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Evaluation of chemical composition, *in situ* degradability and *in vitro* gas production of ensiled and sun-dried mulberry pomace*

B. Zhou[1], Q.-X. Meng[1,3], L.-P. Ren[1], F.-H. Shi[1], Z. Wei[1,2] and Z.-M. Zhou[1]

[1] State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University
100193, Beijing, P.R. China
[2] Human Resources and Development Center, Ministry of Agriculture
100027, Beijing, P.R. China

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ABSTRACT

The study was conducted to evaluate the nutritive value of mulberry pomace (MP) treated with sun-drying (SD), ensiling (EN), and ensiling with a *Lactobacillus* additive (ELA), respectively. The chemical composition, *in situ* degradabilities, and *in vitro* gas production were determined for all treated MP samples. The results showed that there were significant differences ($P<0.05$) in dry matter (DM), crude protein (CP) and water-soluble carbohydrates (WSC) among SD, EN and ELA treatments. Although some fermentation parameters of two ensiled MP products were significantly different, both of them fell within the normal fermentation range. The *in vitro* gas production of the SD treatment was higher than of the EN and ELA treatments, while the proportion of methane produced by MP treated with EN and ELA was significantly lower ($P<0.05$) than that of MP treated with SD; MP treated with SD had significantly higher acetate production than the two ensiled MPs. *In situ* DM and protein effective degradabilities of MP treated with SD were higher than in MP treated with EN and ELA. In conclusion, mulberry pomace ensiled with or without *Lactobacillus* additives exhibited good fermentation traits, suggesting that ensiling is more acceptable than sun-drying in practice.

KEY WORDS: mulberry pomace, sun-drying, ensilage, *in vitro*, *in situ,* ruminant

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[3] Corresponding author: e-mail: qxmeng@cau.edu.cn

INTRODUCTION

As the global population is soaring, the conventional feed for animal production, such as grains, legumes, is in shortage and highly priced in many parts of the world. Feeding by-products from agricultural and food processing industries to livestock can be one of the solutions. These by-products, such as apple pomace, grape pomace, citrus pulp, tomato pomace, and other vegetables or fruits, not only provide good energy sources for feeding animals, but also eliminate the need for costly waste management in fruit processing.

Mulberry has been a traditional Chinese fruit for thousands of years. Its annual production in Beijing is approximately 6000 tons. It has been widely used as a raw material for making juice and in liquor industries, which produced nearly 1000 tons of mulberry pomaces (MP). The juice-making factory usually sun dries these by-products and local farmers make sealed bales or silages using fresh MP products for feeding their animals. Although studies have reported the chemical composition of mulberry fruit (Lale and Ozcagiran, 1996; Gerasopoulos and Stavroulakis, 1997; Ozdemir and Topuz, 1998; Elmaci and Altug, 2002), no information has been available on the ruminal fermentation characteristics of sun-dried or ensiled mulberry fruit by-product as an animal feed. We hypothesized that mulberry pomace treated with EN and ELA had comparative rumen fermentation parameters and digestibilities with that of the traditional SD treatment. The objective of the present study was to compare the difference in chemical composition, *in situ* degradability, and *in vitro* gas production of mulberry pomace treated with sun-drying (SD), ensiling (EN), or ensiling with a *Lactobacillus* additive (ELA).

MATERIAL AND METHODS

Treatment of mulberry pomace

Mulberry pomace (MP) was obtained from a mulberry (*Morus nigra* L.) fruit juice factory located in Daxing District, Beijing. The MP product was mixed well and then subjected to sun-drying (SD), ensiling (EN), or ensiling with *Lactobacillus* additives (ELA). The average DM content of fresh MP was 270 g/kg. For making silage, a pressed bale (60 kg per bale) was made with a special pressing machine (Model DK 600A, Baoding Jintudi Biological Engineering Co., Ltd. Hebei, China) and wrapped with a double layer of polyethylene sheet for sealing. The bale had a packing density of 0.9 kg/l. The ELA treatment of MP followed the same procedure, but included a specialized silage inoculant (containing *Lactobacillus plantarum* and *Streptococcus faecium*) obtained from

Lvkangyuan Biotechnology Co., Ltd. Beijing (China). Inoculants were mixed with water and applied according to the label instructions to supply 3.6 x 10^4 cfu of lactic acid bacteria/g of fresh material. All ensiled bales were kept outdoors for at least 50 days during the autumn. For sun-drying, 200 kg of fresh MP were uniformly spread into a thin layer on a cement ground to dry for two days under sunlight, and then baled for use in the following experiments.

