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Effect of Ensiling Time on Microbial Composition and Aerobic Stability of Total Mixture Ration#

ABSTRACT

Objective: In this study, the effects of ensiling total mixed ration (TMR) on aerobic stability properties were investigated.

Material and Methods: In the study, half of the TMR with 55% DM content was fresh and the other half was subjected to aerobic stability test after being ensiled for 30, 60 and 90 days. The analyses of chemical and microbiological parameters in feed samples were carried out at 0, 12, 24, 48, 72 and 96 hours of aerobic stability. At the same time, making the T200 IR imaging brand evaluation results in each treatment group at 1 m from the silage samples were recorded with a thermal imaging camera.

Results: In the study, crude protein (CP), crude ash (CA), ether extract (EE), starch, neutral detergent fiber (NDF) and acid detergent fiber (ADF) values of TMR decreased as the ensiling time increased (P<0.001). The pH, dry matter (DM), lactic acid (LA) values decreased but NH_3-N and NH_3-N/TN values increased with the duration of aerobic stability. Ensiling had positive effects on microbial composition and yeast and mould values decreased (P<0.000).

Conclusion: As a result of this research, although 55% KM TMR is best consumed fresh, ensiling is better in terms of aerobic stability.

Keywords: Aerobic stability, ensiling time, temperature sensor, thermal camera, total mixed ration

Silolama Süresinin Toplam Rasyon Karısımının Mikrobiyal Kompozisyonu ve Aerobik Stabilitesi Üzerine Etkisi

ÖZ

Amaç: Bu araştırmada, toplam rasyon karışımı (TRK)'nın silolanmasının aerobik stabilite özellikleri üzerine etkileri araştırılmıştır.

Materyal ve Metot: Araştırmada %55 KM içeriğine sahip TRK'nın yarısı taze olarak, diğer yarısı 30, 60 ve 90 gün silolandıktan sonra, aerobik stabilite testine tabi tutulmuşlardır. Aerobik stabilitenin 0., 12., 24., 48., 72. ve 96. saatlerinde yem örneklerinde kimyasal ve mikrobiyolojik parametrelere ilişkin analizler yürütülmüştür. Aynı zamanda, T200 IR marka termal kamera ile 1 m mesafeden silaj örneklerinde her muamele grubunda görüntüleme yapılarak değerlendirme sonuçları kaydedilmiştir. Daha sonra elde edilen veriler ThermaCAM software programında değerlendirilmiştir.

Bulgular: Araştırmada silolama süresi artıkça TRK'nın ham protein (HP), ham kül (HK), ham yağ (HY), nişasta, nötr deterjanda çözünmeyen lif (NDF), asit deterjanda çözünmeyen lif (ADF) değerleri düşmüştür (P<0.001). Aerobik stabilite süresine bağlı olarak pH, kuru madde (KM), laktik asit (LA) değerleri düşmüş ancak NH3-N ve NH3-N/TN değerleri artmıştır. Mikrobiyal kompozisyon üzerine silolamanın olumlu etkileri olmuş maya ve küf değerleri düşmüştür (P<0.000).

Sonuç: Araştırma sonucunda %55 KM TRK'nın taze olarak tüketilmesi en ideali olmakla birlikte, aerobik stabilite açısından silolanmasının daha iyi olduğu söylenebilir.

Anahtar Kelime: Aerobik stabilite, silolama süresi, sıcaklık sensörü, termal kamera, toplam rasyon karışımı

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INTRODUCTION

A total mixed ration (TMR) is a balanced food supply that satisfies the daily dietary needs of animals kept indoors throughout the year. It includes grass, cereal grains, protein sources, by-products, minerals, vitamins, and additives (Türkgeldi et al., 2023). A significant part of TMR consists of roughage (silage, pulp, etc.) with high water content. Therefore, the combination of materials with high water content makes TMR favourable for aerobic spoilage. The microorganism load from each of the materials is transferred to the TMR (Ashbell et al., 2002). In recent years, it has become a common practice to ferment, i.e. ensiling, TMRs consisting of high-moisture by-products. Previous studies have shown that the aerobic stability of ensiled TMRs is better than fresh TMRs (Nishino and Hattori, 2007; Wang and Nishino, 2008). This also facilitates longer storage and transport of TMR. On the other hand, the microbial composition of TMR is one of the important factors on aerobic stability (Weinberg et al., 2011; Tian et al., 2023).

