

RESEARCH ARTICLE

Investigating the effect of apple pomace silage as fodder source on performance and residues of its toxins in milk and some rumen fermentation in Mahabadi lactation goats in early lactation period

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The aim of this study was to evaluate effect of apple pomace silage as fodder source on performance and residues of its toxins in milk and some rumen fermentation in Mahabadi lactation goats in early lactation period. There was no significant difference within treatments apple pomace mixed silage had no effect on dairy goat's average daily feed intake. There were no significant differences about pH changes between treatments. The protozoa population tended to decrease and ammonia nitrogen has increased significantly in different treatments ($P \leq 0.05$). According to the increase of volatile fatty acids production, the proportion of acetate did not differ even though that of Propionate and iso-butyrate increased but none significantly. The molar ratios of acetate and propionate and iso-butyrate in the goats rumen containing apple pomace was differed in respect to the availability for fermentation by rumen microflora. In regard to this information, the inclusion of apple pomace silage in experimental goat's diet was associated with a slight lower rumen pH, higher concentration of acetate and propionate and total volatile fatty acids in rumen. The potential value of byproducts in animal feeding and better dry matter and organic matter digestibility depends on their nutritive characteristics. But the livestock producers are responsible for ensuring that the animals and products that they market do not contain unacceptable chemical residues. Anyone intending to feed waste materials to ruminant animals such as cattle, sheep and goats or to pigs must ensure that it is free of prohibited or restricted substances.

Key words: Apple pomace silage; Toxins residues; Rumen fermentation; Lactation goats

Introduction

Byproducts from the food industry can be utilized as feed for small ruminants such as goats and sheep to the benefit of both farmers and animals. Furthermore, the utilization of the byproducts can reduce waste production from the fruit industry, and thus also improve the resource efficiency to the good of the environment. The inclusion of industrial byproducts such as citrus pulp in the composition of animal diets has been widely recommended due to sustainability aspects and their high level of carbohydrates. Notwithstanding low nitrogen and high free sugar content whose fermentation in the rumen may eventually lead to alcoholemia, apples (*Malus domestica*) as an alternative feed for cattle, sheep and goats and have also been considered to help solving the economical disposal of industrial residues and fruits without the required characteristics to commercialization. Studies showed that apple pomace supplemented with natural protein was comparable to protein enriched corn silage.

Apple contains about 12 percentage carbohydrate, most of which is in the form of readily fermentable carbohydrates such as glucose, fructose and sucrose. 30 or 40 percent of total world production of apples are broken, damaged, culled, dropped and therefore not marketed. Also 20 or 40 percent of them are processed for juice extraction. The residue left after extraction of the juice, called apple pomace is the solid residue that remains after milling and pressing of apples for cider, apple juice or puree production. It could be used as a livestock feed (Kafilzadeh et al., 2008).

The dried apple pomace contains 7 percent crude protein and 5 percent ether extract. It has 1.86 Mcal metabolizable energy per each kg DM and 1.12 Mcal net energy (NE)/kg DM for lactating dairy cows (NRC, 2001). The ensiled apple pomace, incorporated up to 30 percent in the diet of lactating multiparous Holstein cows, did not show any adverse effects on milk yield or its composition (Ghoreishi, Pirmohammadi and Yansari, 2007). Alibes et al., (1984) showed that apples pomace and apple product could be included in diets containing alfalfa hay and soya bean meal as supplements. Gasa et al., (1992) reported that ensiled apple pomace could be safely incorporated in roughage diets that were properly supplemented with degradable nitrogen. Some researchers have reported data suggesting that structural carbohydrates can also be used as substrate for micro-organisms during ensiling (McDonald et al., 1991; Yahaya et al., 2000). Fang et al., (2016) indicate that feeding fermented apple pomace to finishing pigs increases the feed efficiency and affects the meat quality and fatty acid composition of back fat. Fayed (2016) conclude that replacement of berseem hay with apple pomace silage up to 50 percent in the diets of dairy goats could improve milk yield and composition without any adverse effect on their performance.

