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Hygienic Profile and Nutritive Value of Boot Stage Wheat Silage Treated with Acid-Based Preservative

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Abstract: The current study was undertaken to investigate the effects of acid-based preservative (AP) on fermentation, hygienic and post-storage quality as well as digestibility of low dry matter (DM) direct cut wheat silages. Wheat was harvested at boot stage of maturity (265 g DM kg^{-1}). Silages with no additive served as control (AP0). The AP was applied at 2 mL kg^{-1} (AP2), 3 mL kg^{-1} (AP3) and 4 mL kg^{-1} (AP4) of fresh forage weight. After AP treatment, the chopped forages were ensiled in 1.5 L anaerobic jars. Four jars per treatment were sampled on d 90 after ensiling, for chemical and microbiological analysis. Overall, AP treatment did not affect (P>0.05) the concentrations of lactic and acetic acid of the wheat silages. In contrast, inclusion of AP increased the concentrations of formic and propionic acid whereas decreased the concentrations of butyric acid, ethanol, ammonia-N and fermantation losses when compared to the control silage (P<0.05). Under aerobic conditions, AP treated silages had lower pH, CO_2 production and the numbers of yeast and mold than AP0 (control) silage (P<0.05). Fibrous fractions were decreased (P<0.05) with the application of AP, but 96 h in vitro gas production and in vitro organic matter digestibility of the silages were not affected (P>0.05) by AP inclusion. In conclusion, the AP administration in to direct-cut wheat silage apparently improved fermentation properties and post storage quality. Its antimicrobial properties caused a reduction in proteolysis.

Keywords: Acid preservatives, boot-stage, hygienic profile, feed value, wheat silage.

Asit Bazlı Bir Koruyucunun Başaklanma Döneminde Hasat Edilen Buğday Silajlarinin Mikrobiyal Özellikleri ve Besleme Değeri Üzerine Etkisi

Öz: Bu çalışma, asit bazlı bir koruyucunun (AP) düşük kuru maddeli buğday silajlarında fermantasyon, mikrobiyal yapı ve depolama sonrası kalite özellikleri ile besleme değeri üzerine olan etkilerini belirlemek amacı ile düzenlenmiştir. Buğday, başak bağlama döneminde hasat edilmiştir. Parçalanmış olan taze materyale asit bazlı koruyucu 2 mL kg-1 (AP2), 3 mL kg-1 (AP3) ve 4 mL kg-1 (AP4) düzeylerinde katılmıştır. Katkı maddesi içermeyen silajlar kontrol grubu olarak ele alınmıştır. Çalışmada 1.5 L anaerobik kavanozlar kullanılmıştır. Silolamadan 90 gün sonra açılan silajlarda kimyasal ve mikrobiyolojik analizler yapılmıştır. Genel olarak, AP katkısı silajların laktik ve asetik asit konsantrasyonlarını etkilememiştir. Diğer yandan, katkılı silajların formik ve propiyonik asit konsantrasyonlarının daha yüksek (P<0.05), bütrik asit, etanol, amonyak-N ve gaz kayıplarının ise kontrol silajlarına göre daha düşük olduğu gözlenmiştir (P<0.05). Aerobik koşullarda, AP katkılı silajlarda pH, CO2 üretimi ile maya ve küf sayıları daha düşük bulunmuştur (P<0.05). AP katkılı silajlarda sellüloz fraksiyonlarında azalma gözlenirken, AP katkısı 96. saat in vitro gaz üretimini ve organik madde sindirilebilirliğini etkilememiştir (P<0.05). Sonuç olarak, düşük kuru maddeli buğday silajlarında AP ilavesi; eriyebilir şekerleri arttırıp, proteolizi azaltmış ve antimikrobiyal etkisi sayesinde fermantasyon ve depolama sonrası silaj kalitesini iyileştirmiştir.

