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Effects of additives to improve aerobic stability of TMR

Effects of Propionic acid, Sodium benzoate, Salvana TMR and FreshFOSS on aerobic stability and microbial composition of total mixed rations for dairy cattle

Introduction

Aerobic spoilage of total mixed rations (TMR) during the summer months is a problem that is commonly observed in the Danish dairy industry. However, problems with aerobic spoilage vary considerably among herds. In some herds, aerobic spoilage might be apparent on only a few days in a year and other herds have pronounced spoilage for months, if the TMR is not treated with appropriate additives. Any heating of TMR in the bunk is to be considered as an important feeding problem and it is strongly recommended to check leftovers for heating on a regular basis during summer.

If a ration has been extensively mixed e.g. Compact TMR (Kristensen 2017), the feed can be warm already when unloaded, however, this should not be confused with aerobic spoilage because it is caused by friction in the mixer and not microbial spoilage.

Sodium benzoate is not approved for use in TMR, it is only approved for treatment of silage. Many farmers have wondered why the substance is not allowed for use in TMR when it legally can be used in food and is extensively used by the food industry. The aim of the present study was to test the efficacy of Sodium benzoate when compared to other additives used for the prevention of aerobic spoilage in TMR. It was also the aim of the study to compare “dry acid products” Salvana TMR and FreshFOSS with propionic acid.

Materials and methods

Experimental license to feed sodium benzoate in an on-farm experiment was obtained from The Danish Veterinary and Food Administration (Fødevarestyrelsen; J.no. 2018-29-79-02739).

The experiment was carried out on a farm feeding a single TMR to all milking cows. Experimental treatments were applied by splitting the daily allowance of TMR into 4 different batches.

Experimental treatments were: Control = untreated TMR; Propionic acid = 3 L propionic acid/ton TMR; Sodium benzoate = 1 kg sodium benzoate/ton TMR (R2 Agro A/S, Hedensted, Denmark; Sodium Benzoate Feed Grade); Salvana TMR = 1 kg Salvana TMR/ton TMR (product 1051028, Acid TMR Fresh Plus; Linds A/S) and FreshFOSS = 1 kg FreshFOSS/ton TMR (Vilofoss, Denmark).

Propionic acid, Salvana TMR and FreshFOSS were used without predilution in water. Sodium benzoate was dissolved in tap water with a final concentration of 33% (weight/volume), and 3 L of 33% solution/ton TMR were applied.

The protocol was repeated on 3 different days in a herd with approx. 260 milking cows (Viking Red, Viking Holstein and crossbreds). On each day, the feeding was split into 4 batches – each treated with one of the additives being tested. Untreated TMR was sampled from the first batch on each day before application of the additive. The feed bunk was divided into 4 separately marked sections and each batch of TMR was unloaded in a predefined section. The combination of sections and treatments was randomized between days (Table 1).

The composition of the TMR was (% of DM): corn silage, 46.1; canola cake and meal, 25.7, grass-clover silage, 18.4; caustic rye, 8.5; mineral premix and salt, 1.3. The ration was added 9 kg water/ration.

On each day, the total amount of corn silage to be used in all 4 batches was premixed in the feed mixer and unloaded. Corn silage was sampled on each day from the corn silage premix.

TMR was mixed using a horizontal auger mixer (Seko Samurai 7 Power 600/230, Seko Industries Srl., Italy). The main advantage of this mixer was that both large and small batches could be mixed with full impact due to the horizontal augers and extended counter blade system.

The mixing protocol was a standard Compact TMR protocol (Kristensen, 2017) except that the soaking and structuring phases were combined and all ingredients except corn silage were mixed into a common premix and unloaded.

Each of the 4 daily TMR batches were mixed by combining the same relative amount of the structuring premix and corn silage premix in order to obtain closely similar batches of TMR.

