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

F. Erdem[1#], N. Cetinkaya[2], C. Nisbet[3] & E. Altin[3]

[1] Department of Molecular Microbiology, Public Health Laboratory, Ministry of Health, 55060 Samsun, Turkey
[2] Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ondokuz Mayis University, 55139 Samsun, Turkey
[3] Department of Biochemistry, Faculty of Veterinary Medicine, Ondokuz Mayis University, 55139 Samsun, Turkey

(Received 6 February 2015; Accepted 29 October 2015; First published online 4 December 2015)

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, ME_{OMD} and ME_{GP} 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 $T_{1/2}$ 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
[#] Corresponding author: fundaerdemtr@gmail.com

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

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v45i5.7

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 ºC. 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 ºC overnight), ash (525 ºC 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 D_3 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 ºC 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 CO_2 at 39 ºC 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 $T_{1/2}$ = Ln2/c, $T_{1/2}$ = 0.693/c (Menke *et al.,* 1979). The OMD %, $MEGP_{,}$ and ME_{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):

ME_{GP} (MJ/kg DM) = 2.2+0.136 GP+0.057 CP+0.0029 EE
OMD (%) = 14.88+0.889 GP+0.45 CP+ 0.0651 CF
GP (mL/200 mg DM)
ME_{OMD} (MJ/kg DM) = 0.16 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 results were expressed in 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).

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

	Juncus acutus	
	Stem (n = 20) ($\bar{x} \pm S_{\bar{x}}$)	**Seed (n = 20) ($\bar{x} \pm S_{\bar{x}}$)**
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

^{a,b}Row means with different superscripts differ significantly at P <0.01.
n: number of sample; SE: standard error.

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 that 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. ME_{OMD} 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 $T_{1/2}$ value of *J. acutus* stems calculated from the Ørskov & McDonald (1979) model was higher than that of *J. acutus* seeds, though the $T_{1/2}$ 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 (ME_{OMD}, MJ/kg DM) estimated from OMD and ME estimated from (GP ME_{GP}, MJ/kg DM) of stems and seeds of *Juncus acutus*

	Juncus acutus	
	Stem (n = 20)	**Seed (n = 20)**
Gas production	$25.6^{b} \pm 1.21$	$31.9^{a} \pm 0.90$
Organic matter digestibility	$40.3^{b} \pm 1.07$	$47.7^{a} \pm 0.94$
ME_{OMD}	$6.44^{b} \pm 0.17$	$7.63^{a} \pm 0.15$
ME_{GP}	$5.96^{b} \pm 0.18$	$7.07^{a} \pm 0.15$
A	$35.1^{b} \pm 0.60$	$47.2^{a} \pm 0.66$
c	$0.055^{b} \pm 0.0027$	$0.092^{a} \pm 0.0034$
$T_{1/2}$	$12.6^{a} \pm 0.11$	$7.53^{b} \pm 0.15$

^{a,b} Row means with different superscripts differ significantly at $P < 0.05$.
n: number of sample; A: potential gas production (mL); c: gas production rate constant for the insoluble fraction (%); $T_{1/2}$: time taken to produce half of the total gas pool (h).

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 (Fratianni *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 (Jawhara 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 viscida* 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 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 *Oroxylum 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	0.63^{b} ± 0.04	1.20^{a} ± 0.04
Total phenolic	19.70^{b} ± 1.11	40.99^{a} ± 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.

Acknowledgements

The authors would like to extend their sincere appreciation to the Ondokuz Mayis University for Research Fund Project number: OMU/ PYO.VET.1906.011.004.

References

Ajayi, F.T., Akande, S.R., Odejide, J.O. & İdowu, B., 2010. Nutritive evaluation of some tropical under-utilized grain legume seeds for ruminant's nutrition. J. Am. Sci. 7, 1-7.

Akinfemi, A., Adesenya, A.O. & Aya, V.E., 2009. Use of an *in-vitro* gas production technique to evaluate some Nigerian feedstuffs. American-Eurasian J. Sci. Res. 4, 240-245.

