzaei Aghsaghali et al. (2011) and cottonseed meal by Nezarati et al. (2014). High fermentation rates indicate high nutrient availability for rumen microorganisms. Besides, a high NDF level in feedstuff may be because of a low fermentation rate (Fievez et al., 2005). The T1/2 value of J. acutus stems calculated from the Ørskov & McDonald (1979) model was higher than that of J. acutus seeds, though the T1/2 value of J. acutus stems was similar to those reported for wheat straw, oat straw and berseem by Sing et al. (2010).

Table 2  Mean (± SE) cumulative gas production volume at 24 h (GP, mL/200 mg DM), organic matter digestibility (OMD %), metabolizable energy (MEOMD, MJ/kg DM) estimated from OMD and ME estimated from (GP MEGP, MJ/kg DM) of stems and seeds of Juncus acutus

| Juncus acutus        | Stem (n = 20) | Seed (n = 20) |
|----------------------|--------------|--------------|
| Gas production       | 25.6 ± 1.21  | 31.9 ± 0.90  |
| Organic matter digestibility | 40.3 ± 1.07  | 47.7 ± 0.94  |
| MEOMD                | 6.44 ± 0.17  | 7.63 ± 0.15  |
| ME GP                | 5.96 ± 0.18  | 7.07 ± 0.15  |
| A                    | 35.1 ± 0.60  | 47.2 ± 0.66  |
| c                    | 0.055 ± 0.0027 | 0.092 ± 0.0034 |
| T1/2                 | 12.6 ± 0.11  | 7.53 ± 0.15  |

a,b Row means with different superscripts differ significantly at P <0.05.

In the present study the total flavonoid and phenolic concentrations, total flavonoid : phenolic ratio and total antioxidant activity of the J acutus stems and seeds are shown in Table 3. Total phenolic concentration of the seeds was higher than that of the stems (P <0.01), though the concentrations of the stems and seeds were lower than those reported for Vicia fava L. (Baginsky et al., 2013), Onopordon acanthium L. seeds (Zare et al., 2014), Trigonella foenum graecum seeds (Seasotiya et al., 2014) and Albizia lebbeck and Cicer arietinum seeds (Imran et al., 2014). Total phenolic concentrations of J. acutus seeds and stems were higher than those reported for some varieties of potatoes (Solanum tuberosum L.) (Hesam et al., 2012). The phenol concentration of plants depends on intrinsic and extrinsic factors (Fratani et al., 2007).

The total flavonoid concentration of J. acutus seeds was higher than that in other parts of the plant (P <0.01). Total flavonoid concentrations of the J. acutus stems and seeds were lower than those reported for safflower (Carthamus tinctorius) varieties (Jawahra and 104) flowers (Salem et al., 2014), Brassica seeds (Bors et al., 2014) and Ginkgo biloba leaf (Yang et al., 2015). Total flavonoid concentrations of J. acutus stems and seeds were higher than those reported for Pseudarthria viscosa root (Fabaceae) and Hygrophila schulli by Sulaiman & Balachandran (2012). Flavonoids are polyphenolic compounds that play an important role in balancing lipid oxidations, and are associated with antioxidant activity (Yen et al., 1993).

The total flavonoid : phenolic ratio of J. acutus stems was similar to that of the seeds. This ratio in J. acutus stems and seeds was similar to that reported for Nerium oleander leaf (Srivistava et al., 2013). The ratio of total flavonoid : phenolic of J. acutus stems and seeds was higher than that reported for Oroxyllum indicum L. bark (Sulaiman & Balachandran, 2012). The flavonoid : phenolic ratio shows the importance of flavonoids in total phenolic content and its antioxidant activity. Phytochemical concentration and antioxidant potential in plant extracts are affected by factors such as parts of plant, types of solvents, method of extraction and variety in plant material (Tiwari et al., 2011).
Table 3: Mean (± SE) total flavonoid (mg Qe/100 g), total phenolic (mg GAE/100 g), the ratio of total flavonoid: phenolic and total antioxidant activity (IC50 mg/mL) of Juncus acutus stems and seeds

|                      | Juncus acutus |
|----------------------|--------------|
|                      | Stem (n = 20) | Seed (n = 20) |
| Total flavonoid      | 0.63b ± 0.04  | 1.20a ± 0.04  |
| Total phenolic       | 19.70b ± 1.11 | 40.99a ± 0.77 |
| Total flavonoid / total phenolic | 0.031 ± 0.001 | 0.029 ± 0.001 |
| Total antioxidant activity | 88.5 ± 0.84  | 88.5 ± 0.79   |

a,b Row means with different superscripts differ significantly at P < 0.01.

n: number of sample.

Total antioxidant activities of the stems and seeds of J. acutus were similar. The total antioxidant activity and free radical scavenging activity of J. acutus stems and seeds were better than those reported for Morus nigra seeds by Shukla et al. (2014) and Trigonella foenum graecum seeds (Seasotiya et al., 2014). Total antioxidant activities of J. acutus seeds and stems were quite close to that reported for some varieties of potato (Solanum tuberosum L.) (Hesam et al., 2012). In general, plants with a high phenolic content show a high antioxidant activity. Therefore, there is a positive correlation between total phenolic compound and antioxidant activity (Chanda & Dave, 2009). However, some researcher reported no correlation between phenolic content and antioxidant capacity (Yu et al., 2002; Norshazila et al., 2010). Souri et al. (2008) found that there was no significant correlation between antioxidant activity and phenolic content of the studied plants. However, nonphenolic components in plants such as trace elements can reduce the antioxidant activity of the phenolic compounds (Vinson et al., 1998). The antioxidant activity of a plant extract cannot be based only on its phenolic content, but must include its chemical characterization. It is important to know the character of the phenolic compound in each plant extract to assign antioxidant activities. The phenols could show activity synergistically with nonphenolic compounds. For this reason, phenolic compounds would not be the only ones responsible for the antioxidant activity (Onyeneho & Hettiarachchy, 1992). The high total phenolic concentration in some plant extracts may be due to the presence of saponin (Grover et al., 2001), amino acid (Uchikoba et al., 1998) and triterpenoids (Shih et al., 2005). Because of these, phenolic concentrations of plant are not always good indicators of antioxidant capacity. The seeds of J. acutus showed a similar antioxidant activity to the stems. However, the total phenolic concentration of seeds was higher than that of the stems. From these results it can be inferred that the seed of J. acutus contains high molecular weight phenolic compounds. Paixaa et al. (2007) reported that DPPH is known to react specifically with low molecular weight phenolic compounds. Therefore, the molecular weight for each individual phenolic compound of J. acutus stems and seeds must be identified and estimated.

Although the nutritive value (chemical composition, gas production parameters, OMD and ME values) of J. acutus seeds was better than that of the stems, both parts may be used as alternative feedstuffs for ruminants. Total antioxidant activities of the stems and seeds were found to be similar, while the total phenolic and total flavonoid concentrations of the seeds were higher than those of the stems. Both parts of J. acutus contain considerable amounts of phenolic and flavonoid and are good sources of antioxidants.

Conclusion
Both stems and seeds of J. acutus may be considered as alternative feed sources for ruminants. The use of these stems and seeds as an energy and protein source in ruminant nutrition would provide phenolic compounds to display antioxidant value.

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