The studies on this subject have shown that yeasts are microorganisms that affect aerobic stability and yeast counts above 5 $\log_{10}$ cfu g-1 are associated with a decrease in the aerobic stability of silage and TMR (Pahlow et al., 2003; Wilkinson and Davies, 2013; Soycan Önenç et al., 2019). Kung (2005) reported a negative correlation between the aerobic stability of TMR and yeast counts. There are studies suggesting that ensiling of TMR decreases yeast counts and improves aerobic stability. In a study by Wang and Nishino (2008), it was reported that yeast counts decreased to levels below the detection limit ($<10^2$ cfu/g) when the ensiling period was extended to 30 days or more. In this study, the chemical and microbiological composition of fresh and ensiled TMR and its effects on aerobic stability were evaluated.

MATERIAL and METHODS

Material

The material for the experiment was obtained from Namık Kemal University, Faculty of Agriculture Faculty of Science, Research and Application Farm. A total of 120 kg of TMR with 55% DM content, the content and composition of which are given in Table 1, was transported to the laboratory.

Table 1. Ingredients and composition of the TMR
Table 1. TRK'nın içerik ve kompozisyonu

Item	DM%
Corn silage	24.41
Corn grain (high moisture)	18.23
Alfalfa hay	13.08
DDGS (corn)	5.36
Cottonseed	5.89
Canola meal	5.04
Barley	3.37
Rice bran	3.98
Wheat bran (fine)	2.43
Sugar beet pulp	0.57
Wheat straw	2.47
Sunflower meal	1.5
Sunflower grain	0.92
Soybean peel	0.93
Molasses	0.89
Orange pulp	3.46
Maceration water	5.12
$CaCO_3$	0.62
Vit + Min premix	0.42
Ecomass	0.42
Buffer (Sodium bicarbonate)	0.3
NaCl	0.21
K_2CO_3	0.20
OmniGen AF	0.14
Toxin binder	0.04
TOTAL	**100.00**

TMR: Total mixed ration, DM: Dry matter

The material was then divided into 2 treatment groups (fresh and ensiled TMR) of 60 kg. TMR samples of 20 kg in 3 replicates were exposed to the open air and chemical and microbiological analyses were carried out at

0, 24, 48, 72 and 96 hours of aerobic stability. In the other treatment group, TMR samples were ensiled in 500 g packages for 30, 60 and 90 days. CAS CVP 260 PD vacuum machine was used for vacuum packaging of the feeds.

Chemical and Microbiological Analyses

The pH, dry matter (DM), lactic acid (LA), water-soluble carbohydrate (WSC), lactic acid bacteria (LAB), yeast and mould counts were performed at 0, 12, 24, 48, 72 and 96 hours of aerobic stability period. Chemical and microbiological analyses were performed on triplicate samples. Dry matter was determined by oven drying for 48 h. The pH in fresh material and silage samples was measured according to the British Standard method (Anonymous, 1986). The ammonia nitrogen (NH_3-N) content of silages was determined, according to Anonymous (1986). The WSC content of silages was determined Dubois et al. (1956). Lactic acid was determined by the spectrophotometric method (Anonymous, 1986). Crude protein (CP), crude ash (CA), eter extract (EE) and starch analysis were determined following the procedure of Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the method of Van Soest (1991). Lactic acid bacteria (LAB), yeast and mould counts were determined according to the methods reported by Seale et al. (1990).

