

2022, 28 (3) : 430 – 437 Journal of Agricultural Sciences (Tarim Bilimleri Dergisi)

> J Agr Sci-Tarim Bili e-ISSN: 2148-9297 jas.ankara.edu.tr





Effect of Supplementation of Urea on the Nutritive Value and Fermentation Characteristics of Apple Pulp Silages

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ARTICLE INFO

Research Article Corresponding Author: Önder CANBOLAT, E-mail: canbolat@uludag.edu.tr Received: 16 April 2021 / Revised: 27 July 2021 / Accepted: 28 July 2021 / Online: 01 September 2022

Cite this article

CANBOLAT Ö (2022). Effect of Supplementation of Urea on the Nutritive Value and Fermentation Characteristics of Apple Pulp Silages. Journal of Agricultural Sciences (Tarim Bilimleri Dergisi), 28(3):430-437. DOI: 10.15832/ankutbd.917540

ABSTRACT

The aim of current experiment was to determine the effect of supplementation of urea on the nutritive value and fermentation characteristics of apple pulp silage. Apple pulp obtained from apple with Granny Smith (*Malus domestica*) varieties was used in the research. Apple pulp were ensiled in special glass jars with 1.5 L capacity. Urea (0, 0.5, 1.0, 1.5, 2.0 and 2.5% on DM basis) was added homogeneously to apple pulp in triplicate. The experimental silos were placed in a room until opening after 45 day of preservation to determine chemical composition and silage fermentation parameters. The addition of urea to apple pulp reduced the water soluble carbohydrate (WSC), content of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) whereas the supplementation of urea significantly (P<0.01)

increased crude protein (CP) contents of resultant silages. The addition of urea to apple pulp significantly (P<0.01) increased pH, lactic acid (LA), propionic acid (PA), butyric acid (BA) and ammonia nitrogen (NH3-N) contents of the resultant silages whereas the supplementation of urea significantly (P<0.01) reduced the acetic acid (AA) contents. Addition of urea to apple pulp increased in vitro gas production, digestible organic matter (DOM), metabolic energy (ME) and lactic acid bacteria (LAB), and significantly reduced yeast and mold count (P<0.01). The urea supplementation also increased the aerobic stability of the resultant silages. It can be concluded that supplementation of urea to apple pulp at 2.0 and 2.5% can be recommended to improve the nutritive value and fermentation parameters of the resultant silages.

Keywords: Apple Pomace, Silage, Urea, In Vitro Gas Production, Silage Fermentation

1. Introduction

Recently apple production increased from 2.6 million tons in 2010 to 4.3 million tons in 2021 due to an increased demand for apple consumption in Turkey (Anonymous 2022). Apple is consumed not only fresh but used to produce apple juice in Turkey. Therefore, considerable amount of apple pulp is produced during apple juice production. The pulp constitutes 25-30 % of total processed apple and consists of a crust, flesh and seeds (Ajila et al. 2015; Skinner et al. 2018). Although the moisture content of the apple pulp is very high at the time of production, pulp contains considerable amount of cellulose, pectin, minerals, vitamin C, anti-oxidant, water soluble carbohydrate (WSC) and some organic acids (Alibes et al. 1984; Vrhovsek et al. 2004; Varzakas et al. 2016; Islam et al. 2018a). In dry matter (DM), the crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of apple pulp ranges from 19-65 g/kg Carson et al. 1994), 300-482 g/kg and 250-420 g/kg, respectively (Wolter et al. 1980; Singhal et al. 1991). The metabolisable energy (ME) value of apple pulp may vary from 7.7 to 9.1 MJ/kg DM (MAFF 1984). The mean protein degradability of apple pulp reported by NRC (2001) was 68.4%.

Generally, apple pulp is used in the feeding of ruminant animals in the form of fresh, dry and silage (Fontenot et al. 1977; Alibes et al. 1984; Gasa et al. 1992; Kennedy et al. 1999; Taasoli & Kafilzadeh 2008; Ajila et al. 2015; Islam et al. 2018a). It is very difficult to store the apple pulp due to high moisture content (Alibes et al. 1984; Fang et al. 2016; Islam et al. 2018b). Therefore, the apple pulp can be preserved by ensiling (McDonald et al. 1991; Fang et al. 2016; Islam et al. 2018a), since that contains water soluble carbohydrates which is required by lactic acid bacteria to produce lactic acid which is responsible for the decrease in pH of silage (Afzal et al. 2015; Skinner et al. 2018). Previous investigations clearly showed that apple pulp silage is an ideal feed for ruminant animals (Rumsey 1978; Pirmohammadi et al. 2006; Ajila et al. 2015; Islam et al. 2018a; Islam et al. 2018b; García-Rodríguez et al. 2019). Although apple pulp has high energy value, its protein content is low (Gasa et al. 1992; Taasoli and Kafilzadeh 2008). The fact that apple pulp contains low digestible protein is an important factor that limits the nutritive value (Rumsey 1978; Ajila et al. 2015). Therefore, urea can be used as silage additive to increase the crude protein content of the resultant silage (Alibes et al. 1984; McDonald et al. 1991; Filya et al. 2004; Canbolat et al. 2014). It was recommended that urea can be added to silage material with a low protein content at level of 40-50 kg/ton DM to increase the crude protein content of silage (Kaiser 2004). Supplementation of urea not only improve the crude protein content but also improve the aerobic stability of the resultant silage (McDonald et al. 1991; Filya et al. 2004; Canbolat et al. 2014). Therefore, the aim of current experiment was to determine the effect of supplementation of urea on the nutritive value and fermentation properties of apple pulp silage.

