

Chemical and Microbiological Evaluation of Total Mixed Ration in a Dairy Farm

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Abstract

The study was conducted over a 10-month period on a private dairy farm implementing a Total Mixed Ration (TMR) feeding system. The research material consisted of TMR samples taken from the mixer wagon and feed bunks. The mixer wagon used in the farm had an average capacity of 1 ton, and the TMR composition included grass hay, straw, triticale, sunflower meal, corn silage, molasses, salt, a vitamin-mineral premix, and dairy concentrate feed. In the collected TMR samples, several analyses were conducted, including pH, dry matter (DM), water-soluble carbohydrates (WSC), crude protein (CP), and microbiological evaluations such as lactic acid bacteria, yeast, and mold counts. Additionally, during each sampling period, ambient temperature and temperature fluctuations at the feed bunks were recorded. The results of the study highlighted the critical importance of feed bunk monitoring, particularly during periods of high ambient temperatures. These findings emphasize the need for more frequent and attentive feed management practices under elevated thermal conditions to preserve feed quality and optimize animal performance.

Bir Süt Sığırcılığı İşletmesinde Toplam Karma Rasyonun Kimyasal ve Mikrobiyolojik Değerlendirmesi

Özet

Bu çalışma, toplam karma rasyonun (TKR) sisteminin uygulandığı özel bir süt sığırcılığı işletmesinde 10 aylık bir süre boyunca yürütülmüştür. Araştırmanın materyalini, mikser vagon ve yemliklerden alınan TKR örnekleri oluşturmuştur. İşletmede kullanılan mikser vagonun ortalama kapasitesi 1 ton olup, TRK; çayır otu, saman, tritikale, ayçiçeği küspesi, mısır silajı, melas, tuz, vitamin-mineral premiksi ve süt yemi içermektedir. TKR örneklerinde pH, kuru madde (KM), suda çözünabilir karbonhidratlar (SÇK), ham protein (HP) gibi analizlerin yanı sıra laktik asit bakterisi, maya ve küf sayımlarını içeren mikrobiyolojik değerlendirmeler de yapılmıştır. Her numune alım döneminde çevre sıcaklığı ve yemliklerdeki sıcaklık değişimleri kaydedilmiştir. Çalışmanın bulguları, özellikle çevre sıcaklığının yüksek olduğu dönemlerde yemlik kontrollerinin kritik öneme sahip olduğunu ortaya koymuştur. Bu bulgular, yem kalitesini korumak ve hayvan performansını sürdürmek amacıyla yüksek sıcaklık koşullarında daha sık ve düzenli yem yönetimi uygulamalarına duyulan gereksinimi vurgulamaktadır.

1. INTRODUCTION

The basic aim of the feeding programme applied in a dairy cattle farm is to meet the nutrient requirements of the animals in a suitable and balanced way. In order to provide accurate and balanced feeding, it is important to select appropriate feeding systems. In dairy farming practice, different feeding systems such as standard (rational), strategic and total ration mix (TMR) are applied. Among these systems, the most applicable feeding system in terms of milk yield, fertility and cost is the total ration mixture application, which is defined as a feeding system in which all roughage and concentrate feeds in the ration prepared according to the requirements of the animals are mixed and offered free of charge.

Amaral-Philips et al. (2001) stated that one of the most important problems in feeding of TMR is the storage of roughages and silages with high composition in ensiled silos. The researchers suggested that such feeds should be checked more than once a week. Since roughages with high water content deteriorate quickly, they stated that if they are used in feeding animals, they will cause health problems and decrease in milk production.

Aerobic deterioration is a serious problem for silage because it not only reduces the nutritional value of the feed, but also affects human health as well as animal health (Driehuis and Elferink, 1990). To the present day, many studies have been carried out on silages and it has been stated that aerobic spoilage is a microbial process carried out by aerobic microorganisms that cannot proliferate in the anaerobic environment of a contained ensilage (Courtin et al. 1990). In a well-preserved silage, the activities of microorganisms are greatly restricted by the rapid decrease in pH and low pH, in addition to oxygen-free conditions. However, after the silo is opened, contact with oxygen is effective in the proliferation of aerobic microorganisms and in the spoilage of silages. Yeasts are reported to initiate aerobic spoilage of silage (Pahlow et al. 2003).