Temperature Measurement of TMR

During aerobic stability, temperature changes in feed samples and ambient temperature were monitored with a hobo pentant data logger every 2 hours for 96 hours (Ranjit and Kung, 2000).

Statistical Analyses

In the statistical evaluation of the data obtained in the study, analysis of variance was used and Duncan multiple comparison test was applied to determine the difference between groups (Soysal, 1993). Statistica package programme (1999) was used for this analysis.

RESULTS

The results of chemical and microbiological analyses of TMR samples before ensiling

The results of chemical and microbiological analyses of TMR samples before ensiling are given in Table 2. The pH (4.72), DM (55.09% FM), CP (17.00% DM), CA (7.01 % DM), EE (3.86% DM), NDF (39.19% DM), ADF (20.16% DM), WSC (6. 59 g/kg DM), NH_3-N (0.89 g/kg DM), NH_3-N/TN (38.62 g/kg DM), LA (28.00 g/kg DM), LAB (4.59 cfu/g DM) and yeast contents (4.99 cfu/g DM). No mould was detected in the fresh material.

Table 2. Chemical and microbiological analysis values of TMR before ensiling

Tablo 2. Silolama öncesi TRK'nın kimyasal ve mikrobiyolojik analiz değerleri

Chemical composition	
pH	4.72
DM, FM%	55.09
CP, DM%	17.00
CA, DM%	7.01
EE, DM%	3.86
NDF, DM%	39.19
ADF, DM%	20.16
WSC, g/kg DM	6.59
NH_3-N, g/kg DM	0.89
NH_3-N/TN g/kg DM	38.62
LA, g/kg DM	28.00
Microbiological composition	
LAB, cfu/g DM	4.59
Yeast, cfu/g DM	4.99
Mould, cfu/g DM	0

DM: Dry matter, FM: Fresh material, CP: Crude protein, CA: Crude ash, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, WSC: Water soluble carbohydrate, NH3-N: Ammonia nitrogen, TN: Total nitrogen, LA: Lactic acid, LAB: Lactic acid bacteria, cfu: colony forming unit

Results on crude nutrient and cell wall contents of fresh and ensiled TMR

The results of the analyses of crude nutrient and cell wall contents of fresh and ensiled TMR are given in Table 3. The DM % content of TMR was between (55.73-57.80). The highest DM value was obtained in TMR ensiled for 60 days ($P<0.001$). The CP content of TMR changed between 13.60-17.42 (% DM), the highest value was found on the 30th day of ensiling, while the lowest value was found on the 90th day, the difference between the groups was statistically significant ($P<0.001$).

The highest CA content was found in the fresh samples at the beginning of ensiling, this value decreased with ensiling and the lowest value was determined at 90th day, the difference between the groups was found statistically significant ($P<0.001$). Eter extract values ranged between 3.72-4.03 (% DM), although there was an increase in EE value with ensiling, the lowest value was determined at 90th day and the difference between the groups was statistically significant ($P<0.001$). The starch content decreased with the ensiling of TMR and the lowest value was determined on the 90th day and the difference between the groups was statistically significantly ($P<0.001$).

Table 3. Values related to crude nutrient and cell wall contents of TMR (DM%)
Tablo 3. TRK'nın ham besin madde ve hücre çeperi içeriklerine ilişkin değerler (%KM)

Ensiling Time	Parameters						
	DM, %	**CP**	**CA**	**EE**	**Starch**	**ADF**	**NDF**
0	55.88b	16.39b	7.01a	3.86c	23.99a	20.16c	28.24c
30 day	55.73b	17.42a	6.73b	3.93b	21.08c	21.84b	29.18b
60 day	57. 80a	16.01c	6.74b	4.03a	21.13b	22.23a	29.45a
90 day	55.85b	13.60d	6.41c	3.72d	18.67d	18.93d	26.98d
SEM	0.262	0.422	0.064	0.034	0.567	0.400	0.292
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000

DM: Dry matter, CP: Crude protein, CA: Crude ash, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, a-d The difference between the means shown with different letters in the same column is statistically significant ($P<0.001$).