2. Material and Methods

2.1. Feed and silo material

The feed material of the research was composed of apple pulp (*Malus domestica*: Granny Smith) left from apple juice production in Bursa Uludag University Agricultural Research and Application Center. Urea was obtained from a commercial firm. 1.5 L special glass silos (Weck®, Germany) were used for ensiling the apple pulp.

2.2. Animal material

In order to apply the in vitro gas production technique, rumen fluid was taken from 3 Merino rams after slaughtering.

2.3. Preparation of silages

Apple pulp were ensiled in special glass jars with 1.5 L capacity. Urea (0, 0.5, 1.0, 1.5, 2.0 and 2.5% on DM basis) was added homogeneously to apple pulp in triplicate. The experimental silos were placed in a room until opening after 45 day of preservation.

2.4. Chemical analyses

The experimental silos of apple pulp silages were opened on the 45th day of silo. Apple pulp silage was dried in the oven at 65 °C for 48 hours. Dried silages were grinded to a 1 mm sieve diameter and used in chemical analyses. Dry matter, crude ash, ether extract and crude protein contents of silages were determined using the methods described by AOAC (2000). Neutral detergent fiber, acid detergent fiber and acid detergent lignin contents of silages were determined using the method suggested by Van Soest et al. (1990).

The pH of the silages was determined using the digital pH meter (Sartorius PB-20, Goettingen, Germany) and ammonia nitrogen (NH₃-N) contents of silages were determined using the method described by AOAC (2000). Lactic acid, acetic, propionic and butyric acid contents of silages were determined with gas chromatography device (Agilent Technologies 6890N, column properties: Stabilwax-DA, 30 m, 0.25 mm ID, 0.25 um df. Max. Temp: 260 °C. Cat. 11023) using the spectrophotometric method (Barker & Summerson 1941). Water soluble carbohydrate (WSC) contents were determined according to the phenol sulphuric acid method (Dubois et al. 1956).

Lactic acid bacteria (LAB), yeast and mold counts of silages were determined according to the method reported by Seale et al. (1990). De Man, Rogosa and Sharpe (MRS) agar for LAB, Malt Extract agar for yeast and molds were used as a cultivation medium. Lactic acid bacteria, yeast and mold counts of the silages were incubated for 3 days at 30 °C. The LAB, yeast and mold numbers were explained as coliform unit (cfu/g). Aerobic stability of silages was performed according to the method developed by Ashbell et al. (1991).

2.5. Determination of in vitro gas production of apple pulp silages

In vitro gas production of apple pulp silages were determined using the *in vitro* gas production technique reported by Menke et al. (1979). Approximately 200 mg silage samples were incubated in special 100 mL glass syringes (Model Fortuna, Häberle Labortechnik, Lonsee-Ettlenschie, Germany) in triplicate. 30 mL of buffered rumen fluid was transferred into glass syringes containing silage samples. Glass syringes were incubated in a water bath at 39 °C and *in vitro* gas production was measured at 24 hours after incubation.

Organic matter digestibility and metabolisable energy contents of apple pulp silages were calculated using the following equations (Menke & Steingass 1988).

OMD,
$$\% = 15.38 + 0.8453 \text{ x GP} + 0.0595 \text{ x CP} + 0.0675 \text{ x CA}$$

ME, MJ/kg DM = $2.20 + 0.1357 \text{ x GP} + 0.0057 \text{ x CP} + 0.0002859 \text{ x CF}^2$

(GP: Net gas amount produced by 200 mg feed sample for 24 hours. CP: crude protein, EE: ether extract and CA: crude ash, g/kg DM).

2.6. Statistical analysis

Data obtained in the current experiment were subjected to variance analysis (ANOVA) using the General Linear Model) (Minitab 2013). The significance between the treatment groups were determined by Duncan multiple comparison test (Snedecor & Cochran 1976).

