Silage Quality, Aerobic Stability, Gas Production and *in vitro* Digestibility of Pumpkin Waste Silages

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Research Article

Keywords: pumpkin waste, silage quality, gas production, aerobic stability

Posted Date: June 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-383669/v1>

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Abstract

This experiment was carried out to determine the silage quality of pumpkin waste with wheat straw (S) and alfalfa hay (A). The treatment groups as follows: 1) 100% pumpkin waste (PR), 2) 80%PR and 20% A (PW80+A20), 3) 80%PW and 20%S (PW80+S20), 4) 60%PW and 40%A (PW60+A40), 5) 60%PW and 40%S (PW60+S40), 6) 80%PW+10%A+10%S (PW80+A10+S10) and 7) 60%PW + 20%A + 20%S (PW40+A20+S20). The lowest dry matter content was observed in PW group (39.0 g/kg). The crude protein ratio in the alfalfa groups were higher than those of S and PW groups. The crude cellulose was lower and NDF higher in the PW group than those of other groups and ADF was highest in the 40%S group. Water soluble carbohydrate ratio in PW and PW60+A20+S20 groups were higher than those of other groups. In the PW group, the lactic acid ratio was higher than PW80+S10+A10 group. In the PW80+S20 group the highest gas production was observed at 48th, 72nd and 96th hours. The energy values of PW group were higher than those of other groups. The aerobic stability of pumpkin silage was improved with hay addition especially alfalfa addition in 400 g/kg concentration.

Statement Of Novelty

Pumpkin wastes remaining after harvest are left on the field, causing deterioration and the proliferation of some insects (house flies, mosquitoes). In the study, pumpkin waste was ensiled and thus an environmentally damaging substance as waste was evaluated an inexpensive feed. In addition, wheat straw hay, which is a poor-quality resource, has been made better quality. In addition, it is the first study in which the spoilage test (aerobic stability) of pumpkin waste silage was carried out.

Introduction

Pumpkin (*Cucurbita pepo*) have been producing to get seed, seed oil and snack. Pumpkin fruits contain a high carotenoid, digestible fiber and carbohydrates, sugar, and pectin, also they are rich in vitamin and minerals [1, 2]. It is reported that pumpkin fruits have about 9 to 12% dry matter, 11 to 16% crude protein, 14 to 19% crude cellulose [3, 4] and 58% total digestible nutrient content [4]. After separation of seed from pumpkin fruit a large amount of waste material emerging (fruit meat and small seeds constitute 95% of the whole fresh fruit, determined by own). Fresh pumpkin wastes cannot be stored a long time due to the high-water content (87.5 to 95%) [3, 4] and microbial deteriorations. Its sugar and pectin content, which most of them can be fermented by silage bacteria [5]. When it considers as a feed source it can benefit for ruminants a nutritive feed material especially in winter, which livestock may feed with poor quality forages (such as cereal straws) which have lack carotenoids and vitamin A. There were some efforts on suitability for silage and feed value of pumpkin waste [6–8], however get high quality silage from various vegetables, dry matter content of materials should be kept about 28 to 35% [9]. Thus, pumpkins are described difficult ensiling material due to high moisture content [10]. In practice, to get optimal dry matter, increase dry matter content and to prevent loss of nutrients with leakage; silage material should be admixed cereal hay or another hays. The aim of this study is to evaluate the nutritional, fermentation value and quality of pumpkin waste silage, ensiled with wheat straw and alfalfa hay and their combinations at various ratios.

Material And Methods

Ensiling Process

The pumpkin wastes were obtained from a pumpkin seed harvest machine (Özkanlar Tarım, Kayseri, Turkey). The machine chopped of pumpkin fruits about 3–8 cm length and width. Before to ensiling process, moisture content of pumpkin fruits was measured (two days ago in the same field pumpkin samples) and dry matter (DM) content was found between 4 to 7% (in experimental samples was 4.7%, Table 1). Pumpkin wastes were mixed with straw and alfalfa hay in different levels in order to increase dry matter. Pumpkin wastes were ensiled alone, with straw and alfalfa (20 and 40% and their combinations as 10 and 20%). The samples were ensiled in 5 liters of jars and remained for two months for fermentation at room temperature (between 20 to 24°C).

Table 1
Chemical composition of pumpkin waste before ensiling

Item	DM, g/kg	CP, g/kg DM	OM, g/kg DM	NDF, g/kg DM	ADF, g/kg DM
Pumpkin waste	47.0	137.6	879.9	516.3	420.5
DM: Dry matter; CP: Crude protein; OM: organic matter; NDF: Neutral detergent fiber; ADF: Acid detergent fiber.					

Chemical Analysis

The jars were opened at after 60 days and pH of samples were measured according to method of Akyildiz [8] with a benchtop pH meter (Thermo Scientific Orion 3-Star, Waltham, MA, USA). The dry matter (DM), organic matter (OM), crude protein (CP), crude cellulose (CC) and ether extract (EE) were determined according to methods specified in AOAC [11]. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest and Robertson [12]. Water soluble carbohydrate (WSC) contents were determined by the phenol sulphuric acid method according to Dubois et al. [13] and aerobic stability were determined according to Kung et al. [14]. Lactic acid (LA) was determined by Lepper's

methods [10]. The acetic, propionic and butyric acid analysis were done in a gas chromatograph with a capillary column (30 m × 0.25 mm × 0.25 µm, Restek), in a Shimadzu GC-2010+ (Kyoto, Japan).

