

Intake, digestibility and rumen fermentation pattern in wethers fed total mixed ration silage containing dry or fresh apple pomace

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³ Corresponding author: e-mail: fang@hirosaki-u.ac.jp ABSTRACT. The fermentation quality of total mixed ration silage (TMRS) prepared with dry apple pomace (DAP) or fresh apple pomace (FAP), as well as their effects on digestibility and rumen fermentation in feeding wethers were evaluated. Maize, wheat bran, soyabean meal, timothy and alfalfa hay were used to compose TMRS. Proportions of DAP and FAP in TMRS were 200 g/kg dry matter (DM) each, and the moisture content of TMRS was adjusted to 550 g/kg and ensiled for 60 days. The mixture of TMR with DAP without ensiling was used as a control diet, and experimental treatments were TMRS with DAP and TMRS with FAP. The TMRS with DAP and FAP were well fermented, with low pH and high lactic acid content. Ethanol was produced in both TMRS treatments and was significantly higher in that with FAP. Nitrogen retention and digestibility of DM, organic matter and crude protein were significantly lower in the treatments with DAP and FAP than in control one. Four hours after feeding TMRS with DAP and FAP, the rumen molar proportion of acetic acid was higher, while those of propionic acid and ammonia nitrogen content were lower, with more pronounced changes in the treatment with FAP. The results of this study suggest that the proportion of DAP or FAP in TMRS should not exceed 200 g/kg of diet DM.

Introduction

Apple pomace is a by-product that remains after milling and pressing apples for juice and cider production. It is usually a seasonal product available after the autumn harvest and is produced in large amounts in many countries (Perussello et al., 2017). Apple pomace has many uses, such as for food, pectin, citric acid and dietary fibre production (Shalini and Gupta, 2010). Demand for it as feed is expected to increase with a global supply shortage of feed grains and rising feed prices. As a feed material, the chemical composition of fresh apple pomace (FAP) is characterized by high moisture and fermentable sugar contents and low crude protein (CP) content (Gasa et al., 1992). It was shown

that FAP promotes silage fermentation (Fang et al., 2016). However, it was also indicated that silage containing FAP produces not only organic acids but also ethanol. Ethanol contains high energy and volatilizes easily, however when its content in silage is high, a loss of energy and dry matter (DM) may occur through volatilization (Pedroso et al., 2005). Furthermore, in some studies it was observed that ethanol affects cow milk composition and flavour (Randby et al., 1999) and reduces feed digestibility in ruminants (Emery et al., 1959; Fang et al., 2016). Because of high moisture content, FAP is challenging to transport over long distances, limiting its widespread use. In practice, apple pomace as feed material is circulated in a dry state. Because most of the circulated dry apple pomace (DAP)

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has been dried at temperatures of more than 100 °C, the microorganisms attached to the apple pomace that affect fermentation, such as lactic acid bacteria and yeast, may decrease or die out. Consequently, this may result in different fermentation patterns for total mixed ration silage (TMRS) containing DAP or FAP, particularly regarding ethanol production. Although ethanol production via fermentation of DAP under industrial conditions has been widely evaluated (Magyar et al., 2016), its effects on feed silage fermentation are not fully estimated. So, fermentation characteristics, particularly ethanol production of total TMRS prepared with either DAP or FAP were evaluated in the present study. Feed intake, digestibility, nitrogen (N) retention and rumen fermentation were also estimated in wethers to determine which form (dry or fresh) of apple pomace is more beneficial for ruminants.

Material and methods

TMRS preparation

Apple pomace was obtained from a commercial juice factory (Aoren, Aomori, Japan), and FAP was used to prepare TMRS immediately after production. The DAP needed for the experiment was prepared in a commercial factory (Nihon-Kouken, Aomori, Japan), where it was dried with hot air at 110 °C while being stirred for 4 h. Experimental diets were prepared with FAP or DAP together with TMRS with maize, wheat bran, soyabean meal, timothy hay, alfalfa hay and a vitamin-mineral supplement (Snow Brand Seed, Sapporo, Japan). The moisture content of DAP was adjusted to be equal to the calculated value of FAP treatment (550 g/kg). The proportion of DAP or FAP in the TMRS was 200 g/kg DM; CP content and proportion of roughage were determined according to Xu et al. (2008). The mixture of TMRS material with DAP without ensiling was used as a control diet. When preparing the TMRS, timothy and alfalfa hay were cut into 2-cm pieces. All materials were well stirred using a heavy mixer, and the moisture content of DAP was adjusted by gradually adding water. Overall, 300 kg of TMRS with FAP and DAP were prepared and ensiled equally into three 200-l sealing-type drum silos (Maeda Manufacturing Co. Ltd., Ichihara, Japan) after being fully compressed. Then the TMRS was stored outdoors (4.4–25.8 °C) to ferment for 60 days.

