Fermentation control and ethanol production of total mixed ration prepared with apple pomace and microbial and chemical additives

Fang, J.*; Cai, Y. †

* Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Japan; †Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

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Abstract. The objective of this study is to evaluate the impacts of moisture adjustment, lactic acid bacteria (LAB) inoculant and chemical additives on fermentation characteristics and ethanol production of a total mixed ration (TMR) containing apple (*Malus domestica*) pomace. The TMR was prepared with apple pomace, corn, wheat bran, soybean meal, timothy, and alfalfa hay. In Experiment 1, the proportion of apple pomace was 150 g/kg of dry matter (DM), and the moisture of the TMR was unadjusted (control) or adjusted to 450, 500, and 550 g/kg, respectively. In Experiment 2, the same ingredient proportions as in experiment 1 were used and the TMR moisture was adjusted to 550 g/kg. The treatments were no additive (Control), homo-fermentative LAB (*Lactobacillus plantarum*, LP), hetero-fermentative LAB (*Lactobacillus buchneri*, LB) and calcium propionate (CP). The small-scale fermentation system was used to prepare the TMR, and their fermentation characteristics were analyzed after 60 days of ensiling. In Experiment 1, the pH of various TMRs was around 4.1. With the moisture decrease, the lactic acid increased ($P<0.05$), and the ammonia nitrogen decreased ($P<0.05$). The ethanol decreased significantly with moisture adjustment compared to the control, and the TMR with moisture of 500g/kg showed the lowest ethanol concentration ($P<0.05$). In Experiment 2, LP treatment increased lactic acid and decreased acetic acid and ammonia nitrogen significantly ($P<0.05$), while LB treatment had no effect on fermentation. Both LP and LB had no effect on the ethanol concentration. The TMR treated with CP significantly decreased the ethanol and acetic acid concentrations ($P<0.05$) but did not inhibit the lactic acid production compared to control. The results confirmed that adjusting moisture to 500 g/kg and adding CP could effectively inhibit the excessive production of ethanol in TMR containing apple pomace. Homo-fermentative LAB can better improve the fermentation quality of TMR than hetero-fermentative LAB, but neither can inhibit the production of ethanol.

Introduction

With the development of the world economy and the continuous increase of the population, the global demand for food production is constantly rising. Therefore, the shortage of food and feed has become a serious problem in many countries of the world, including Japan. Effective utilization of food by-products as feed resources may be an ideal solution to this problem.

Apple pomace is a by-product that remains after milling and pressing apples for juice production and is produced in large amounts in many countries. Because of high moisture, apple pomace spoils readily and is not suitable for long-distance transportation. These factors render apple pomace expensive and limit its extensive utilization for feed. In recent years, total mixed rations (TMR) have become popular in many countries as a way to use high-moisture food by-products in ruminant feed, enabling widespread use of apple pomace. When apple pomace is used for preparing a TMR, it produces not only organic acids but also ethanol. Ethanol ingestion affects cow milk composition and flavor, causes malformation and stillbirth, and reduces feed digestibility in ruminants. Ethanol in silage is usually produced by yeast fermentation, thus it is necessary to use microbial or chemical additives to inhibit the production of ethanol in apple pomace TMR. In recent years, propionic acid or its salt and some lactic acid bacteria (LAB) have been used successfully to inhibit yeast and ethanol production in high-sugar silage (Carvalho *et al.,* 2012). Furthermore, some studies have shown that the TMR moisture level affects the fermentation pattern (Antonio *et al.,* 2020). If moisture adjustment can inhibit yeast and ethanol production in TMR containing apple pomace, it would be a safe and economical strategy. This study evaluated the impacts of moisture adjustment and chemical and microbial additives on the fermentation characteristics of TMR containing apple pomace, especially the inhibition of ethanol production.

