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Nutritive value of fermented apple pomace silage and its effect in Suffolk ewes

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ABSTRACT

The experiment was carried out to assess the nutritive value of fermented apple pomace silage (APS) and its effect on blood parameters in Suffolk ewes. Three Suffolk ewes (weighing 51.1±1.5 kg) were used in 3×3 Latin Square Design including three dietary periods with three dietary treatments: Hay diet, low ethanol APS (L-APS, 48.7 g kg^{-1} DM ethanol) diet and high ethanol APS (H-APS, 87.2 g kg^{-1} DM ethanol) diet. Alfalfa hay cube and APS provide half of the total digestible nutrient (TDN) requirement for APS diets but control group fed only alfalfa hay cubes. The apparent digestibility of dry matter, organic matter, crude protein, nitrogen-free extract, hemicellulose were higher ($P < 0.01$) and crude fibre, ether extract and neutral detergent fiber were also higher ($P < 0.05$) in APS groups. The digestible organic matter, digestible crude protein, total digestible nutrient and metabolizable energy values were also higher ($P < 0.01$) in APS diets. But there was no significant difference between the L-APS and H-APS for all of the digestible nutrients. Nitrogen intake and faecal nitrogen excretion were higher ($P < 0.01$) and total nitrogen excretion was also higher ($P < 0.05$) in hay diet. The concentration of postprandial plasma ethanol, lactate and β-hydroxybutyrate were significantly increased ($P < 0.05$) in ewes fed APS diets. Fermented APS can be used as ruminant feed in combination with hay cube or dry roughage up to 50% of the TDN requirement and ewes are capable of using the 15.1 g/d ethanol intake without any adverse side effect.

Keywords: Apple pomace silage, digestibility, nutritive value, ethanol, ewe

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INTRODUCTION

Global intensification of food production has led to the creation of large quantities of agro-industrial by-products and wastes. More efficient utilization of agro-industrial by-products and wastes is the crucial demand throughout the world because of social, economic and environmental concern. A huge quantity of fruit and vegetable wastes and by-products from the fruit and vegetable processing industry is available throughout the world. Large proportions of these immense quantities of wastes are dumped in landfills or rivers, causing environmental hazards and emit harmful greenhouse gases (Venkat 2011; Vilariño et al., 2017). Alternatives to such disposal methods the agro-industrial by-products could be recycling through livestock as feed resources and further processing to extract or develop value-added products that can reduce the environmental impact of the food industry and improves profitability and valorization of the agricultural by-products. Apple pomace (AP) is a by-product of apple processing industry, consisting of peel, pulp, and seeds. Approximately, 160,000 tons of apple

pomace (AP) are generated annually in Japan (Takahashi and Mori, 2006). It accounts for 25-30% of the dry mass of apple (Gullón et al., 2007) which contains high moisture (80-85%), fiber-rich concentrates, pectin, minerals, vitamin C, anti-oxidant with rich fermentable carbohydrate and organic acid also (Alibes et al., 1984). Apple pomace is a palatable and effective energy source, but poor in protein; could be used as ruminant feed economically in the apple growing area (Fontenot et al., 1977; Alibes et al., 1984; Gasa et al., 1992; Taasoli and Kafilzadeh, 2008). However, some studies were undertaken to utilize the AP with urea and other ingredients supplementation to balance the low protein and high moisture content (Rumsey, 1978; Nikolić and Jovanović, 1986; Pirmohammadi, 2006) but the performance was not satisfactory. On the other hand, silage mixed ration has been widely used in the livestock industry for the last decade worldwide to stabilize the microbial activity and improve the utilization of energy and protein in the rumen (Coppock et al., 1981). The factory wet by-products contained a large amount of moisture, a small amount of lactic acid and lower pH, but prolonged ensiling can improve the silage mixed ration stability. If AP would be ensiled with other feed ingredients to absorb the high moisture and balance the low protein content similar to a concentrate mixer, then that will be of good quality silage, and high amount of ethanol would become minimize. Although, limited researches are available about the nutritive value of AP and apple pomace silage (APS), but information on the nutritive value of nutritionally balanced fermented APS and its effect on blood biochemistry in sheep is still unknown. Therefore, this current study was undertaken to evaluate the fermentation characteristics, digestibility and nutritive value of APSs which were low ethanol containing APS (L-APS) and high ethanol containing APS (H-APS). After feeding of nutritionally balanced APSs, the effect on blood parameters of Suffolk shorn ewes was also investigated.

MATERIALS AND METHODS

Animal, experimental design and management

All procedures involving animals in this study were approved by the Institutional Animal Care and Use Committee at Hirosaki University, Japan (A08023). Three Suffolk shorn ewes (aged 11 months on average and weighing 51.1 ± 1.5 kg) were used in a 3 × 3 Latin Square Design over three 21 day periods, including initial seven days for dietary adaptation and the last seven days for sample collection. The ewes were randomly assigned to three dietary treatments: alfalfa hay cube and either L-APS or H-APS provided as half of total digestible nutrient (TDN) requirement for L-APS or H-APS diet, and hay diet receiving group fulfill their TDN requirement from alfalfa hay cubes. The daily allowance was offered as a TDN requirement for 110% maintenance (Ministry of Agriculture, Forestry, and Fisheries, 1996; Japan) in 2 equal meals at 10:00 and 18:00. The required TDN amount was adjusted based on their body weight (BW). The BW was measured before the morning feeding at the beginning of each period. The ewes were housed in individual pens with sawdust bedding in an indoor animal room during the feed adjustment period (first seven days) and then moved to metabolic cages for next 14 days where the last seven days was for sample collection. According to the dietary adaptation period BW, same feed allowance was offered for the last 14 days. Trace mineralized salt block and water were offered access throughout the experimental period.