Aerobic Stability Measurement

Silage samples (150 g) were incubated in glass jars at 24°C in an incubator (Nuve EN300, China). A Pt-100 probe (TMC6-HD) connected to a data logger measured the temperature in the centre of the material hourly. Aerobic stability was defined as the time needed to increase the temperature 2°C above the temperature of a reference silage inoculated to prevent aerobic spoilage completely [14].

In vitro Gas Production, Energy Values and Organic Matter Digestibility

Organic matter digestibility (OMD) and metabolisable energy (ME) and net energy lactation (NEL) contents of silages in *in vitro* conditions were made according to the *in vitro* gas production technique reported by Menke and Steingass [15]. The tubes were incubated in a shaking water bath at 39°C and the amounts of gas formed by fermentation at 0 (start of incubation), 3, 6, 9, 12, 24, 48, 72 and 96 hours.

Cumulative gas production data were fitted to the model of Ørskov and McDonald [16] by NEWAY computer package program.

$$y = a + b(1 - e^{-ct})$$

Where, a, the gas production from the immediately soluble fraction (mL); b, the gas production from the insoluble fraction (mL); c, the gas production rate constant for the insoluble fraction (mL/h); a + b, potential gas production (mL); t, incubation time (h); y, gas produced at time "t". Organic matter digestibility (OMD), metabolizable energy (ME) [21] and net energy lactation (NEL) [15] contents of forages were estimated using equations given below:

- OMD, % = $15.38 + 0.8453 \times GP + 0.0595 \times CP + 0.0675 \times CA$
- ME, MJ kg⁻¹ DM = $2.20 + 0.1357 \times GP + 0.0057 \times CP + 0.0002859 \times EE^2$
- NEL, MJ kg⁻¹ DM = $0.101 GP + 0.051 CP + 0.112 EE$

in the formula: CP: % crude protein, EE: % ether extract, and CA: % crude ash (%).

Statistical Analysis

For statistical analysis of the data obtained from the study, one-way Anova was applied by using SPSS [17] program and Duncan multiple comparison test was used to determine the differences among the treatment groups. Probability was accepted as P < 0.05.

Results

For the pumpkin silages, it was observed that in the physical evaluation of the smell, color and structure, they had a pleasant and aromatic scent, a color changed natural yellow to light yellow and in the groups except pure pumpkin, the particles generally did not lose the structure. It was determined that the silage made with pure pumpkin showed a watery and gaseous structure in which the particles were scattered but there was no significant loss in the color. In this case a total of 7 points, "middle" degree has received. Nutrient composition of fresh pumpkin waste is given in Table 1. The dry matter of fresh pumpkin waste was very low (4.70%). The pH, chemical composition, water soluble carbohydrate, aerobic stability and fleig score of pumpkin waste silage alone or with alfalfa and wheat straw are shown in Table 2. The pH value (4.54) in 40% alfalfa group was significantly higher than other groups and PW group's pH (3.90) was lower than that of other groups (P < 0.01). Dry matter content of silage samples was the lowest in ensiled alone pumpkin (7.27%) group and highest in PW60 + A40 and PW60 + S40 groups (37.87 and 36.13%) (Table 2, P < 0.01). It was observed that the dry matter was low when it was added to the pumpkin waste with 20% concentration of alfalfa or straw hay. However, PW60 + A20 + S20 group pH value was similar PW60 + A40 and PW60 + S40 groups. The crude protein content of alfalfa groups was higher than those of other treatment groups and in the S groups was lower than those of other groups while the lowest protein content was found in the 200 and 400 g kg⁻¹ S groups. Pumpkin waste protein content was 9.61% and this ratio higher than those of S groups. There was no significant effect in terms of ether extract content of samples between the treatment groups. Crude cellulose (CC) percentage and its ratio in organic matter was highest in the 400 g kg⁻¹ S group. Ensiled alone pumpkin silage had lowest CC content (P < 0.01). The highest NDF content was observed in the pumpkin waste group and NDF content lowered by straw addition (P < 0.01), however, the ADF content of straw groups was higher than those of other groups (P < 0.05). Also, 20% and 40% straw addition to PW caused an increase in organic matter content compared to other groups (P < 0.01). Crude protein ratio in organic matter 20 and 40% alfalfa group were higher than those of 20 and 40% straw groups (P < 0.01). In this study, fleig score value of silages was classified good (only PW) and the other groups have "very good" class. The fleig score of PW60 + S40 group higher than of those other groups, whereas the fleig score in the control group was significantly lower than those of the other groups (P < 0.01).