Corn silage is frequently used in dairy cow rations, which deteriorates easily after exposure to air during the feeding period. It has been reported that materials with high moisture and water soluble carbohydrate (WSC) content exhibit better aerobic stability when formulated as a total ration mixture (TMR) than when used alone (Hu et al. 2015, Wang et al. 2016). Especially in corn silage, it was reported that aerobic stability improved with the decrease in yeast count (Tabacco et al., 2011).

In previous studies, it was found that the aerobic stability of TRCs increased with the decrease in yeasts (Hu et al., 2015, Hao, 2015). There are also evidences that yeast species may contribute more to aerobic spoilage than the number of yeasts (Hu et al., 2015). Previous research has indicated that yeasts are intimately associated with the early onset of aerobic spoilage in TMR silages (Dunière et al., 2013; Wang et al., 2016). However, few studies have focused on yeast dynamics during different periods and their characteristics associated with aerobic stability.

In this study, the chemical and microbiological composition of the feeds prepared for 10 months in a farm where TMR

was applied was evaluated. In addition, the dominant yeast and mould species in TMR were determined.

2. MATERIAL AND METHOD

This study was carried out in a private dairy cattle farm in Koruköy in the centre of Kırklareli Province, which implements TMR and uses the related mechanisation. The period of data collection in the study included a total period of 10 months between May 2018 and June 2019. The material of the study consisted of feed samples taken from mixers, wagons and feeders from TMR feeds prepared in the dairy farm. The mixer wagon capacity of the facility where the research was conducted was 1 tonne on average, and the composition of the TMR consisted of meadow grass, straw, triticale, sunflower meal, corn silage, molasses, salt, vitamin mineral mixture and milk feed. The same ration was used throughout the experiment.

2.1. Sample Collection

Collecting samples from the mixer wagon:

During the preparation of TMR, samples for chemical and microbiological analyses were taken from the mixer wagon twice during the time until the mixture of the final ration composition was put inside the mixer, taking into account the mixing periods.

Collecting samples from the paddock feeders:

For this purpose, considering the length of the paddock feeder, samples (3 replicates for each feeder area) taken from the beginning, middle and end of the paddock feeder from a section corresponding to the distance of the feeder to the head of the animal were divided into two sections, taking care not to mix them again. After weighing, the two parts of the sample groups corresponding to the mutual cross were immediately placed in sealed bags and placed in a thermostatic bag containing an ice pack. The samples were taken to the laboratory for chemical and microbiological analyses.

2.2. Chemical and Microbiological Analyses

During the study, pH, dry matter (DM), lactic acid (LA), water soluble carbohydrate (WSC), lactic acid bacteria (LAB), yeast and mold counts were performed in feed samples. In the study, pH was determined by Chen et al. (1994), DM, CA and CP analyses by Akyıldız (1984), WSC analyses by Anonymous (1986), and LA analysis by the spectrophotometric method reported by Koç and Coşkuntuna (2003). LAB, yeast and mold counts were performed according to the methods reported by Seale et al. (1990). MRS Agar was used as the nutrient medium for LAB, and Malt Extract Agar was used for yeast and molds. LAB counts of the samples were performed following incubation periods at 30 °C for 3 days and at 30 °C for 5 days for yeast and molds. Each month, microbiological analyses were performed on 10 samples collected separately from the mixer wagon and the feed bunk.

2.3. Identification of Yeast and Mold Isolated from TMR Samples

Malt agar was used as a medium to examine the total yeast and mold load in TMR samples. After weighing 10 g of each feed sample, it was homogenized in a shaker for 30 minutes in 90 ml of sterile pure water. After preparing dilution series from 10⁻¹ to 10⁻⁴, inoculation was performed on the medium by spreading with a sterile spatula in 3 repetitions. The inoculated petri dishes were kept in the incubator at 24 °C for 7-10 days to be left for incubation. In order to identify fungi with high growth rate such as *Aspergillus niger*, petri dishes were checked from the 3rd day onwards.

2.4. Temperature Measurement of TMR

Ambient temperature, temperature changes in the feed trough and wagon were also recorded during each sampling period (Ranjit and Kung, 2000).

2.5. Statistical Analyses

The data obtained at the study were evaluated using Statistica (Statistica for the Windows Operating System 1999; Sta Soft, Inc., Tulsa, OK, USA) statistical package programme. Duncan multiple comparisons test was used to evaluate the differences between group averages (Soysal, 1993).