3. Results

Effect of urea supplementation on chemical composition of apple pulp silages was given in Table 1. Urea supplementation had a significant (P<0.01) effect on chemical composition of the resultant apple pulp silages.

| Urea Treatment (%) | DM | СР | EE | CA | NDF | ADF | ADL | WSC |
|--------------------|--------------------|--------------------|---------------------|-------------------|---------------------|---------------------|-------------------|-------------------|
| 0.0 | 26.74 ^f | 7.64 ^f | 4.40 ^a | 2.43 ^f | 47.68 ^a | 38.96ª | 7.24 ^a | 7.37ª |
| 0.5 | 27.09 ^e | 8.57 ^e | 4.40^{a} | 2.53 ^e | 46.42 ^b | 38.77 ^a | 7.20 ^a | 6.59 ^b |
| 1.0 | 27.72 ^d | 9.18 ^d | 4.37 ^{ab} | 2.62 ^d | 45.73 ^{bc} | 37.92ªb | 7.15 ^a | 6.41 ^c |
| 1.5 | 27.93° | 10.14 ^c | 4.35 ^{abc} | 2.69 ^c | 44.88 ^{cd} | 37.22 ^{bc} | 7.06 ^b | 6.30 ^c |
| 2.0 | 28.22 ^b | 10.75 ^b | 4.30 ^{bc} | 2.79 ^b | 43.93 ^d | 36.47° | 7.04 ^b | 5.11 ^d |
| 2.5 | 28.79 ^a | 11.29 ^a | 4.29 ^c | 2.90 ^a | 42.63 ^e | 34.32 ^d | 6.95° | 4.83 ^e |
| *SD | 0.081 | 0.090 | 0.042 | 0.029 | 0.638 | 0.590 | 0.263 | 0.091 |

| Table 1- Effect of urea supplementation on chemical | l composition of | f apple pulp silages, 9 | % |
|---|------------------|-------------------------|---|
|---|------------------|-------------------------|---|

a,b,c,d,e,f: The differences between the means indicated by different letters in the same column are significant (P<0.01); *SD: Standard deviation; Dry matter; CP: Crude protein; CA: Crude ash; EE: Ether extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; WSC: Water soluble carbohydrate

The DM, CP and CA contents of the resultant silage increased (P<0.01) in a dose dependent manner of urea whereas NDF, ADF, ADL and WSC contents decreased (P<0.01).

Effect of urea supplementation on apple pulp silage fermentation characteristics are given in Table 2.

Table 2- Effect of urea supplementation on apple pulp silages fermentation characteristics

| | | g/kg DM | | | | | |
|--------------------|-------------------|--------------------|---------------------|-------------------|-------------------|--------------------|--------------------|
| Urea Treatment (%) | pH | Lactic acid | Acetic acid | Propionic acid | Butyric acid | Ethanol | NH ₃ -N |
| 0.0 | 3.72 ^f | 42.25 ^e | 26.07 ^a | 3.13 ^e | 1.35 ^a | 2.85 ^a | 13.14 ^f |
| 0.5 | 3.85 ^e | 44.44 ^d | 25.36 ^{ab} | 3.23 ^d | 1.17 ^b | 2.12 ^b | 14.37 ^e |
| 1.0 | 3.90 ^d | 48.85 ^c | 24.15 ^{bc} | 3.30 ^c | 1.16 ^b | 1.70 ^c | 15.61 ^d |
| 1.5 | 3.94 ^c | 51.51 ^b | 23.56 ^c | 3.40 ^b | 1.01 ^c | 1.61 ^{cb} | 16.46 ^c |
| 2.0 | 4.13 ^b | 53.69 ^a | 20.95 ^d | 3.45 ^b | 0.78^{d} | 1.51 ^d | 17.89 ^b |
| 2.5 | 4.21 ^a | 54.62 ^a | 18.61 ^e | 3.59 ^a | 0.64 ^e | 1.30 ^e | 19.07 ^a |
| *SD | 0.058 | 1.184 | 0.771 | 0.032 | 0.065 | 0.073 | 0.365 |

a,b,c,d,f: The differences between the means indicated by different letters in the same column are significant (P<0.01); *SD: Standard deviation; NH₃-N: Ammonia nitrogen (NH₃-N is given as % of total N)

Urea supplementation had a significant (P<0.01) effect on fermentation characteristics of the resultant apple pulp silages. The pH, lactic acid and NH₃-N, propionic acid, also contents of the resultant silage increased (P<0.01) in a dose dependent manner of urea whereas acetic, butyric acid and ethanol contents decreased (P<0.01). Accordingly, the most effective urea dose on silage parameters was 2.5%.