Table 2

The pH, chemical composition, water soluble carbohydrate and fleig score of pumpkin wastes ensiled alone or with alfalfa and straw hay

Treatments	pH	DM, g/kg DM	CP, g/kg DM	EE, g/kg DM	CC, g/kg DM	aNDF, g/kg DM	ADF, g/kg DM	OM, g/kg DM	OMCP, g/kg DM	OMCC, g/kg DM	WSC, g/kg DM	Fleig score	Quality class*
PW	3.90 ^c	72.7 ^c	96.1 ^b	17.0	295.8 ^f	522.4 ^a	382.0 ^{abc}	902.3 ^d	106.5 ^b	327.8 ^f	24.5 ^a	63.53 ^c	good
PW80 + A20	4.15 ^{bc}	210.0 ^b	132.7 ^a	20.5	329.5 ^e	483.3 ^b	346.6 ^c	900.7 ^d	147.3 ^a	365.8 ^e	8.6 ^b	80.87 ^b	very good
PW80 + S20	3.97 ^{bc}	232.0 ^b	57.3 ^d	13.3	452.5 ^b	314.0 ^f	465.3 ^{ab}	930.3 ^a	61.6 ^d	486.3 ^b	11.8 ^b	92.47 ^b	very good
PW60 + A40	4.54 ^a	378.7 ^a	132.7 ^a	22.2	374.6 ^d	486.7 ^b	365.3 ^{bc}	897.0 ^d	148.0 ^a	417.6 ^d	6.7 ^b	99.00 ^b	very good
PW60 + S40	4.03 ^{bc}	361.3 ^a	52.2 ^d	17.7	490.8 ^a	351.4 ^e	473.3 ^a	929.7 ^{ab}	56.2 ^d	528.0 ^a	10.3 ^b	116.20 ^a	excellent
PW80 + A10 + S10	3.98 ^{bc}	237.3 ^b	72.1 ^c	15.7	409.3 ^c	418.7 ^d	389.3 ^{abc}	919.0 ^{bc}	78.5 ^c	445.4 ^c	7.7 ^b	93.27 ^b	very good
PW60 + A20 + S20	4.34 ^b	302.7 ^{ab}	86.8 ^b	19.8	403.9 ^c	441.3 ^c	328.7 ^c	913.0 ^c	95.0 ^b	442.4 ^c	28.7 ^a	91.93 ^b	very good
SEM	0.05	2.36	0.69	0.09	1.14	1.58	1.55	0.31	0.79	1.42	2.2	3.78	-
P	0.001	0.001	0.001	0.043	0.001	0.001	0.043	0.001	0.001	0.001	0.001	0.001	-
PW: pumpkin waste, A: alfalfa, S: wheat straw, DM: dry matter, CP: crude protein, EE: ether extract, CC: crude cellulose, aNDF: neutral detergent fiber, ADF: acid detergent fiber, OM: organic matter, OMCP: crude protein in organic matter, OMCC: crude cellulose in organic matter, SEM: standard error of means, P: probability. *Quality classes determined by Fleig score (100+: excellent, 100 – 81: very good, 80 – 61: good, 60 – 41: satisfactory, 40 – 21: medium and 20 – 0: bad). ^{a,b,...,f} : The differences between means with different letters in the same column are significant.													

Pumpkin waste silages lactic, acetic, butyric and propionic acid and ethanol concentrations are given in Table 3. The lactic acid concentration (45.01 g kg⁻¹ DM) of K60 + S40 group was lower than those of PW and PW80 + S10 + A10 groups. The ratio of acetic acid, butyric acid and propionic acid in the silage samples were not significantly differ among the groups. Ethanol ratio was highest in the PW group and this group's ethanol concentration significantly differ from PW80 + A20, PW80 + A10 + S10 and PW60 + A20 + S20 groups.

Table 3

The fermentation parameters of pumpkin waste ensiled alone or with alfalfa and straw hay

Treatments	Fermentation parameters, g/kg DM				
	LA	AA	PA	BA	Ethanol
PW	53.90 ^a	16.13	3.56	0.00	5.45 ^a
PW80 + A20	50.23 ^{ab}	15.89	2.65	0.97	3.13 ^b
PW60 + A40	53.56 ^a	15.74	2.85	0.82	3.49 ^{ab}
PW80 + S20	47.62 ^{ab}	18.12	2.66	1.26	3.97 ^{ab}
PW60 + S40	45.01 ^b	15.63	2.69	0.00	3.54 ^{ab}
PW80 + S10 + A10	52.67 ^a	17.03	3.53	0.48	4.24 ^b
PW60 + S20 + A20	48.29 ^{ab}	15.91	3.40	0.00	4.39 ^b
SEM	0.93	0.33	0.15	0.17	0.18
P	0.042	0.379	0.382	0.234	0.001
PW: pumpkin waste, A: alfalfa, S: wheat straw, LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid; SEM: Standard error of means; P: probability; ^{a,b} : The differences between means with different letters in the same column are significant.					

Time-dependent gas production rate of silage samples is given in Table 4. In the group PW the gas production from quickly soluble fractions (a) (-10.22) was very high and in the PW60 + S20 + A20 group (4.21) lower (P < 0.05). According to the time dependent gas production, the maximum gas production (b) was in the PW80 + S20 (60.70) group and the lowest in the PW60 + A40 (48.91) group (P < 0.01).