Animals and feeding trial

The experiment was approved by the committee of animal experimentation and performed under the institutional guidelines for animal experiments of Hirosaki University (Japan). Four Suffolk wethers (body weight = 65.3 ± 3.4 kg) were used for the experiment and were fed control, DAP and FAP diets in three separate experiments. The wethers were housed individually in metabolic cages ($150 \times 60 \times 100$ cm, with a function of collecting faeces and urine separately) and fed diet *ad libitum* to ensure 10% residual. The moisture content of control diet was also adjusted to 550 g/kg just before feeding to ensure consistent texture and mouthfeel with the TMRS.

The diets were fed in two halves at 07:00 and 19:00, and refusals were weighed before the morning feedings. The wethers had free access to water and mineral blocks during the experiments. Each experiment lasted 21 days, including a 14-day adaptation period followed by a 7-day faecaluria collection period. During each collection period, samples of faeces were collected twice a day, in the morning and afternoon, urine was also collected into a bottle containing 0.3 ml hydrochloric acid (100 mg/g), then 3% of the faeces and urine by weight was stored at -20 °C.

At the end of each period, the stored faeces and urine were combined and mixed fully, and then a sample was collected for chemical analyses. Rumen fluid was collected using a rumen catheter (Sanshin Industrial, Yokohama, Japan) at 0^{th} (before feeding) and 4^{th} h after feeding on the last day of each period. Rumen fluid pH was measured immediately, and samples were separated from feed particles by filtration through four layers of gauze followed by centrifugation at 1200 g for 15 min at $4 \, ^{\circ}$ C. The sample fluid was stored frozen ($-20 \, ^{\circ}$ C) for further analyses of volatile fatty acid (VFA) and ammonia N.

Sampling and chemical analysis

Samples of TMRS for chemical analyses and fermentation quality were taken equally from the upper, middle and lower parts of each drum silo, combined and mixed fully. The moisture content of the TMRS was measured using the toluene distillation method according to Dewar and McDonald (1961). Diet and faeces samples were dried in a forced-air oven at 60 °C for 48 h and ground to pass a 1-mm screen with a sample mill. Dry matter, CP, ether extract (EE) and ash were analyzed according to AOAC (1990) methods 934.01, 976.05, 920.39 and 942.05, respectively. Urinary N was determined using the Kjeldahl procedure (AOAC, 1990). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined following the methods of Van Soest et al. (1991) with amylase and sodium sulphite;

the presented results include residual ash. Gross energy (GE) was determined using an automatic bomb calorimeter (CA-4PJ, Shimadzu Co. Ltd., Kyoto, Japan). The TMRS fermentation products were determined from the liquid extract. Wet samples (100 g) were homogenized with 300 ml sterilized distilled water for 5 min using a mixer, then filtered through four gauze layers (Cai et al., 1999). Filtrate pH was measured using a glass electrode pH meter (D-21; Horiba Co. Ltd., Tokyo, Japan). Lactic acid and ammonia N were determined according to Barker and Summerson (1941). The VFA was steam-distilled and measured qualitatively and quantitatively by gas chromatography (G-5000A; Hitachi Co. Ltd., Tokyo, Japan). The ethanol content of the TMRS was determined using the gasification balance method according to Xu et al. (2001). The VFA and ammonia N contents of rumen fluid were measured using the same methods as for TMRS filtrates.