When mixing the first and largest TMR batch of the day, a sample of untreated TMR (control) was obtained after mixing for 10 min. The sample was collected after unloading approx. 100 kg TMR to clean the door and conveyor belt and then a 65 L sample was collected directly from the conveyor. The mixer was turned off and the sample reduced to 4 L by coning and quartering. The TMR from cleaning the door and residue from subsampling was scoped into the bucket of the front loader, the additive to be used in the firsts treatment of the day was applied on top of the TMR in the bucket and the bucket emptied into the mixer. The mixer was turned on and allowed to mix for 20 min. before unloading the mix.

For the 3 subsequent applications of additives, the additive was applied to the bucket of the front loader during loading of the structuring mix to the mixer. The final mixing time was 20 min. for all treatments. Table 1 shows the different batches of TMR.

The treated TMR was sampled upon unloading at the feed bunk. A 65-L bucket was placed on the floor and filled with TMR. A subsample was obtained by sequential coning and quartering. The final 4 L sample was bagged and placed in insulated boxes with cooling elements and transported to the laboratory for processing on the day after feeding.

On the day after feeding, leftovers were scored in the bunk for signs of uneven feed intake and heating of leftovers was evaluated by palpation.

Aerobic stability of TMR was determined by incubating approx. 1,000 g TMR in a 2,000 mL plastic bucket. The buckets were incubated in temperature-controlled chambers with a target temperature of 20 °C. The temperature in the center of the buckets was continuously logged using Powerlab (Kristensen et al., 2010). The threshold for stability was 2.5 °C above chamber temperature that also was continuously logged.

Parallel to samples incubated for determination of aerobic stability, another set of samples was incubated under the same conditions for determination of microbial profile after 48 hours of incubation at 20 °C. Microbial composition of samples was analyzed by Eurofins Agro Testing Denmark A/S. Aerobic bacteria were assayed by NMKL method 86:2006 and yeast and fungi by NMKL 98:2005.

Silage and TMR were analyzed by NIR following drying at 60 °C and milling at 1 mm (Kvægbrugets ForsøgsLaboratorium, SEGES).

Treatment effects were analyzed by ANOVA using PROC MIXED in SAS.

Table 1. TMR batches used for testing the effects of Propionic acid, Sodium benzoate, FreshFOSS and Salvana TMR on aerobic stability, chemical composition and microbial composition of TMR. A sample of untreated control was obtained from the largest batch on each day. The largest batch was mixed first. ‘Section’ refers to the feed bunk section where the batch was unloaded.

Treatment Date	Propionic acid, 3 L/ton	Sodium benzoate, 1 kg/ton	FreshFOSS, 1 kg/ton	Salvana TMR, 1 kg/ton
20180724	4017 kg	3667 kg	3667 kg	3667 kg
	Section 4	Section 1	Section 2	Section 3
20180726	2240 kg	8400 kg	2240 kg	2240 kg
	Section 3	Section 4	Section 1	Section 2
20180731	2250 kg	2250 kg	8400 kg	2250 kg
	Section 1	Section 3	Section 4	Section 2

Results

The present udy was undertaken during a period with warm weather in the summer of 2018. The 24-h average temperatures on the 3 mixing days were 21.2, 21.5 and 21.1 °C and the precipitation was zero in the study period.

Evaluating the quality of leftovers with a simple palpation test indicated differences between treatments. Leftovers of TMR treated with propionic acid and sodium benzoate were found to warm in 3 of 3 days. TMR treated with FreshFOSS did not warm on any of the experimental days. Leftovers from Salvana TMR was found to be in-between and leftovers were found to warm on 2 of 3 days (Table 2).

Table 2. Evaluation of leftovers in the feed bunk the day after feeding (approx. 18-20 h after feeding).

Treatment	Propionic acid	Sodium benzoate	FreshFOSS	Salvana TMR
Date				
20180725	Warm	Warm	Not warm	Warm
20180727	Warm	Warm	Not warm	Warm
20180801	Warm	Warm	Not warm	Not warm

There were no signs on any selection for or against any of the additives when evaluated from the amount of leftovers in each section the day after unloading the TMR.