The ADF content ranged between 18.93-22.23 (% DM), the lowest ADF value was determined at day 90 and the highest at day 60 and the difference between the groups was statistically significant ($P<0.001$). NDF content ranged between 26.98-29.45 (% DM), the lowest NDF value was detected on day 90 and the highest on day 60, in this context, it was similar to ADF and the difference between the groups was found statistically significant ($P<0.001$).

Results on chemical and microbiological parameters during aerobic stability of fresh and ensiled TMR

The results of the analysis of fermentation parameters of fresh and ensiled TMR are given in Table 4. The pH value decreased with the ensiling of TMR, the highest pH value was found in fresh TMR and the lowest pH value was found on the 30th day of ensiling and the difference between the groups was statistically significant ($P<0.001$). The highest NH_3-N content was detected at 96 h of aerobic stability in unensiled and 90 days ensiled TMR groups.

The lowest NH_3-N/TN values were determined at the 0th hour of the ensiled materials and at the 0th hour of the materials ensiled for 30 and 90 days, and at the 0th and 24th hours of 60 days ensiling. The difference between the groups was statistically significant ($P<0.001$). The NH_3-N/TN content of ensiled and unensiled TMR varied between (36.04-55.21 g/kg DM), the lowest value was found at 30 days of ensiling (0th hour) and the highest value was found at 90 days of ensiling (96th hour) and the difference between the groups was statistically significant ($P<0.001$).

Table 4. Changes in pH, DM, LA, WSC, NH_3-N and NH_3-N/TN content of TMR during aerobic stability

Tablo 4. Aerobik stabilite süresince TRK'nın pH, KM, LA, SÇK, NH_3-N ve NH_3-N/TN içeriğindeki değişiklikler

Ensiling Time	AS (hour)	pH	DM	LA	WSC	NH_3-N	NH_3-N/TN
0	0	4.72d	55.00h	28.00a	6.59j	0.89b	38.62ef
	24	4.76cd	64.07fg	23.89b	6.81i	0.93ab	40.62def
	48	4.81ab	63.78fg	23.80b	13.2a	0.96ab	41.11def
	72	4.78bc	70.16de	21.84bc	8.40e	0.94ab	42.02def
	96	4.85a	71.93cde	21.62bc	11.7b	1.02a	44.50bcde
30 day	0	4.33l	59.33gh	27.34a	6.00n	0.89b	36.04f
	24	4.54f	74.02cd	20.69cde	6.45l	0.93ab	37.91ef
	48	4.59e	80.45ab	18.99defg	6.56k	0.96ab	38.36ef
	72	4.62e	84.16a	18.17efgh	5.40ö	0.94ab	39.21ef
	96	4.59e	83.33a	18.97defg	4.95p	1.02a	39.99def
60 day	0	4.40k	57.80h	20.16cdef	8.58d	0.89b	39.70ef
	24	4.51fgh	69.60de	16.72ghıi	7.62g	0.89b	41.53def
	48	4.53fg	76.33bc	15.35ıi	9.79c	0.99ab	43.78cdef
	72	4.53fg	81.28ab	14.36i	7.65g	0.98ab	44.35bcde
	96	4.49ghı	72.09cde	16.30hıi	7.34ı	1.01a	45.27bcde
90 day	0	4.38k	55.85h	20.81cd	7.59h	0.89b	47.92abcd
	24	4.45ij	68.93def	16.74ghıi	5.88o	0.93ab	50.38abc
	48	4.46ıi	68.30ef	17.05ghı	5.40ö	0.96ab	50.39abc
	72	4.41jk	81.28ab	17.70fghı	6.33m	0.93ab	52.12ab
	96	4.48hıi	69.07def	16.90ghıi	8.29f	1.02a	55.21a
SEM		0,024	1.427	0.594	0.328	0.009	0.857
P							
Ensiling Time (ET)		0,000	0.000	0.0000	0.000	0.998	0.000
Air Exposure Time (T)		0,000	0.000	0.0000	0.000	0.004	0.004
ET x T		0,000	0.000	0.0002	0.000	0.153	0.090