Statistical analysis

The data of fermentation products of the two TMRS were analyzed using the independent t-test. Data of chemical composition, nutrient digestibility, N retention and rumen fermentation of three treatments were analyzed using one-way ANOVA. Statistical comparisons were made using Tukey-Kramer tests. Differences were considered significant at a threshold of P < 0.05. Statistical analyses were performed using SPSS software ver. 25 (SAS Institute Inc., Cary, NC, USA).

Results

Chemical composition of materials and TMRS

Very low DM and CP contents of 151 and 76.1 g/kg DM, respectively were noted in FAP (Table 1). Almost no differences in chemical composition between DAP and FAP were observed, and their NDF content was close to that of alfalfa hay, which is a very good quality feed for ruminant nutrition. Since ingredient proportions of TMRS were almost identical except for the apple pomace, the three diets had similar chemical compositions (Table 2).

Fermentation quality of TMRS

Low pH and high lactic acid contents were found in TMRS with DAP and FAP (Table 3), ethanol was generated in both DAP and FAP treatments but was significantly higher in FAP one (P < 0.05). Conversely, the lactic acid, acetic acid

Table 1. Chemical composition of materials used in the experiment

Indices, g/kg DM	DAP	FAP	Soyabean meal	Maize	Wheat bran	Timothy hay	Alfalfa hay
DM, g/kg	924.8	151.0	868.8	855.1	862.3	874.3	875.7
Organic matter	964.1	963.9	931.1	987.0	942.2	935.0	896.5
Crude protein	75.9	76.1	484.0	81.3	178.7	105.2	216.7
Ether extract	59.6	60.0	15.2	34.8	46.9	9.3	14.7
Acid detergent fibre	344.8	342.2	90.2	32.7	141.3	383.4	312.2
Neutral detergent fibre	431.1	428.9	148.1	123.2	463.1	641.7	441.8
Ash	35.9	36.1	68.9	13.0	57.8	65.0	103.5

DM – dry matter, DAP – dry apple pomace, FAP – fresh apple pomace

Table 2. Ingredient proportions and chemical composition of the experimental diets

Indices	Control	FAP	DAP	SEM	P-value
Ingredient, g/kg DM			-		
fresh apple pomace	-	200	-	-	-
dry apple pomace	200	-	200	-	-
soyabean meal	78	78	78	-	-
maize	132	132	132	-	-
wheat bran	93	93	93	-	-
timothy hay	243	243	243	-	-
alfalfa hay	240	240	240	-	-
vitamin-mineral supplement	14	14	14	-	-
Chemical composition, g/kg	g DM				
DM, g/kg	449.1	444.7	446.7	1.27	0.593
organic matter	940.9	936.2	939.4	1.39	0.182
crude protein	162.8	161.2	159.2	1.04	0.395
ether extract	29.8	28.1	30.7	0.76	0.457
acid detergent fibre	260.3	258.8	259.7	0.44	0.991
neutral detergent fibre	439.7	442.5	431.2	3.40	0.573
gross energy, MJ/kg DM	19.0	18.9	18.9	0.03	0.971

DM — dry matter, Control — DAP treatment without ensiling, FAP — treatment as total mixed ration silage containing fresh apple pomace, DAP — treatment as total mixed ration silage containing dry apple pomace, SEM — standard error of the mean

Table 3. Fermentation quality of total mixed ration silages (n = 3)

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Indices	DAP	FAP	SEM	P-value
pH	3.98b	4.11a	0.06	0.022
Ethanol, g/kg FM	8.93b	36.1ª	13.59	<0.001
Lactic acid, g/kg FM	19.2ª	17.6 ^b	0.80	0.023
Acetic acid, g/kg FM	10.4ª	6.8 ^b	1.82	<0.001
Propionic acid, g/kg FM	ND	ND	-	-
Butyric acid, g/kg FM	ND	ND	-	-
Ammonia nitrogen, g/kg TN	18.0ª	16.6⁵	0.70	0.016

DAP - treatment as total mixed ration silage containing dry apple pomace, FAP - treatment as total mixed ration silage containing fresh apple pomace, SEM - standard error of the mean, FM - fresh matter, ND - not detected, TN - total nitrogen; $^{\text{ab}}-$ values within the same row with different superscripts are significantly different at P < 0.05

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and ammonia N contents were higher in the DAP treatment (P < 0.05). Neither propionic nor butyric acid were detected.