Table 4
pH, gas composed of readily soluble fraction, gas production and rate of pumpkin waste ensiled alone or with alfalfa and straw hay

Treatments	pH	a	b	c	RSD
PW	6.73 ^b	-10.22 ^b	58.25 ^{ab}	0.11 ^a	1.327
PW80 + A20	6.77 ^a	-4.40 ^a	48.99 ^c	0.09 ^a	1.537
PW80 + S20	6.64 ^d	-5.64 ^a	60.70 ^a	0.05 ^b	0.943
PW60 + A40	6.79 ^a	-6.17 ^a	48.91 ^c	0.09 ^a	1.370
PW60 + S40	6.65 ^d	-5.70 ^a	59.81 ^a	0.04 ^b	1.673
PW80 + S10 + A10	6.69 ^c	-4.65 ^a	55.09 ^{abc}	0.06 ^b	0.800
PW60 + S20 + A20	6.72 ^b	-4.21 ^a	51.63 ^{bc}	0.05 ^b	1.117
SEM	0.012	1.326	2.112	0.006	-
P	0.001	0.081	0.004	0.001	-
PW: pumpkin waste, A: alfalfa, S: wheat straw, a: the gas production from the quickly soluble fraction (mL); b: the gas production from the insoluble fraction (mL); c: the gas production rate constant for the insoluble fraction (mL h ⁻¹); SEM, standard error of means; P: probability. RSD: residual standard error. ^{a,b,c,d} : The differences between means with different letters in the same column are significant.					

The rate of gas production in the alone PW group was higher than those of the other groups in the 3, 6, 9, 12 and 24 h, however, PW80 + A20 The amount of gas released during the 96-hour fermentation period in the study material pumpkin waste silages is given in Table 5. The gas production rate of PW80 + S20 group was highest at 48th, 72nd and 96th hours (P < 0.01) and the gas production rate of PW60 + A40 group was lower at 72 and 96 hours (P < 0.01).

Table 5
Time dependent gas production (mL) of pumpkin waste ensiled alone or with alfalfa and straw hay

Treatments	Fermentation time, h							
	3	6	9	12	24	48	72	96
PW	40.50 ^a	52.17 ^a	64.33 ^a	75.17 ^a	88.67 ^a	96.67 ^{ab}	97.67 ^{bc}	99.33 ^{cd}
PW80 + A20	41.67 ^a	53.33 ^a	64.33 ^a	71.33 ^{ab}	82.50 ^{abc}	92.33 ^{abc}	94.67 ^{cd}	97.50 ^d
PW80 + S20	37.33 ^{bc}	45.17 ^{cd}	53.50 ^c	61.66 ^d	83.16 ^{ab}	99.17 ^a	103.50 ^a	107.00 ^a
PW60 + A40	38.33 ^b	48.67 ^b	58.83 ^b	67.33 ^{bc}	79.50 ^{bc}	88.17 ^c	91.17 ^d	93.50 ^e
PW60 + S40	35.67 ^d	40.67 ^e	47.67 ^d	54.83 ^e	76.83 ^{bc}	93.67 ^{abc}	99.83 ^{ab}	104.00 ^{ab}
PW80 + S10 + A10	37.83 ^{bc}	47.00 ^{bc}	56.67 ^{bc}	64.00 ^{cd}	82.50 ^{abc}	95.67 ^{abc}	99.50 ^{abc}	102.83 ^{bc}
PW60 + S20 + A20	36.33 ^{cd}	43.67 ^d	53.00 ^c	59.50 ^d	76.17 ^c	89.50 ^{bc}	94.83 ^{bcd}	98.67 ^d
SEM	0.51	0.71	1.25	1.52	2.02	2.34	1.54	1.22
P	0.001	0.001	0.001	0.001	0.010	0.050	0.001	0.001
PW: pumpkin waste, A: alfalfa, S: wheat straw, SEM: standard error of means; P: probability, ^{a,b,c,d} : the differences between means with different letters in the same column are significant.								

The organic matter digestibility (OMD), metabolic energy (ME) and net energy lactation (NEL) values calculated using the *in vitro* gas production volumes of silages are given in Table 6. In the control group's the OMD, ME and NEL values were significantly higher than those of other groups (without PW80 + A20 group, P < 0.05).

Table 6
Nutritional parameters of pumpkin waste ensiled alone or with alfalfa and straw hay

Treatments	OMD, g/kg DM	ME, MJ/kg DM	NEL, MJ/kg DM
PW	581.5 ^a	8.61 ^a	5.03 ^a
PW80 + A20	545.5 ^{ab}	8.02 ^{ab}	4.62 ^{ab}
PW80 + S20	518.8 ^b	7.72 ^b	4.29 ^b
PW60 + A40	533.6 ^b	7.83 ^b	4.54 ^b
PW60 + S40	510.7 ^b	7.48 ^b	4.28 ^b
PW80 + S10 + A10	521.1 ^b	7.73 ^b	4.34 ^b
PW60 + S20 + A20	506.2 ^b	7.48 ^b	4.22 ^b
SEM	15.99	0.244	0.182
P	0.027	0.031	0.033
PW: pumpkin waste, A: alfalfa, S: wheat straw, ME: metabolizable energy; NEL: net energy lactation; OMD: organic matter digestibility; SEM: standard error of means; P: probability, ^{a,b} : The differences between means with different letters in the same column are significant.			