ANKOM, 2011. RF Gas Info. pdf. Available at http://www.ankom.com[Verified 27 February 2012].

AOAC, 2006. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Inc., Arlington, Virginia, USA.

Baginsky, C., Pena-Neira, A., Caceres, A., Hernandez, T., Estrella, I., Morales, H. & Pertuze, R., 2013. Phenolic compound composition in immature seeds of fava bean (*Vicia faba* L.) varieties cultivated in Chile. J. Food Compos. Anal. 31, 1-6.

Blummel, M. & Ørskov, E.R., 1993. Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting of food intake in cattle. Anim. Feed Sci. Technol. 40, 109-119.

Blummel, M., Karsli, A. & Russell, J.R., 2003. İnfluenceof diet on growth yields of rumen micro-organisms *in vitro* and *in vivo*: influence on growth yield of variable carbon fluxes to fermentation products. Br. J. Nutr. 90, 625-634.

Boga, M., Yurtseven, S., Kilic, U., Aydemir, S. & Polat, T., 2014. Determination of nutrient contents and *in vitro* gas production values of some legume forages grown in the Harran plain saline soils. Asian Australas. J. Anim. Sci. 27, 825-831.

Bors, M.D., Socaci, S., Tofană, M., Muresan, V., Pop, A.V., Nagy, M. & Vlaic, R., 2014. Determination of total phenolics, flavonoids and antioxidant capacity of methanolic extracts of some brassica seeds. Food Sci. Technol. 71, 205-206.

Carr, A.C., Zhu, B.Z. & Frei, B., 2000. Potential anti-artherogenic mechanism of ascorbate (vitamin C) and alpha-tocopherol (vitamin E). Circ. Res. 87, 349-354.

Chanda, S. & Dave, R., 2009. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. Afr. J. Microbiol. Res. 3, 981-996.

Chen, Y., Zhao, Y., Zhu-Yan, F., Zhao-Wei, M., Fa-Chen, Q., Anwaier, A., Bin, Y., Reyanggu, A., Xiao-Li, X. & Aniwaer, A., 2011. Chemical composition and *in vitro* ruminal fermentation characteristics of tetraploid black locust (*Robinia pseudoacacia* L.). Asian J. Anim. Vet. Adv. 6, 706-714.

Dai, J. & Mumper, R.J., 2010. Plant Phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15, 7313-7352.

Dimins, F., Kuka, P. & Augspole, I., 2010. Characterisation of honey antioxidative properties. International Conference of Food Innova. 28-29 Oct., Latvia.

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

Fievez, V., Babayemi, O.J. & Demeyer, D., 2005. Estimation of direct and indirect gas production in syringes: A tool to estimate short chain fatty acid production that requires minimal laboratory facilities. Anim. Feed Sci. Technol. 123-124, 197-210.

Fratianni, F., Tucci, M., De-Palma, M., Pepe, R. & Nazzaro, F., 2007. Polyphenolic composition in different parts of some cultivars ofglobe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) *Fiori*). Food Chem. 104, 1282-1286**.**

Grover, J.K., Adiga, G., Vats, V. & Rathi, S.S., 2001. Extracts of *Benicasa hispida* prevent development of experimental ulcers. J. Ethnopharmacol. 78, 159-164.

Gungor, T., Basalan, M. & Aydogan, I., 2008. The determination of nutrient contents and metabolizable energy levels of some roughages produced in Kirikkale region. J. Fac. Vet. Med., Ankara Univ. 55, 111-115.

Hesam, F., Balali, G.R. & Tehrani, R.T., 2012. Evaluation of antioxidant activity of three common potato *(Solanum tuberosum)* cultivars in Iran. Avicenna J. Phytomed. 2, 79-85.

Imran, I., Zia-Ul-Haq, M., Calani, L., Mazzeo, T. & Pellegrini, N., 2014. Phenolic profile and antioxidant potential of selected plants of Pakistan. J. Appl. Bot. Food Qual. 87, 30-35.

Kilic, U. & Garipoglu, A.V., 2009. *In situ* rumen degradability, *in vitro* digestibility and *in vitro* gas production of full fat canola seeds. Asian J. Anim. Vet. Adv. 4, 200-208.