Material and methods

Fresh apple pomace obtained from a juice factory (Aomori Morita Apple Juice Company, Aomori, Japan) was immediately used to prepare the TMR (Fig. 1.) The ingredient proportions and chemical composition of the TMR are shown in Fig. 2 and Table 1, respectively. In Experiment 1, the proportion of apple pomace in the TMR was 150 g/kg on a dry matter (DM) basis. The moisture in the control TMR was not adjusted, and the calculated moisture was 400 g/kg. Then, TMRs with moisture of 450, 500, and 550 g/kg were prepared by adding distilled water and labeled M450, M500, and M550 as treatments, respectively.

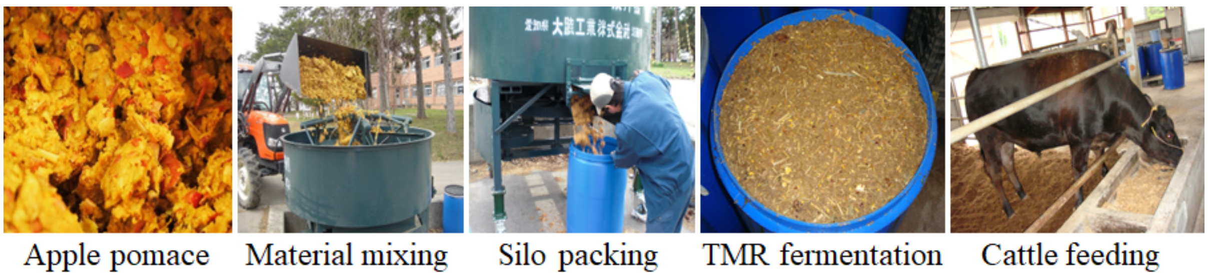

Fig. 1. Apple pomace TMR preparation and cattle feeding. TMR, total mixed ration.

In Experiment 2, the ingredient proportions of TMR materials and moisture adjustment were the same as that of M550 in Experiment 1, and the control TMR had no additive. As the treatment, homo-fermentative LAB (*Lactobacillus plantarum* Chikuso-1; Snow Brand Seed, Sapporo, Japan), hetero-fermentative LAB (*Lactobacillus buchneri* 11A44; Pioneer EcoScience CO., LTD, Tokyo, Japan), and calcium propionate (Granular feed grade, concentration: 92.5%; Niacet, Netherlands) were used and labeled LP, LB and CP as treatments, respectively. All additives were dissolved in distilled water and then added, and the moisture was adjusted to 550 g/kg. For each treatment, 3 kg of the TMR was prepared and packed equally into three plastic bag silos, degassed, sealed using a vacuum packing machine, and stored at 20-25°C for 60 days.

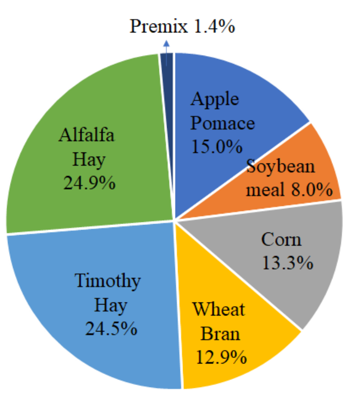

Fig. 2. Ingredient proportion of TMR. TMR, total mixed ration, percentage shows mix ratio.

Table 1. Chemical composition of apple pomace and control TMR in experiment 1.

Item	Apple pomace	TMR
DM g/kg	218	596
Organic matter (g/kg DM)	974	938
Crude protein (g/kg DM)	46	151
Ether extract (g/kg DM)	34	21
Acid detergent fiber (g/kg DM)	257	259
Neutral detergent fiber (g/kg DM)	347	422

TMR, total mixed ration; DM, dry matter.