3. RESULTS

The chemical and microbiological composition of TMR prepared in a dairy cattle farm operating within the borders of Kırklareli province and using TMR-based feeding system in a 10-month period was tried to be presented. As it is shown in Table 1, the pH content of TMR varied between 4.43-5.12 in the wagon and 4.54-5.13 in the feeders. In this study, the lowest pH content was determined in June and the highest pH value was determined in October in both the feeder and the wagon. As a result of the statistical analysis, no difference was detected on the pH value of the samples taken from the feeder and wagon. However, the pH values of the feeds differed

depending on the months ($P < 0.001$). The DM content of TMR varied between 47.42-66.74% in wagon and 46.07-67.29% in feeders. In this study, the lowest %DM content was determined in June and the highest %DM content was determined in December in both feeders and wagons. As a result of the statistical analyses, no difference was detected on the %DM value of the samples taken from feeder and wagon. However, the %DM values of the feeds differed depending on the months ($P < 0.001$).

The CA content of TMR changed between 6.19-8.95 in the wagon and 6.79-8.48 in the feeders. In the research, the lowest CA% content of the wagon was determined in October and the highest CA% content was determined in May. In feeders, the lowest CA% content was determined in December and the highest CA% content was determined in April. As a result of the statistical analysis, no differences were detected on the CA% value on the samples taken from feeders and wagons. However, the CA values of the feeds differed depending on the months ($P < 0.001$).

The WSC contents of TMR varied between 13.77-51.02 g/kg DM in the wagon and 11.11-45.74 g/kg DM in the feeders. In the research, the lowest WSC content of the wagon was determined in December and the highest WSC content was determined in June. In the feeders, the lowest WSC content was determined in January and the highest WSC content was determined in June. However, the WSC values of the feeds differed depending on the months ($P < 0.001$).

The CP content of TMR varied between 13.6-15.6%DM in the wagon and 13.8-15.3%DM in the feeders. In the study, the lowest CP% content of the wagon was determined in June, and the highest CP% content was determined in December. The lowest CA% content in the feeders was determined in June, and the highest CP% content was determined in December. As a result of the statistical analysis, no difference was detected in the CP% value in the samples taken from the feeders and wagons ($P > 0.001$).

Table 1. Results of chemical analysis of TMR feeds

Place	Month	pH	DM, %	CA, DM%	WSC, g/kg, DM	CP, DM%
Wagon		4.80	54.66	7.52	24.97	14,8
Feeder		4.84	54.57	7.43	29.34	14,5
SEM		0.03	0.43	0.09	1.98	0.52
	September	4.75 ^b	51.03 ^{de}	7.10 ^{cd}	28.82 ^b	14.9
	October	5.04 ^a	52.01 ^{de}	6.87 ^d	31.37 ^b	14.6
	November	5.06 ^a	51.41 ^{de}	7.07 ^d	20.33 ^b	14.4
	December	4.80 ^b	67.02 ^a	7.13 ^{cd}	23.80 ^b	14.3
	January	4.74 ^b	60.91 ^b	7.06 ^d	17.01 ^b	15.0
	February	4.65 ^{bc}	57.67 ^c	7.35 ^{cd}	22.82 ^b	14.7
	March	4.62 ^{bc}	57.21 ^c	7.68 ^{bc}	30.10 ^b	14.5
	April	5.02 ^a	52.80 ^d	8.10 ^{ab}	21.92 ^b	14.2
	May	5.05 ^a	49.36 ^{ef}	8.10 ^{ab}	27.02 ^b	14.6
	June	4.48 ^c	46.75 ^f	8.28 ^a	48.38 ^a	13.8
SEM		0.06	0.96	0.19	4.43	0.45
	September	4.72 ^{d-f}	49.18 ^{e-g}	6.90 ^{d-f}	28.45 ^{b-e}	15.1
	October	5.12 ^a	52.90 ^{de}	6.19 ^f	28.07 ^{b-e}	14.8

