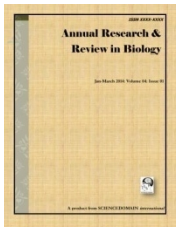

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In vitro Rumen Fermentation and Gas Production: Influence of Different by-product Feedstuffs

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To determine the chemical composition and estimation of nutritive value of different by-product feedstuffs (BPF) using in vitro gas production technique.
Place and Duration of Study: Department of Animal Science, between February 2013 and June 2013.
Methodology: In an anaerobic batch culture system, 50 ml of buffered rumen fluid was dispensed into a 125-ml serum bottle containing 0.2 g dry matter (DM) of the experimental treatments. Experimental treatments included five by-products (pomegranate peel and seed, apple pomace, walnut hull, almond hull). All bottles were purged with anaerobic CO_2, sealed and placed in a shaking water bath for 96 h at 38.6ºC. Gas production of each bottle was recorded at 3, 6, 9, 12, 24, 36, 48, 72 and 96h of the incubation and then gas released. The batch cultures were repeated in three incubation runs. The biomass residues were centrifuged and the pellet was dried at 65ºC for the determination of the residual DM and in vitro DM disappearance (IVDMD).
Results: The total tannins and phenol content were higher (P<0.01) in almond hull and pomegranate peel than in the other BPF. The total tannins content ranged from 0.34% in apple pomace to 9.78% in almond hull. The total phenol of pomegranate peel, pomegranate seed, apple pomace, walnut hull and almond hull were 10.9, 1.20, 0.76, 3.80,

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and 10.6%, respectively. The rate (c) and cumulative gas volume (b) was significantly higher ($P<0.01$) for apple pomace than the other feedstuffs. There were significant differences ($P<0.01$) among feedstuffs about lag time. Apple pomace showed higher ($P<0.01$) organic matter digestibility (OMD), in vitro dry matter digestibility (IVDMD), metabolizable energy (ME), short chain fatty acid (SCFA) and lower ($P<0.01$) pH than the other feedstuffs.

Conclusion: The higher values obtained for the potential gas production in apple pomace will indicate a better nutrient availability for rumen microorganisms ($P<0.01$).

Keywords: Almond hull; apple pomace; pomegranate peel; pomegranate seed; walnut hull.

1. INTRODUCTION

Most by-product feedstuffs (BPF) result from the processing of commercial crops, the food processing industry, and the fiber industry [1]. Increased disposal costs in many parts of the world lead to increase interest in BPF as alternative feeds for ruminants [2]. By-products feedstuffs utilization as animal feed not only lowers feed shortage in the area but also reduce the risk of environmental pollution [2,3]. While some by-products have been successfully fed for decades (e.g., corn distiller's grains or sugar beet pulp), other by-products (e.g., apple pomace, pomegranate hull, almond hull and walnut hull) are not consistently used because of the uncertainty regarding availability, poor handling characteristics, storage properties, palatability or intake [1]. Increasing agricultural industrial units for producing pomegranate juice leads to the accumulation of pomegranate peel and the annual production of this by-product approximately 120,000 metric tons in Iran [3]. Pomegranate fruit is consists of three parts: the seeds, the juice and the peels which include the husk and interior network membranes [4]. Apple pomace is the main by-product resulting from apple juice processing and containing peel, seeds and solid parts [5]. Apple pomace is a rich source of pectin besides other nutrients like carbohydrates, dietary fibres, minerals and vitamin C [5]. Almond hull is the by-product obtained by drying that portion of the almond fruit that surrounds the hard shell [6]. Low moisture content makes almond hull attractive for livestock feed by reducing transportation costs and allowing for long-term storage [6]. Walnut processing yields several by-products (walnut meal and hull). A fibrous product called walnut hull possibly a mixture of kernel particles and ground shells [7]. There is a very little information available regarding the nutritive value of pomegranate peel, pomegranate seed, apple pomace, almond hull and walnut hull produced in Iran. The aim of this study was to determine the chemical composition including tannin content of BPF and gas production characteristics using in vitro gas production technique.