AS: Aerobic stability (Air exposure time), DM: Dry matter, LA: Lactic acid, WSC: Water soluble carbohydrate, NH3-N: Ammonia nitrogen, TN: Total nitrogen, [a-p]: The difference between the means shown with different letters in the same column is statistically significant (P<0.001), SEM: Standard error of means

The content of WSC ranged between (4.95-13.2 g/kg DM), the lowest amount of WSC was observed at the 30th day, 96th hour of ensiling, while the highest amount was determined at the 48th hour in the fresh group and the difference between the groups was found statistically significant (P<0.001). Lactic acid content varied between (14.36-28.00 g/kg DM) and LA content decreased with ensiling. The lowest LA content was observed at 72 hours of the 60th day of ensiling, while the highest was determined at 0 hour in fresh samples. The difference between the groups was statistically significant (P<0.001).

Results on microbiological parameters during aerobic stability of fresh and ensiled TMR

The microbiological parameters of fresh and ensiled TMR during aerobic stability are given in Table 5. The amount of LAB ranged between (3.25-6.66 cfu/g DM), the highest LAB content was found in fresh feeds at 96th hour, while the lowest value was found at 90th day, 0th hour. The difference between the groups was statistically significant (P<0.001). Yeast content varied between (2.65-6.32 cfu/g DM).

The yeast values of ensiled TMRs were found to be lower (P<0.001). Ensiling time positively affected the yeast content and the lowest yeast values were detected in forages ensiled for 30 days (P<0.001). The ensiling time positively affected the mould content and no mould was detected in feed samples ensiled for 90 days (P<0.001).

Table 5. Microbiological analysis values of TMR during aerobic stability (cfu/g DM)

Tablo 5. TRK'nın aerobik stabilite süresince mikrobiyolojik analiz değerleri (kob/g KM)

Ensiling Time	AS (hour)	LAB	Yeast	Mould
0	0	4.86b	5.12abc	0.00e
	24	5.09b	5.18ab	0.00e
	48	4.51bc	4.71abcd	0.99d
	72	4.34bcde	4.15bcde	0.99d
	96	6.66a	6.32a	2.18a
30 day	0	4.81b	4.23bcde	0.00e
	24	4.67b	3.49cde	0.00e
	48	4.71b	2.65e	0.00e
	72	4.53bc	4.03bcde	1.37c
	96	4.04bcde	4.05bcde	1.94b
60 day	0	5.00b	4.55bcd	0.00e
	24	5.06b	4.44bcd	0.00e
	48	4.64bc	3.77bcde	0.00e
	72	4.46bcd	4.13bcde	0.99d
	96	4.50bc	4.45bcd	0.99d
90 day	0	3.25e	3.44de	0.00e
	24	4.45bcd	4.42bcd	0.00e
	48	3.56cde	3.47de	0.00e
	72	4.45bcd	3.44de	0.00e
	96	3.38de	3.62bcde	0.00e
SEM		0.116	0.132	0.112
P				
Ensiling Time (ET)		0.000	0.000	0.000
Air Exposure Time (T)		0.021	0.001	0.000
ET x T		0.000	0.014	0.000

AS: Aerobic stability (Air exposure time), DM: dry matter, LAB: Lactic acid bacteria, cfu: colony forming unit, [a-e] The difference between the means shown with different letters in the same column is statistically significant ($P<0.001$), SEM: Standard error of means.