In vitro gas production, fermentation parameters and gas composition

In vitro incubation was carried out according to the procedure of Menke et al. (1979). The MP samples (200 mg DM) were in weighed in triplicate into 100 ml calibrated glass syringes (HFT000025, Häberle Maschinenfabrik Gmbh, Germany) with pistons lubricated with vaseline, then 30 ml buffered rumen liquor (Menke and Steingass, 1988) was pumped with a pipet into each of the pre-warmed syringes (39°C). Rumen fluid was obtained from 3 Limousin x Fuzhou crossbred steers through their permanent rumen canulas before the morning feeding. The rumen fluid was immediately carried to the laboratory and mixed with the buffer solution under a continuous flush of CO_2. The syringes were then incubated in a water bath shaker at 39°C. Gas production in each syringe was measured at time intervals of 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40 and 48 h. At the end of incubation, the syringes were placed in ice water to terminate fermentation. About 10 ml of the fermentation liquid was sampled for pH measurement, another 10 ml for VFA and ammonia N determination prior to centrifugation (4°C; 8.000 *g*; 15 min). To assess the gas composition, duplicate syringes were incubated following the above procedure, but the incubation was terminated at 24 h. For analysis of gas composition, a 5 ml disposable syringe equipped with a long needle was used to sample the gas through the top of the incubated syringes. The dynamics of gas production was computed by the nonlinear equation (Ørskov and McDonald, 1979):

$$Y = b\ (1\text{-}e^{-ct})$$

where: Y - the volume of gas produced at time t, b - the potential gas production (ml g^{-1} DM), and c - the fractional rate of gas production.

In situ dry matter and protein degradation

In situ DM and protein degradability were determined by the nylon bag technique (Ørskov et al., 1980). Four rumen fistulated Limousin x Fuzhou crossbred steers of approximately 400 kg liveweight were fed *ad libitum* a ration consisting of maize silage, wheat bran, ground maize and mineral-vitamin premix

with a 70.30 of forage-to-concentrate ratio (DM basis). Three treated MP samples were dried and ground to pass a 0.5-mm screen in a Wiley mill. Then, 5 g of each sample were placed in nylon bags and incubated in the rumen for 3, 6, 12, 24, 48 or 72 h prior to the morning feeding. In each steer, three bags were used per time interval. At each incubation time, bags were removed from the rumen, rinsed immediately in cold water and frozen. After incubation during all time intervals was completed, the bags were thawed and washed for 5 min in a washing machine until the water was clear. Washed bags were dried at 72°C for 48 h and weighed. Zero-time washing losses were obtained by soaking 2 bags for 10 min and washing with water until it became clear, then dried as before. The residue in each bag was assayed for DM and crude protein (CP), and the degradability at each time interval was calculated on the mean value of four bags from each steer. Degradation (Y) of DM and CP at time (t) was calculated from an exponential equation (Ørskov and McDonald, 1979):

$$Y= a +b (1-e^{-ct})$$

where: a - rapidly degraded fraction, b - insoluble but potentially degradable component in time t, c - degradation rate constant of b fraction.

Chemical analyses

The dry matter, crude protein, and organic matter of EN and SD were determined by the procedures of AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest et al. (1991). Silage fermentation pH was measured by a portable pH meter equipped with a glass electrode (Model PHS-3C, Shanghai Leici Scientific Instrument Co., Ltd., China). Ammonia N was determined according to Broderick and Kang (1980). Volatile fatty acids (VFAs) were analysed with an Agilent 6890 gas chromatograph fitted with an HP-INNOwax capillary column (30 m × 0.32 mm) according to Erwin et al. (1961). Water-soluble carbohydrates (WSC) were determined colorimetrically using an anthrone reagent according to Thomas (1977). The Flieg indexes of EN and ELA were calculated according to Zimmer (1966). Flieg index = (lactate/total acid) point + (acetate/total acid) point + (butyrate/total acid) point.