Effect of urea supplementation on silage microbiology of apple pulp silages was given in Table 3.

| Table 3- | · Effect of ure | ea supplementation | on silage | microbiology | of apple | pulp silages |
|----------|-----------------|--------------------|---------------------------------------|--------------|---------------------------------------|---|
| | | | · · · · · · · · · · · · · · · · · · · | | · · · · · · · · · · · · · · · · · · · | r · · · · · · · · · · · · · · · · · · · |

| Urea Treatment (%) | LAB (cfu/g FM) | Yeast (cfu/g FM) | Mold (cfu/g FM) |
|--------------------|--------------------|---------------------|--------------------|
| 0.0 | 10.47 ^d | 3.73ª | 1.67ª |
| 0.5 | 12.15 ^c | 3.62 ^{ab} | 1.33ª |
| 1.0 | 13.89 ^b | 3.56 ^{abc} | 1.00 ^{ab} |
| 1.5 | 14.63 ^b | 3.19 ^{bc} | 1.00 ^{ab} |
| 2.0 | 15.55ª | 3.07 ^{cd} | 0.33 ^b |
| 2.5 | 16.30ª | 2.65 ^d | 0.33 ^b |
| *SD | 0.507 | 0.287 | 0.471 |

a,b,c,d: The differences between the means indicated by different letters in the same column are significant (P<0.01); *SD: Standard deviation; LAB: Lactic acid bacteria; FM: Fresh material

Urea addition affected the LAB production of apple pulp silages. Depending on the dose of urea addition to apple pulp, the number of LAB increased significantly (P<0.01). In addition, adding urea to apple pulp silage decreased the number of yeast and mold (P<0.01). In addition, depending on the dose of urea addition to apple pulp, the number of yeast and mold significantly decreased (P<0.01). The most effective urea dose was 2.0% and above.

Effect of urea supplementation on aerobic stability of silage is given Table 4.

| Urea Treatment (%) | pH | $CO_2 (g/kg DM)$ | Mold (cfu/g FM) |
|--------------------|-------------------|----------------------|-------------------|
| 0.0 | 3.92° | 29.74 ^a | 3.99 ^a |
| 0.5 | 4.12 ^b | 27.51 ^b | 3.53 ^b |
| 1.0 | 4.26 ^a | 26.18 ^c | 3.17° |
| 1.5 | 4.32 ^a | 22.53 ^d | 3.09 ^c |
| 2.0 | 4.33ª | 21.19 ^e | 3.01° |
| 2.5 | 4.35 ^a | 18.85^{f} | 2.59 ^d |
| *SD | 0.064 | 0.728 | 0.095 |

Table 4- Effect of urea supplementation on aerobic stability of silages

a,b,c,d,f: The differences between the means indicated by different letters in the same column are significant (P<0.01); *SD: Standard deviation; CO₂: Carbon dioxide; FM: Fresh material

Urea supplementation decreased (P<0.01) the CO₂ (g/kg DM) and mold number. The most effective urea dose on carbon dioxide (CO₂) and mold production was 2.5% and reduced these parameters.

Effect of urea supplementation on *in vitro* gas production, digestible organic matter and metabolic energy contents of apple pulp silages are given Table 5.

| Table 5- Effect of urea supplementation on in vitro gas production, organic matter digestibility and metabolic energy contents |
|--|
| of apple pulp silages |

| Urea treatment (%) | Gas production | OMD | ME |
|--------------------|--------------------|--------------------|--------------------|
| 0.0 | 43.86 ^e | 65.43 ^f | 9.14 ^f |
| 0.5 | 45.69 ^d | 67.96 ^e | 9.45 ^e |
| 1.0 | 46.00 ^d | 69.09 ^d | 9.56 ^d |
| 1.5 | 48.15 ^c | 71.98° | 9.91° |
| 2.0 | 50.43 ^b | 74.70 ^b | 10.24 ^b |
| 2.5 | 51.31 ^a | 76.21ª | 10.41 ^a |
| *SD | 0.427 | 0.404 | 0.059 |

a,b,c,d,e,f: The differences between the means indicated by different letters in the same column are significant (P<0.01). *SD: Standard deviation; Gas production (mL/200 mg DM); OMD: Organic matter digestibility (%); ME: Metabolisable energy (ME/kg DM)

The gas production, OMD and ME contents of the resultant silage increased (P<0.01) in a dose dependent manner of urea. Depending on the urea dose added to the silage, *in vitro* gas production, ME and OMS increased, and the most effective urea dose was 2.5%.

4. Discussion

4.1. Chemical composition of feed and silages

Urea supplementation to apple pulp significantly increased CP content of silage (P<0.01). These results are consistent with those reported by Filya et al. (2004), Pirmohammadi et al. (2006), Celik et al. (2009) and Canbolat et al. (2014). Crude protein content of apple pulp silages obtained in the current study was lower than that reported by Islam et al. (2014) whereas it was higher than that reported by Kara et al. (2018). The differences among the studies might be associated with variety of apple used for pulp.