The aerobic stability of silages is given in Table 6. Aerobic stability was lowest in PW group silage ($P < 0.05$). The aerobic stability of straw and alfalfa groups was increased. It was observed that aerobic stability achieved the best results when straw, alfalfa or straw and clover were added with 40% of pumpkin silage ($P < 0.05$).

Discussion

Silage quality can be predicted objectively as a tool for preliminary assessment [9]. Subjective evaluation may give a clue for silage quality without any chemical analysis results. In this experiment there was no substantial change of pumpkin waste particle size in the silage samples. DLG score [18] in silages made with corn varieties sum of the smell, structure and color scores may contribute to predict silage quality classification and it can be detected by the sensory organs. The additives used to reduce the amount of water in the pumpkin waste silages changed the physical appearance of the silage, which made it possible to get a high score in terms of this criterions. Konca et al. [19] found that silage quality class was satisfactory in pea silages in unusual feed materials in silage production, bad and satisfactory in triticale silages, good for artichoke silage, and satisfactory for corn silages.

The pH value ranges of this experiment can be evaluated for success a good silage quality. According to recommendation of good quality silage pH ranges should be 3.5 to 4.0 [9]. It has been reported that when pH is not low enough, there was not a sufficient fermentation, so, it is presumably assumed silage is not harvested at an appropriate time, or insufficient carbohydrate provided for fermentation and therefore lactic acid bacteria are not developed [9, 20]. Especially, when occurred insufficient fermentation, the pH of the silage does not fit decrease sufficiently, not enough lactic acid formation but undesirable butyric and propionic acids increase in the silo [9]. In the current experiment the pH value of silages was generally around 4.0 and it can be said to be sufficient fermentation well done.

The dry matter of ensiled alone pumpkin waste was very low (47.0 g/kg). Dry mater of silages was increased by the alfalfa hay and straw supplemented groups. Scharrer et al., [6] reported that the dry matter of fresh pumpkin and silage pumpkin 88.1 and 80.1 g/kg respectively. Also, Hashemi and Razzagzadeh [8] added 286% straw and 0, 10 and 20 molasses and 5% urea to pumpkin waste and they found that 10% molasses addition increased DM content of silages (37.68%). Church [4] reported that the pumpkin fruit dry matter content was 90 g/kg and Mokhtarpour [3] found 12.5%. Both of these researcher results of DM values are higher than the values in the current study. However, it has been reported that the variety of pumpkin and harvest time may significantly affect the content of DM [8]. Silage DM is accepted as an important criterion in silage compaction and aneorobic fermentation and harvest time signal. The expected rate of DM in a good quality maize silage is accepted as 300 to 350 g/kg [15]. From this point of view, it can be said that the DM rates in the silage samples high and low values from these ranges are not suitable for good quality silages. On the other hand, in the silages obtained from seasonally grown plants and sometimes insufficient maturation, so, lower DM ratio than that of desired values and dry rough feeds such as hay or straw were mixed into the silage material to prevent seepage losses due to high moisture content. In this study, wheat straw and alfalfa hay were used to reduce the high humidity content of pumpkin wastes and it was observed that the content of DM reached to the desired (DM > 350 g/kg) levels especially in groups with 40% straw and alfalfa (Table 2). Kara et al. [21] reported that broccoli, parsley, lettuce, leek, cauliflower, cabbage and spinach contain low dry matter than that of pumpkin wastes and from these reports it has been determined that pumpkin waste is a silo material with better quality of crude nutrient content than most of vegetable wastes.

Fleig score calculate based on DM and pH values of silage, the silages samples were classified generally good (only PW) and the most of groups have "very good" class quality. Hashemi and Razzagzadeh [8] found that ensiling of PW with molasses and urea pH value ranges between 7.61 and 6.24 while Invanchuk [22] determined the pH value in the pumpkin silage between 4.4 and 4.5 and Mokhtarpour [3] reported that pH of pumpkin prior to

ensile was 5.9 and after fermentation ranged from 3.87 to 4.54 and these pH values similar as the current experimental pH values. Konca et al. [19] reported that fleig scores in the silages produced (such as peas, artichoke, triticale, and corn) in dairy cattle farms of Turkey were between 11.0 and 98.5 and they have been classified between the "bad" and the "very good" quality. Kara et al. [21] found that the quality class of lettuce, leek, cauliflower and cabbage was "satisfactory" and celery, broccoli and parsley and spinach was not suitable for make silage. The highest fleig score was 83.94 in leek, and the lowest in 15.66 broccoli. These values obtained are more satisfactory than the values obtained for the other materials which are ensiled as by-products. In the commercial farms, maize silage quality may be changed between 'middle' and 'famously' quality classes [23].

The crude protein content alone PW group was 96.1 g/kg and their results were similar to Enishi et al. [24] results who reported that pumpkin waste crude protein content was 94 g/kg. Alfalfa added groups' crude protein ratio were higher than those of other groups. Alfalfa hay contains a high crude protein ratio compared to straw (132 vs 40.2 g/kg, respectively (analyzed results). Bakshi et al. [25] noted that protein content of pumpkin was 151 g/kg and Mokhtarpour [3] and Church [4] noted that crude protein of pumpkin silage about 110 to 160 g/kg. Other researchers reported low CP content of pumpkin and corn grits mixture (2015). However crude protein content was higher than that of corn silages [19] and similar to NDF and ADF content and crude ash.