Wagon	November	4.99 ^{a-d}	50.73 ^{ef}	6.77 ^{ef}	19.77 ^{de}	14.5
	December	4.76 ^{b-f}	66.74 ^a	7.46 ^{c-e}	13.77 ^{de}	15.6
	January	4.73 ^{c-f}	62.84 ^b	7.13 ^{de}	22.91 ^{c-e}	15.3
	February	4.66 ^{c-g}	56.06 ^{cd}	7.17 ^{de}	18.12 ^{de}	14.9
	March	4.63 ^{c-g}	58.52 ^c	8.44 ^{ab}	17.04 ^{de}	14.7
	April	5.01 ^{a-c}	52.83 ^{de}	7.73 ^{b-d}	23.72 ^{c-e}	14.4
	May	4.96 ^{a-d}	49.37 ^{e-g}	8.95 ^a	26.85 ^{b-e}	14.6
	June	4.43 ^g	47.42 ^{fg}	8.44 ^{ab}	51.02 ^a	13.6
Feeder	September	4.77 ^{b-f}	52.88 ^{de}	7.31 ^{c-e}	29.19 ^{b-e}	14.9
	October	4.97 ^{a-d}	51.12 ^{ef}	7.55 ^{c-e}	34.67 ^{a-d}	14.6
	November	5.13 ^a	52.08 ^{de}	7.38 ^{c-e}	20.90 ^{de}	14.4
	December	4.84 ^{b-e}	67.29 ^a	6.79 ^{ef}	33.83 ^{a-d}	15.3
	January	4.75 ^{b-f}	58.98 ^{bc}	6.98 ^{d-f}	11.11 ^e	15.0
	February	4.64 ^{c-g}	59.27 ^{bc}	7.53 ^{c-e}	27.52 ^{b-e}	14.7
	March	4.61 ^{c-g}	55.91 ^{cd}	6.93 ^{d-f}	43.17 ^{a-c}	14.5
	April	5.03 ^{ab}	52.78 ^{de}	8.48 ^{ab}	20.11 ^{de}	14.2
	May	5.14 ^a	49.35 ^{e-g}	7.26 ^{c-e}	27.19 ^{b-e}	14.6
	June	4.54 ^{fg}	46.07 ^g	8.12 ^{a-c}	45.74 ^{ab}	13.8
SEM		0.09	1.36	0.27	6.27	0.58
Place		0.289	0.889	0.492	0.121	0.137
Month		<0.001	<0.001	<0.001	<0.001	0.14
Place X Month		<0.001	<0.001	<0.001	<0.001	0.18

DM: Dry matter, CA: Crude ash, WSC: Water soluble carbohydrate, CP: Crude protein

Values in the same column with different letters are significantly different (P <0.001)

Table 2 shows that LAB content of TMR ranged between 2.01-4.84 cfu/g DM in wagon and 1.92-5.04 cfu/g DM in fodder troughs. In this study, the lowest LAB content of the wagon was determined in February and the highest LAB content was determined in October. In the feeders, the lowest LAB content was detected in January and the highest LAB content was detected in September. As a result of the statistical analysis, no difference was detected on the LAB value on the samples taken from feeders and wagons. However, LAB values of the feeds differed depending on the months (P<0.001). The yeast content of TMR varied between 2.01-5.01 cfu/g DM in the wagon and between 2.53-5.31 cfu/g DM in the feeders as shown

in Table 2. In the research, the lowest yeast content of the wagon was determined in February and the highest yeast content was determined in September. In the feeders, the lowest yeast content was detected in February and the highest yeast content was detected in September. As a result of the statistical analysis, no difference was detected on the yeast value of the samples taken from the feeder and wagon. However, yeast values of the feeds differed depending on the months (P<0.001).

In the study carried out for the identification of yeasts, mainly *Saccharomyces cerevisiae* species were identified. The yeast species in the feed samples and their appearance on malt agar are shown in Figure 1.



Figure 1. Growth appearance of *Saccharomyces cerevisiae* colonies on malt agar from feed sample isolates

The mould content of TMR ranged between 0.00-3.82 cfu/g DM in the wagon and 0.00-4.94 cfu/g DM in the feeders as shown in Table 2. In the research, the

lowest mould content of the wagon was determined in December, March and June, and the highest mould content was determined in September. In the feeders, the lowest mould content was detected in November,

December and March and the highest mould content was detected in September. Figure 2 shows the time-dependent mould change in feeders and wagons. As a result of the statistical analysis, no difference was

detected in the mould values of the samples taken from the feeder and wagon. However, the mould values of the feeds differed depending on the months ($P < 0.001$).