Results and Discussion

In Experiment 1, the four kinds of TMR had a low pH and high lactic acid concentration, indicating that they were well fermented (Table 2), but their fermentation patterns were affected by the moisture level. Compared to the control, the lactic acid concentration was highest with the M450 treatment, followed by the M500 treatment (*P*<0.05), whereas the M550 treatment was similar to the control. Acetic acid and ammonia nitrogen also increased with the moisture concentration and peaked with the M550 treatment (*P*<0.05). Ethanol was decreased by adding water and was lowest in the M500 treatment, while the control had the highest value (*P*<0.05). No propionic or butyric acid was detected in any treatment. In Experiment 2, all additives affected fermentation of the TMR, except LB (Table 3). Compared to the control, LP significantly increased lactic acid, and inhibited acetic acid and ammonia nitrogen, and had the lowest ammonia nitrogen concentration (*P*<0.05). Of the LAB, LP had the ideal effect. CP had no effect on lactic acid and ammonia nitrogen, but significantly inhibited acetic acid production compared to the control (*P*<0.05). Propionic acid was detected only with CP treatment. Importantly, CP significantly inhibited ethanol production (*P*<0.05), whereas two kinds of LAB had no effect. All TMRs were well fermented based on their pH and lactic acid concentration, and there were no problems with long-term preservation.

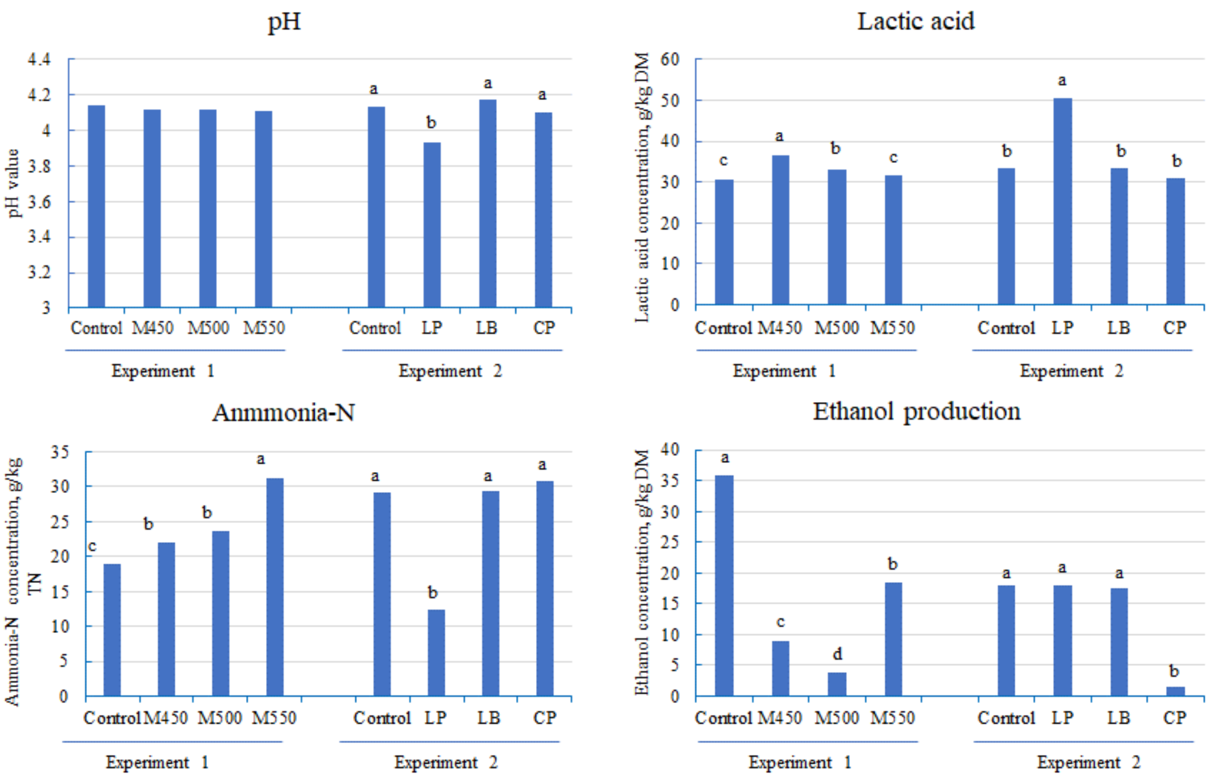

Fig. 2. Fermentation characteristics and ethanol production of TMRs in Experiment 1 and Experiment 2. DM, dry matter; M450, 500 and 550, the moistures of the TMRs were adjusted to 450, 500 and 550 g/kg, respectively. TMR, total mixed ration; N, nitrogen; TN, total nitrogen. [a,b,c,d] Different letters in the same experimental group show significant differences (*P*<0.05).