The wheat straw addition decreased crude ash content of pumpkin waste silages. Crude fat content was not influenced by the treatments. Lozicki et al. [5] reported that pumpkin fruit ash was 86.4 g/kg. Hashemi and Razzagzadeh [8] found that dry matter content of pumpkin (71.4%) + straw (28.6%) silage was 8.38%; and found the highest in the group with 10% molasses added as 37.68%. Enishi et al. [24] reported that pumpkin waste NDF content was 94 and 251 g/kg respectively and higher digestibility in goat rumen compared to sugar beet pulp and carrot juice waste. Lozicki et al. [5] determined that NDF and ADF content of pumpkin alone was 217 and 180 g/kg, respectively.

It is known that short chain volatile fatty acids such as acetic, propionic and butyric acid prevent aerobic deterioration in silage by suppressing the growth of yeast and mold in silage. The lowest lactic acid concentration (45.01 g/kg DM) was obtained in PW60 + S40 group and highest lactic acid ratio (58.97 g/kg DM) observed in the PW80 + A10 + S10 group. The ratio of acetic acid, butyric acid and propionic acid in the silage samples were not significantly differ between the groups. Ethanol ratio was highest in PW80 + A20 group and lowest ethanol concentration was obtained in PW90 + A10 group. Ivanchuck [22] found 10.2 g of lactic acid, 1.3 g of acetic acid and 0.83 g of butyric acid per kilogram of pumpkin silage. Brune et al. [7] noted that when pumpkin ensiled with stubble alfalfa and sunflower up to 1/3 ratio evaluated "very good quality" in terms of acid content, digestibility and feeding value. Researcher claimed that pumpkin and corn silage was better quality than corn alone, higher crude protein digestibility with 2: 1 or 1: 1 ratio. Butyric acid was not detected in the silage of PW alone, PW60 + S40 and PW80 + S20 + A20 groups. Butyric acid is generally low in feeds made with good silos.

Improvement of rumen conditions is important in terms of cellulase hydrolysis. In terms of cellulasis, it can be desired pH values should be changed between 6.6 and 6.8, and if increase or decrease occurs in the rumen pH value it may affect cellulose digestibility. In particular, the pH value is less than 5.9, which suggests a decrease in cellulite activity [26]. In this experiment, the pH value was higher than 6.0 which create desirable range. As seen in the Table 2, while depend WSC content of samples in group C the gas production from quickly soluble fractions (a) (-10.22) was very high and in the PW60 + S20 + A20 group (4.21) lower gas production was observed ($P < 0.05$). According to the time dependent gas production, the maximum gas production was in the PW80 + S20 (60.70) group and the lowest gas production was in the PW60 + A40 (48.91) group. The rate of gas production in the control group was higher than those of the other groups. The amount of gas released during the 96-hour fermentation period in the study material pumpkin waste silages is given in Table 4. The gas production rate of PW80 + S20 group was the highest at 48th, 72nd and 96th hours ($P < 0.01$) and the gas production rate of PW60 + A40 group was low at 72 and 96 hours ($P < 0.01$). In the control group's the OMD, ME and NEL values were significantly higher than those of other groups ($P < 0.05$).

Examining Table 2, it will be seen that there is no statistically significant difference between the groups in terms of WSC values for pumpkin waste silages. However, the WSC values obtained in this study were found to be closer to the values determined in legumes [27] than the WSC values of corn silage reported by other researchers [28]. In many cases, materials with high WSC content have the advantage of providing suitable fermentation development [29]. If there is not enough WSC in the medium, silage quality will decrease. In order to get a better-quality silage, it must be contained at least 30 to 50 g/kg fermentable carbohydrate in DM, especially hexoses. From this point, it can be said that the WSC content of the pumpkin waste silages is sufficient to get good quality silage.

The time-dependent gas production rates are given in Table 4. When the *in vitro* gas production kinetics are considered, the highest group was the PW60 + S40 (pH = 6.79) group and the lowest value was in the PW80 + S20 (pH = 6.64) group. However, it has been found that the pH change intervals are very narrow and vary between 6.79 and 6.64. Differences in nutrient content of feeds may affect *in vitro* gas production parameters, energy values and OMD in silages [30]. Similarly, in this study, it can be seen that between the gas production values of the groups varied in different silage groups. The silages OMD values were lower than in another study PW ensiled with wheat bran and urea [31]. In the present study, organic matter values were higher in the PW80 + S20 and PW60 + S40 groups and NDF values were lower than those of other groups. At the same time, the PW80 + S20 group's gas production at 48th, 72nd and 96th hours significantly higher than the other groups. In this matter, differences of silage samples nutrient compositions may explain of difference of gas production values. On the other hand, protein concentration of samples may affect of gas production and high percentage of feeds negatively effect of gas production [30]. Similarly, in this study, the PW60 + A40 group could explain the fact that it has less gas production value than those of the other groups.

Digestibility of organic matter, metabolisable energy and net energy lactation values were higher in PW alone silage samples these values were higher than those of other groups (without PW80 + A20 group). It shows PW alone silage have a better digestibility, NDF ratio and higher gas production. Both alfalfa hay and wheat straw mixture decreased these values. Especially, as well-known straw hay lower digestibility and feed value. So, pumpkin waste can be used a valuable silage material in terms of higher digestibility and energy value.