Table 2. The microbiological analysis results of TMR feeds (cfu/g DM)

Place	Month	LAB	Yeast	Mold
	Wagon	3.62	3.79	0.93
	Feeder	3.63	3.74	1.04
SEM		0.08	0.08	0.11
	September	4.81 ^a	5.16 ^a	4.38 ^a
	October	4.77 ^a	4.55 ^b	2.26 ^b
	November	4.66 ^a	4.56 ^b	0.34 ^{de}
	December	3.26 ^c	3.23 ^{de}	0.00 ^c
	January	2.21 ^d	2.95 ^c	1.09 ^c
	February	2.30 ^d	2.27 ^f	0.91 ^{cd}
	March	2.90 ^c	3.28 ^{de}	0.00 ^c
	April	3.33 ^c	3.63 ^{cd}	0.31 ^{de}
	May	4.00 ^b	4.01 ^c	0.29 ^{de}
	June	4.04 ^b	4.06 ^c	0.29 ^{de}
	SEM	0.19	0.17	0.15
Wagon	September	4.58 ^{a-c}	5.01 ^{ab}	3.82 ^b
	October	4.84 ^{ab}	4.78 ^{a-c}	2.63 ^c
	November	4.62 ^{a-c}	4.61 ^{a-d}	0.68 ^{ef}
	December	3.28 ^{d-f}	3.32 ^{f-i}	0.00 ^f
	January	2.49 ^{fg}	3.27 ^{g-j}	0.93 ^{d-f}
	February	2.01 ^g	2.01 ^k	0.63 ^{ef}
	March	2.99 ^f	3.28 ^{g-j}	0.00 ^f
	April	3.31 ^{d-f}	3.60 ^{e-h}	0.29 ^{ef}
	May	4.11 ^{b-d}	3.98 ^{d-g}	0.29 ^{ef}
	June	4.01 ^{b-e}	4.08 ^{c-f}	0.00 ^f
Feeder	September	5.04 ^a	5.31 ^a	4.94 ^a
	October	4.70 ^{a-c}	4.31 ^{b-e}	1.89 ^{cd}
	November	4.69 ^{a-c}	4.51 ^{b-d}	0.00 ^f
	December	3.24 ^{ef}	3.13 ^{h-j}	0.00 ^f
	January	1.92 ^g	2.64 ^{i-k}	1.24 ^{de}
	February	2.60 ^{fg}	2.53 ^{jk}	1.18 ^{d-f}
	March	2.82 ^f	3.28 ^{g-j}	0.00 ^f
	April	3.35 ^{d-f}	3.65 ^{e-h}	0.33 ^{ef}
	May	3.89 ^{c-e}	4.04 ^{c-g}	0.29 ^{ef}
	June	4.06 ^{b-e}	4.04 ^{c-g}	0.58 ^{ef}
	SEM	0.27	0.24	0.36
Place		0.960	0.637	0.462
Month		<0.001	<0.001	<0.001
Place X Month		<0.001	<0.001	<0.001

LAB: Lactic acid bacteria; Values in the same column with different letters are significantly different ($P < 0.001$)

In the present study, *Aspergillus* spp. and *Penicillium* spp. were predominantly identified among the mould species isolated. The morphological

appearance of these fungi on Malt Agar medium is presented in Figure 2.



Figure 2. Growth appearance of *Penicillium spp.* and *Aspergillus spp.* colonies on malt agar from feed sample isolates

Recorded Temperature Variations in the Environment, Mixer Wagon, and Feed Bunk During the Experimental Period

According to the temperature values for the period in which the research was conducted, the highest environmental, wagon and feeder temperatures were

determined in September. Feed sample temperature values also varied depending on the environmental temperature. The lowest temperature and moisture content were determined in January (Figure 3).