Contamination of food and agricultural commodities by various types of toxigenic molds (fungi) is a serious and a widely neglected problem. Patulin is a mycotoxin produced by several species of *Penicillium*, *Aspergillus* and *Byssochlamys* molds that may grow on a variety of grains and fruits. The presence of residual patulin in apple juice shows as an indicator of the quality of fruit used in its production since an appreciable concentration of the mycotoxin remains in product after processing.

It is also reported to be present in silage intended for ruminants and it has been reported to be responsible for the deaths of cattle. Schneweis et al., (2000) reported that lower concentrations of patulin found in silage will rarely cause the typical neurotoxic signs in animals, but might exert detrimental effects on the rumen microflora, mainly because of its antimicrobial activity. Diazinon is an organophosphate insecticide with agricultural, commercial, and household uses.

It is used in agriculture to control insects on fruit and crops. Feeding Diazinon to pregnant animals has caused a decrease in the endurance, coordination, and growth of their offspring. In addition, the sexual development of offspring of both sexes was delayed. Diazinon also has caused reproductive problems in animals. Dogs fed Diazinon developed atrophied testicles.

There is no publicly available information about the reproductive effects of commercial Diazinon containing products on other animals. Therefore, the aim of the current experiment was to explore the effect of apple pomace silage as fodder source and residual pesticides determination on performance and residues of toxins in milk and some rumen fermentation in Mahabadi lactation goats in early lactation period.

Materials and Methods

This experiment was carried out at the goat barn of Urmia University in Iran. The effects of apple pomace silage on milk yield, milk composition and some nutrients digestibility were measured using 30 multiparous early lactating Mahabadi goats. They were held in individual stalls and the normal herd management practices were followed during the experiment.

The iso-caloric and iso-energetic dietary treatments were formulated according to the nutrition requirements for Sheep and Goats (NRC, 2007) guidelines by small ruminant nutrition system software (SRNS) version 1.9.4468 with constant amounts of dry alfalfa, corn silage, soybean meal, barley and apple pomace silage. The treatments were control include apple pomace silage with no add-ons. The second treatment using microwave (900 watts and frequency 2450 MHz for 20 minutes), in the third treatment, 50 g of "Mycofix-Plus" absorbent supplement per each goat was used and in the 4 and 5 treatments "Bio-Tox" absorbent supplement and "Bio-Acid" acidifier per ton of apple pomace silage were used respectively. The experimental diets in the form of a total mixed ration TMR were given daily in two equal feeds at 08:00 and 16:00 h.

Analysis the chemical composition

Dry samples were analyzed for ash (no. 942.05, AOAC 1990) and total N as Kjeldahl N (no. 954.01, AOAC 1990). Neutral detergent fiber (NDF) acid detergent fiber (ADF) and ADL fractions were determined by the detergent procedures of Robertson and Van Soest (1981) and Van Soest et al., (1991) without the use of sodium sulphite. Neutral detergent fiber and acid detergent fiber were expressed exclusive of residual ash. The acid detergent insoluble nitrogen (ADIN) was determined according to Goering and Van Soest (1970). Non fiber carbohydrates (NFC) were calculated according to NRC (2001).

Dry matter intake measurement

The amount of dry matter consumed was measured by taking into account the amount of dry matter left in the feeder and the amount of dry matter consumed daily.

Evaluation apparent digestibility of nutrients, organic matter and dry matter

To evaluate the digestibility of nutrients, during the 25 to 28 days of the experiment (for 4 days), the amount of feed consumed and the total feces and the feed remaining per day were measured.

100 g from food and stool residues per each day was placed inside the nylon bags, and the samples were mixed together and dried in the oven at 60 °C for 48 hours. Dry matter, crude protein and crude fat of feed samples were evaluated according to the method described by (Van Keulen and Young 1977). Finally, the apparent digestibility of nutrients was determined using acid insoluble ash as an internal marker described by (McDonald et al., 1990).