Aerobic stability is an important factor that determines the silage quality and feed value [32]. Aerobic stability indicates the capacity of oxygen-exposed silage to resist microbial growth. Silage is exposed to oxygen during storage or animal feeding. A number of changes occur in the silage material that has been exposed to oxygen. These changes are determined by following the silage temperature. In the method used to determine the aerobic stability of the silage material, the temperature change caused by the silage material exposed to oxygen is measured [33]. In addition to these measurements, pH measurements are also evaluated together [32]. As shown in Table 7, when the pH values of spoiled silages were examined, it was observed that spoilage occurred in all groups. When the groups were examined, the aerobic stability of the groups of straw and alfalfa was affected positively. It was observed that aerobic stability was best when 40% straw, alfalfa or straw and alfalfa were added to pumpkin waste silage alone. However, when the results are examined, it can be said that alfalfa is more effective than wheat straw to increasing the aerobic stability.

Table 7
Aerobic stability of pumpkin waste ensiled alone or with alfalfa and straw hay

Treatments	pH before spoilage	pH after spoilage	Aerobic stability, h
PW	3.90 ^c	8.1	27.00 ^c
PW80 + A20	4.15 ^{bc}	8.74	99.33 ^a
PW80 + S20	3.97 ^{bc}	9.69	74.33 ^{ab}
PW60 + A40	4.54 ^a	7.68	102.67 ^a
PW60 + S40	4.03 ^{bc}	9.31	49.00 ^{bc}
PW80 + S10 + A10	3.98 ^{bc}	9.16	74.00 ^{ab}
PW60 + S20 + A20	4.34 ^b	9.32	107.67 ^a
SEM	0.05	0.23	7.66
P	0.001	0.194	0.01
PW: pumpkin waste, A: alfalfa, S: wheat straw; SEM: standard error of means; P: probability, ^{a,b,c} : The differences between means with different letters in the same column are significant.			

Conclusions

Agricultural wastes and agricultural industry by products remain in the production areas and may cause environmental pollution. This study showed that the pumpkin waste materials may be ensiled with 40% alfalfa hay and/or wheat straw. However, more research is needed for the effects of animal performance as in vivo studies.

Declarations

Conflict of interest

There is no conflict of interest for the publication of this article.

Ethical Approval

No need.

Consent to Participate

All authors agree to participate in the current work. Consent for Publication All authors agree to publish the findings of the current research.

Author Contributions

Conceptualization by YK, SBB; methodology by MB, MK, SBB; investigation and validation by MK, MB; Writing original draft, review editing by YK, MB and SBB.