The addition of urea to apple pulp silage reduced the NDF, ADF and ADL contents of the silages (P<0.01). The decrease in the NDF, ADF and ADL content of the silages in the addition of urea to apple pulp can probably be attributed to an increase in the number of lactic acid bacteria (LAB) that use urea as a nitrogen source. It can be said that the amount of LAB in silage increases due to the breakdown of cell wall components.

Indeed, Filya et al. (2004), Canbolat et al. (2014) and Kang et al. (2018) explained that the decrease in the amount of NDF and ADF of the silage by increasing the number of lactic acid bacteria and some anaerobic bacteria in the silages of the nitrogen source urea, increasing the degradability of NDF, ADF and crude cellulose. Similar results Celik et al. (2009), Demirel and Yildiiz. (2001) and Islam et al. (2014) were also reported by. Although the NDF and ADF content detected in apple pulp silage was lower than those determined by Fang et al. (2016), it was found higher than the data determined by Kara et al. (2018). The amount of NDF (45.3%) and ADF (38.0%) found in this study were similar to the data reported by Abdollahzadeh et al. (2010).

The addition of urea to the silage also significantly reduced the WSC content of the silages (P<0.01). Increasing the amount of WSC in the feed structure improves the ensilability of the feeds (McDonald et al. 1991; Kaiser 2004; Canbolat et al. 2014). For lactic acid fermentation and development of LAB, the WSC content in the feed should be higher than 2.5% (Kaiser 2004). The high content of WSC (39.58%) in apple pulp increases the ensilability of apple pulp. Due to this feature, apple pulp is feed quite suitable for ensiling. The WSC content of apple pulp used in this study was lower than the values reported by Skinner et al. (2018) and similar to the research results reported by Islam et al. (2018a) (40.4 \pm 0.4%). The water soluble carbohydrate content (39.58%) of apple pulp decreased significantly by fermentation in the silo. The addition of urea to apple pulp significantly reduced the WSC content of silages and regardless of that improved silage fermentation (Table 2). Findings in this research with apple pulp silage were similar to the results of Canbolat et al. (2014) working with pomegranate pulp silage.

4.2. Fermentation properties of silages

It was found that increasing doses of urea in apple pulp significantly affected the VFA contents of the silage (P<0.01). With the greater content of urea in apple pulp silage, the amount of acetic acid, butyric acid and ethanol decreased, and the amount of lactic acid and propionic acid increased significantly (P<0.01). In other words, the larger amounts of urea positively affected the fermentation of apple pulp silage.

One of the most important parameters that determine the quality of ensiled feeds is the lactic acid level. As the amount of lactic acid increases in ensiled silo feed, silage quality also increases (McDonald et al. 1991; Kaiser 2004). The addition of urea to ensiled apple pulp increased the amount of lactic acid by 2.5%, compared with ensiled material that was not supplemented with urea. The addition of urea to apple pulp silages increased the amount of lactic acid in by silage by about 29.28%. Increasing the urea dose decreased the amount of acetic acid (28.62%) and butyric acid (52.59%) of apple silage.

The high content of WSC (Table 1) of apple pulp increased lactic and propionic acid production and reduced butyric acid production in apple pulp silage (McDonald et al. 1991; Filya et al. 2004; Kaiser 2004; Yalcinkaya et al. 2012; Canbolat et al. 2014). It can be said that the addition of urea to apple pulp can provide high quality silages with high lactic and propionic acid content, as well as with low acetic and butyric acid content. It is reported that the addition of straw and urea to apple pulp increases the amount of lactic acid and decreases the amount of acetic acid and butyric acid (Yalcinkaya et al. 2012). These findings are similar to those of Canbolat et al. (2014), which added urea to pomegranate pulp silage and Filya et al. (2004)24, which added urea to corn silage.

The addition of urea to apple pulp significantly increased silage pH and ammonia nitrogen (NH₃N) (P<0.01). Supplementation of urea to apple pulp increased the NH₃-N content of silages by increasing proteolysis (Filya et al. 2004; Canbolat et al. 2014). In this way, it can be said that it causes nitrogen loss in ensiled feeds. The highest NH₃N was detected in apple pulp silage with 2.5% added urea. The amount of NH₃N detected in apple pulp silage was found similar to pomegranate pulp silage supplemented with urea (Canbolat et al. 2014).

The addition of urea has increased ammonia nitrogen, which is alkaline (Table 2). This situation increased the silage pH of apple pulp silages. However, the apple pulp silage pH remained at the recommended level for ensiled feeds (McDonald et al. 1991; Kaiser 2004). The highest pH was found in the apple pulp silage group, in which 2.5% urea was added. It has been demonstrated by many studies that urea increases pH in silo feeds (Filya et al. 2004; Celik et al. 2009; Canbolat et al. 2014, Kang et al. 2018).