Patulin and diazinon determination

Determination of Patulin and Diazinon from apple pomace was performed by high performance liquid chromatography model Agilent-1200. Steps of method for Patulin and Diazinon determination included: Preparations of test sample, sample extraction, extract purification, extract evaporation to dryness, residue re-dissolution, HPLC detection of Patulin and Diazinon. Patulin and Diazinon extraction was achieved in acetonitrile, and purification of the obtained extract, using cleanup (C.U.) Patulin and Diazinon columns (MycoSep®228).

Statistical analysis

Data were analyzed in a form of completed randomized design, with five treatments and three replications each, by the general linear models procedure of SAS (2000). When significant differences occurred, Duncan test was used to compare means at level of ($P \leq 0.05$).

Results

The results of ruminal protozoal population means among different treatments are presented in the Table 1. As result revealed that there were no significant differences about pH changes between treatments. The protozoa population tended to decrease and ammonia nitrogen has increased significantly in different treatments ($P \leq 0.05$).

Table 1. Effect of experimental treatments on pH, protozoa and ruminal ammonia nitrogen concentration.

Treatments	pH	Protozoa population (ml.10 ⁻⁵)	Ammonia nitrogen (mg.dl)
1	6.42	16.22 ^{ab*}	21.42 ^{dc}
2	6.48	16.20 ^a	21.68 ^c
3	6.50	16.11 ^a	22.12 ^a
4	6.36	16.18 ^b	21.76 ^{bc}
5	6.08	16.18 ^c	21.90 ^b
P-value	0.001	0.221	0.001
SEM	0.042	0.015	0.065

*_{a,b,c} Means within rows with different superscripts are significantly different ($P \leq 0.05$).

According to the increase of volatile fatty acids production, the proportion of acetate differed even though that of Propionate and iso-butyrate increased but none significantly. The molar ratios of acetate and propionate and iso-butyrate in the goats rumen containing apple pomace was differed in respect to the availability for fermentation by rumen microflora. It was suggested that the progress of fermentation about four substrates by the rumen microflora was influenced by the amount of sugar, especially reducing sugar in apple pomace substrates (Tables 2 and 3).

Table 2. Effect of experimental treatments on ruminal fatty acids concentration.

Treatments	Total fatty acids (L/mmol)	Acetate (% molar)	Propionate and isobutyrate (%)	Butyrate (%)	Valerate (%)
1	91.27 ^{d*}	61.25	21.66	13.90 ^e	2.15
2	91.80 ^c	60.59	22.68	15.39 ^b	2.19
3	93.17 ^a	60.80	23.50	15.87 ^a	2.13
4	90.63 ^e	61.03	24.00	14.77 ^c	2.14
5	92.71 ^b	61.33	24.24	14.60 ^d	2.13
P-value	0.001	0.606	0.058	0.001	0.11
SEM	0.24	0.15	0.33	0.23	0.073

*_{a,b,c} Means within rows with different superscripts are significantly different ($P \leq 0.05$).

Table 3. The amount of residual imidacloprid toxin measured in different treatments.

Treatments	Imidacloprid toxin (mg.Kg)
1	4.86 ^{a*}
2	4.23 ^b
3	2.81 ^e
4	3.90 ^c
5	3.73 ^d
P-value	0.001
SEM	0.180

*_{a,b,c} Means within rows with different superscripts are significantly different ($P \leq 0.05$).

Discussion

Results of this study suggest that apple pomace can improve the performance, protozoa population and ammonia nitrogen of experimental goats. There was no significant difference within treatments apple pomace mixed silage had no effect on dairy goat's average daily feed intake.