Anahtar Kelimeler: Asit bazlı koruyucu, başaklanma dönemi, besleme değeri, buğday silajı, mikrobiyal yapı

1. Introduction

As an economical source of silage, wheat, has recently been taken into account as a replacement for the traditional resource of silages such as grass and corn. Inaccurate feeding value information may be part of the reason why wheat has not been utilized to its full potential. Good quality whole-crop wheat silage could be a good fit for high

lactating dairy cows or beef cattle (Kennelly and Weinberg, 2003). Harvesting wheat at the earlier stage of maturity, such as late vegetative or boot stage will provide the highest protein content and moderate metabolizable energy (ME) content which are about 8–18 percent dry matter (DM) and 9.5–10.5 MJ/kg DM, respectively (Kaiser et al., 2003). Research conducted by Beck et al. (2009) demonstrated that DM and neutral detergent fibre (NDF) digestibility of boot-stage wheat forage diets were greater ($P<0.01$) than diets containing forage harvested in dough stage. Moreover, including wheat silage in the diet of dairy (Huhtanen et al., 2007) and beef cattle (Walsh et al., 2005; Keady et al., 2007) increased intakes than grass silages.

It's known that ensiling whole crop cereals often result in silages with high concentrations of butyric acid. Furthermore, poor aerobic stability is a big problem in whole crop cereal silages (Kaiser et al., 2003). Strategies which minimize losses during storage and feedout phase may be beneficial for low DM and water soluble carbohydrate (WSC) small grain silages when silage is the large proportion of diet (0.50 to 0.60 of ration DM). Addition of acid-based preservative (AP) to silage combats microorganisms, including pathogenic bacteria and some fungi, which would otherwise cause spoilage and reduce its nutritive value by metabolizing the starch and protein therein (Grajewski et al., 2005; Filya and Sucu, 2007a). Besides, AP promotes higher production by modifying rumen fermentation pattern (Jaakkola et al., 2006 a,b; Seppälä et al., 2016) and increasing digestibility of silages (Lorenzo and O`Kiely, 2008; Sucu et al., 2009).

At boot stage of maturity, direct cut wheat forage is difficult to preserve because its high moisture and protein contents which could cause excessive fermentation during ensiling (Nadeu, 2007). We hypothesized that when adding a an acid-based preservative (blend of formic acid, ammonium format, propionic acid, benzoic acid and ester of benzoic acid) to low dry matter direct cut wheat forage would improve fermentation, preservation, and nutritive value of silage. Therefore, this study was designed to identify the effects of an acid-based preservative on the silage quality and nutritive value of wheat which was harvested at boot stage of maturity.

2. Materials and Methods

Forage and additive

Turkish wheat (*Triticum aestivum* L., var. *aestivum*) cultivar 'Pehlivan', was used in this study. Wheat was grown the Agricultural Experimental Station (40º14′ N, 28º50′ E). Wheat was harvested by hand (21 weeks after sowing) at boot stage and chopped with a laboratory type chopper to about 1.5 cm. The analysis samples and ensiling materials were taken from four replicates, respectively. Liquid formulation of AP was used as additive 860 g kg^{-1} active ingredients containing formic acid (590 g kg^{-1}) and propionic acid (200 g kg^{-1}), ammonium formate (45 g kg^{-1}), benzoic acid/K-sorbate (25 g kg^{-1}) and water (140 g kg^{-1}) at the rate of 2, 3 and 4 mL kg^{-1} of fresh forage weight.

Ensiling procedures

The chopped forage ensiled in 1.5 l anaerobic jars (Weck®, Wher-Oflingen, Germany) equipped with a lid that enables gas release only. Each jar was filled with about 1074 g (fresh weight) of chopped forage, without a headspace. The obtained wet and dry densities were 477 kg and 126.5 kg of DM/m^3 wheat, respectively. The experiment had 4 treatments (untreated control and 3 doses of AP) with 4 parallels (jars). Jars were stored at ambient temperature (24 to 29°C). Fresh and ensiled forages were sampled for further analysis on d 90 after ensiling. Within 1 h of chopping, the following treatments were applied to fresh forage: control (no additive, AP0); AP2 (applied at 2 mL kg^{-1} fresh weight); AP3 (3 mL kg^{-1}); AP4 (4 mL kg^{-1}). The amount of chopped forage for a given jar was weighed out, sprayed with the appropriate AP using a plant sprayer, mixed by hand, and then placed into the jar by hand with periodic tamping. Jars were weighed before and after filling to determine the actual amount ensiled.