Figure 1 shows examples of temperature profiles from incubation of feed samples in the temperature-controlled cabinets. The warm weather was a challenge for keeping the planned temperature of 20 °C and the actual incubation temperature was approx. 21 °C. As shown in Figure 1 and 2, all incubated samples were found to heat during incubation.

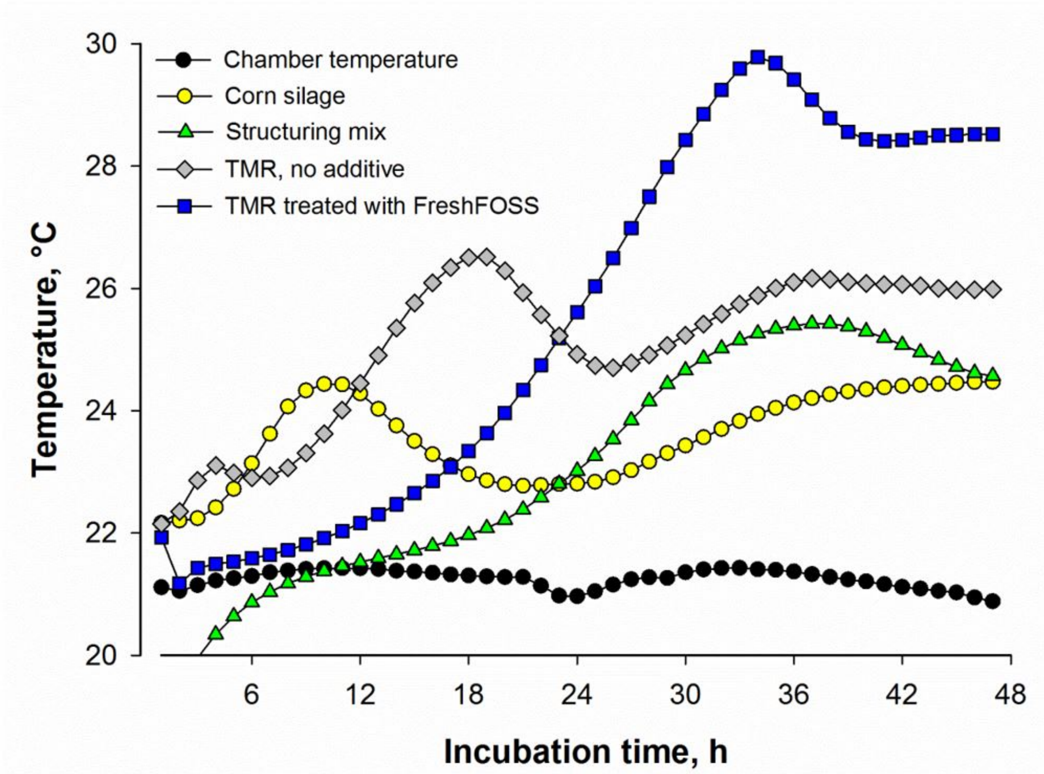

Figure 1. Temperature in incubation chamber (circle, black fill), corn silage (circle, yellow fill), structuring mix (triangle, green fill), TMR without additive (rhomb, grey fill) and TMR treated with FreshFOSS (square, blue fill). The temperature was logged continuously in the center of the sample. Each observation is the mean temperature during 1 h.

Figure 2 shows the aerobic stability of corn silage, structuring mix and TMR. Aerobic stability of corn silage (9 ± 1 h) had only half the stability observed for structuring mix (19 ± 4 h). The final TMR obtained from mixing the structuring mix with corn silage premix had a stability of 8 ± 2 h. The overall analysis including all treatments did not detect any treatment effect on aerobic stability of TMR ($P > 0.10$). Aerobic stability of TMR treated with propionic acid, sodium benzoate, and Salvana TMR was very similar (11 to 14 h). For TMR treated with FreshFOSS, the stability was greater (24 ± 2 h) and if tested to each of the other treatments, FreshFOSS had higher stability ($P < 0.05$) compared with Control and all other additives.