4.3. Microbiological properties of silages

Adding urea to apple pulp has been found to affect and increase LAB values of apple pulp silage (P<0.01). The highest LAB value was found in apple pulp silage with 2.5% urea added. Due to the increase in the urea dose added to apple pulp, the CP level of silages increased and the level of NDF and ADF decreased (Table 1). This provided more nutrients for LAB, and therefore LAB values in the silage increased. The LAB levels determined in this study were found to be higher than the values obtained from the studies of Filya et al. (2004) and Canbolat et al. (2014).

The use of urea in ensiled apple pulp caused a decrease in the number of yeast and mold of the silage (P<0.01). The decrease in the number of yeast and mold effected the development of fermentation in the desired direction. It is also the result of the antifungal effect of urea by turning into ammonia in silage (Table 3) (McDonald et al. 1991; Filya et al. 2004; Canbolat et al.

2103). The numbers of yeasts and molds found in actual research were similar to the results reported by Filya et al. (2004) and Canbolat et al. (2014).

4.4. Aerobic stability characteristics of silages

The pH values were determined after the aerobic stability test of apple pulp silages. The pH in the control group was significantly higher than the other silage groups with urea supplemented (P<0.01). Adding urea to ensiled material increased the pH value of silage. This increase can be explained by hydrolysis of urea to ammonia that increased the buffer capacity of silages (McDonald et al. 1991).

The addition of urea to apple pulp significantly reduced the CO_2 production of silages (P<0.01). There was found the lowest value of CO_2 (18.85 g/kg DM) in the silage with the greater added amount of urea (2.5%). Due to the addition of urea, the CO_2 level decreased by about 36.62%. In this study, it can be said that 2.5% urea improves the aerobic stability of apple pulp silage. These research findings were similar to studies with urea added to the corn silage (Filya et al. 2004) and pomegranate pulp silage (Canbolat et al. 2014).

The addition of urea to apple pulp silages also significantly reduced the number of molds (P<0.01). This indicates that the addition of urea to apple pulp will protect silage during feed (McDonald et al. 1991; Kaiser 2004).

4.5. In vitro gas production features of silages

Adding urea to apple pulp silage increased *in vitro* gas production (P<0.01). The highest *in vitro* gas production was determined in the silage added with 2.5% urea and the lowest in the control group (P<0.01). The addition of urea to apple pulp increased the CP content of silage and decreased the NDF and ADF content (Table 1), which likely could increase *in vitro* gas production. The effect of added urea to ensiled material has been demonstrated by studies in which *in vitro* gas production also was increased (Canbolat et al. 2014; Kang et al. 2018). *In vitro* gas production of apple pulp silages was lower than the results reported by Mirzaei-Aghsaghali et al. (2011). It was found to be higher than *in vitro* gas production reported by García-Rodríguez et al. (2019) in apple pulp. Determined values were higher than *in vitro* gas production detected in pomegranate pulp silage (Canbolat et al. 2014).

Supplementing urea to apple pulp increased the silage DOM and ME contents. This situation increased *in vitro* gas production (24 hours). As a result of increased *in vitro* gas production, there were increased the DOM and ME levels in apple pulp silages. This situation was found similar to the findings of Canbolat et al. (2014) adding urea to pomegranate pulp silage. In addition, Kang et al. (2018) reported that the supplementation of urea to cassava silage increased the DOM content of silages. Apple pulp ME contents were found similar to those reported by Pirmohammadi et al. (2006), Mirzaei-Aghsaghali et al. (2011) and García-Rodríguez et al. (2019).

The digestible organic matter content of apple pulp has been consistent with the research findings reported by Mirzaei-Aghsaghali et al. (2011), while the content of apple pulp silage DOM was higher than the results reported by Abdollahzadeh et al. (2010) and Kara et al. (2018).

5. Conclusions

As a result, it can be said that apple pulp is a quality silage material due to the high WSC content. It has been revealed that urea can be used as a nitrogen source in increasing the crude protein content of apple pulp. In addition, the supplementation of urea to ensiled apple pulp has been found to improve silage nutrient composition, fermentation properties, aerobic stability, *in vitro* gas production, DOM and ME levels. According to the findings obtained from the research, it was concluded that 2.5% urea can be used in apple pulp silage.