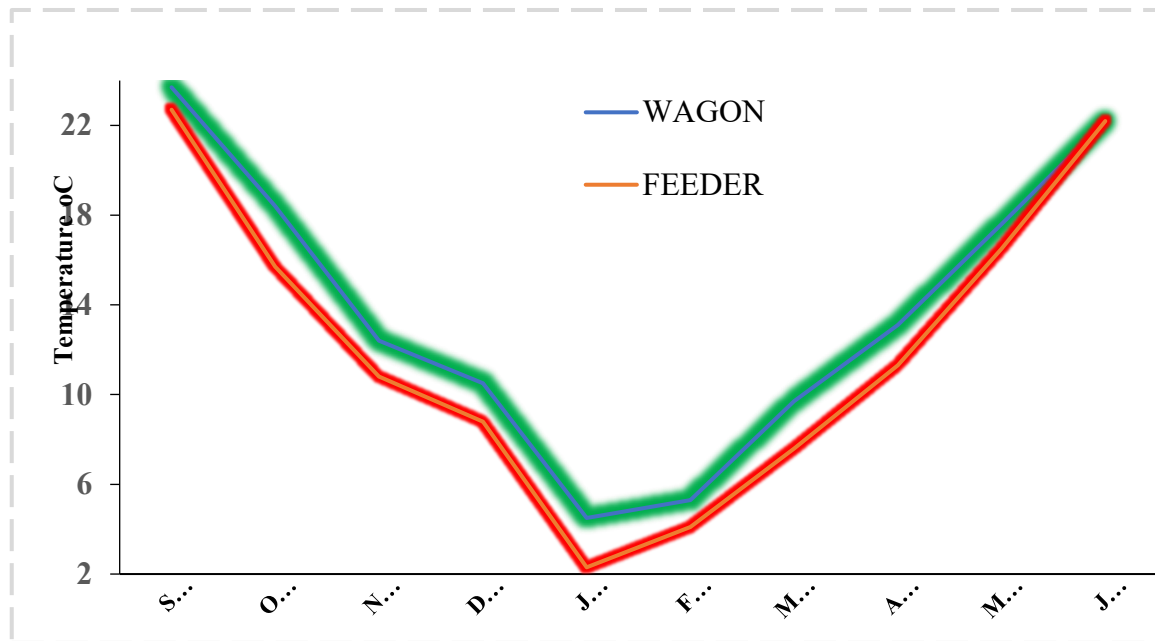


Figure 3. The temperature change in wagons and feeders

Table 3. Temperature and humidity values for the trial period

Month	Temperature maximum °C	Temperature minimum °C	Temperature average °C	Moisture average %
September	30.65±5.59 ^a	13.73±1.98 ^{ab}	21.48±3.31 ^a	51.45±10.15 ^c
October	23.63±3.32 ^{a-c}	9.98±1.93 ^{bc}	15.85±2.70 ^{ab}	70.35±6.89 ^b
November	15.88±1.14 ^{d-f}	6.68±3.71 ^c	10.03±2.29 ^{bc}	80.60±7.41 ^{ab}

December	12.98±7.65 ^{ef}	5.68±6.94 ^c	8.43±6.74 ^{cd}	78.13±11.52 ^{ab}
January	2.90±2.83 ^g	-2.53±3.29 ^e	0.10±3.40 ^e	89.90±9.51 ^a
February	8.90±5.78 ^{fg}	-0.70±4.17 ^{de}	2.88±4.15 ^{de}	80.45±14.16 ^{ab}
March	11.35±3.43 ^{ef}	4.30±2.39 ^{cd}	7.28±2.02 ^{cd}	83.38±10.33 ^{ab}
April	18.83±6.49 ^{c-e}	5.13±4.06 ^c	11.10±4.97 ^{bc}	68.13±14.53 ^b
May	22.58±1.78 ^{b-d}	9.98±2.36 ^{bc}	15.70±1.95 ^{ab}	69.00±11.03 ^b
June	27.15±5.59 ^{ab}	16.43±2.47 ^a	21.03±3.42 ^a	73.58±7.78 ^{ab}
P	<0.001	<0.001	<0.001	<0.002

*Values in the same column with different letters are significantly different (P <0.002)

4. DISCUSSION

This study was conducted over a 10-month period in a dairy farm utilizing the TMR system, with the objective of determining the chemical and microbiological characteristics of the feed. The findings revealed that the composition of the TMR varied significantly under the influence of seasonal environmental conditions, which played a crucial role in determining feed quality and animal health (Zhao et al., 2020).

The TMR system involves the uniform mixing of roughage and concentrate components of the diet, aiming to prevent feed selection by animals and to ensure consistent nutrient intake. The effectiveness of this system is closely related not only to the physical properties of the TMR (e.g., dry matter content, pH, moisture level) but also to its microbial stability (Heinrichs and Kononoff, 2002).