Statistical analysis

To observe the effects of three treatments, the data on chemical composition, fermentation parameters, gas production, and degradation of DM and CP were subjected to analysis of variance using the following model:

$$y_{ij} = \mu+\alpha_i+\varepsilon_{ij}$$

where: y_{ij} - the experimental data; μ - the general mean; α - the treatment effect (i - SD, EL, ELA); and ε - the error term.

Parameters b and c in the gas production study and parameters a, b and c in the *in situ* study were estimated by an iterative least square method using a non-linear regression procedure. All statistical analyses were performed for a completely randomized design using general linear models (GLM) procedures of SAS (2000).

RESULTS AND DISCUSSION

Chemical composition. The data on DM, OM, CP, ADF, and NDF are presented in Table 1. The treatments resulted in significant (P<0.05) differences in DM and CP contents. Due to removal of most of the moisture from MP samples through sun drying, the SD treatment had a significantly higher (P<0.05) DM content than the EN and ELA treatments, but the OM contents were similar among the three treatments. These observations are in agreement with the study on apple pomace by Pirmohammadi et al. (2006).

Table 1. Chemical composition of mulberry pomace, n=3

Item	Treatment			SEM
	SD	EN	ELA	
DM, g/kg fresh sample	855.9[a]	451.1[b]	450.3[b]	4.3
OM, g/kg DM	876.0	897.5	878.0	0.9
CP, g/kg DM	218.6[a]	197.9[b]	198.8[b]	1.4
WSC, g/kg DM	208.5 [a]	156.4 [b]	149.3 [b]	1.8
NDF, g/kg DM	490.6	515.4	506.7	15.1
ADF, g/kg DM	379.6	382.3	379.0	10.1

SD - sun-dried; EN - ensiled; ELA - ensiled with *Lactobacillus* additives; DM - dry matter; OM - organic matter; CP - crude protein; ADF - acid detergent fibre; NDF - neutral detergent fibre; WSC - water soluble carbohydrates; SEM - standard error of means. Means in the same row with different superscripts letters differ significantly (P<0.05)

For EN and ELA, dry matter contents were similar to those obtained from mango and lemon silages (350 g/kg fresh sample) reported by Aguilera et al. (1997). In comparison with the EN and ELA treatments, the SD treatment had a higher CP content. The reduced CP content in the EN and ELA treatments was expected because anaerobic fermentation of MP by bacteria would convert some true proteins into non-protein nitrogen such as ammonia and amino acids. The WSC in treatment SD was significantly higher (P<0.05) than in EN and ELA, which was close to the data of dried citrus pulp (241 g/kg DM) reported by Bampidis and Robinson (2006). The low WSC in EN and ELA was accounted for by glycolysis

and other fermentation processes of microorganisms, which would produce lactate and volatile fatty acids. The treatments seemed to be unchanged in NDF and ADF.

The fermentation parameters of MP treated with EN and ELA are shown in Table 2. The pH of treatment ELA was lower than in treatment EN. The lower pH for treatment ELA may be accounted for by the addition of *Lactobacillus* additives, which can accelerate WSC fermentation. As shown in Table 2, the lactate content of treatment ELA was 42 g/kg DM, significantly higher ($P < 0.05$) than in treatment EN. Leroy and Zelter (1954) reported that the high sugar content in MP silages would lead to rapid production of total VFA and lactic acid. The ammonia N concentration was numerically higher in ELA than EN, although the difference was not significant ($P>0.05$). Ammonia N was generated mainly through proteolysis by *Clostridium butyricum*, which is an indicator of good silage. In this study, both EN and ELA had an anticipated ammonia N concentration (112.3 and 114.5 g/kg total N, respectively) that was comparable to that (100 g/kg total N) reported by Pirmohammadi et al. (2006) on apple pomace silage. Moreover, acetate and propionate were significantly higher in the ELA treatment than in EN, with a very low concentration of butyrate, indicating good fermentation in the EN and ELA treatments. Moreover, the Flieg index of MP with EN and ELA was rather high, approaching 100, indicating a very good quality of the silages.