Chemical and microbiological analysis procedures

Chemical analyses of fresh forage and silages were performed in quadruplet per treatments for each replicate and presented on DM basis. The silage pH was measured directly from the silage juice using a pH meter (Sartorius PB-20, Germany). The DM content of the fresh forage and silages was determined by drying at 60°C for 48 h in a fan-assisted oven (Procedure number: 930.15; AOAC, 1990). Fresh forage and silages were analyzed for crude protein (CP) and ash according to AOAC (984.13 and 942.05, respectively; 1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analyzed by using the sodium sulphite addition method without α-amylase and expressed with residual ash (Van Soest et al., 1991). Wet samples stored at –20°C were extracted for 3 min in a blender in water or in ethyl acetate (1:9) for WSCs and fermentation products analysis, respectively. The WSCs were determined by the phenol sulphuric acid method (Dubois et al., 1956). Lactic acid was determined by a spectrophotometry method (Barker and Summerson, 1941). The VFAs and ethanol were determined in aqueous extracts by means of a GLC with a semi-capillary FFAP (nitroterephthalic acid-modified polyethylene glycol) column (Hewlett-Packard, Wardbronn, Germany), over a temperature range 45 to 230°C. Ammonia-N was determined by using Kjeltech auto analyzer (Gerhardt, Bonn, Germany) without a digestion step according to AOAC (1990). The concentration of fermentation products (FEP) was calculated as the sum of lactic, acetic, propionic, formic, butyric acids and ethanol. Ammonia –N values were corrected by subtracting on the DM basis, the ammonia and N added by the additives (2, 3 and 4 mL kg^{-1} AP). Gas losses during storage were estimated by weight loss, calculated separately for each silo the difference in the weight at the beginning and end of the ensiling period. Microbiological analyses of fresh forage and silages were performed in quadruplet (per treatment for each replicate) and presented on fresh and wet silage basis. Microbiological evaluation included enumeration of lactobacilli on pour-plate rogosa agar (Oxoid CM627, Oxoid, U.K.), and yeast and mold on spread-plate malt extract agar (Difco, Detroit, MI) acidified with lactic acid to pH 4.0. Aerobic bacteria were counted on nutrient agar (Nissui-seiyaku Ltd, Japan). Plates were incubated for 3 d at 30°C. All microbiological data were transformed to $\log_{10}$.

Aerobic stability measurement

At the end of the ensiling period (d 90), the silages were subjected to an aerobic stability test at room temperature (29°C), which lasted 5 d, in a "bottle" system developed by Ashbell et al. (1991). Analyses were carried out on the silage samples after 5 d exposure to air.

In vitro gas production procedure

Gas productions of the silages were measured by the *in vitro* procedure of Menke and Steingass (1987). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979). Digestible organic matter (OMD), metabolizable energy (ME) and net energy lactation (NE_L) values in silages were calculated using equations of Menke and Steingass (1987):

OMD (%) = 0.9042xGP + 0.0492xCP + 0.0387xCA + 16.49,

ME (MJ/kg KM) = 0.136xGP + 0.0057xCP + $0.000286xEE^2$ + 2.20,

NE_L (MJ/kg DM) = 0.096xGP + 0.0038xCP + $0.000173xEE^2$ + 0.54,

where GP is 24-h net gas production (mL 200 mg^{-1} DM), and CP, EE, CA are crude protein, ether extract, crude ash (% DM), respectively.

Statistical analysis

The data were analyzed as a completely randomized design with four replications and subjected to analysis of variance by the GLM procedure of SAS (2005). Differences among means were tested using Tukey's test and significance was declared at $P<0.05$.

3. Results

The chemical and microbiological compositions of the fresh wheat forage are presented in Table 1. The numbers of initial LAB, yeast and mold in the fresh forage were high, each being >4 log cfu/g.