Koyama, N., Suzuki, K., Furukawa, Y., Arisaka, H., Seki, T., Kuribayashi, K., Ishii, K., Sukegawa, E. & Takahashi., 2009. Effects of safflower seed extract supplementation on oxidation and cardiovascular risk markers in healthy human volunteers. Br. J. Nutr. 101, 568-575.

Lin, J.Y. & Tang, C.Y., 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 101, 140-147.

Meda, A., Lamien, C.E., Romito, M., Millogo, J. & Nacoulma, O.G., 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well at their radical scavenging activity. Food Chem. 91, 571-77.

Melesse, A., 2012. Assessing the feeding values of leaves, seeds and seeds-removed pods of Moringa stenopetala using *in vitro* gas production technique. Afr. J. Biotechnol. 11, 11342-11349.
Menke, K.H. & Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in-vitro* gas production using rumen fluid. Anim. Res. Dev. 28, 7-55.
Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. & Schneider, W., 1979. The estimation of the digestibility and metabolisable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor. J. Agric. Sci. 93, 217-222.
Meot-Duros, L., Le Floch, G. & Magne, C., 2008. Radical scavenging, antioxidant and antimicrobial activities of halophytic species, J. Ethnopharmacol. 116, 258-262.
Mirzaei-Aghsaghali, A., Maheri-Sis, N., Mansouri, H., Razeghi, M.E., Shayegh, J. & Aghajanzadeh-Golshani, A., 2011. Evaluating nutritional value of apple pomace for ruminants using *in vitro* gas production technique. Ann. Biol. Res. 2, 100-106.
Muraina, I.A., Suleiman, M.M. & Eloff, J.N., 2009. Can MTT be used to quantify the antioxidant activity of plant extracts? Phytomedicine 16, 665-668.
Nezarati, S, Maheri-Sis, N, Salamatdoust-Nobar, R. & Aghajanzadeh-Golshani, A., 2014. *In vitro* fermentation characteristics and nutritive value of Iranian oil seed meals for ruminants. Greener J. Biol. Sci. 4, 53-58.
Noipa, T., Srijaranai, S., Tuntulani, T. & Ngeontae, W., 2011. New approach for evaluation of the antioxidant capacity based on scavenging DPPH free radical in micelle systems. Food Res. Int. 44, 798-806.
Norshazila, S., Syed Zahir, I., Mustapha Suleiman, K., Aisyah, M.R. & Kamarul Rahim, K., 2010. Antioxidant levels and activities of selected seeds of Malaysian tropical fruits. Malays. J. Nutr. 16, 149-159.
Onyeneho, S.N. & Hettiarachchy, N.S., 1992. Antioxidant activity of durum wheat bran. J. Agric. Food Chem. 40, 1496-1500.
Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurement weighed according to rate of passage. J. Agric. Sci. 92, 499-503.
Paixão, N., Perestrelo, R., Marques, J.C. & Câmara, J.S., 2007. Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. Food Chem. 105, 204-214.
Riasi, A., Golizadeh, M., Fathi, M.H., Asadzadeh, N. & Taghizadeh, A., 2014. Determination of the nutritive value of unheated vs. heat processed grass pea seed in ruminants. J. Agric. Sci. Technol. 16, 527-536.
Rodrigues, M.A.M., Lourenco, A.L., Cone, J.W., Numes, F.M., Santos, A.S., Cordeiro, J.M.M., Guades, C.M.V. & Ferreira, L.M.M., 2014. Evaluation of the nutritive value of muiumba *(Baikiaea plurijuga)* seeds: chemical composition, *in vitro* organic matter digestibility and *in vitro* gas production. SpringerPlus. 3, 311.
Sallam, S.M.A., 2005. Nutritive value assessment of alternative feed resources by gas production and rumen fermentation *in-vitro*. Res. J. Agric. Biol. Sci. 1, 200.
Salem, N., Msaada, K., Dhifi, W., Sriti, J., Mejri, H., Limam, F. & Marzouk, B., 2007. Effect of drought on safflower natural dyes and their biological activities. EXCLI Journal 2014, 13, 1-18.
SAS, 2007. SAS Statistic Software, SAS campus DRİVE. Cary NC, USA.
Seasotiya, L., Siwach, P., Bai, S., Malik, A., Bhartı, P. & Dalal, S., 2014. Free radical scavenging activity, phenolic contents and phytochemical analysis of seeds of *Trigonella foenum graecum.* Asian Pac. J. Health Sci. 1, 219-226.
Seifdavati, J. & Taghizadeh, A., 2012. Effect of moist heat on *in-vitro* gas production parameters of some of legume seeds. Journal of Petroleum and Gas Exploration Research 2, 61-68.
Shih, P.W., Lai, P.L. & Jen, H.W.K., 2006. Antioxidant activities of aqueous extracts of selected plants. Food Chem. 99, 775-783.
Shukla, R.K., Painuly, D., Shukla, A., Singh, J., Porval, A. & Vats, S., 2014. *In vitro* biological activity and total phenolic content of *Morus nigra* seeds. J. Chem. Pharm. Res. 6, 200-210.
Sing, B., Tomar, S.K. & Kundu, S.S., 2010. *In vitro* gas production technique for feed evaluation. 1. Ed: Karnal-132 001, Haryana, İndia İntech Printers & Publishers #.353, Mughal Canal Market. 1-115.
Souri, E., Amin, G., Farsam, H. & Barazandeh Tehrani, M., 2008. Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. DARU J. Pharmaceutical Sci. 16, 83-87.
Srivastava, M.P., Tiwari, R. & Sharma, N., 2013. Assessment of phenol and flavonoid content in the plant materials. J. New Biol. Reports. 2, 163-166.
Sulaiman, C.T. & Balachandran, I., 2012. Total phenolics and total flavonoids in selected Indian medicinal plants. Indian J. Pharm. Sci. 74,258-260.
Swapana, N., Lokendrajit, N., Warjeet, S., Laitonjam, C. & Singh, B., 2013. Antioxidant activities of the rhizomes of different Zingiberaceae plants of north-east India. Asian J. Biol. Life Sci. 2, 19-22.