Apple pomace silage preparation

Fresh apple pomace (AP) was obtained from an apple juice factory of the Farm village industry federation of Aomori prefectural agricultural cooperatives at Hirosaki, Japan. The L-APS was prepared with fresh AP, soybean meal, wheat bran and beet pulp to balance TDN contents similar to a commercial concentrate (Table 1). The H-APS was also prepared with the intention of having the same overall ingredient composition exception of fermented AP of the different ethanol content. Because remaining sugars in fresh AP is fermented rapidly and efficiently turned into ethanol under solid-state fermentation condition (Hang et al., 1981), fresh AP was stored alone in an anaerobic condition for two months to secure higher ethanol contents before ensiling of H-APS. For ensiling of L-APS, fresh AP within two days of production was used. The AP and all other ingredients were mixed thoroughly with a mechanical mixer and stuffed into a plastic container, pressed sufficiently to fill properly, topped with air-tight cover and ensiled for at least two months before use in the feeding experiment. Fermented components of APSs were manipulated by ensiling with either fresh AP or fermented AP.

Analysis of feed and feed refusal samples

One hundred grams of AP or APS sample was extracted with 300 g of distilled water at 4ºC with occasional gentle swirling. After 18 h of extraction, the aqueous solution was strained through four layers of gauze and further filtered through a filter paper (Quantitative ashless 5A type, Advantec, Japan). The pH was measured immediately by using a digital pH meter (KR5E, AS-PRO, Japan) and then extracted solution was stored at -30ºC for analysis of volatile basic nitrogen (VBN), organic acids and ethanol. The VBN content was determined as described by Conway, (1962). Ethanol was determined by a specific gravity meter (DA-310, Kyoto Electronics, Japan) after direct distillation of the extracted sample and organic acids were analyzed by a bromothymol blue post-column method using HPLC (D-

Table 1. Ingredient compositions of APS and chemical composition of feed ingredients used in the APSs fed to the ewes.

Item	Fresh AP[1]	Fermented AP[2]	Beet pulp	Soybean meal	Wheat bran
Ingredients (%, fresh basis)					
L-APS	70	0	12	06	12
H-APS	0	70	12	06	12
Chemical composition of feed ingredients					
Dry matter (%)	21.4 ± 0.1	14.4 ± 0.2	92.0 ± 2.5	92.1 ± 3.2	91.8 ± 4.8
Nutrient composition (%, DM basis)					
Organic matter	97.7 ± 0.1	96.3 ± 0.2	93.9 ± 0.2	92.9 ± 0.2	94.2 ± 0.2
Crude protein	4.5 ± 0.2	7.5 ± 0.3	6.9 ± 0.4	47.9 ± 0.4	17.4 ± 0.4
Crude fiber	13.7 ± 0.4	21.1 ± 0.2	18.5 ± 0.3	5.6 ± 0.3	9.2 ± 0.3
Ether extract	3.2 ± 0.0	5.9 ± 0.0	0.7 ± 0.0	1.5 ± 0.0	4.7 ± 0.0
NFE	76.3 ± 0.7	61.8 ± 0.3	67.7 ± 0.9	37.8 ± 0.9	62.8 ± 0.9
Crude ash	2.3 ± 0.0	3.7 ± 0.2	6.1 ± 0.1	7.1 ± 0.1	5.8 ± 0.1
ADF	20.3 ± 0.1	31.4 ± 0.6	25.3 ± 0.3	8.3 ± 0.3	12.6 ± 0.3
NDF	27.9 ± 1.2	42.5 ± 0.2	38.2 ± 0.2	11.8 ± 2.6	41.9 ± 1.7
Hemicellulose	7.5 ± 1.3	11.1 ± 0.4	12.8 ± 0.5	3.4 ± 2.8	29.3 ± 2.0
NFC[3]	62.1 ± 1.4	40.4 ± 0.4	48.1 ± 0.4	31.7 ± 1.5	30.2 ± 1.7

[1] Fresh apple pomace within 2 days of production was used. [2] Fermented AP was stored fresh AP for 2 months in an anaerobic condition. [3] NFC = (100 - CP - EE -NDF – Ash). AP, apple pomace; L-APS, low ethanol containing apple pomace silage; H-APS, high ethanol containing apple pomace silage; DM, dry matter; NFE, nitrogen free extract; ADF, acid detergent fibre; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrate.

2000 HSM, Hitachi, Japan). All feed and feed refusal samples were dried in a forced-air oven at 60ºC for 48 h, ground through a 1 mm screen using a Willey mill and kept in a plastic airtight container for chemical analyses. Dry matter (DM), crude protein (CP), ether extract (EE), ash, crude fibre (CF) and organic matter (OM) were determined according to the methods of the Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the procedures of Van Soest et al. (1991). To determine the NDF, 20 ml distilled water was added to 1 g of ground sample and heat to boiling, cooled at room temperature, 20 ml α-amylase (015-03731, Wako, Japan; 1 mg heat-stable α-amylase: 20 ml phosphate buffer) solution was added, shake and incubate at 40°C for overnight. After 16 h of incubation, the solution was filtered through the pre-weighed filter paper (Quantitative ashless 5A type, Advantec, Japan). Filtered residue was transferred in the beaker and added 100 ml of neutral detergent solution to each sample, heat to boiling and refluxed for one h from the onset of boiling. Filtered residues were washed with boiling water and acetone and then ashed in a muffle furnace for two h at 600°C. ADF analysis procedure was similar to NDF except for incubation with α-amylase buffer solution and acid detergent solution was used instead of a neutral detergent solution. The chemical composition of feed ingredients used in the L/H-APSs is presented in Table 1.

Analysis of faeces and urine samples

On the last seven days of each 21 day period, urine and feces were collected. Urine was collected in a plastic pot containing 50 ml of 6N H_2SO_4 solution to prevent nitrogen (N) loss. The total amount of daily excreted urine and feces by each animal was measured, recorded and 10% of well-mixed sub-sample was stored at 4°C during the collection period. On the last day of each period, total excreted urine and faeces sub-samples were well mixed and again 10% of sub-sample was taken for further analysis. All faeces samples were dried in a forced-air oven at 60ºC for 48 h, ground through a 1 mm screen using a Willey mill and kept in a plastic airtight container for chemical analyses. DM, CP, EE, crude ash, CF, NDF and ADF contents were determined according to the methods of AOAC, (1990). Urinary nitrogen (N) content was determined by Kjeldahl method (AOAC, 1990).

Blood parameters and analytical methods

On the last day of each 21 day period, series of blood samples were obtained by jugular venipuncture at pre-feeding (0) and at 0.5, 1, 2 and 3 h after the morning

Table 2. The pH value, organic acids and others fermented products of apple pomace and apple pomace silages.