References

- Abdollahzadeh F, Pirmohammadi R, Farhoomand P, Fatehi F & Pazhoh F F (2010). The effect of ensiled mixed tomato and apple pomace on holstein dairy cow. Italian Journal of Animal Science 9(2): 212-216. https://doi.org/10.4081/ijas.2010.e41
- Afzal B Y, Ganai A M & Ahmad H A (2015). Utilisation of apple pomace as livestock feed: A review. *Indian Journal of Small Ruminants* 21(2): 165-179. https://doi.org/10.5958/0973-9718.2015.00054.9

Ajila C M, Saurabh S J, Brar S K, Godbout S, Cote M, Guay F, Verma M & Valéro J R (2015). Fermented apple pomace as a feed additive to enhance growth performance of growing pigs and its effects on emissions. Agriculture 5(2): 313-329. https://doi.org/10.3390/agriculture5020313

Alibes X, Munoz F & Rodriguez J (1984). Feeding value of apple pomace silage for sheep. Animal Feed Science and Technology 11(3): 189-197. https://doi.org/10.1016/0377-8401(84)90062-2

AOAC (2000). Official Methods of Analysis, 17th Edition, Association of Official Analytical Chemist International, Washington DC.

Ashbell G, Weinberg Z G, Azrieli A, Hen Y & Horev B (1991). A simple system to study the aerobic deterioration of silages. Canadian Agricultural Engineering 33: 391-393

Anonymous (2022). Statistical tables. Fruits, nuts and beverage crops balance sheets. https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1

- Barker S B & Summerson W H (1941). The colorimetric determination of lactic acid in biological material. *Journal of Biological Chemistry* 138(2): 535-554. https://doi.org/10.1016/s0021-9258(18)51379-x
- Canbolat Ö, Kamalak A & Kara H (2014). The effects of urea supplementation on pomegranate pulp (Punica granatum L.) silage fermentation, aerobic stability and in vitro gas production. *Ankara Universitesi Veteriner Fakultesi Dergisi* 61 (3): 217-223
- Carson K J, Collins J L & Penfield M P (1994). Unrefined, dried apple pomace as a potential food ingredient. Journal of Food Science 59(6): 1213-1215. https://doi.org/10.1111/j.1365-2621.1994.tb14679.x
- Celik S, Budag C, Demirel M, Bakici Y & Celik S (2009). The Effects of adding urea and molasses to corn harvested at dough stage on silage fermentation quality, in vitro organic matter digestibility and metabolic energy contents. *Journal of Animal and Veterinary Advances* 8(10): 1921-1924
- Demirel M. & Yildiz S (2001). The effect of whole-crop barley harvested at milk stage by adding urea and molasses on silage quality and nutrient degradability in the rumen. *Yuzuncu Yil University Journal of Agricultural Sciences* 11(1): 55-62
- Dubois M, Giles K A, Hamilton J K, Rebes P A & Smith F (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28(3): 350-356. https://doi.org/10.1021/ac60111a017
- Fang J, Cao Y, Matsuzaki M, Suzuki H & Kimura H (2016). Effects of apple pomace-mixed silage on growth performance and meat quality in finishing pigs. Animal Science Journal 87(12): 1516-1521. https://doi.org/10.1111/asj.12601
- Filya I, Sucu E & Hanoğlu H (2004). Effects of urea application on the silage fermentation, aerobic stability ruminal degradability and fattening performance of lambs. *Journal of Agricultural Sciences* 10 (3): 258-262
- Fontenot J P, Bovard K P, Oltjen R R, Rumsey T S & Priode B M (1977). Supplementation of apple pomace with non-protein nitrogen for gestating beef cows. Feed intake and performance. *Journal of Animal Science* 45(3): 513-522. https://doi.org/10.2527/jas1977.453513x
- García-Rodríguez J, Ranilla M J, France J, Alaiz-Moretón H, Carro M D & López S (2019). Chemical composition, in vitro digestibility and rumen fermentation kinetics of agro-industrial by-products. Animals 9(11): 1-13. https://doi.org/10.3390/ani9110861
- Gasa J, Castrillo C, Guada J A & Balcells J (1992). Rumen digestion of ensiled apple pomace in sheep: effect of proportion in diet and source of nitrogen supplementation. Animal Feed Science and Technology 39(3-4): 193-207. https://doi.org/10.1016/0377-8401(92)90041-4
- Islam S, Islam M & Matsuzaki M (2014). Apple pomace silage ethanol intake and its effect on sheep. *Bangladesh Journal of Animal Science* 43(3): 224-231. https://doi.org/10.3329/bjas.v43i3.21655
- Islam S, Islam M N & Matsuzaki M (2018a). Nutritive value of fermented apple pomace silage and its effect in Suffolk ewes. *Journal of Agricultural Science and Food Technology* 4(4): 80-91
- Islam S, Islam M N & Matsuzaki M (2018b). Effect of apple pomace silage on blood parameters in Suffolk ewe. *Bangladesh Journal of Animal Science* 47(2): 51-60. https://doi.org/10.3329/bjas.v47i2.40234
- Kaiser A G (2004). Silage additives. Chapter 7 in Successful Silage. Kaiser AG, Piltz JW, Burns HM, Griffiths NW. (eds). Dairy Australia and New South Wales Department of Primary Industries. New South Wales, Australia.
- Kang S, Wanapat M & Nunoi A (2018). Effect of urea and molasses supplementation on quality of cassava top silage. *Journal of Agricultural Science and Food Technology* 27(1): 74-80. https://doi.org/10.22358/jafs/85544/2018
- Kara K, Kocaoglu Guclu B, Baytok E, Aktug E, Karakas Oguz F, Kamalak A & Atalay A I (2018). Investigation in terms of digestive values, silages quality and nutrient content of the using pomegranate pomace in the ensiling of apple pomace with high moisture contents. *Journal of Applied Animal Research* 46(1): 1233-1241. https://doi.org/10.1080/09712119.2018.1490300
- Kennedy M, List D, Lu Y, Newman R H, Sims I M & Bain P J S (1999). Apple pomace and products derived from apple pomace: uses, composition and analysis. In: Analysis of Plant Waste Materials, vol. 20. Springer-Verlag, Berlin, pp. 75-119
- MAFF (Ministry of Agriculture, Fisheries and Food) (1984). Energy allowances and feeding systems for ruminants, ADAS Reference Book 433, HMSO, London
- McDonald P, Henderson A R & Heron S J E (1991). The Biochemistry of Silage (2rd ed.). Chalcombe Publ., Church Lane, Kingston, Canterbury, Kent, UK.
- Menke K H & Steingass H (1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research and Development (28): 7-55
- Menke K H, Raab L, Salewski A, Steingass H, Fritz D & Schneider W (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. *Journal of Agricultural Science* 93(1): 217-222. https://doi.org/10.1017/s0021859600086305
- Minitab (2013). Minitab® 17 Statistical Software.
- Mirzaei-Aghsaghali A, Maheri-Sis N, Mansouri H, Razeghi ME, Shayegh J & Aghajanzadeh-Golshani A. (2011). Evaluating nutritional value of apple pomace for ruminants using in vitro gas production technique. Annals of Biological Research 2(1):100-106
- NRC (National Research Council). (2001). Nutrient Requirements of Dairy Cattle. 7th revised edn. National Research Council, National Academy of Sciences, Washington, D.C., U.S.A.
- Pirmohammadi R, Rouzbahan Y, Rezayazdi K & Zahedifar M (2006). Chemical composition, digestibility and in situ degradability of dried and ensiled apple pomace and maize silage. Small Ruminant Research 66(1-3): 150-155. https://doi.org/10.1016/j.smallrumres.2005.07.054
- Rumsey T (1978). Ruminal fermentation products and plasma ammonia of fistulated steers fed apple pomace-urea diets. *Journal of Animal Science* 47(4): 967-976. https://doi.org/10.2527/jas1978.474967x
- Seale D R, Pahlow G, Spoelstra S F, Lindgren S, Dellaglio F & Lowe J F (1990). Methods for the microbiological analysis of silage. Proceeding of The Eurobac Conference, 147, Uppsala.
- Singhal K K, Thakur S S & Sharma D D (1991). Nutritive value of dried and stored apple pomace and its further processing for improved utilization. Indian Journal of Animal Nutrition 8(3): 213-216
- Skinner R C, Gigliotti J C, Ku K M & Tou J C (2018). A comprehensive analysis of the composition, health benefits, and safety of apple pomace. Nutrition Reviews 76(12): 893-909. https://doi.org/10.1093/nutrit/nuy033