Table 2. Fermentation parameters of ensiled mulberry pomace (n=3)

Item	EN	ELA	SEM
NH_3-N, g/kg total N	112.3	114.5	1.3
Flieg index	98	97	-
Lactate, g/kg DM	36^b	42^a	0.35
Acetale, g/kg DM	8.3^b	10.8^a	0.28
pH	4.5^a	4.0^b	0.08
Propionate, g/kg DM	2.5^b	5.3^a	0.12
Butyrate, g/kg DM	0.02	0.01	0.01

EN - ensiled mulberry pomace; ELA - ensiled mulberry pomace with additives; SEM - standard error of means. Means in the same row with different superscripts letters differ significantly ($P<0.05$)

In vitro gas production, fermentation parameters and gas composition. In vitro gas production and gas composition results are presented in Table 3. It was found that the different treatments had a significant influence on 48 h gas production ($P<0.05$) and potential gas production (B), but no influence on the gas production rate (C). The 48 h gas production and potential gas production (B) of treatment SD was significantly ($P<0.05$) different from EN, but there was no significant ($P>0.05$) difference in this parameter between treatments SD and ELA. Gas production was highly correlated with the content of fermentable components of feedstuffs (Menke et al., 1979), thus the SD treatment may have had a higher fermentable component content than treatments EN and ELA. In addition, compared with treatments EN and ELA, sun-drying may destroy some phenolic components in

the MP products, which may explain the low gas production of treatments EN and ELA due to the relatively high phenolic content of treatments EN and ELA that may inhibit growth of rumen microorganisms (El Hassan et al., 1995).

The gas from *in vitro* incubation was composed mainly of H_2, CH_4, and CO_2 (Table 3). The H_2 percentage ranged from 1.6 to 11.6%, CO_2, 35.0 to 40.2%, while the CH_4 content varied from 63.3 to 58.7%. These lower percentages of H_2 and CO_2 and the higher percentage of CH_4 in the treatments are indicative of effective utilization of H_2 and CO_2 by methanogenic bacteria in the rumen. The proportion of methane in total gas production was significantly higher ($P<0.05$) in the SD treatment than in treatments EN and ELA, indicating that ensiled MP, irrespective of the addition of *Lactobacillus*, has a positive effect on potential reduction of greenhouse gas emission when used as a ruminant feedstuff.

Table 3 *In vitro* gas production parameters and gas composition for mulberry pomace

Item	SD	EN	ELA	SEM
48 h GP, ml/0.2 g DM	25.3[a]	18.7[b]	21.2[ab]	0.57
B, ml/0.2 g DM	23.6[a]	17.0[b]	22.4[a]	0.40
C, ml/h	0.05	0.08	0.06	0.01
H_2, % total gas	1.6[b]	2.4[b]	11.6[a]	0.56
CH_4, % total gas	63.3[a]	48.2[c]	58.7[b]	0.67
CO_2, % total gas	35.0[c]	38.9[b]	40.2[a]	0.18

GP - gas production (ml) of 0.2 g sample (DM basis) at time t; B - potentially maximum gas production (ml) of 0.2 g sample (DM basis); C - fractional rate of gas production (ml/h); Lag -delaying time of gas production (h). Means in the same row with different superscripts letters differ significantly ($P<0.05$)

The *in vitro* ruminal fermentation parameters of MP treated with SD, EN and ELA are shown in Table 4. Most of these parameters were unaffected by the treatment, with the exception of total VFA and molar proportion of

Table 4. *In vitro* ruminal fermentation parameters (48 h) of mulberry pomace with three different treatments

Item	SD	EN	ELA	SEM
pH	6.93	6.90	6.91	0.01
NH_3-N, mg/100 ml	7.08[a]	6.75[ab]	6.47[b]	0.11
Total VFA, mmol/l	48.59[a]	43.23[b]	42.28[b]	0.58
VFA, mol%				
acetate	65.86[a]	60.84[b]	61.42[b]	1.73
propionate	21.10	19.61	21.13	1.49
isobutyrate	1.89[a]	1.98[a]	3.06[b]	0.53
butyrate	9.65	9.30	9.49	0.14
isovalerate	2.83	2.61	2.47	0.17
valerate	1.66	1.56	1.42	0.33
Acetate/propionate	3.12	3.10	2.90	0.35