Results on sensor data during aerobic stability of fresh and ensiled TMR

The results of the sensor data of fresh and ensiled TMR during aerobic stability are given in Table 6. According to the results of the evaluation of the sensor data of fresh and ensiled TMR during aerobic stability, it can be said that all feed samples were stable during the aerobic period.

Table 6. Results related to sensor data during aerobic stability of fresh and ensiled TMR

Tablo 6. Taze ve silolanmış TRK'nın aerobik stabilite süresince sensör verileri ile ilgili bulgular

Ensiling Time	Temperature °C	Air Exposure Time (hour)				
		0	24	48	72	96
Fresh TMR	Ambient	18.81	17.80	17.38	17.56	16.70
	Sensor	18.71	16.86	16.40	16.64	16.13
30 day	Ambient	15.86	15.00	14.71	15.38	15.57
	Sensor	13.41	12.45	12.69	13.99	14.13
60 day	Ambient	23.20	16.62	18.43	17.76	17.48
	Sensor	23.58	15.09	17.00	16.05	16.05
90 day	Ambient	19.09	15.19	16.81	16.90	17.95
	Sensor	19.52	13.61	14.10	14.55	15.99

Results of thermal camera images during aerobic stability of fresh and ensiled TMR

Thermal camera images of fresh and ensiled TMR during aerobic stability are presented in Figure 1.

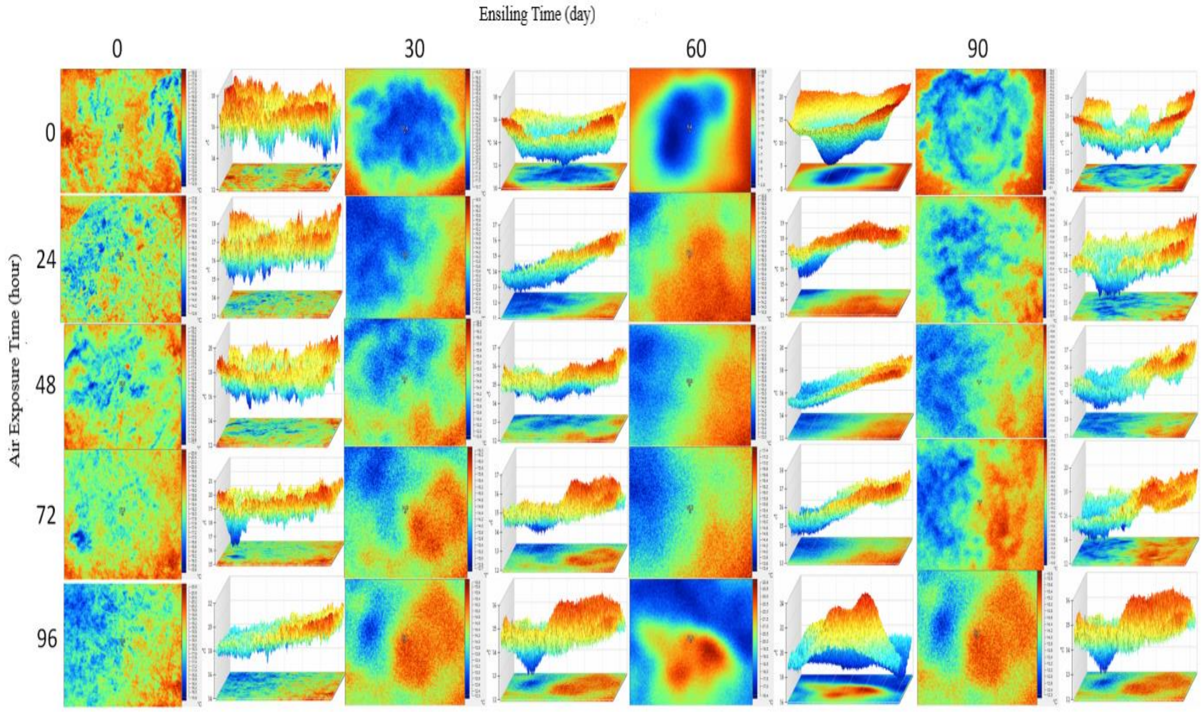

Figure 1. Thermal camera images of fresh and ensiled TMR during aerobic stability
Şekil 1. Taze ve silolanmış TRK'nın aerobik stabilite süresince termal kamera görüntüleri