2. MATERIALS AND METHODS

2.1. Samples Collection and Treatments

Fresh BPF (pomegranate peel, pomegranate seed, apple pomace, walnut hull, almond hull) samples were collected from the agro-industrial unites in Khorasan-Razavi province, Iran. Air-dry samples were ground in a Wiley mill to pass a 1-mm screen. Chemical analysis and in vitro gas production (GP) evaluated at the laboratories of Department of Animal Science, Ferdowsi University of Mashhad, Iran. The by-product samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extracts (EE) and ash by standard procedures [8]. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined according to Van Soest et al. [9]. The total phenolics (TP) content was determined according to the Folin-Ciocalteu assay and total tannins were determined by methods previously described [10]. Tannic acid (Merck GmbH, Darmstadt, Germany) was used as the standard to express the amount of total phenols and total tannins.

Rumen fluid was collected manually before the morning feeding from three adult ruminallyfistulated sheep (49.5 ± 2.5 kg, body weight) and was maintained at 39ºC until strained through 3 layers of cheesecloth. Animals were fed 0.6 kg of alfalfa hay and 0.4 kg of concentrate (24% corn grain, 20.4 barley grains, 27% soybean meal, 13.8% canola meal, 13.8% wheat bran, 0.3% calcium carbonate, 0.5% mineral and vitamin premix, and 0.2% salt) mixture. The filtrated rumen fluids from the three animals were mixed in equal proportion and held under CO_2 in a water-bath at 39ºC prior to in vitro inoculation. Procedure of in vitro batch culture was performed according to the Menke and Steingass [11]. In an anaerobic condition, 50 ml of buffered rumen fluid was dispensed with pipetor pump into a 125-ml serum bottle containing 0.2 g DM of the experimental treatments. Treatments were: 1) pomegranate peel, 2) pomegranate seed, 3) apple pomace, 4) walnut hull, and 5) almond hull. For each treatment, 200 mg of the sample was placed in a bottle (5 bottles per treatment) each filled with 50 ml of buffered rumen fluid [ratio of buffer to rumen fluid was [2:1]. Bottles were gently shaken after each recording.

All bottles were purged with anaerobic CO_2 for 5 second, sealed with rubber stoppers and aluminums caps and placed in a shaking water bath for 96 h at 39ºC. To prevent accumulation of gas produced, head space gas pressure of each bottle was recorded using a pressure transducer [12] at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of the incubation and then gas released. The batch cultures were repeated in three incubation runs. Gas pressure was measured manually by using a digital pressure gauge (model SEDPGB-0015-PG5 sensor unit, SenSym, Milpitas, Calif). After 96 h incubation, the bottles were respectively transferred to refrigerator to stop fermentation, and then opened. Total in vitro gas produced was corrected to blank incubations which contained only rumen fluid. In addition to in vitro GP, the bottles were transferred to an ice bath to stop fermentation after 24 h of the incubation, and then opened to measure medium pH using a pH meter (Metrhom pH meter, Model 691). The filtrated (42 µm pore size) residual was oven dried (60ºC for 48 h) and used to calculate in vitro dry matter disappearances (IVDMD).

2.2. Calculations and Statistical Procedure

Gas pressure was converted into volume using an experimentally calibrated curve. Data of cumulative GP data were fitted to the exponential equation $Y=B\ (1-e^{-Ct})$, where B is the GP from the fermentable fraction (mL), the GP rate constant C (mL/h), t the incubation time (h) and Y is the gas produced at time t. In vitro DM disappearance (IVDMD) was calculated as the difference between initially incubated DM and residual DM, corrected by blanks. Metabolizable energy (MJ/kg) content and feed OM digestibility (g/kg^{-1} OM) were estimated using equations [11], given below:

Metabolizable energy (MJ/kg^{-1} DM) = 2.20 + 0.136GP + 0.057CP + 0.0029CP^2
Organic matter digestibility (OMD) (%) = 14.88+0.889GP+0.45CP+0.065 ash

Where, GP is 96 h net gas production (mL/ 200 mg of incubated DM), CP is crude protein (%).
Short chain fatty acids (SCFA) (mmol/ 200 mg of incubated DM) were calculated according to the equation from Getachew et al. [13].

$$SCFA\ (mmol/200\ mg\ of\ incubated\ DM) = 0.0222\ GP - 0.00425$$

Where, GP - 96 h net gas production (ml/200 mg of incubated DM). Data were statistically analyzed using GLM procedure of SAS [14] with flowing statistically model; $y=\mu+T_i+ e_{ij}$, where y= depended variable, μ= overall mean, T_i= effect of BPF and e_{ij}= residual error. Significant means were compared using the Duncan's multiple range tests. Mean differences were considered significant at P<0.05.