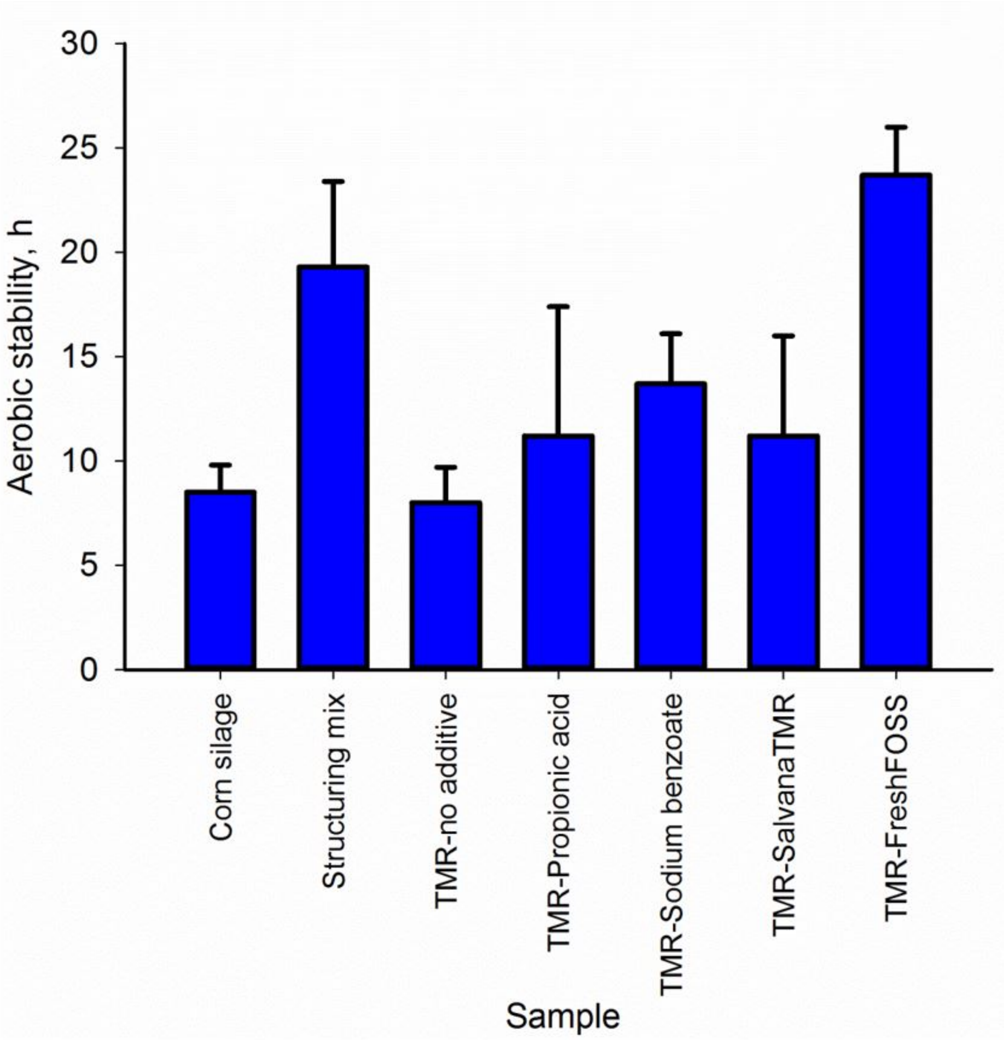

Figure 2. Aerobic stability of corn silage, structuring mix and TMR determined as hours for center temperature to increase 2.5 °C relative to chamber temperature. Samples were incubated in plastic buckets of 2.000 mL. Each bar is the mean of 3 observations ± SEM.