DISCUSSION

Aerobic stability is characterised as the length of time an opened silage or TMR remains unheated and unspoilt. The methods of monitoring TMR temperature are the same as the methods used to measure silage aerobic stability. In studies on this subject, different methods are used. In some studies, feed temperature was measured once a day and evaluated (Saarisalo et al., 2006; Pursiainen and Tuori, 2008; Heikkila et al., 2010; Jaakkola et al., 2010). In other studies, the ambient temperature and TMR temperature over a 7-day period were evaluated by measuring the temperature twice a day manually or at 10-minute intervals by a data logger (Pursiainen and Tuori, 2008). Monitoring temperature automatically with sensor data allows more detailed temperature curves to be determined and aerobic stability to be defined in terms of hours required for a given temperature increase. The upper limit for aerobic stability may vary according to researchers. According to some studies, aerobic spoilage was evaluated when the feed temperature exceeded the ambient temperature by 1 °C (Adesogan and Salawu, 2004), 2 °C (Xie et al., 2022) or 3 °C (Pauly and Wyss, 2018). In this study, 1 °C above the ambient temperature was considered as a condition for aerobic spoilage. The graphs of the sensor data showed that fresh and ensiled TMR samples remained stable throughout the aerobic stability period. In the studies carried out on this subject, it was determined that aerobic stability was lower especially in TMRs with a DM content lower than 45% (Coşkuntuna et al., 2022). In recent years, thermal cameras have started to be used in the evaluation of aerobic stability of both silage and TMRs during aerobic stability. The results of the studies indicate that thermal cameras can be used as an evaluation method during the aerobic stability period (Addah et al., 2012; Koç et al., 2018; Ünal et al., 2018; Okur et al., 2022).