In this study, dry matter (DM) content in the mixer wagon ranged from 47.42% to 66.74%, while it varied from 46.07% to 67.29% at the feed bunk. Correspondingly, moisture levels fluctuated. For optimal ration homogeneity and to minimize feed sorting, a moisture content between 33% and 55% is recommended (Kononoff et al., 2003; Felton & DeVries, 2010). While the findings largely align with this recommendation, elevated ambient temperatures during summer months led to increased moisture levels, which in turn raised the risk of microbial contamination.

The pH values of the TMR ranged between 4.43–5.12 in the mixer wagon and 4.54–5.13 at the feed bunk. This pH range reflects the characteristics of well-fermented silage and may exert an inhibitory effect on microbial growth (Kung et al., 2018). However, exposure to oxygen after silo opening promotes the proliferation of yeasts and molds (Pahlow et al., 2003). A seasonal increase in yeast and mold levels was observed in this study, particularly during the summer.

Yeasts play a primary role in the aerobic spoilage of TMR silages (Wang et al., 2016). Yeast counts ranged between 2.01–5.01 cfu/g DM in the mixer wagon and 2.53–5.31 cfu/g DM at the feed bunk. The highest yeast count was recorded in September, and the lowest in February, supporting the temperature-dependent growth behavior of yeasts (Hu et al., 2015). *Saccharomyces cerevisiae* was identified as the dominant species, which thrives in warm, humid

environments and consumes lactic acid, raising the pH and creating favorable conditions for spoilage microorganisms (Kızıllışımşek et al., 2016). Therefore, rapid feed consumption and routine cleaning of feed bunks are particularly important during warmer months.

Mold counts ranged from 0–3.82 cfu/g DM in the mixer wagon and 0–4.94 cfu/g DM at the feed bunk. The highest levels of mold contamination were again observed in September. The most frequently identified genera were *Aspergillus* spp. and *Penicillium* spp., both of which are known for their ability to produce mycotoxins (especially aflatoxins), posing direct risks to feed quality and animal health (Wambacq et al., 2016; Zhao et al., 2020). Aflatoxins, particularly those produced by *A. flavus* and *A. parasiticus*, are hepatotoxic and carcinogenic. Their production is influenced by environmental factors such as temperature, relative humidity, and substrate water activity (Parvin and Nishino, 2010; Kung and Muck, 2015; Zhao et al., 2020).

Although aflatoxin analysis was not conducted in this study, the elevated mold loads observed under high temperature and humidity conditions suggest a favorable environment for aflatoxin formation. Supporting this, Xiang et al. (2018) reported that 59.7% of milk samples in China contained aflatoxin M1 (AFM1), potentially linked to the consumption of spoiled summer feed.

Environmental temperature data further corroborated these findings. The highest microbial loads coincided with peak temperatures in the mixer wagon and feed bunk during September. In contrast, microbial growth was limited at lower temperatures. Studies on aflatoxin production have indicated that maximum synthesis occurs at 24–30 °C with relative humidity exceeding 80% (Chelkowski et al., 1983; Christensen and Kaufman, 1969), consistent with the conditions observed in this study. More recent studies support this temperature-humidity relationship; for instance, Kebede et al. (2020) emphasized that *Aspergillus flavus* proliferation and aflatoxin B1 synthesis are significantly enhanced at 28–30 °C and 85% relative humidity. Similarly, Al-Wadai et al. (2013) reported optimal aflatoxin production under warm and humid environmental conditions, aligning with typical late-summer feed storage climates. These findings highlight the critical need for environmental

monitoring in feed management to mitigate mycotoxin risks.

CONCLUSION

Microbiological load of feeds constitutes a very important parameter in the process from the harvesting of the materials from which the feeds are obtained from the field to the addition to the feed after harvesting. The adverse conditions have an effect on the deterioration of feeds and even more badly on the process up to animal health. The research data show that the microbial activity is high in feeds, especially in periods when the ambient temperature is high. In this sense, it is important to carry out the required controls in both mixer wagons and feeders. Particularly, not leaving the remaining feed in the feeders, controlling the raw materials that make up the TMR, homogenised mixing of the feed raw materials in the wagon seem to be one of the important factors in this regard.

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