3. RESULTS AND DISCUSSIONS

The chemical composition of the BPF is presented in Table 1. The chemical composition of a given BPF varied with source of the feedstuffs in the current study (Table 1). The CP content ranged from 4.80 % in pomegranate peel to 11.2% in pomegranate seed. The CP content was higher (P<0.01) in pomegranate seed than in the other BPF. Chemical compositions of pomegranate seeds in the current study were inconsistent with findings of Taher-Maddah et al., [15] and Mirzaei-Aghsaghali et al., [3]. Feizi et al., [16] reported that DM, OM, CP, crude fiber, and EE values of pomegranate seeds were 94.8, 96.8,11.4, 38.9, and 1.0%, respectively. These differences in chemical composition of by-products may be due to a difference in cultivar, growing conditions, varieties, and different de-hulling process methods [15]. The CP concentration of the pomegranate peel, pomegranate seed, walnut and almond hulls, used in this experiment would be inadequate for the requirements of maintenance and production of ruminants. Thus, it is recommended that treating these BPF with urea as a cheap source of nitrogen would reduce the need to provide supplementary ruminal CP for these classes of livestock. The NDF and ADF contents were higher (P<0.01) in pomegranate seed than in the other BPF. The TT of pomegranate peel, pomegranate seed, apple pomace, walnut hull and almond hull were 9.73, 0.66, 0.34, 2.34, and 9.78%, respectively. The total phenol content ranged from 0.76% in apple pomace to 10.9% in pomegranate peel. The total tannins and phenol content were higher (P<0.01) in almond hull and pomegranate peel than in the other BPF. This result is in agreement with findings of Taher-Maddah et al [15]. Kamalak et al. [17] reported that total and soluble condensed tannins, NDF and ADF were negatively correlated with estimated parameters of gas production. The results in our study are consistent with those of Feizi et al. [16] who obtained that pomegranate peel tannins have negative effect on in vitro rumen fermentation.

Table 1. Chemical composition of by-product feedstuffs

Item	Treatment					S.E.M	P-value
	Pomegranate peel	**Pomegranate seed**	**Apple pomace**	**Walnut hull**	**Almond hull**		
DM	93.4^c	94.6^b	95.2^a	95.1^a	94.5^b	0.13	0.01
CP	4.80^d	11.2^a	6.80^b	5.40^c	6.40^b	0.17	0.01
EE	0.63^c	0.70^c	3.60^a	1.30^b	3.90^a	0.12	0.01
Ash	6.40^b	11.2^a	6.80^b	5.40^c	6.40^b	0.14	0.01
ADF	21.2^e	46.0^a	36.4^b	24.4^d	27.6^c	0.17	0.01
NDF	31.6^d	64.0^a	48.0^c	48.5^c	58.6^b	0.31	0.01
TP	10.9^a	1.20^c	0.76^d	3.80^b	10.6^a	0.37	0.01
SP	1.17^b	0.56^{cd}	0.42^d	1.50^a	0.82^c	0.09	0.01
TT	9.73^a	0.66^c	0.34^d	2.30^b	9.78^a	0.17	0.01

DM= Dry matter; CP= Crude protein; EE= Ether extract; ADF= Acid detergent fiber; NDF= Neutral detergent fiber; TP= Total phenol; SP= Simple phenol; TT= Total tannins.