Item	Fresh AP[1]	Fermented AP[2]	L-APS	H-APS
pH	4.01	4.08	3.85 ±0.0	3.86 ±0.2
Organic acids and others fermented products (g kg^{-1}DM)				
Lactic acid (g kg $^{-1}$ DM)	15.3	72.9	35.0 ± 10.0	36.0 ± 22.6
Acetic acid (g kg $^{-1}$ DM)	5.0	40.6	15.0 ± 2.5	23.6 ± 15.0
Iso-butyric acid (g kg $^{-1}$ DM)	0.9	ND	ND	ND
Butyric acid (g kg $^{-1}$ DM)	ND	ND	ND	ND
Propionic acid (g kg $^{-1}$ DM)	ND	ND	ND	ND
Total VFA[3](g kg $^{-1}$ DM)	5.9	40.6	15.0 ± 2.5	23.6 ± 15.0
Ethanol (g kg $^{-1}$ DM)	1.3	242.5	48.7^{a} ± 5.1	87.2^{b} ± 14.9
VBN in TN (g kg $^{-1}$ DM)	0.06	0.13	0.33 ± 0.1	0.65 ± 0.3
Moisture (g kg $^{-1}$ DM)	767.0	856.9	633.8^{a} ± 6.9	663.1^{b} ± 5.6

[1] Fresh apple pomace within 2 days of production was used.[2] Fermented AP was stored fresh AP for 2 months in an anaerobic condition.[3] Total VFA= (Acetic acid + Iso-butyric acid + Butyric acid + Propionic acid) [a,b] Different letters indicate significant difference among the treatments (at 5% level). AP, apple pomace; L-APS, low ethanol containing apple pomace silage; H-APS, high ethanol containing apple pomace silage; DM, dry matter; ND, not detectable; VFA, volatile fatty acid; VBN, volatile basic nitrogen; TN, total nitrogen.

feed. On each occasion, blood was collected into two 7 ml evacuated tube containing sodium heparin as an anticoagulant, chilled on ice and centrifuged (3000 × g for 15 min at 4°C) to harvest plasma sample. Plasma was aliquotted and stored at –30°C until analyzed for ethanol, lactate, β-hydroxybutyrate (BHBA) and glucose. Plasma ethanol concentration was measured by UV method using an enzymatic F-kit ethanol kit (R-biopharma, Germany). Plasma lactate and BHBA were determined by enzymatic colorimetric methods using medical test instruments, Lactate Pro 2 (Arkray, Japan) and Precision Xceed Pro (Abbott, Japan), respectively. Plasma glucose was determined with an enzymatic colorimetric method using Glucose CII-test Wako (Wako pure Chemicals, Japan). The area upper or under the curve (AUC) for 3 h after feeding of plasma parameters was calculated as an indication of the responses to feeding in each parameter.

Calculation

Hemicellulose was calculated as the difference between the NDF and ADF. Non-fibrous carbohydrate (NFC) was calculated by using the equation of NRC (2001): NFC = 100- (CP + EE + NDF + Ash). Average body weight gain (BWG) and average TDN intake were calculated for each experimental period. Ethanol intake was calculated from APS intake and ethanol contents of the APSs. The digestibility of L-APS and H-APS feeds were calculated by difference, according to Schneider and Flatt, (1975). The ME values of the feeds were calculated using the following equation (AFRC, 1993):

ME (MJ/kg DM) = 0.016DOMD.
Where, ME, metabolizable energy
MJ, mega joule
Kg, kilogram
DOMD, digestible organic matter as dry matter basis.

Statistical analysis

Least significant difference (LSD) test of SPSS was used to compare the treatment means for fermentative products, chemical composition, apparent digestibility and nutritive values of feeds. Digestibility trial was analyzed as a replicated 3×3 Latin square using the general linear model (GLM) procedure of SPSS, with ewe, period and diet were included in the model. Serial data for blood parameters were analyzed with Polynomial contrasts to determine the slop response of the time influence and their interaction with diet. Tukey test (SPSS) was used to identify differences ($P < 0.05$) between means. The relationship among the AUC responses for plasma measurements was analyzed by Pearson's correlation coefficient.

RESULTS

Organic acids and other fermentation profiles

Fermented components of APSs were manipulated by ensiling with either fresh AP or fermented AP. Fermented AP contained the largest amount of ethanol and thus ethanol contents of L-APS and H-APS were successfully differentiated. The pH, moisture, organic acid contents and other fermentation characteristics of AP and APSs are presented in Table 2. The H-APS contained higher amount of moisture than that of L-APS. Fresh AP had a

Table 3. Chemical composition of low ethanol containing apple pomace silage and high ethanol containing apple pomace silage.

Item	L-APS	H-APS	P value
Dry matter	37.7[b] ±0.1	34.6[a] ±0.2	0.001
Nutrient Composition (%, DM basis)			
Organic matter	94.5[b] ± 0.3	94.1[a] ± 0.1	0.049
Crude protein	17.2[a] ± 0.3	18.3[b] ± 0.4	0.019
Crude fiber	12.9[a] ± 0.3	14.2[b] ± 0.5	0.014
Crude ash	5.5[a] ± 0.3	5.9[b] ± 0.1	0.049
Ether extract	3.3 ± 0.4	3.5 ± 0.3	0.503
NFE	61.1[b] ± 0.8	58.1[a] ± 0.4	0.018
ADF	20.7 ± 0.2	20.8 ± 0.4	0.792
NDF	38.6[a] ± 0.2	42.3[b] ± 0.2	0.001
Hemicellulose	17.9[a] ± 0.4	21.5[b] ± 0.4	0.001
NFC[1]	35.4[b] ± 0.9	30.0[a] ± 0.6	0.001

[1]NFC = (100 - CP - EE -NDF – Ash).[a,b,c] Different letters indicate significant difference among the treatments (at 5% level).L-APS, low ethanol containing apple pomace silage; H-APS, high ethanol containing apple pomace silage; DM, dry matter; NFE, nitrogen free extract; ADF, acid detergent fibre; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrate.

negligible amount of ethanol (0.1% DM) and during ensiling ethanol content was increased (4.9% DM) in L-APS. Moreover, ethanol content of L-APS and H-APS were significantly remarkable. The APSs did not find bad smell and fungal growth during the experimental period.