The microbial composition of fresh samples and samples incubated for 48 h at 20 °C is shown in Table 3. Incubation for 48 h increased aerobic bacteria and yeast (log10 CFU/g) compared with the fresh sample. The CFU of fungus did not increase with incubation at 20 °C for 48 h. No differences between treatments could be detected for 48-h increase in aerobic bacteria and yeast ($P > 0.10$). With FreshFOSS, the increase in bacteria and yeast was numerically lower compared with the effect of incubation in Control and samples treated with other additives.

Table 3. Microbial composition (log10 CFU/g) of silage, structuring mix and TMR of samples collected on the day of feed mixing and in samples incubated for 48 h at 20 °C. No differences could be detected for TMR treated with propionic acid, sodium benzoate, Salvana TMR or FreshFOSS when incubated for 48 timers at 20 °C.

	Variable		
Item	Aerobic bacteria	Yeast	Fungi
Corn silage	6.5 ± 0.2	3.9 ± 1.2	3.0 ± 0.3
Corn silage – 48 h 20 °C	7.9 ± 0.4	8.0 ± 0.1	-
Structuring mix	6.5 ± 0.3	5.4 ± 0.4	2.7 ± 0.2
Structuring mix – 48 h 20 °C	> 8.4	7.6 ± 0.2	-
TMR	6.5 ± 0.3	5.0 ± 0.9	3.1 ± 0.2
TMR – Control – 48 h 20 °C	> 8.4	8.1 ± 0.1	-
TMR – Propionic acid – 48 h 20 °C	8.0 ± 0.4	7.5 ± 0.3	2.3
TMR – Sodium benzoate – 48 h 20 °C	8.4 ± 0.1	7.8 ± 0.2	2.2 ± 0.2
TMR – Salvana TMR – 48 h 20 °C	8.0 ± 0.4	7.7 ± 0.4	2.0
TMR – FreshFOSS – 48 h 20 °C	7.4 ± 0.2	7.1 ± 0.3	2.3 ± 0.3

Table 4 shows the dry matter concentration and chemical composition of TMR. Treatment affected ($P < 0.05$) organic matter digestibility (OMD), soluble crude protein, starch concentration and pH of TMR. The pH of TMR was lower for propionic acid treatment compared with all other treatments.

Table 4. Dry matter concentration and chemical composition of TMR analyzed by drying at 60 °C and NIR analysis. Treatments were Control (no additive), Propionic acid, Sodium benzoate, Salvana TMR, and FreshFOSS. Data are presented as mean of 3 observations with the standard error of the mean (SEM). The overall treatment effects are given (P-value). Different letters in the same row indicate that means differ (P < 0.05).

	Treatment						
Variable	Control	Propionic acid	Sodium benzoate	Salvana TMR	Fresh-FOSS	SEM	P-value
Dry matter, g/kg	412	419	414	418	415	4.1	0.89
Ash, g/kg DM	77	76	74	76	77	1.5	0.75
OMD, %	77.0a	78.0b	76.6a	77.8b	77.2a	0.2	0.01
Crude protein, g/kg DM	155	157	155	156	156	1.6	0.93
Sol. crude protein, g/kg DM	63.0a	63.4a	59.8b	63.2a	61.7ab	0.7	0.02
Crude fat, g/kg DM	39.2	39.5	39.3	39.2	38.6	0.6	0.87
NDF, g/kg DM	321	318	322	322	318	4	0.87
Starch, g/kg DM	176a	175a	183b	179ab	181b	1.5	0.01
Sugar, g/kg DM	11.5	11.5	4.9	12.0	9.4	2.8	0.40
pH	4.73a	4.64b	4.76a	4.74a	4.71a	0.02	< 0.01

The differences between analyzed composition of leftovers and fresh TMR are shown in Table 5. No treatment effects were observed for any of the differences between leftovers and fresh TMR. Overall, the concentration of NDF, starch, and crude fat as well as pH was greater in leftovers (P < 0.05) compared with TMR. The concentration of reducing sugars and OMD was lower in leftovers compared with TMR (P < 0.05). The concentration of dry matter, ash, crude protein and soluble crude protein did not differ between leftovers and TMR (P > 0.10).