SEM - standard error of means. Means in the same row with different superscripts letters differ significantly ($P<0.05$)

acetate, both of which were significantly higher (P<0.05) in treatment SD than treatments EN and ELA. The main reason may be related to the higher WSC content in treatment SD (Table 1) than in EN and ELA, in which the WSC were degraded in the process of silage fermentation. The ruminal pH of the *in vitro* fermentation liquor in different treatments was maintained in the range of 6.90-6.93, indicating that the treatment had no significant (P>0.05) effects on rumen buffer capacity. Moreover, there were no differences in the production of ammonia N and other individual VFA among the three treatments.

In situ dry matter and protein degradability. The *in situ* degradability of DM and protein of three treatments of mulberry pomace is presented in Table 5. The soluble fraction (a) of MP treated with SD was significantly higher (P<0.05) than in treatments EN and ELA, suggesting that easily fermentable carbohydrates from MP may be fermented by microorganisms. The insoluble, but potentially fermentable, component (b) and its rate of fermentation (c) among three treatments showed no differences (P>0.05). This observation is compatible with the data in Table 1.

Table 5. *In situ* dry matter and protein degradation (%) parameters of mulberry pomace with three different treatments

Item	SD	EN	ELA	SEM
DM				
a	16.03[a]	9.92[b]	10.87[b]	0.91
b	60.26	64.02	62.54	0.65
c (/h)	0.048	0.055	0.05	0.02
CP				
a	35.18	32.55	34.92	1.62
b	49.85[a]	41.65[b]	42.98[b]	0.61
c (/h)	0.049	0.045	0.048	0.03

DM - dry matter; CP - crude protein; a - rapid degraded fraction (%); b - slowly degraded fraction (%); c - rate of degradation (/h); the constant in exponential equation $p = a + b\,(1\text{-}e^{-ct})$. SEM -standard error of means. Means in the same row with different superscripts letters differ significantly (P<0.05)

The ruminal protein degradabilities of MP treated with SD, EN, and ELA differed from the data on DM degradation. The soluble fractions (a) of three treated mulberry pomaces did not differ (P>0.05), but the protein degradation of fraction b was significantly higher (P<0.05) in the SD treatment than in treatments EN and ELA. Although the mechanism by which ruminal protein degradation was increased in sun-dried MP is unclear, the degradation of certain anti-nutritional factors (such as anthocyanins) may be responsible. Arnmwit et al. (2010) reported that light and heat exposure of mulberry fruit extract at 70°C for 10 h significantly deteriorated total anthocyanins.

Use of mulberry pomace in ruminant feeding. As far as we know, mulberry pomace (fresh or sun-dried) as an alternative feedstuff is widely fed to local

animals such as sheep, goats, dairy and beef cattle in Beijing, owing to its competitive pricing against the main feedstuffs. Indeed, sun-drying is a traditional and convenient way to store MP, but limitations occur due to changeable weather and large production in practice. So ensilage of MP can be an effective way for long-term storage and industrial utilization.

High feeding levels of MP may harm ruminants due to its high concentrations of digestible energy, especially nonstructural carbohydrates and starches. Cullen et al. (1986) reported that high feeding levels of citrus pulp can increase the risk of lactic acidosis in dairy cattle. Orange has been used as a flavouring agent for sheep (Ralphs et al., 1995). MP may also be used as such an agent. Nonetheless, additional studies based on feeding different kinds of MP are needed in order to elaborate guidelines for its use in different ruminants.

CONCLUSIONS

Although ensilage of mulberry pomace showed lower *in vitro* gas production, fermentation parameters, and *in situ* ruminal degradation of dry matter and protein than sun-drying, its ensiling with or without a *Lactobacillus* inoculant revealed good fermentation traits and significantly reduced methane production in ruminal fermentation, which suggests that ensilage is more acceptable than sun -drying in practice. The effects of feeding mulberry pomace to ruminants on their performance require further studies.

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