The comparison of chemical and microbiological changes between AP treated silages and control silage is shown in Table 2. The silages were relatively well fermented but the extent of fermentation differed depend on the AP rate. The addition of AP decreased the silage pH than control silage (P<0.05). Compared with the AP0 (control) or AP2, residual WSCs were higher (P<0.05) in the silages treated with AP3 and AP4. Crude protein level was similar in all silages. The concentration of lactic and acetic acid ranged from 34 to 35 (g kg^{-1} DM) and 5.9–6.2 (g kg^{-1} DM), respectively, but there was no obvious effect of AP application (P>0.05). Thereafter, the concentrations of formic and propionic acids increased with the higher application rate of AP whilst the concentration ethanol and butyric acid decreased (P<0.05). The AP treatment significantly affected the ammonia-N and corrected ammonia-N concentrations of the silages relative to values in the control. The ammonia-N and corrected ammonia-N concentrations decreased as the rate of AP application increased (P<0.05). The use of AP significantly lowered the NDF and ADF contents of silages. The highest reduction was observed in the AP4 treated silages (P<0.05). After 90 d of ensiling, lactobacilli numbers of the wheat silages were not affected by treatments. On the other hand, treatment with acid additive resulted in a drastic reduction (P<0.05) in yeast and mold counts. The

numbers of yeast and mold were the lowest in the AP4 treated silage than the control silage or AP2 treated silages (P<0.05).

Table 1. Chemical and microbiological composition of direct-cut wheat forage before ensiling (mean ± SD)
Çizelge 1. *Buğdayın silolanmadan önceki kimyasal ve mikrobiyolojik özellikleri (ortalama±standart sapma)*

Item	
DM g kg^{-1}	264.9±0.69
pH	6.2±0.09
WSC g kg^{-1} DM	62.4±1.61
CP g kg^{-1} DM	150.3±0.18
EE g kg^{-1} DM	28.3±0.60
Ash g kg^{-1} DM	87.5±0.11
NDF g kg^{-1} DM	555.5±0.43
ADF g kg^{-1} DM	372.7±1.07
ADL g kg^{-1} DM	50.4±2.11
HC g kg^{-1} DM*	182.8±0.75
C g kg^{-1} DM*	322.3±1.59
NNDFC g kg^{-1} DM	178.4±0.54
LAB log cfu/g	4.6±0.34
Aerobic bacteria log cfu/g FM	7.0±0.33
Yeasts log cfu/g FM	4.4±0.24
Molds log cfu/g	4.3±0.17

SD – standard deviations; FM – fresh matter; WSC – water-soluble carbohydrate; CP – crude protein; EE – ether extract; NNDFC – non-NDF carbohydrates [100 – (CP + NDF + EE + Ash)]; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; HC – hemicellulose; C – cellulose; LAB – lactic acid bacteria; log – logarithm of the numbers; cfu – colony-forming units. * – HC was calculated as the difference between NDF and ADF and C as the difference between ADF and ADL.

The results of the aerobic stability test of the wheat silages are presented in Table 3. The pH and the release of CO_2 were lower in the AP treated silages than the control silage (P<0.05). The most significant improvement in aerobic stability was seen in AP4 treated silage because of less activity of yeast and mold than the other silages (P<0.05).

Gas production volumes (mL 200 mg^{-1} DM) in different incubation times and calculated metabolizable energy (ME), net energy lactation (NE_L), OM digestibility (OMD) of wheat silages are presented in Table 4. After 3, 6 and 12 h of incubations, silages treated with AP had the highest gas production values than the control silage (P<0.05). The 24, 48, 72 and 96 h of gas production and in vitro OM digestibility of the silages were not affected by AP treatment (P>0.05). Calculated ME or NE_L values of the wheat silages was not affected by treatments (P>0.05). On the other hand, AP inclusion numerically increased energy density of the

silages by 0.4 MJ kg^{-1} DM or 4.5% and organic matter digestibility (OMD) by 4.4% (Table 4).

4. Discussion

Good-quality whole-crop wheat silage can serve as adequate forage for high-lactating cows or beef cattle. Stage of maturity is a key determinant of the nutritional quality of whole crop wheat (Kennelly and Weinberg, 2003). Late-boot/early-heading is the best time to cut wheat for silage because energy and protein are optimized at this stage (Marsalis, 2007). In the present study, we found that DM content (270 g kg^{-1} DM), WSC (62.4 g kg^{-1} DM) and ADF (372.7 g kg^{-1} DM) values were low whilst protein content was high being between 150 g kg^{-1} DM (Table 1), which is characteristic for boot stage whole-crop wheat (Chen and Weinberg, 2009). Xie et al. (2012) reported that wheat silage harvested in the flowering stage of maturity contained 110.3 g kg^{-1} CP and 334.3 g kg^{-1}ADF compared with 93.1 g kg^{-1} CP and 229.9 g kg^{-1} ADF for wheat silage harvested in the milk stage of maturity.