Gas production volumes (ml/200mg DM) from in vitro incubation of different BPF samples at different incubation times are shown in Table 2 and Fig. 1. The cumulative volume of gas production increased with increasing time of incubation. Ranges of GP characteristics reported in this study may partly due to difference in CP, NDF and ADF contents. The rate and cumulative gas volume at each sampling time was affected by different BPF (Table 2). These finding indicate that fraction of substrate and degradability of BPF are different. The gas produced is directly proportional to the rate at which substrate degraded [18]. The maximum gas volume (b) was highest for apple pomace (P<0.01), greater for pomegranate seed (P<0.01) than for almond hull or walnut hull and lowest for pomegranate peel. The reason of more GP volume (b) in apple pomace may be caused by present of high level of pectin and non-structural carbohydrates [1]. Maheri-Sis et al. [19] reported that variation in chemical components (i.e., starch, soluble sugars, non-fiber carbohydrate, OM, CP, and NDF) of same feeds in different studies can be result in variation of in vitro gas production volume.

Table 2. Gas production characteristics and estimated parameters of different by-product feedstuffs

Item	Treatments					S.E.M	P-value
	Pomegranate seed	Pomegranate peel	Apple pomace	Walnut hull	Almond hull		
B	64.37^b	48.59^e	83.17^a	58.66^d	61.86^c	1.51	0.01
C	0.034^e	0.071^b	0.073^a	0.046^d	0.062^c	0.001	0.01
Lag time, h	0.76^a	0.47^d	0.38^e	0.62^b	0.56^c	0.05	0.01
ME	8.27^d	8.93^c	11.39^a	7.81^e	9.53^b	0.14	0.01
OMD	43.24^e	59.74^c	70.40^a	51.63^d	64.78^b	0.91	0.01
SCFA	0.88^d	1.05^c	2.25^a	0.86^d	1.82^b	0.02	0.01

B= the gas production from the fermentable fraction (mL g^{-1} DM), C= Rate constant of gas production during *incubation (ml/h^{-1}); ME= Metabolizable energy (MJ/kg^{-1}DM); OMD= Organic matter digestibility (%DM); SCFA= Short chain fatty acid (mmoL). Within rows, means with different letters are significantly different (P < 0.05). S.E.M= standard error of the mean.*

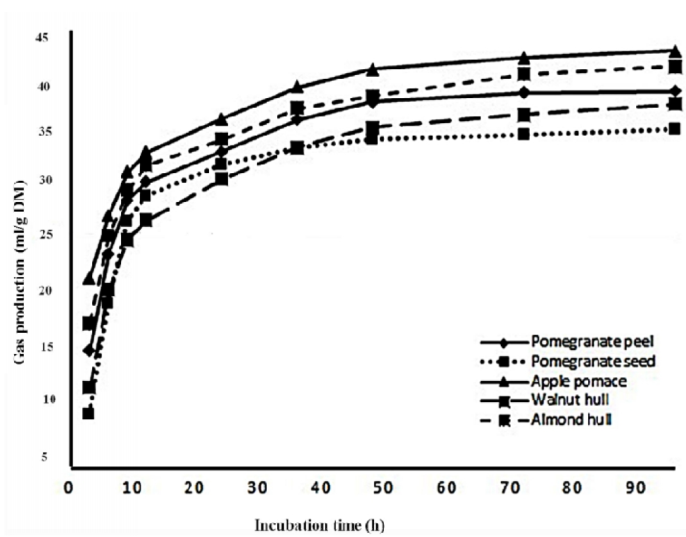

Fig. 1. Pattern of in vitro gas production (fitted with exponential model) affected by different by-product feedstuffs at different incubation times.

The rate of GP (c) was highest for apple pomace (P<0.01), followed by pomegranate peel, almond hull or walnut hull (P<0.01), and lowest for pomegranate seed (P<0.01). High rate of GP possibly affected by carbohydrate fractions which readily available to the microbial population. There were significant differences among the experimental feedstuff about the lag time. Lag time ranged from 0.76 in pomegranate seed to 0.38 in Apple pomace. Lag time probably is not affected by the time required for bacterial attachment to new substrate per se, which occurs very rapidly; lag time probably is related more to proper digestion of feed and subsequent, long-term colonization by microbes. Ude´n reported that in vitro lag time generally decreased as particle size of forages decreased. The extent of digestion in vitro may be decreased by addition of concentrate or by low pH. However, increasing buffer strength to buffer VFA production can increase lag time, probably because of high osmolality (20). So, Ranges of lag time reported in this study may partly due to difference in chemical composition of feedstuff.