Feed intake and digestibility

The feed intake of DM was not affected by diet treatment (P > 0.05) (Table 4). Digestibilities of DM, organic matter, CP and GE were similar in DAP and FAP treatments but lower than that of the control treatment (P < 0.05). Digestibility of EE, ADF and NDF were the highest in the control treatment, followed by the DAP and FAP treatments (P < 0.05). No differences were found in N intake or urinary N among the treatments (P > 0.05), but faecal N was higher in DAP and FAP treatments, while retained N was higher in the control treatment (P < 0.05).

Table 4. Feed intake, nutrient digestibility and nitrogen balance in wethers fed experimental diets (n = 4)

Indices	Control	DAP	FAP	SEM	P-value
Feed intake, dry matter g/day	1211.6	1208.0	1210.5	1.07	0.753
Digestibility, %					
dry matter	73.8ª	71.5b	70.4b	1.00	0.036
organic matter	73.3ª	70.6 ^b	70.4b	0.94	0.028
crude protein	77.2ª	73.7b	73.6b	1.18	0.033
ether extract	76.5ª	71.9b	61.2°	4.53	< 0.001
acid detergent fibre	61.3ª	59.2b	44.8°	5.92	< 0.001
neutral detergent fibre	63.9ª	60.7b	50.7℃	3.98	< 0.001
gross energy	72.6ª	70.4b	70.1 ^b	0.78	0.034
Nitrogen retention, g/day	1				
nitrogen intake	31.6	30.8	31.2	0.23	0.531
urinary excretion	16.3	15.8	16.2	0.15	0.655
faecal excretion	7.2 ^b	8.1ª	8.2ª	0.32	0.029
nitrogen retention	8.1ª	6.9b	6.8b	0.42	0.008

Control – DAP treatment without ensiling, DAP – treatment as total mixed ration silage containing dry apple pomace, FAP – treatment as total mixed ration silage containing fresh apple pomace, SEM – standard error of the mean; abc – values within the same row with different superscripts are significantly different at P < 0.05

Rumen fermentation

The diet treatment did not affect rumen pH or total VFA concentration at 0^{th} and 4^{th} h after feeding (P > 0.05) (Table 5). Molar proportions of acetic and propionic acids, and the ratio of acetic acid to propionic acid (A:P) did not differ among the treatments (P > 0.05) at the 0^{th} h. However, the acetic acid molar proportion increased whereas that of propionic acid decreased in FAP and DAP in comparison to control treatment at the 4^{th} h after feeding and differences were found among the treatments (P < 0.05). The A:P ratio has changed

in accordance with significant differences among the treatments (P < 0.05). The diet treatments also affected molar proportions of butyric, valeric and caproic acids, and significant differences were observed at 0^{th} and 4^{th} h after feeding (P < 0.05). The rumen ammonia N concentrations were similar for the three treatments at the 0^{th} h (P > 0.05).

Table 5. Rumen fermentation of wethers fed experimental diets (n = 4)

Indices	Control	DAP	FAP	SEM	P-value		
рН							
$0^{th} h$	6.20	6.54	6.51	0.109	0.749		
$4^{\text{th}} h$	6.21	6.22	6.43	0.072	0.826		
Total volatile fatty acid, mmol/l							
$0^{th} h$	75.9	76.6	75.2	0.40	0.625		
$4^{\text{th}} h$	80.4	81.2	81.0	0.24	0.586		
Acetic ac	id, % mol						
$0^{th} h$	59.2	60.2	61.4	0.64	0.670		
$4^{\text{th}} h$	55.5°	59.3⁵	63.3ª	2.25	<0.001		
Propionio	acid, % mo	ol					
$0^{th} h$	22.8	21.6	21.5	0.42	0.627		
$4^{\text{th}} h$	25.3a	21.5⁵	17.8°	2.17	<0.001		
Butyric a	cid, % mol						
$0^{th} h$	13.3ª	13.2ª	10.9 ^b	1.20	0.006		
$4^{\text{th}} h$	14.3ª	14.0ª	11.9 ^b	0.75	0.009		
Valeric ad	Valeric acid, % mol						
$0^{th} h$	3.4 ^b	3.6 ^b	4.9a	0.47	0.012		
$4^{\text{th}} h$	3.5 ^b	3.7 ^b	5.1a	0.50	0.010		
Caproic acid, % mol							
$0^{th} h$	1.3	1.4	1.3	0.03	0.682		
$4^{\text{th}} h$	1.4 ^b	1.5⁵	1.9ª	0.15	0.021		
Acetic acid/Propionic acid							
$0^{th} h$	2.59	2.79	2.85	0.079	0.664		
$4^{\text{th}} h$	2.20°	2.75b	3.55ª	0.392	<0.001		
Ammonia nitrogen, mg/dl							
$0^{th} h$	13.2	13.2	12.3	0.30	0.628		
$4^{th} h$	17.5ª	15.7⁵	14.3°	0.93	<0.001		