Previous studies indicated that changing the moisture level can affect TMR fermentation, but the result varied with each material and no fixed relation between the fermentation pattern and moisture (Antonio *et al.,* 2020). In experiment 1, moisture level affected the levels of lactic acid, acetic acid, and ammonia nitrogen, but their production was all in the good range. However, the moisture level strongly affected ethanol, with the most produced in control treatment that without moisture adjustment. Adding water inhibited ethanol, with the maximum inhibition at a moisture level of 500 g/kg. Since the microorganisms count was not determined in the present study, and the study about moisture level and yeast activity is lacking, the reason for it is unclear. Further study is needed to establish the causal relationship between moisture and ethanol production in TMRs containing apple pomace. Some studies found that LAB may be inhibited when the ethanol concentration exceed 20 g/kg DM. In Experiment 1, the ethanol concentration in the control was 35.9 g/kg DM. The higher ethanol in TMRs of the control may have inhibited LAB activity, resulting in lower lactic acid than in M450 and M500 treatment. The lowest acetic acid level in TMRs of the control may be also caused by ethanol inhibiting the activity of acetic acid-producing bacteria. Regarding the ammonia nitrogen, ethanol in TMRs may also affect the activity of proteolytic bacteria, and previous studies reported that ammonia nitrogen in tofu-cake silage decreased with the ethanol addition. Thus, the lowest ammonia nitrogen observed in TMRs of the control may have been due to the inhibition of protein degradation by ethanol during fermentation. In Experiment 2, LP increased lactic acid, decreased acetic acid and ammonia nitrogen, and had no effect on ethanol, whereas LB had no effect on fermentation. Previous studies have shown that the compatibility of the silage material and inoculated strain determines the success of microbial additive, and incompatible strains may have no effect on fermentation (Yang *et al.,* 2010). The fermentation result indicated that LP was compatible with apple pomace-based materials, whereas LB was incompatible. Some studies showed that when LAB were used to inhibit ethanol in sugarcane, LB was more effective than LP because LP fermentation produces lactic acid only, while LB fermentation produces lactic and acetic acid (Carvalho *et al.,* 2014). Since lactic acid has a much weaker inhibitory effect on yeast than acetic acid, LB better inhibits ethanol than LP. This may explain that while LP significantly increased lactic acid, ethanol was not inhibited in this experiment. To develop LAB that can inhibit ethanol in apple pomace, the LAB strains in apple pomace should be analyzed to find effective LB strains. Propionic acid and its salt are chemical preservatives with excellent antifungal effects. They are often used to inhibit yeast and mold in sugarcane or maize silage (Carvalho *et al.,* 2012). In Experiment 2, CP significantly inhibited ethanol and acetic acid compared with the control, while lactic acid was not affected. Therefore, CP is an ideal chemical additive for TMR preparation.

The results showed that the proportion of apple pomace in the TMR should be moderate or low to ensure room for moisture regulation, and moisture in the range of 450-500 g/kg has a better inhibitory effect on ethanol production. Future studies are needed to assess the combined effects of chemical additive additions in this moisture range.

Conclusions

Moisture adjustment in TMR containing apple pomace can inhibit the production of ethanol, and the best inhibition effect is when the moisture of TMR is 500 g/kg. Compared with LB, LP inoculant could increase the lactic acid concentration in the TMR and improve its fermentation quality, but neither could inhibit the production of ethanol. CP can effectively inhibit ethanol production in TMR, but it has no inhibitory effect on lactic acid fermentation, thus confirming that it is an ideal chemical additive.

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