Table 5. Differences in dry matter concentration and chemical composition between leftovers and TMR (leftovers – TMR). Leftovers were collected in the feed bunk 18-20 h after feeding. Treatments were Propionic acid, Sodium benzoate, Salvana TMR eller FreshFOSS. Data are presented as mean of 3 observations with the standard error of the mean (SEM). The overall treatment effects are given (P-value).

	Treatment					
Variable	Propionic acid	Sodium benzoate	Salvana TMR	Fresh-FOSS	SEM	P-value
Dry matter, g/kg	2.4	-1.8	-3.5	12.0	6.2	0.36
Ash, g/kg DM	2.3	3.7	2.6	2.2	2.7	0.98
OMD, %	-0.8	-1.2	-1.1	-0.3	0.4	0.43
Crude protein, g/kg DM	-1.2	0.3	0.3	-0.5	2.0	0.93
Sol. crude protein, g/kg DM	-0.5	2.1	0.3	2.5	1.5	0.49
Crude fat, g/kg DM	2.4	2.4	1.3	-0.3	0.9	0.22
NDF, g/kg DM	7.6	9.5	8.1	6.5	4.2	0.97
Starch, g/kg DM	6.3	2.2	6.4	-0.4	2.4	0.21
Sugar, g/kg DM	-18	-21	-16	-9	5	0.41
pH	0.16	0.11	0.13	0.12	0.08	0.95

Samples incubated at 20 °C for 48 h were analyzed as TMR, and Table 6 shows the differences between dry matter concentration and chemical composition for 48-h samples – TMR. No effects of the applied treatments were detected for any of the variables shown in Table 6. Concentrations of NDF, starch, crude protein, and crude fat were greater (P < 0.05) for samples incubated at 20 °C compared with concentrations in fresh TMR. The concentrations of dry matter, reducing sugars and OMD were lower (P < 0.05) in samples incubated for 48 compared with fresh TMR. Only soluble crude protein was not affected (P > 0.10) by incubation for 48 h.

Table 6. Differences in dry matter concentration and chemical composition between samples of TMR incubated at 20 °C for 48 h and fresh TMR (48 h sample – fresh TMR). Treatments were Control (no additive), Propionic acid, Sodium benzoate, Salvana TMR, and FreshFOSS. Data are presented as mean of 3 observations with the standard error of the mean (SEM). The overall treatment effects are given (P-value).

	Treatment						
Variable	Control	Propionic acid	Sodium benzoate	Salvana TMR	Fresh-FOSS	SEM	P-value
Dry matter, g/kg	-11	-9	-11	-11	-8	1.7	0.65
Ash, g/kg DM	0.4	0.1	0.6	0.7	-0.7	1.1	0.91
OMD, %	-1.3	-0.9	-1.6	-1.1	-1.2	0.4	0.83

Crude protein, g/kg DM	2.8	2.8	2.2	3.1	0.8	1.4	0.78
Sol. crude protein, g/kg DM	-2	2	1	1	4	1.7	0.22
Crude fat, g/kg DM	2.9	3.3	2.7	3.0	2.7	0.7	0.98
NDF, g/kg DM	9	9	12	6	6	5.3	0.91
Starch, g/kg DM	9	6	3	9	4	2.4	0.28
Sugar, g/kg DM	-26	-21	-30	-31	-24	3.4	0.28
pH	0.35	0.31	0.26	0.41	0.31	0.16	0.92

Discussion

The present study was carried out in the summer of 2018 during a period with warm and dry weather. The situation at the experimental farm was very well suited for the test, as the TMR was not stable for a 24-h feeding period without the use of stabilizing additives.