In general wheat silages were well preserved containing moderate levels of LA and low levels of ammonia, butyric acid and ethanol. Using an acid-based preservative containing formic acid affected silage fermentation. Formic acid appears in many commercial proprietary silage additives either as a sole ingredient or in combination with other chemicals (Kennelly and Weinberg, 2003). Acid-based preservatives enhance pH reduction and thereby reduce plant enzyme and microbial-mediated proteolysis (Jaakkola et al., 2006 b). Moreover, acid-based additives result in limited fermentation and reduction in organic acids but a greater amount of WSC content of silage (Jaakkola et al., 2006a; Lorenzo and O'Kiely, 2008; Seppälä et al., 2016). Indeed, addition of AP limited the fermentation resulting in a significant pH drop and in making silages high in WSC (Table 2).

Table 2. Chemical and microbiological composition of wheat silages after a 90-day storage period
Çizelge 2. *Buğday silajlarının silolamadan 90 gün sonra kimyasal ve mikrobiyolojik özellikleri*

	Treatment				
Item	AP0	AP2	AP3	AP4	SEM
DM g kg^{-1}	271.5	273.9	275.0	273.8	0.25
pH	4.1a	3.9b	3.8bc	3.7c	0.03
WSC g kg^{-1} DM	19.7b	22.7b	32.6a	35.0a	1.19
EE g kg^{-1} DM	28.7	28.0	26.8	28.7	0.08
CP g kg^{-1} DM	143.0	148.0	149.0	149.0	0.11
Lactic acid g kg^{-1} DM	34.4	35.0	34.1	34.7	0.32
Acetic acid g kg^{-1} DM	6.2	6.2	6.0	5.9	0.21
Formic acid g kg^{-1} DM	0.1d	6.16c	7.4b	8.4a	0.07
Propionic acid g kg^{-1} DM	0.8d	3.6c	4.4b	5.8a	0.05
Butyric acid g kg^{-1} DM	5.9a	5.1b	3.9c	2.4d	0.04
Ethanol g kg^{-1} DM	9.2a	9.0a	7.7b	6.5c	0.11
FEP g kg^{-1} DM	56.6c	65.1a	63.5b	63.7b	0.13
Ammonia-N g kg^{-1} TN	43.8a	38.5b	37.8b	33.7c	0.44
Corrected Ammonia-N g kg^{-1} TN	43.8a	34.1b	31.2c	35.2d	0.40
Weight loss g kg^{-1} DM	7.6a	6.7b	4.2c	3.5d	0.36
NDF g kg^{-1} DM	549.2a	535.0b	526.2c	512.7d	0.90
ADF g kg^{-1} DM	369.8a	359.7b	354.6bc	351.8c	1.20
ADL g kg^{-1} DM	39.7	38.7	38.6	36.3	0.21
HC g kg^{-1} DM*	179.4a	175.3ab	171.6b	160.9c	1.79
C g kg^{-1} DM*	330.1a	321.0b	316.0b	315.5b	1.30
LAB log cfu g^{-1}	5.11	5.02	5.06	4.97	0.33
Yeasts log cfu g^{-1}	4.5a	4.4a	3.4b	3.4b	0.29
Molds log cfu g^{-1}	3.7a	2.8b	2.0c	1.1d	0.16

AP0 – control; AP2 – acid-based preservative (2 mL kg^{-1}); AP3 – acid-based preservative (3 mL kg^{-1}); AP4 – acid-based preservative (4 mL kg^{-1}); SEM – standard error of the mean; DM – dry matter; WSC – water soluble carbohydrate, EE – ether extract; CP – crude protein; FEP – fermentation products (lactic + acetic + formic + propionic + butyric + ethanol); TN – total nitrogen; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; LAB – lactic acid bacteria; log – logarithm of the numbers; cfu – colony-forming units.* – HC – hemicellulose was calculated as the difference between NDF and ADF and C – cellulose as the difference between ADF and ADL. Means in the same row with different superscript letters differ significantly ($P<0.05$).