Chemical composition of APSs

The chemical composition of L-APS and H-APSs are presented in Table 3. High moisture containing H-APS had lower DM, OM, NFE and NFC content ($P < 0.01$, $P < 0.05$, $P < 0.05$ and $P < 0.01$; respectively) than those of L-APS. Besides, the L-APS had lower CP, CF, ash, NDF and hemicellulose content ($P < 0.05$, $P < 0.05$, $P < 0.05$, $P < 0.01$ and $P < 0.01$; respectively) than those of H-APS.

Chemical composition of diets

The chemical composition of experimental diets is shown in Table 4. The hay diet contained higher ($P < 0.01$) DM content than that of other two APS diets. On the other hand, the H-APS diet contained lower DM ($P < 0.05$) content than that of L-APS diet. The OM, NFE and NFC content of hay diet were lower ($P < 0.01$) than that of APSs. The L-APS diet contained slightly higher ($P < 0.05$) OM, NFE and NFC content than that of H-APS diet. The hay diet contained higher ($P < 0.01$) CF and ADF content with lower ($P < 0.05$) EE than that of APSs. However, CF, EE and ADF contents of L-APS and H-APS did not differ significantly ($P > 0.05$) between each other. The highest ($P < 0.01$) crude ash was observed for hay diet compare to APSs diets and lowest ($P < 0.05$) content was for a L-APS diet. The hay diet contained higher ($P < 0.01$) NDF than that of others. No notable difference was observed in ADF content for both APS diets. However, the H-APS diet contained higher ($P < 0.05$) NDF content than that of L-APS diet. The H-APS diet contained highest ($P < 0.01$) hemicellulose than that of other two diets and L-APS diet also contained comparatively higher ($P < 0.05$) hemicellulose content than that of hay diet.

Nutrient digestibility and nutritive value of diets

Apparent digestibility and nutritive value of different experimental diets are shown in Table 5. The digestibility of DM, OM, CF, EE, NFE, NDF and hemicellulose were significantly lower ($P < 0.01$, $P < 0.01$, $P < 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.05$ and $P < 0.01$; respectively) in hay diet than those on both APS diets. But there was no significant ($P > 0.05$) difference between the L-APS and H-APS diets. The digestibility of CP was also lower in hay diet compared to APSs diets. However, slightly ($P < 0.05$) lower digestibility of CP was observed in the L-APS diet than that of H-APS diet. The CF and ADF digestibility did not differ significantly ($P > 0.05$) among the diets.
The DOM, TDN and ME values were significantly ($P < 0.01$) higher for the both APS diets than that of hay diet. But there was no significant difference between the L-APS and H-APS diets.

Nutrient digestibility and nutritive value of L-APS and H-APS

Table 4. Chemical composition of experimental diets.

Item	Hay diet	L-APS diet	H-APS diet	P value
Dry matter	92.1[c] ± 0.6	58.2[b] ± 0.3	56.3[a] ± 0.2	0.001
Nutrients Composition (%, DM basis):				
Organic matter	88.5[a] ± 0.2	90.9[c] ± 0.1	90.6[b] ± 0.1	0.001
Crude protein	17.1 ± 1.0	17.1 ± 0.5	17.5 ± 0.6	0.333
Crude fiber	27.8[b]± 0.7	21.8[a] ± 0.5	22.6[a] ± 0.2	0.001
Ether extract	1.8[a] ± 0.2	2.4[b] ± 0.2	2.4[b] ± 0.2	0.032
Crude ash	11.5[c] ± 0.2	9.1[a] ± 0.1	9.4[b] ± 0.1	0.001
NFE	41.8[a] ± 0.8	49.6[c] ± 0.2	48.0[b] ± 0.6	0.007
ADF	31.6[b] ± 0.4	27.3[a] ± 0.2	27.5[a] ± 0.3	0.002
NDF	46.4[c] ± 0.8	43.3[a] ± 0.5	44.9[b] ± 0.5	0.004
Hemicellulose	14.8[a] ± 0.9	16.0[b] ± 0.5	17.4[c] ± 0.7	0.008
NFC[2]	23.2[a] ± 0.6	28.1[c] ± 0.4	25.8[b] ± 0.6	0.005

[1] Values are means with SD of three ewes per treatment.[2] NFC = (100 - CP - EE -NDF – Ash).[a,b,c] Different letters indicate significant difference among the treatments (at 5% level).L-APS, low ethanol containing apple pomace silage; H-APS, high ethanol containing apple pomace silage; DM, dry matter; NFE, nitrogen free extract; ADF, acid detergent fibre; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrate.

Table 5. Apparent digestibility and nutritive value of different experimental diets.

Item	Hay diet	L-APS diet	H-APS diet	P value
Apparent digestibility of Nutrients (%)				
Dry matter	58.1[a] ± 0.9	66.7[b] ± 1.9	65.6[b] ± 0.2	0.010
Organic matter	60.2[a] ± 0.5	68.9[b] ± 1.9	67.9[b] ± 0.2	0.008
Crude protein	72.3[a] ± 2.0	73.2[b] ± 1.6	73.6[c] ± 1.7	0.003
Crude fiber	45.1 ± 1.0	54.3 ± 4.5	54.2 ± 1.9	0.043
Ether extract	28.3[a] ± 7.5	47.1[b] ± 1.5	45.9[b] ± 2.6	0.019
NFE	66.7[a] ± 0.9	74.8[b] ± 1.2	73.4[b] ± 0.8	0.010
ADF	42.5 ± 1.1	49.2 ± 3.7	48.0 ± 1.7	0.139
NDF	44.4[a] ± 1.8	53.1[b] ± 2.9	53.7[b] ± 0.8	0.018
Hemicellulose	48.2[a] ± 5.3	59.7[b] ± 3.6	62.7[b] ± 4.3	0.002
Nutritive value (%, DM basis)				
DOM	53.3[a] ± 0.4	62.6[b] ± 1.6	61.6[b] ± 0.1	0.006
DCP	12.4 ± 1.0	12.5 ± 0.6	12.9 ± 0.7	0.158
TDN	54.0[a] ± 0.5	64.0[b] ± 1.7	63.0[b] ± 0.3	0.004
ME (MJ/kg DM)	8.5[a] ± 0.1	10.0[b] ± 0.7	9.90[b] ± 0.2	0.006

[1] Values are means with SD of three ewes per treatment.[a,b,c] Different letters indicate significant difference among the treatments (at 5% level).L-APS, low ethanol containing apple pomace silage; H-APS, high ethanol containing apple pomace silage; DM, dry matter; NFE, nitrogen free extract; ADF, acid detergent fibre; NDF, neutral detergent fibre; DOM, digestible organic matter; DCP, digestible crude protein; TDN, total digestible nutrient; ME, metabolizable energy; MJ, mega joule; kg, kilo gram; SD, standard deviation.