Control – DAP treatment without ensiling, DAP – treatment as total mixed ration silage containing dry apple pomace, FAP – treatment as total mixed ration silage containing fresh apple pomace, SEM – standard error of the mean; abc – values within the same row with different superscripts are significantly different at P < 0.05

However, at the 4th h after feeding, the concentration was highest in control, followed by DAP, then FAP treatment (P < 0.05).

Discussion

Fermentation quality of TMRS. In some studies it was shown that FAP is a fermentative material due to its high sugar content (Fang et al., 2016). In the present study, both TMRS with DAP and FAP were well fermented if evaluated by low pH and high lactic acid, and there were no problems

with long-term preservation. However, high ethanol production in TMRS causes a high DM loss so the fermentation cannot be regarded as a good quality one. Ethanol is produced in silage containing ingredients such as sweet sorghum (Gallagher et al., 2018), sugar cane (Pedroso et al., 2005) and pineapple (Nisarani et al., 2015), and its production in silage is affected by yeast count (Weiss et al., 2016). In the present study, the DAP was dried for 4 h at 110 °C, which exceeds the sterilization temperature of 60 °C for yeast (Arthur and Watson, 1976); therefore, most of the yeast may have died out during the drying process, resulting in significantly lower ethanol production in the DAP TMRS. The lactic acid in silage is mainly produced by lactic acid bacteria using water-soluble carbohydrates, and its production is affected by the activity of lactic acid bacteria (Cai et al., 1999). In the present study, maize, DAP and FAP containing high watersoluble carbohydrates (sugar or starch) were used in high proportions in the TMRS (132.2, 200 and 200 g/kg DM, respectively). They provided an adequate substrate for lactic acid fermentation. Predicted from this condition, there should be no difference in lactate acid; still, lactic acid production differed between the FAP and DAP treatments. Because ethanol has an antibacterial effect, its concentration may have affected the lactic acid bacteria activity. Fang et al. (2016) studied the fermentation quality of TMRS containing FAP at different proportions. They found that lactic acid production peaked when the ethanol concentration was less than 20 g/kg but decreased significantly at ethanol concentrations above 20 g/kg. In the present study, the ethanol concentrations in TMRS with FAP and DAP were 36.1 and 8.93 g/kg, respectively. The higher ethanol in TMRS with FAP may have inhibited lactic acid bacteria activity, resulting in lower lactic acid than in the DAP treatment. The lower acetic acid levels in TMRS with FAP may be also caused by ethanol inhibiting the activity of acetic acid-producing bacteria. The acetic acid in silage is produced by plural course (Limin et al., 2018), and as it was shown in our previous experiment (Fang et al., 2016; 2020), the effect of ethanol concentration on acetic acid production is often variable in TMRS containing FAP. Thus, it was not clear why acetic acid production differed between FAP and DAP treatments in the present study. Regarding the ammonia N, ethanol in TMRS may also affect the activity of proteolytic bacteria, and Xu et al. (2001) reported that ammonia N in tofu-cake silage decreased with the ethanol addition.

Thus, the lower ammonia N observed in TMRS of FAP may have been due to the inhibition of protein degradation by ethanol during fermentation.