Snedecor G W & Cochran W (1976). Statistical methods. The Iowa State Univ. Pres Amer IA.

- Taasoli G & Kafilzadeh F (2008). Effects of Dried and Ensiled Apple Pomace from Puree Making on Performance of Finishing Lambs. Pakistan Journal of Biological Sciences 11(2): 294-297. https://doi.org/10.3923/pjbs.2008.294.297
- Van Soest P J, Robertson J B & Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74(10): 3583-3597. https://doi.org/10.3168/jds.s0022-0302(91)78551-2
- Varzakas T, Zakynthinos G & Verpoort F (2016). Plant Food Residues as a Source of Nutraceuticals and Functional Foods. Foods 5(4): 1-32. https://doi.org/10.3390/foods5040088
- Vrhovsek U, Rigo A, Tonon D & Mattivi F (2004). Quantitation of polyphenols in different apple varieties. *Journal of Agricultural and Food Chemistry* 52(21): 6532-6538. https://doi.org/10.1021/jf049317z

Wolter R, Durix A, Letourneau J C, Carcelen M & Veis T (1980). Estimation of total digestibility of soybean hulls, apple pomace, carob husk, grape seed oil meal. Annales de zootechnie 29(4): 377-385

Yalcinkaya M Y, Baytok E & Yörük. (2012). Some Physical and Chemical Properties of Different Fruit Pulp Silages. *Journal of The Faculty of Veterinary Medicine Erciyes University* 9(2): 95-106



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