Acknowledgements

References

1. Korzeniewska, A., Sztangret, J., Seroczynska, A., Niemirowicz-Szczytt, K. Zawartość związków karotenoidowych w owocach dyni olbrzymiej (*Cucurbita maxima*). *Zeszyty Problemowe Postępów Nauk Rolniczych*, **1**, 497 (2004).
2. Niewczas, J., Mitek, M., Korzeniewska, A., Niemirowicz-Szczytt, K.: Characteristics of selected quality traits of novel cultivars of pumpkin (*Cucurbita Maxima*). *Pol. J. Food Nutr. Sci.* **64.2** (2014).
3. Mokhtarpour, G.H.: Selecting the best method of preparing silage of Pumpkin residues. Final Report. Natural resources and animal affairs research center of Golestan Province, Iran (1994).
4. Church, D.C.: Livestock feeds and feeding. 2nd. Edition, Princeton Hall, New Jersey (1996).
5. Lozicki, A., Koziorzebska, A., Halik, G., Dymnicka, M., Arkuszewska, E., Niemiec, T., Bogdan, J.: Effect of ensiling pumpkin (*Cucurbita maxima* D.) with dried sugar beet pulp on the content of bioactive compounds in silage and its antioxidant potential. *Anim Feed Sci Tech.* **206**, 108-113 (2015).
6. Scharrer, K., Schreiber, B., Kuhn, H.: Suitability for silage and feed value of oil pumpkin (*Cucurbita pepo*). *Zeitschrift fur Tierphysiologie, Tierernahrung und Futtermittelkunde* **15**, 265-273 (1960).
7. Brune, H., Zaddach, H.: Silages of pumpkin and pumpkin mixtures. Difference of digestibility of silage with pumpkin added and the drainage of fluid from pumpkin silages. *Zeitschrift fur Tierphysiologie, Tierernahrung und Futtermittelkunde* **18**, 321-340, (1996).
8. Hashemi, A.J., Razzaghzadeh, S.: Investigation on the possibility of ensiling cucurbit (*Cucurbita pepo*) residues and determination of best silage formula. *J Anim Vet Adv.* **6**, 1450-1452 (2007).
9. Ozen, N., Çakir, A., Haşımoğlu, S., Aksoy A.: Feed Science and Technology (In Turkish). Atatürk Üniversitesi Ziraat Fakültesi Ders Kitabı, Erzurum, pp. 50 (1986).
10. Akyildiz, A.R.: Feed Analyses Laboratory Technics Manual (In Turkish). Ankara Üniversitesi Zir. Fak., Basımevi, Ankara. p 236 (1984).
11. AOAC: Association of Official Analytical Chemists. Official Methods of Analysis, 15th ed., vol. 1. AOAC, Washington, DC, pp. 69-79 (1995).
12. Vansoest, P.J., Robertson, J.B.: Systems of Analyses for Evaluation of Fibrous Feed. In W.J. Pigden, C. C. Balch and M. Grahm (Eds). *Proc. Int. Workshop on Standardization of Analytical Methodology for Feeds*. Int. Dev. Res. Center, Ottawa (1979).
13. Dubois, M., Giles, K.A., Hamilton, J.K., Rebes, P.A., Smith, F.: Colorimetric method for determination of sugars and related substances. *Anal Chem.* **28**, 350-356 (1956).
14. Kung, L., Robinson, J.R., Ranjit, N.K., Chen, J.H., Golt, C.M., Pesek, J.D.: Microbial populations, fermentation end products, and aerobic stability of corn silage treated with ammonia or a propionic acid-based preservative. *J Dairy Sci.* **83**, 1479-1486 (2000).
15. Menke, K.H., Steingass, H.: Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Anim Res Dev.* **28**, 7-55 (1988).
16. Ørskov, E.R., McDonald, I.: The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *J Agric Sci.* **92**, 499-503 (1979).
17. SPSS: Version 17.00 for Windows. SPSS Inc., Chicago, IL. USA (1988).
18. DLG: Futterwerttabellen für Wiederkäuer. DLG Verlag, Frankfurt (1997).
19. Konca, Y., Alçiçek, A., Yaylak, E.: Determination of quality of the silage made in dairy cattle farms. *J Anim Prod.* **46**, 6-13 (2005).
20. Kutlu, H.R.: Silage and silage feeding in all aspects (in Turkish). Çukurova Üniversitesi, Ziraat Fakültesi Zootekni Bölümü yayınları, Adana. <http://www.zootekni.org.tr/upload/File/SILAJ%20EI%20KTABI.pdf> Access: 21 April 2020 (2014).
21. Kara, B., Ayhan, V., Akman, Z., Adıyaman, E.: Determination of silage quality, herbage and hay yield of different triticale cultivars. *Asian J Anim Vet Adv.* **4**, 167-171 (2009).
22. Ivanchuk, V.A.: Vitamin rich silage a valuable feed for pigs. *Problemy Kormleniya. Sovremennom Zhivotnovodstve*, Russia (1984).
23. Alçiçek, A.: Silo feed; importance and factors affecting quality (in Turkish). E.Ü.Z.F. Agricultural Application and Research Center Publications, İzmir. pp. 22 (1995).
24. Enishi, O., Yoshioka, T., Nakashima, K., Saeki, M., Kawashima, T.: Analysis of *in situ* ruminal digestive characteristics and nutritive value of pumpkin and carrot juice residue for ruminant feeds. *Grassland Sci.* **50**, 360-365 (2004).
25. Bakshi, M.P.S., Wadhwa, M., Makkar, H.P.: Waste to worth: vegetable wastes as animal feed. *Reviews* **11**, 1-26 (2016).
26. McDonald, P., Henderson, A.R., Heron, S.J.E.: The Biochemistry of Silage. (2nd ed.). Chalcombe Publ., Church Lane, Kingston, Canterbury, Kent, UK, (1991).
27. Keleş, G., Alataş, M.S., Ateş, S.: Nutrient value and fermentation characteristics of alfalfa and Hungarian clover silages ensiled with silage additives. VII. National Animal Feed Congress, 26-27 September, Ankara, Turkey (2013).
28. Polat, C., Koç, F., Özdüven, M.L.: The Effects of Lactic Acid Bacteria and Lactic Acid Bacteria+Enzyme Mixture Silage Inoculants on Maize Silage Fermentation and Nutrient Digestibility in Lambs. *J Tekirdag Agri Fac.* **2**, 3-22 (2005).

29. Davies, D.R., Merry, R.J., Williams, A.P., Bakewell, E.L., Leemans, D.K., Tweed, J.K.S.: Proteolysis during ensilage of forages varying in soluble sugar content. *J Dairy Sci.* **81**, 444-453 (1988).
30. Kılıç, Ü., Boğa, M.: Determining the effects of sunflower varieties with different protein contents on results of in vitro gas production and enzyme techniques. *J Tübbav Sci.* **2**, 231-238 (2009).
31. Seifdavati, J., Seifzadeh, S., Ramezani, M. et al. Wastes Valorization of Wheat Straw and Wheat Bran Treated with Urea, Probiotic or Organic Acids to Enhance Ruminal Gas Production and Digestibility of Pumpkin By-product. *Waste Biomass Valor* (2021).
32. Filya, I.: Nutritive value and aerobic stability of whole crop maize silage harvested at four stages of maturity. *Anim Feed Sci Tech.* **116**, 141-150 (2004).
33. Adesogan, A.T., Salawu, M.B.: The effect of different additives on the fermentation quality, aerobic stability and in vitro digestibility of pea/wheat bi-crop silages containing contrasting pea to wheat ratios. *Grass Forage Sci.* **57**, 25-32 (2002).

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