Apparent digestibility and nutritive value of APSs which were calculated by difference method is shown in Table 6. No significant difference was observed among the APSs for the apparent digestibility and nutritive values.

Nitrogen retention

Nitrogen retention of ewes fed different experimental diets is presented in Table 7. The ewes fed on APS diets showed lower nitrogen intake and total nitrogen excretion ($P < 0.01$ and $P < 0.01$; respectively) than the ewes fed on hay diet. The ewes fed on hay diet showed significantly ($P < 0.01$) higher faecal nitrogen excretion than the ewes fed on L-APS and H-APS diets. Besides, faecal nitrogen excretion of ewes fed on diet L-APS was slightly higher ($P < 0.05$) than that of H-APS diet. No significant results were observed among the diets for the urinary nitrogen excretion. All ewes used in the experiment showed a positive nitrogen balance.

Performance traits of ewes fed different experimental diets

Table 6. Apparent digestibility and nutritive value of low ethanol containing apple pomace silage and high ethanol containing apple pomace silage (Estimated by difference).

Item	L-APS	H-APS	P value
Apparently digestibility (%)			
Dry matter	79.5 ± 5.0	77.9 ± 1.3	0.603
Organic matter	80.9 ± 4.3	79.6 ± 1.4	0.656
Crude protein	74.6 ± 6.1	75.7 ± 5.9	0.844
Crude fiber	83.5 ± 21.2	83.0 ± 8.4	0.969
Ether extract	63.2 ± 7.4	61.2 ± 9.5	0.792
NFE	83.1 ± 1.4	81.2 ± 1.5	0.170
ADF	64.3 ± 11.8	61.5 ± 3.4	0.714
NDF	68.8 ± 10.7	70.4 ± 5.5	0.825
Hemicellulose	74.2 ± 13.1	79.2 ± 11.4	0.642
Nutritive value (%, DM basis)			
DOM	76.5 ± 4.1	74.9 ± 1.2	0.563
DCP	12.9 ± 0.9	13.9 ± 1.2	0.296
TDN	79.1 ± 4.1	77.6 ± 1.4	0.596
ME (MJ/kg DM)	12.2 ± 0.7	12.0 ± 0.2	0.562

L-APS, low ethanol containing apple pomace silage; H-APS, high ethanol containing apple pomace silage; DM, dry matter; NFE, nitrogen free extract; ADF, acid detergent fibre; NDF, neutral detergent fibre; DOM, digestible organic matter; DCP, digestible crude protein; TDN, total digestible nutrient; ME, metabolizable energy; MJ, mega joule; kg, kilo gram; SD, standard deviation.

Table 7. Nitrogen retention of ewes fed different experimental diets.

Item	Hay diet	L-APS diet	H-APS diet	P value
Nitrogen intake (g/d)	31.0^{b} ± 2.3	26.0^{a} ± 0.6	25.5^{a} ± 1.3	0.002
Nitrogen in faeces (g/d)	8.6^{c} ± 0.4	7.0^{b} ± 0.4	6.7^{a} ± 0.1	0.001
Nitrogen in urine (g/d)	17.2 ± 2.9	12.6 ± 0.8	13.5 ± 1.6	0.056
Total nitrogen excretion (g/d)	25.7^{b} ± 3.2	19.6^{a} ± 0.4	20.2^{a} ± 1.6	0.029
Nitrogen retention (%)	16.5 ± 14.1	24.5 ± 3.0	20.4 ± 7.9	0.163

[1] Values are means with SD of three ewes per treatment.[a,b,c] Different letters indicate significant difference among the treatments (at 5% level).L-APS, low ethanol containing apple pomace silage; H-APS, high ethanol containing apple pomace silage; DM, dry matter; %, percent; g/d, gm per day ; SD, standard deviation.

Table 8. Body weights and Performance traits of ewes[1] fed different experimental diets.

Item	Hay diet	L-APS diet	H-APS diet	P value
Initial BW(kg)	51.3 ± 2.0	51.3 ± 1.7	50.7 ± 1.5	0.239
Final BW(kg)	51.7 ± 2.8	52.0 ± 2.8	51.4 ± 1.6	0.649
BWG (g/d)	19.4 ± 64.5	33.0 ± 69.3	33.0 ± 10.6	0.926
DM intake (g/d)	1136^{b} ± 59.5	948^{a} ± 32.1	908^{a} ± 21.9	0.008
TDN intake (g/d)	574 ± 21.0	573 ± 15.1	564 ± 19.4	0.357
Ethanol intake[2] (g/d)	0.0^{a} ± 0.0	10.6^{ab} ± 2.2	15.1^{b} ± 2.6	0.040

[1] Values are means with SD of three ewes per treatment.[2] Ethanol intakes were calculated from APS intake and ethanol contents of the APSs.[a,b,c] Different letters indicate significant difference among the treatments (at 5% level). L-APS, low ethanol containing apple pomace silage; H-APS, high ethanol containing apple pomace silage; BW, body weight; BWG, body weight gain; TDN, total digestible nutrient; g/d, gm per day; SD, standard deviation.

No health problems were observed in any of the ewes throughout the experimental period. Performance traits of ewes are presented in Table 8. The DM intake was higher ($P < 0.01$) in hay diet than that of APS diets, but there was no significant difference between the APSs diets. Ethanol intake was higher ($P < 0.05$) for the ewes

fed on H-APS diet than that of L-APS diet, whereas ewes on hay diet were assumed to receive any of alcohol.