The values for the ME and OMD ranged from 7.81 in walnut hull to 11.39 (MJ/kg-1 DM) in apple pomace and from 43.24 in pomegranate peel to 70.40 (%DM) in apple pomace, respectively. Menke and Steingass [11] suggested that gas volume at 24 h after incubation has been relationship with ME in feedstuffs. In the current study, low ME content of pomegranate peel and walnut hull can be resulted from its low rate of gas production and extent of gas production. The SCFA was highest for apple pomace (2.25 mmoL) and lowest for walnut hull (0.86 mmoL) in this study. Short chain fatty acids (i. e., acetic, propionic, butyric, and valeric) are produced during rumen fermentation and supply up to 80% of their maintenance energy requirements [3]. There is a highly significant correlation between SCFA and gas production [13,21] and this correlation use to estimate the SCFA from gas values, which is an indicator of energy availability to the animal. Thus, it can be concluded that, higher SCFA production in gas production technique is the reliable index of gas production and energy content of tested materials [15].

The values of IVDMD and pH of different BPF are presented in Table 3. The IVDMD was varied in five BPF particularly high in apple pomace and pomegranate seed had significantly lower (P<0.01) values of IVDMD (Table 3). The higher (P<0.01) IVDMD observed in this study may be due to the low level of tannin in apple pomace which suggest that it could be a valuable protein supplement in ruminant diets [22].

Table 3. In vitro dry matter disappearance (IVDMD) and pH of different by-product feedstuffs using batch culture

Item	Treatments					S.E.M	P-value
	Pomegranate seed	**Pomegranate peel**	**Apple pomace**	**Walnut hull**	**Almond hull**		
IVDMD	39.92^{e}	59.24^{c}	74.88^{a}	52.84^{d}	70.28^{b}	0.62	0.01
pH	6.69^{a}	6.48^{c}	6.35^{e}	6.58^{b}	6.44^{d}	0.02	0.01

Within rows, means with different letters are significantly different (P < 0.05). S.E.M= standard error of the mean.

The presence of tannins and phenolic compounds affected the nutritive value of these BPF to a different degree. Pomegranate seed and apple pomace contained traces of tannins (<1%), almond hull contained medium levels of tannins (>1%) and pomegranate peel and walnut hull contained high levels of tannins (>2%). This result is consistent with the findings of Seresinhe and Iben [23] and Ammar et al. [24], indicate that NDF and ADF were significant and negatively correlated with in vitro digestibility. In the current study, pH was highest for pomegranate seed (6.69) and lowest for apple pomace (6.35) and the lower

(P<0.01) pH observed in apple pomace may be due to its high NSC content. Bae et al., [25] reported that apple pomace contains abundant NSC and energy.

4. CONCLUSION

Chemical composition and in vitro digestibility can be considered useful indicators for the preliminary evaluation of the likely nutritive value of previously uninvestigated BPF. The results of current study based on chemical composition, IVDMD, ME, OMD and SCFA indicated that by-products can be used as replacement feedstuffs in diet for ruminants. It can be said that apple pomace has a potentially relative nutritive value in ruminants under in vitro conditions. However, there is a need for in vivo studies to support the in vitro findings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Maghsoud B, Taghizadeh A. Evaluation of some by-Products using In situ and In vitro Gas Production Techniques. American Journal of Animal and Veterinary Sciences. 2008;3:7-12.
2. Bampidis VA, Robinson PH. Citrus by-products as ruminant feeds: A review. Animal Feed Science and Technology. 2006;128:175–217.
3. Mirzaei-Aghsaghali A, Maheri-Sis N, Mansouri H, Razeghi ME, Mirza-Aghazadeh A, Cheraghi H, Aghajanzadeh-Golshani A. Evaluating nutritional value of apple pomace for ruminants using in vitro gas production technique. ARPN J. Agr. Biol. Sci. 2011;6:45-51.
4. Shabtay A, Eitam H, Tadmor Y, Orlov A, Meir A, Weinberg P, Weinberg ZG, Chen Y, Brosh A, Izhaki I, Kerem Z. Nutritive and antioxidant potential of fresh and stored pomegranate industrial byproduct as a novel beef cattle feed J. Agric. Food Chem. 2008;56:10063–10070.
5. Joshi V.K., Attri, D. A Solid State Fermentation System for Production of Ethanol from Apple Pomace. Natural Production Radiance. 2006;5;289-296.
6. Yalchi T, Kargar S. Chemical composition and in situ ruminal degradability of dry matter and neutral detergent fiber from almond hulls. Journal of Food, Agriculture and Environment. 2010;8:781-784.
7. Federal Register. Walnuts grown in California; Changes to the quality Regulations for shelled walnuts. Federal Register. 2010;75(163):51926-51929.
8. AOAC. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Inc., Arlington, VA; 2000.
9. Van soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 1991;74:3583–3597.
10. Makkar HPS. A Laboratory Manual for the FAO/IAEA Co-ordinated Research Project on 'Use of Nuclear and Related techniques to Develop Simple Tannin Assays for Predicting and Improving the safety and Efficiency of Feeding Ruminants on Tanniniferous Tree Foliage'. Joint FAO/IAEA of Nuclear Techniques in Food and Agriculture. Animal Production and Health Sub-Progrmme, FAO/ IAEA Working Document. IAEA, Vienna, Austria; 2000.

11. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim Res Dev. 1988;28:7-55.
12. Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France J. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Techno. 1994;48:185-197.
13. Getachew G, Makkar HPS, Becker K. Tropical browses: content of phenolic compounds, in vitro gas production and stoichiometric relationship between short chain fatty acid and in vitro gas production. J. Agric Sci. 2002;139:341-352.
14. SAS. SAS/STAT User's guide Statistics, Version 9.1. SAS Inst., Inc., Cary, NC; 2001.
15. Taher-Maddah M, Maheri-Sis N, Salamatdoustnobar R, Ahmadzadeh A. Estimating fermentation characteristics and nutritive value of ensiled and dried pomegranate seeds for ruminants using in vitro gas production technique. Open Veterinary Journal. 2012;2:40-45.
16. Feizi R, Ghodratnama A, Zahedifar M, Danesh-Mesgaran M, Raisianzadeh M. Apparent digestibility of pomegranate seed fed to sheep. Proceeding of British Society of Animal Science. 2005;222.
17. Kamalak A, Canbolat O, Gurbuz Y, Ozay O, Ozkan CO, Sakarya M. Chemical composition and in vitro gas production characteristics of several tannin containing tree leaves. Research Journal of Agriculture and Biological science. 2007;3:983-986.
18. Dhanoa MS, Lopez S, Dijkstra J. Estimating the extent of degradation of ruminant feeds from a description of their gas production profile observed in vitro: comparison of models. Br. J. Nutr. 2000;83:131-142.
19. Maheri-Sis N, Chamani M, Sadeghi AA, Mirza-Aghazadeh A, Aghajanzadeh-Golshani, A. Nutritional evaluation of kabuli and desi type chickpeas. (cicerarietinum L.) for ruminants using in vitro gas. Afr. J. Biotechnol. 2008;7:2946-2951.
20. Jeffrey L. Firkins. Effects of Feeding Nonforage Fiber Sources on Site of Fiber Digestion. J Dairy Sci. 1997;80:1426–1437
21. Beuvink JMW, Spoelstra SF. Interactions between substrate, fermentation end products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms in vitro. Applied Microbiol. Biotechnol. 1992;37:505-509.
22. Aganga AA, Mosase KW. Tannin content, nutritive value and dry matter digestibility of Lonchocarpuscassa, Zizyphusmucronata, Sclerocaryabirrea, Kirkia acuminate and Rhuslancea seeds. Anim. Feed Sci. Technol. 2001;91:107-113.
23. Seresinhe T, Iben C. In vitro quality assessment of two tropical shrub legumes in relation to their extractable tannin contents. J. Anim. Physiol. Anim. Nutr. 2003;84:109-115.
24. Ammar H. Compositión química, digestibilidad y cinética de fermentación ruminal in vitro de arbustos. Ph.D. tesi, Universidad de León, Spain; 2002.
25. Bae DH, Shin CN, Ko KH. Effect of total mixed ration including apple pomace for lactating cows. Korean J. Dairy Sci. 1994;16:295-302.

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