Similar results were also reported by Filya and Sucu (2007b) and Nadeau (2007) who also demonstrated that acid additive decreased pH and increased residual WSC content of wheat silage. In this study, the levels of butyric acid, ethanol, fermentation losses (weight loss) and the growth yeast and mold were reduced, whilst the levels formic and propionic acids were raised by the application of AP (Table 2). The higher concentrations of formic and propionic acid in the silages were expected because the additive used in the current experiment contained both acids. As a consequence, AP increased (P<0.05) fermentation products (FEP) comparing to control silage (Table 2). The results indicate that there is a synergistic effect of formic acid protecting proteins and propionic acid that inhibited the undesirable microorganisms. On the other hand, the result obtained from FEP conflicts with Lorenzo and O'Kiely (2008). They concluded that formic acid treatment (3 mL kg^{-1}) restricted silage fermentation resulting in a reduced production of FEP in the treated silage (28 *vs*. 55 g kg^{-1} DM). Low pH and acidic conditions in the silos discourage lactic-acid utilizing bacteria such as clostridia evidenced by lower butyric acid contents in AP added silages (Table 2). Jatkauskas and Vrotniakiene (2006) determined that acid treated legume-grass silages had a lower concentration of lactic, acetic and butyric acid compared to control silage. The ammonia-N in silages shows the degree of protein degradation. Extensive proteolysis adversely affects the utilization of nitrogen by ruminants (Kaiser et al., 2003). It was observed that ammonia-N and corrected ammonia-N concentrations and fermentation loses were lower in AP4 treated silages than in control silage or AP2 and AP3 treated silages (P<0.05). However, the level of ammonia-N formation in all silages in the present experiment was below the threshold level of 80 g kg^{-1} of total nitrogen for good quality silages (Spörndly, 2003). The CP concentration of the wheat silage was not affected during storage period in relation to the fresh material. This may indicates a controlled fermentation with less proteolysis. We found that, AP treatment influenced the fibrous fractions of the wheat silages (P<0.05). Decomposition of cell walls in AP treated silages supposedly as a result of acid hydrolysis (Jaakkola et al., 2006a) which can improve the availability of energy for fermentation in the silo and in the rumen proven by the increase (78%) in WSC (Table 2) and the increase (4%) in OMD (Table 4) in our AP treated silages. Some researchers also found that even low application rate of formic acid disturbed cell membranes and released soluble cell contents in silages (Sucu et al., 2009).

As silage deterioration indicators, we examined changes in pH, CO_2 production and number of yeast and mold (Table 3).

Table 3. Results of aerobic stability test (5 day) of wheat silages after a 90-day storage period
Çizelge 3. *90 günlük silolama döneminden sonra buğday silajlarına ait aerobik stabilite test (5 gün) sonuçları*

Item	Treatment				
	AP0	AP2	AP3	AP4	SEM
pH	4.33a	3.97b	3.86c	3.73d	0.30
CO_2 g kg^{-1} DM	8.65a	6.15b	4.14c	2.80d	0.58
Yeasts log cfu g^{-1} DM	5.92a	5.07b	4.13c	3.29d	0.41
Molds log cfu g^{-1} DM	4.81a	4.13b	3.25c	2.30d	0.33

AP0 – control; AP2 – acid-based preservative (2 mL kg^{-1}); AP3 – acid-based preservative (3 mL kg^{-1}); AP4 – acid-based preservative (4 mL kg^{-1}); SEM – standard error of the mean; CO_2 = carbon dioxide; DM = dry matter; log – logarithm of the numbers; cfu – colony-forming units. Means in the same row with different superscript letters differ significantly (P<0.05).

The data presented in this paper showed that the application of AP enhanced the stability of wheat silages by reducing pH, CO_2 production and number of yeast and mold during air exposure. Aerobic stability of silages was endangered by metabolic activities and proliferation of yeast and mold. The increased stability is attributed to the inhibitory effect of

acetic and propionic acids on yeast and mold growth (Kaiser et al., 2003). Selwet (2006) reported that the superior oxygen stability of the formic + propionic acid treated Italian and red clover silages resulted from decreased yeast and mold fungal cells and possibly the maintenance of low pH during exposure to air. The same trend was shown in this experiment, as the findings are in agreement with Grajewski et al. (2005) and our previous experiment Filya et al. (2005), Filya and Sucu (2007a), Seppälä et al. (2016) with wheat, sorghum, maize and grass silages.