Time course changes of plasma parameters

Time course changes of plasma ethanol, lactate, BHBA, and glucose concentrations in ewes fed different experimental diets are presented in Figure 1. After ingestion of APSs, plasma ethanol concentration was abruptly increased ($P < 0.01$) and H-APS diet showed a higher value than that of L-APS diet and peaked at 0.5 h and 1 h after feeding for L-APS and H-APS; respectively. These increased concentrations for APS diets were not returned to the previous value until 3 h after feeding and H-APS diet represented the remarkably higher value (3.68 mmol L^{-1}) than those of other diets. After ingestion, plasma lactate concentrations were increased ($P < 0.05$) in APS diets receiving ewes than that of hay diet and peaked at 2 h after feeding of both APSs. That concentration for L-APS diet declined over the last 3rd h after feeding but that was unchanged for the H-APS diet receiving group. After ingestion of APSs, plasma BHBA concentrations were gradually increased ($P < 0.01$) over the first 3 h after feeding for both APS diets. The postprandial hypoglycaemic tendency ($P = 0.056$) occurred in both APS diets receiving ewes. After feeding of APSs, plasma glucose concentrations were declined over the first 2 h in both APS diets and glucose level was increasing very slowly in H-APS receiving ewes at 3 h after the morning feed.

Correlation among AUC responses of plasma measurements

The correlation coefficient among the AUC of plasma ethanol, lactate, BHBA and glucose for 0-3 h sampling windows are presented in Table 9. Plasma ethanol AUC was positively correlated ($P < 0.05$) with the AUC of plasma lactate and the AUC of plasma BHBA was negatively correlated ($P < 0.01$) with that of the plasma glucose.

DISCUSSION

The higher amount of moisture and low protein of AP were in consent with earlier reporters (Alibes et al., 1984; Pirmohammadi et al., 2006; Taasoli and Kafilzadeh; 2008). Therefore, it was required to adjust the high moisture and improve nutritional balance of AP with the addition of other ingredients and for the better understanding of the fermentation products and nutrient efficiency of balanced APSs. In fact, in the present study, the pre-ensiled AP produced higher moisture compared the APSs that blended with other ingredients to minimize the high moisture and improve nutritional balance of AP after two months storage under the similar anaerobic condition. The spongy like nature of AP fiber, with its"easy-to-absorbs" and "easy-to-release" moisture capacity, may be mostly responsible for this. Moreover, high fermentative sugar (Alibes et al., 1984) and more susceptibility to enzymatic hydrolysis (Gullón et al., 2007) of AP lead to the rapid production of ethanol and lactic acid because there is enough soluble carbohydrate available for sugar fermentation by the action of lactic acid bacteria and yeast. Alibes et al. (1984) found the APS ethanol content was 173 g kg^{-1}. In this experiment, the fermented AP was identified as the largest amount of ethanol (242.5 kg^{-1} DM) also. It's indicated that the drained fluid of H-APS was particularly rich in alcohol. But that ethanol content was notably minimized (87.2 kg^{-1} DM) by mixing with other ingredients of H-APS. Moreover, ethanol content of L-APS and H-APS were significantly remarkable by using fermented AP for preparation of balanced H-APS. We succeed to increase its ethanol content by 36% with compared to that of L-APS which indicate that various concentrations of alcohol in silages are depending on the fermentation profile (Kristensen et al., 2010).

The ethanol content of APSs used in this experiment was more than 1.5 to 2.6 times higher than that of our previous RS-APS experiment (Islam et al. 2014a), 3 to 4 times higher than that of corn silage (McDonald et al., 1991; Raun and Kristensen, 2010), 4 to 6 times higher than that of grass silage (Lawrence et al., 2011) and 4 to 5 times higher than that of ensiled TMR silages (Cummins et al., 2009). H-APS contained about 1.4 times higher ethanol than that of sugarcane silage (Daniel et al., 2013). APSs presented a considerable amount of lactic acid, a small amount of acetic acid, especially no butyric acid and lower the pH level, indicating a good fermentation quality. McDonald et al. (1973) also suggested the same indicators for good silage quality. Probably, due to the lower pH and higher lactic acid content the silage storage quality improved and protected the fungal growth.

The main losses in low DM are associated with the ensiling process and effluent loss (McDonald et al., 2011). The DM content of APSs was higher than that reported by others (14.5 - 21.5%, 17.2 - 28.4%, and 28.4% reported by Alibes et al., 1984; Nikolić and Jovanović, 1986 and Pirmohammadi et al., 2006, respectively). The ensiling proteolysis process increases in the proportion of ammonium nitrogen and free $\propto$-amino acid nitrogen in the silage compared with the original material (McDonald et al., 2011) which was the reason of higher CP content in H-APS compared with L-APS. Hang et al. (1981) reported that yeast fermentation increases the amount of protein in AP by over 50%, whereas in this study was 67% for fermented AP and was 6.4% higher in H-APS compare with L-APS. The APSs were formulated to have similar nutrient contents of the commercial concentrate, which has led to increase in CP content (17 - 18%) than that of others (6.7 - 6.8%, 7.2%, 6.4% on