Taher Maddah, M., Maheri-Sis, N., Salamatdoustnobar, R. & Ahmadzadeh, A., 2012. Estimating fermentation characteristics and nutritive value of ensiled and dried pomegranate seeds for ruminants using in vitro gas production technique. Open Vet. J. 2, 40-45.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. & Kaur, H., 2011. Phytochemical screening and extraction: A review. International Pharmaceutical Sciencia 1, 98-106.

Uchikoba, T., Yonezawa, H. & Kaneda, M., 1998. Cucumisin like protease from the sarcocarp of *Benincasa hispida* var. Ryukyu. Phytochem. 4, 2215-2219.

Vinson, J.A., Hao, Y., Su, X. & Zubik, L., 1998. Phenol antioxidant quantity and quality in foods: Vegetables. J. Agric. Food Chem. 46, 3630-3634.

Yang, D., Wang, X.Y., Gan, L.J., Zhang, H., Shin, J.A., Lee, K.T. & Hong, S.T., 2015. Effects of flavonoid glycosides obtained from a Ginkgo biloba extract fraction on the physical and oxidative stabilities of oil-in-water emulsions prepared from a stripped structured lipid with a low omega-6 to omega-3 ratio. Food Chem. 174, 124-131.

Yen, G.C., Duh, P.D. & Tsai, C.L., 1993. Relationship between antioxidant activity and maturity of peanut hulls. J. Agric. Food Chem. 41, 67-70.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J. & Qian, M., 2002. Free radical scavenging properties of wheat extracts. J. Agric. Food Chem. 50, 1619-1624.

Zare, K., Nazemyeh, H., Lotfipour, F., Farabi, S., Ghiamirad, M. & Barzegari, A., 2014. Antibacterial activity and total phenolic content of the *Onopordon acanthium* L. seeds. Pharmaceutical Sci. 20, 6-11.