In the current study, AP inclusion neither affected the OMD values nor influenced the calculated energy contents of wheat silages (Table 4).

The lack of an effect of additive containing formic acid on OMD agrees with Jaakkola et al. (2006a, b) who reported no effect of formic acid treatment on OMD content of alfalfa and grass silages. Additionally, our previous experiments with 3.5 mL kg^{-1} or more formic acid in the laboratory conditions (Filya et al., 2005; Sucu et al., 2009) was shown a significant improvement in DM and OM degradabilities of low DM maize and sorghum silages ($P<0.05$). The energy values of boot stage wheat silage obtained from this study are in accord with Givens et al. (1993) who's reported digestible and metabolizable energy of 9.3–12.0 and 7.4–9.8 MJ kg^{-1} DM, respectively, of two whole-wheat cultivars ensiled at 88 and 84 Zadok growth stage. In contrast, the energy values for wheat silages in the present study were lower than the values of 9.0–9.5 MJ kg^{-1} DM, reported by Kaiser et al. (2003).

Table 4. In vitro gas production and calculated metabolizable energy (ME), net energy lactation (NE_L) and digestible organic matter (OMD) for wheat silages after a 90-day storage period
Çizelge 4. *90 günlük silolama döneminden sonra buğday silajlarının in vitro gaz üretim değerleri ile hesaplanmış metabolik enerji, net enerji laktasyon (NEL) ve sindirilebilir organik maddeler (SOM) içeriği*

	Treatment				
Item	AP0	AP2	AP3	AP4	SEM
	In vitro gas production [ml 200 mg^{-1} DM] after incubation				
3 h	8.5c	11.6ab	11.1ab	12.4a	1.46
6 h	10.0b	16.1a	14.6a	16.9a	2.52
12 h	17.2b	23.2a	24.1a	23.8a	1.29
24 h	40.5	41.8	41.1	43.1	0.94
48 h	59.3	64.2	64.2	64.0	0.87
72 h	67.4	70.6	70.5	70.7	1.56
96 h	70.5	73.6	73.3	73.8	0.64
ME MJ kg^{-1} DM	7.79	7.97	7.88	8.15	0.13
NE_L MJ kg^{-1} DM	4.48	4.61	4.54	4.73	0.09
OMD g kg^{-1}	541.5	553.5	547.2	565.3	0.88

AP0 – control; AP2 – acid-based preservative (2 mL kg^{-1}); AP3 – acid-based preservative (3 mL kg^{-1}); AP4 – acid-based preservative (4 mL kg^{-1}); SEM – standard error of the mean; ME– metabolizable energy; DM – dry matter. NE_L –net energy lactation; OMD –organic matter digestibility. Means in the same row with different superscript letters differ significantly ($P<0.05$).

The wheat silage is of good NE_L value as compared to elected silages commonly fed to cattle (Tudisco et al., 2010) such as corn silage (4.16 MJ kg^{-1} DM) and barley silage (4.14 MJ kg^{-1} DM).

5. Conclusions

Wheat silage was well preserved, with direct ensiled material having pH values 4.1, with limited proteolysis as assessed by ammonia-N content 43.8 g kg^{-1} TN in the current study; however, acid-based additive was found effective in enhancing hygienic quality especially inhibiting the growth of yeast and mold as well as increasing stability of low DM wheat forage. Acid preservatives can lead improved stability and consequently to a higher quality fodder, especially if the starting material is abundant in protein. Acid additive has also led to reduce concentration of the fibrolytic fractions (especially ADF), which is usually digested poorly by ruminants. 96 h *in vitro* gas production and energy values or *in vitro* OM digestibility of the silages were not affected by AP treatment. In terms of fermentation quality

AP2 was found to be an adequate dose. However, when aerobic stability test results were taken into account AP4 was found to be the most effective dose for low DM wheat silages. The data presented in this report on the changes in fermentation quality and nutritive value of AP treated silages and its relevance in improving animal performance warrants further studies.

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