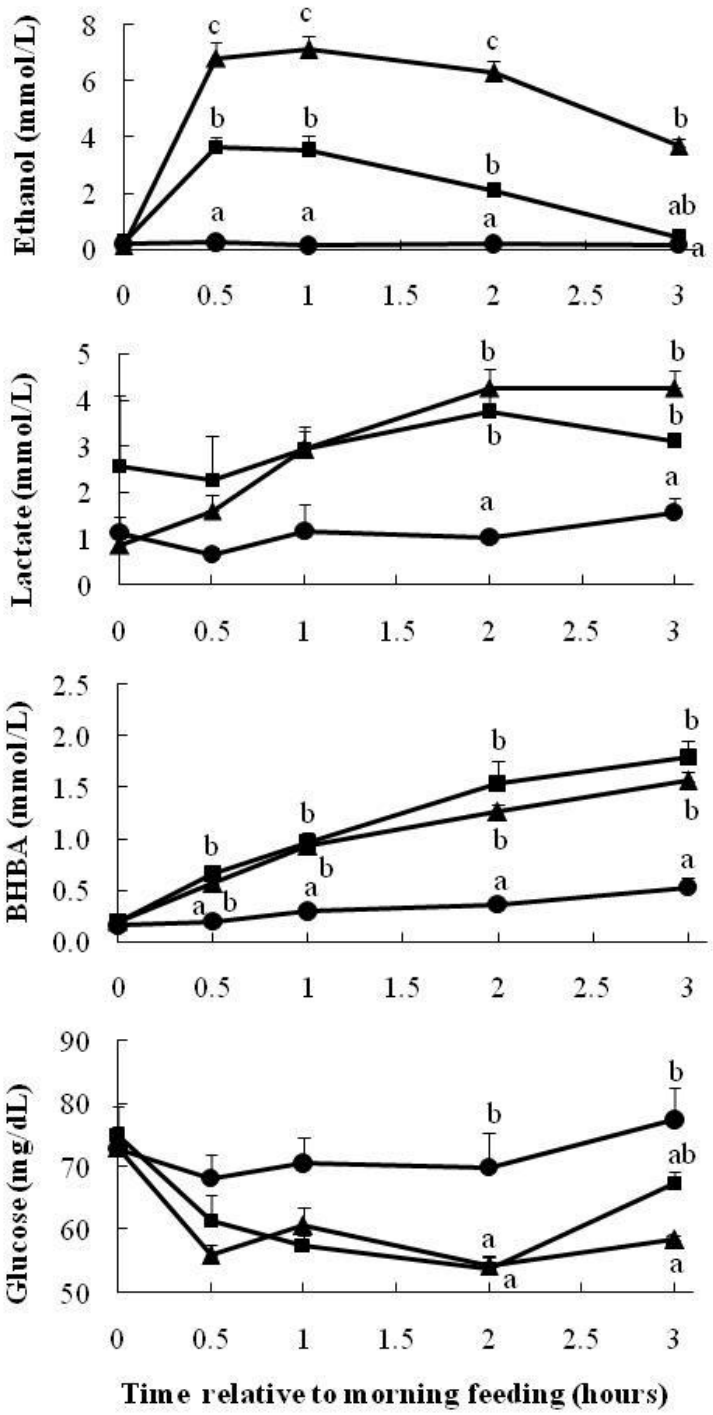

Figure 1. Changes in blood parameters of ewes fed on haydiet (●), L-APSdiet (■) and H-APSdiet (▲). The ethanol intake was calculated to be 10.61 and 15.13 g/d/head for L-APS and H-APS groups, respectively. Each data point is the mean of 3 observations ± standard error of the means. Different letters indicate significant difference within the treatment (at 5% level).

DM basis reported by Alibes et al., 1984, Pirmohammadi et al., 2006 and Taasoli and Kafilzadeh, 2008; respectively). However, Alibes et al. (1984) and Taasoli and Kafilzadeh (2008) reported the similar value for NDF

Table 9. Correlation coefficient among the AUC for 0-3 hours of plasma glucose, lactate, ethanol and BHBA (n=9).

Item	Glucose	Lactate	Ethanol	BHBA
BHBA	-0.9145**	0.3744	0.3744	
Ethanol	-0.5878	0.7275*		
Lactate	-0.2544			

BHBA,β-hydroxybutyrate. The asterisk(s) indicate significante difference (* $P < 0.05$ and ** $P < 0.01$).

of APS (41.3 - 42.7% and 38.6% on DM basis; respectively). During fermentation, even a small amount of water-soluble carbohydrate degraded by lactic acid bacteria could decrease NFE and NFC levels (Cai et al., 2003). The high fiber content of alfalfa hay cube (30% CF as DM basis) increased the dietary DM and CF content of APS diets. The hay diet contained a higher amount of DM, CF, ash, ADF and NDF content and lower amount of OM, EE, NFE, hemicellulose and NFC content compare with the APSs diets due to a higher amount of dry mass in alfalfa hay cube. This is also an indication of the high percentage of the AP material in the APSs.

The digestibility of DM, OM, CP, CF, EE, NFE, NDF and hemicellulose were higher in the ewes fed APSs than those of alfalfa hay diet receiving ewes. Ahn et al. (2002) worked with AP (60% AP + 30% rice bran + 10% concentrate) and reported slightly lower DM values (71.6%) for a Korean goat than those in this study (77.9 - 79.5%). Method of silage making, ensiling condition and type of feed ingredients as the absorbent may have a great impact on the quality of silage and hence the amount that is consumed by animals. Alibes et al. (1984) reported that when diets containing high amount of APS then the OM digestibility of APS diets were 70.1 to 77.7% and estimated OM digestibility for APSs were 74.4 to 80.4%. These estimated values correspond very well to the fact that ewes received 70% fresh or fermented AP containing L/H-APSs and the OM digestibility for APSs were obtained 79.6-80.9%. However APSs diets showed slightly lower values (67.9 - 68.9%) in the present study. Probably the higher amount of hay cube influences the lower OM digestibility for APS diets. However, Taasoli and Kafilzadeh (2008) found slightly lower OM digestibility values (69.9 and 71.8%) for ensiled and dried AP compared to these findings. Fermented APSs were a replacer of concentrate for ruminants, indeed, could increase the estimated apparent CP digestibility of L-APS (74.6%) and H-APS (75.7%) and that level was slightly decreased in the APS diets (73.2 and 73.6% for L-APS and H-APS diets, respectively). However, APS diet having higher digestible CP than that of hay diet (72.3%) and fermented AP influenced slightly higher CP digestibility for H-APS diet than that of L-APS diet. On the other hand, Alibes et al. (1984) found lower estimated apparent CP digestibility for APSs (18.0 - 45.3%) and APS diets (44.9 - 63.4) also. Alibes et al. (1984) reported similar values for estimating apparent CF digestibility (82.2 - 86.2%) of APSs and slightly higher values (63.1 - 83.5%) for APS diets. In addition, fermented APSs had a higher amount of lactic acid, which was extracted out and mixed with EE, resulting in a higher level of EE digestibility in APSs and APS diets. Cellulose digestibility was higher in APSs diets than that of hay diet; probably hydrogen availability was increased by fermented APS ethanol which influenced the cellulose digestion. This result is an agreement with Chalupa et al. (1964), who found that under in-vitro condition ethanol increased cellulose digestion. The special nature of AP improved the digestible OM value (62.6 and 61.6 for L-APS and H-APS diets, respectively) than that of alfalfa hay (53.3%) and the digestible OM value for APSs was obtained 74.9 - 76.5%. However, Pirmohammadi et al. (2006) reported lower digestible OM values (57.5%) than those in the present study. This inconsistent result may be due to differences in variety, environmental conditions, the concentration of cell wall and the technological difference in the juice extraction processes or losses of valuable volatile constituents during dehydration. Daily intake of digestible CP (DCP) were 141.4, 121.8 and 125.8 g in hay diet, L-APS diet and H-APS diet; respectively in this experiment, which was enough to meet the daily requirements (92 g) of 50 kg ewe lambs (Ministry of Agriculture, Forestry, and Fisheries, 1996; Japan). The higher values for digestibility probably contributed to higher TDN value in APSs with fresh AP and fermented AP compared to the alfalfa hay cube. Givens and Barber, (1987) worked with fresh AP and Pirmohammadi et al. (2006) worked with ensiled AP (1 tone AP + 100 kg wheat straw + 5 kg urea) and observed lower values for ME (8.7 and 9.0 MJ/kg DM, respectively) than those in this study (12.0 - 12.2 MJ/kg DM). Such difference might be justified by the contrasting processing methods and additional ingredients. We have not found any significant differences between the L-APS and H-APS for nutrient digestibility and nutritive values. Pirmohammadi et al. (2006) also reported the ME value for maize silages (10.3 MJ kg^{-1} DM) which was lower than those in this study. Such difference indicates that this nutritionally balances fermented APSs are capable to overcome the constrain of low protein content in AP and