In the study, temperature increases were found to be lower in the thermal camera when sensor data and thermal camera images were evaluated. There are points to be considered in the evaluations related to temperature measurements. The specific heat capacity and thermal conductivity of a feed depends mainly on the moisture concentration in the feed (Muck et al., 2003; Berk, 2018). High moisture feed needs more energy than feed with lower moisture content for the same temperature change. Furthermore, the size of the feed and the isolation around the feed affect the measured temperature rise. Therefore, temperature readings are highly dependent on the conditions under which the measurement is performed. The low water activity limits the growth of microorganisms in the feed. At the same time, the generated temperature in the slow deterioration of a small sample can be lost in the environment without causing a detectable temperature rise. In these cases, the feed will spoil, but no deterioration will be detected in the temperature record. Weinberg et al. (2011), a study on aerobic stability of grain feeds (81.6% DM) found that grains were detected to be mouldy even though the temperature increase in the grains was less than 2 ^{o}C. In the present study, the fact that the temperature increase during aerobic stability did not exceed 2 ^{o}C above the ambient temperature and at the same time no mould was detected in the feeds supports the sensor and thermal camera data. The aerobic stability of TMR depends on the composition of the feed, microbial content, ambient temperature, water activity, nutrients and oxygen and the presence of growth inhibitors (Muck et al., 2003; Kung, 2005). The conditions in the field are often different from aerobic stability measurements carried out in the laboratory. However, the results obtained from small scale trials can help to obtain data on the problems to be encountered in practice. Temperature rise in TMR is complex and heterogeneous at the microorganism level. The silages that go into the composition of TMR may have been exposed to oxygen for several days before mixing into TMR. Furthermore, other microorganisms are added together with other feed ingredients present in the TMR. Microorganisms are not evenly spread in the TMR. Different feed ingredients can vary greatly in terms of their microbial composition. Under field conditions it is often not possible to analyse the density of microorganisms in feed. Spoilage is noticeable by a bad odour or visually. Yeast or mould growth are typical signs of spoilage that can be detected at farm level. The TMR ingredients used have a high number of microorganisms that can cause rapid deterioration of TMR. Even feed ingredients with low moisture content can host large numbers of aerobic spoilage microorganisms that are dormant as long as the water activity is low enough (Rose et al., 2012). According to the results of the study, moulds were not detected in the feeds during the 96-hour aerobic stability period. Moulds are eukaryotic, usually aerobic microorganisms (Driehuis and Oude Elferink, 2000). Moulds are harmful microorganisms because most of them can produce mycotoxins. The contamination of TMR by moulds is mostly caused by mouldy silages. They can be visually detected in the surface layers of silages due to poor sealing and compaction of silages (Pahlow et al., 2003). Mould growth occurs in the later stages of silage aerobic spoilage (Driehuis and Oude Elferink, 2000). The consequences of mycotoxin intake by animals can be serious (Koivunen and Huuskonen, 2018; Ogunade et al., 2018), so it is generally accepted practice to completely avoid feeding visually mouldy forages. Removing mouldy spots or the mouldy surface layer from silage results in a significant amount of additional work and feed loss. If separation is not carefully done, mouldy silage is mixed into the TMR. At this point, it is also important to evaluate the aerobic stability of the silages added to TMR. The highest yeast count was detected at 96th hour of aerobic stability in fresh TMRs when the yeast contents of TMRs were evaluated. In ensiled TMRs, yeast values decreased depending on the ensiling time. Yeasts are microorganisms effective on aerobic stability. They are eukaryotic microorganisms that normally reproduce by budding (Pahlow et al., 2003). They can grow under both anaerobic and aerobic conditions and can ferment sugars to ethanol (Pahlow et al., 2003). Yeast counts above 5 log10 cfu g-1 were found to be associated with decreased aerobic stability of silage and TMR (Wilkinson and Davies, 2013; Soycan Önenç et al., 2019; Shah et al., 2024). A negative correlation was found between the aerobic stability of TMR and yeast counts (Kung, 2005). Similar results were found when the research results were evaluated. Rinne et al. (2018) showed in a study that the addition of water can reduce the aerobic stability of TMR. The activity of water is one of the main factors that increase microbial activity. At the at the same time, the silages added to TMR affect the moisture content. During this process aerobic microorganisms multiply. It is assumed that silage stability is linearly related to TMR stability (Kung, 2005). Therefore, the effect of silage DM on TMR stability is much greater than increasing the moisture content of TMR (Holmes and Bolsen, 2009). In this study, 55% DM content of TMR samples may not have caused a significant difference in aerobic stability between fresh and ensiled forages. In general, all factors affecting the aerobic stability of silage and TMR may have multiple interactions. Weiss et al. (2016) also concluded that it is not possible to predict the outcome of silage fermentations with certainty, especially due to the complexity of the silage ecosystem.

CONCLUSION

The results of this study provide strong evidence that ensiling TMR significantly enhances its aerobic stability and extends its shelf life. Ensiled TMR not only demonstrates lower yeast and mold counts but also maintains stable temperature levels, all of which contribute to delaying spoilage. These findings have important practical implications for livestock farmers and feed producers, particularly those who rely on large-scale TMR production and storage. The ability to store TMR for extended periods without compromising its nutritional quality or stability is crucial for efficient feed management and reduces feed wastage.

However, the study also suggests that there may be a balance to strike between aerobic stability and nutrient preservation. While longer ensiling periods enhance stability, they can also result in nutrient degradation, particularly in terms of CP and starch content. Therefore, farmers and feed managers should consider the optimal ensiling duration to maximize both the nutritional value and stability of TMR, depending on their specific feeding schedules and storage needs.

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