Shalini et al., (2010) indicated that apple pomace is used fresh in the vicinity of apple processing plants. Because of its high moisture and high content in fermentable sugars, fresh apple pomace spoils readily and is often ensiled or dehydrated for longer preservation. Zhang et al., (2016) mentioned that unfermented apple pomace lead to better performance, daily gain, feed return and net meat rate in experimental sheep

Fontenot et al. (1977) reported that the mortality of calves and abortion rate of cows may increase if apple pomace is over fed. If too much apple pomace is fed, it may be required to supply more protein, particularly for the growing animals.

Romero-Huelva and Molina-Alcaide, (2013) mentioned that the ruminal pH is one of the most important factors affecting the fermentation and influences its functions and it varies depending on the type of the diets. Increasing the final pH of rumen environment without changes in final ammonia nitrogen concentration substrate degradability and total volatile fatty acid production (2013). Rumsey (1978) reported that feeding apple was associated with a slightly reduction of ruminal pH, a higher acetic to propionic acid ratio and lower proportions of ruminal branched chain fatty acids. Additionally (Oltjen et al., 1977) observed that the lower pH in diets containing apple might be due to the high content of acids components of apple pomace, especially malic and citric acid. Pirmohammadi et al., (2006) showed apple pomace mixed silage replaced 65% corn silage could significantly increase the average milk yield per day, but had no significant effect on blood biochemical indexes, ruminal pH values and ammonia nitrogen concentration.

Ahn et al., (2002) concluded that replacement of concentrates with apple pomace in the rice straw based diets of Korean native goats given separately or by pre-mixing gave satisfactory results of feed intake, digestibility, pH of ruminal fluid and production of $\text{NH}_3\text{-N}$ and VFA in the rumen.

Fu et al. (2001) reported that rumendegradable protein (RDP) was less required for none starch carbohydrates (NSC) fermenting bacteria than that found in many usual diets and it could indicate the rumen microbial population in the goats fed apple pomace was more efficient at scavenging nitrogen from the feed than control.

Paryad and Rashidi, (2009) indicated that the more retention of nitrogen in sheep feedsome by product pomace can explain by reduced ammonia concentrations in the rumen that appeared to result from increased incorporation of ammonia into microbial protein that probably were the direct result of stimulated microbial activity.

Romero-Huelva and Molina-Alcaide, (2013) showed that the increasing rumen volatile fatty acids levels and decreased ruminal ammonia nitrogen in Granadina goats were fed by tomato pomace comparison with the control groups.

The reduction of ammonia nitrogen in the rumen liquor appears to be the result of increased incorporation of ammonia nitrogen into microbial protein and it was considered as an important result to stimulated microbial activity (Romero-Huelva and Molina-Alcaide, 2013). Additionally supplementation of more fermentable carbohydrate to ruminant rations causes a decrease in rumen ammonia probably due to a greater uptake of ammonia by rumen microorganisms in support of enhanced microbial growth (Tagari et al., 1964). Schauff and Clark, (1992) showed that although acetate concentration in the rumen of goats fed 60% apple pomace diet was in a decreasing trend compared to the others. They also indicated that, ruminal ammonia nitrogen ($\text{NH}_3\text{-N}$) may have been used more efficiently for the production of microbial protein due to greater energy intake which would provide for greater energy fermented in the rumen of goats given apple pomace. Acetate to propionate ratio was reduced in diets with the higher levels of apple pomace, likely a consequence of the higher NSC of these diets.

Conclusion

In regard to this information, the inclusion of apple pomace silage in experimental goat's diet was associated with a slight lower rumen pH, higher concentration of acetate and propionate and total volatile fatty acids in rumen. The potential value of byproducts in animal feeding and better dry matter and organic matter digestibility depends on their nutritive characteristics. But the livestock producers are responsible for ensuring that the animals and products that they market do not contain unacceptable chemical residues. Anyone intending to feed waste materials to ruminant animals such as cattle, sheep and goats or to pigs must ensure that it is free of prohibited or restricted substances.

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