Feed intake and digestibility. Some studies have shown that the fermentation products of silage affect feeding preferences and feed intake (Buchanan-Smith, 1990); however, feed intake was not affected by the diet in the present study. The non-roughage proportion of diet was high (520 g/kg); maize, wheat bran, soyabean meal, DAP and FAP were willingly eaten by the animals. These materials with high preferences may have exceeded the effect of fermentation on feed intake, so no differences in the intake were found among treatments. It was observed in some studies that lactic or acetic acids in silage can affect digestibility. However, Hino et al. (1992) reported that continuous ethanol ingestion inhibits the growth of rumen microorganisms, while another study showed that ethanol ingestion reduces digestibility for ruminants (Emery et al., 1959). In the present study, the nutrient digestibility significantly decreased in DAP and FAP treatments in comparison to control.

Furthermore, the digestibility of EE, ADF and NDF in FAP significantly decreased in comparison to those in DAP. These results indicate that the decrease in digestibility may have been caused by ethanol ingestion, and the its effects increased with ethanol intake. This result is consistent with previous reports (Fang et al., 2016; 2020). Another difference among treatments was that the DAP was dried at 110 °C for 4 h. Although a high-temperature drying process can reduce the digestibility of CP (Böttger and Südekum, 2018), no difference in CP digestibility was found between FAP and DAP treatments in this study. Since apple pomace contains very little CP, and most of it is concentrated in seed (Yu et al., 2007), and as shown in our previous study, sheep can barely digest seeds in apple pomace (Fang et al., 2020), no effects of high temperature on CP digestibility were found. Faecal N was higher in the DAP and FAP treatments, but the intake and urine N were similar among treatments, leading to lower N retention. This is consistent with the CP digestibility.

Rumen fermentation. Ethanol produced in TMRS containing FAP increases the molar proportions of acetic, valeric and caproic acids; and decreases the proportion of propionic acid in the rumen fluid of cows, the effects of which increase with ethanol intake (Fang et al., 2016). Similar results were noted when ruminants were fed ethanol (Pradhan and Hemken, 1970; Miyazaki et al., 1991).

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The relationship between rumen fermentation and ethanol ingestion found here is consistent with previous studies, and concerns individual rumen VFA concentrations at the 4th h after feeding and the ethanol content in the diet. Therefore, VFA in the rumen may also be affected by ethanol. The reason for increasing values of acetic, valeric and caproic acids in FAP and DAP treatments is discussed by Miyazaki et al. (1991). Ethanol in feed, in addition to inhibiting the microorganisms of the rumen, can be also generated to acetyl-CoA, which is then used as an intermediate product to generate acetic, valeric and caproic acids. When the effect of ethanol on inhibiting the microorganisms of the rumen is relatively weak, ethanol in feed increases these acids due to the process described above. Ammonia N in the rumen is derived from CP decomposition by microorganisms. The CP content and composition were almost identical among the treatments, so the difference in ammonia N may have been caused by fermentation products. Hino et al. (1992) reported that ethanol ingestion inhibits the growth of rumen microorganisms and rumen protein degradation in goats; thus, changes in rumen ammonia N may be also caused by ethanol ingestion.

Our results indicate that DAP also ferments vigorously and produces ethanol in TMRS. Although the ethanol production was significantly lower in TMRS containing 200 g/kg DM of DAP instead of FAP, its concentration still had a negative impact on digestibility and N retention due to its strong irritant effect. To prevent ethanol negative effects, one should consider the proportion of FAP or DAP in TMRS and employ a means of inhibiting ethanol production when ensiling.

Conclusions

It was indicated that ethanol is also produced in total mixed ration silage (TMRS) containing dry apple pomace (DAP). Although ethanol has no negative effect on feed preservation, it causes dry matter (DM) loss, so TMRS cannot be evaluated as being a good quality one. At the same proportion of 200 g/kg DM, the ethanol content in TMRS with DAP was lower than in TMRS with fresh apple pomace (FAP), but it still decreased digestibility and nitrogen retention, notwithstanding there were potential confunding effects between period and treatment in the feeding trail. If DAP is used to prepare TMRS, its proportion should be reduced. From a practical point of view the ethanol production in TMRS with FAP should be inhibited by reducing proportion, adding

yeast inhibitors or water-absorbing materials, etc. Whereas direct use of DAP without ensiling may be a better option in ruminant feeding.

Conflict of interest

The Authors declare that there is no conflict of interest.

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