will able to fulfill the protein requirement of ruminants. We have formulated daily ration to meet the daily 110% maintenance TDN requirement of growing ewe lambs (Ministry of Agriculture, Forestry, and Fisheries, 1996; Japan) but did not follow the DM requirement because APS had low DM value. Under this condition, higher DM containing alfalfa hay cube intake increased the higher nitrogen intake in the hay diet. In addition, APSs had more nutrients, DCP, TDN and ME, and resulted in less nitrogen excretion in faeces and urine. Nitrogen retention efficiency was also higher in APS diets but the values were non-significant. Under such energy-sufficient condition in APSs, nitrogen utilization was higher in APSs receiving ewes which may have stimulated growth. Although BWG was not significant, APSs receiving ewes had 41% higher growth rate than that of hay diet. Any of performance traits were not affected by a difference in ethanol intake (42%) between L-APS and H-APS diets in this experiment. Therefore, ingestion dose of ethanol (~15 g/d) from APSs in this study would not have any harmful effect on the performance of growing ewes.

After ingestion of APSs, plasma ethanol, lactate and BHBA concentrations were increased, and concentrations were not returned to pre-feeding levels until 3 h after morning feed. Islam et al. (2014a) also found the simultaneous postprandial increase in plasma ethanol, lactate, and BHBA levels by feeding alcoholic fermented APSs. Such increased tendency has been reported by Kristensen et al. (2007) also. This study showed the higher ethanol intake influence the higher plasma ethanol concentration (3.68 and 7.10 mmol/L for L-APS and H-APS, respectively) which corresponds with hypothesis the feeding dietary ethanol led accumulation in plasma ethanol (Kondo et al., 2010, 2011; Raun and Kristensen, 2011). Jean-Blain et al. (1992) worked with the kinetic study of infused ethanol assumed that rumen microflora could metabolize daily ethanol intake (0.2 to 1 g kg-1 BW) and enzymatic system of the host and plasma ethanol level remains below 0.25 g/L. These estimates have exceeded the level in the present study because daily ethanol intake were 0.2 and 0.3 g kg^{-1} BW and the highest plasma ethanol levels were 0.17 - 0.33 g/L. An excess amount of readily fermented carbohydrate in ruminant diets is associated with the higher amount of lactate accumulation (Owens et al., 1998) and thus cause increased plasma lactate in ewes fed on APS diets for this experiment. Islam et al. (2014b) reported that APS ethanol consumption affects the postprandial plasma lactate and BHBA concentrations and we found the similar results in this experiment also. Abrupt glucose supply from silage has often been shown to downregulate hepatic glucose production (Kristensen et al., 2007; Plaizer et al., 2005) in ruminants. Moreover, ethanol inhibits hepatic gluconeogenesis from non-carbohydrate sources such as lactate, glycerol, some amino acids and other substrates (Krebs et al., 1969) which is the cause of postprandial hypoglycemia in sheep fed on APS diets (Kondo et al., 2010; 2011). The similar hypoglycemic condition was also observed in ewes after feeding of APSs in this experiment. Heitmann et al. (1987) reported that the multiparous ewes and high lactating dairy cows walk a fine line between glucose underproduction and ketone body's overproduction. This relation is corresponding very well to the results of highly negative correlation between the AUC of plasma glucose and BHBA which indicating ethanol inhibited gluconeogenesis and result in postprandial hypoglycemia. Positive correlation of lactate with ethanol is strongly supporting this notion also. Therefore, once ingested ethanol increased lactate, suppressed gluconeogenesis and hypoglycemic condition occurred. Although we observed a short-term blood sampling windows in this study all of the responses observed in blood parameters are transient in our previous studied (Islam et al., 2014a) and no toxic effect was observed (Islam et al., 2014b).

The preparation of APSs with grain by-products seems to be promising, as it prevents the loss of drain fluid that is especially rich in nutrients and contains a moderate concentration of fermentation end products, which is a fact of importance as far as ethanol is concerned. Although excess ethanol intake was not detected in this experiment and no health problems were detected, the need for further studies to assess safety and productivity of the use of APS is warranted.

Conclusion

Based on silage characteristics, it was indicated that AP and alcoholic AP could be used for APS preparation and inclusion level of AP would be up to 70% of the fresh and fermented state. It may be concluded that nutritional balanced fermented APS can be used as a replacer of concentrate feed for ruminants in combination with hay cube or dry roughage up to 50% of the TDN requirement and the ewes are capable of using 15.1 g/d ethanol intake without any adverse side effect.

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