The weather was a challenge for cooling of samples, and for maintaining samples sufficiently cooled during transport to the lab, however, all samples were handled similarly, and it is unlikely that the treatment differences were affected by difficulties in the cooling of samples.

The TMR used was mixed using a horizontal feed mixer which was an advantage for the ability to obtain sufficient mixing impact with small batches of TMR. Scoring of samples by the Compact TMR analyzer methods (data not shown) did not indicate any differences between the TMR batches depending on batch size. The ration was formulated with a dry matter concentration of 410 g/kg because a horizontal mixer was used. Had a vertical auger mixer been used, the target dry matter would have been lower (360-375 g/kg) and this might have had an impact on the aerobic stability of the TMR tested.

Testing feed in the bunk

The present study found a high similarity between the simple manual palpation tests for TMR in the bunk compared with determination of aerobic stability in temperature-controlled chambers. The simple palpation test done 18 – 20 h after feeding was sensitive and enabled the person testing the feed to test if there was an insufficient stability of the TMR.

No 100% effect

The study showed that despite the effect of stabilizing additives, none of the tested additives protected TMR from heating under prolonged incubations at 20 °C. Even for FreshFOSS that appeared to be the most effective additive, the heating was only delayed, microbial spoilage was not stopped and all TMR samples heated during incubation.

Corn silage less stable compared with grass silage

The present study confirmed the general observation of lower stability of most corn silages when compared with grass silage and commodities. The structuring mix containing commodities (canola meal and cake as well as caustic rye and minerals) and water had twice the stability of corn silage. However, when the structuring mix was combined with corn silage to the final TMR, the stability was reduced to the level of corn silage. The marked influence of corn silage on the stability of TMR suggests that many farmers that repetitively observe the need for stabilizing additives might consider treating corn silage with heterofermentative lactic acid bacteria as an alternative for using additives in TMR (Kristensen et al., 2010).

Growth of aerobic bacteria and yeast

The incubation of TMR samples at 20 °C was followed by a proliferation of both aerobic bacteria and yeast. Only FreshFOSS was observed to have an impact on aerobic stability and this effect was mirrored by a numerically lower proliferation of aerobic bacteria and yeast in samples of TMR treated with FreshFOSS. It was not possible to detect any differences between additives related to selective inhibition of either aerobic bacteria or yeast. It might be due to the endpoint at 48-h which for most treatments were relatively late, and the samples were found to be markedly affected by microbial growth when the incubation was stopped.

Chemical composition and NIR calibrations

For some of the variables analyzed by NIR, ”treatment” differences were observed which are unlikely to represent true differences in composition between treatments. For TMR treated with Sodium benzoate, it was possible to observe effects on soluble crude protein and starch. The dose of Sodium benzoate was relatively large and only to a limited extend represented in the calibrations used for the analyses. Also the mixing protocol used, where structuring mix and corn silage both were premixed for all batches to be mixed on the same day, suggests that a simple difference in starch without an opposite change in crude protein would not be likely to happen. The mixing precision is underlined by the fact that the dry matter standard deviation for 15 samples of TMR collected over 3 mixing days was only 6 g DM/kg TMR.

Leftovers

Analysis of leftovers indicated only numerically small differences between leftovers and fresh TMR. When considering that cows had access to the TMR for 18-20 hours and that the palpation test indicated that most of the TMR was not completely stable, the findings suggest that an analysis of chemical composition of leftovers is not an appropriate method for evaluation of TMR spoilage. Of the analyzed parameters, only the disappearance of sugar was a strong indicator for the beginning spoilage. The present study showed that a simple palpation test is a much more sensitive test for establishing the need for adding preserving additives compared with analysis of chemical composition of TMR and leftovers. Even the strongly overgrown samples recovered after incubation at 20 °C for 48 h did not show dramatic differences compared with TMR. A decrease in sugar concentration and an increased pH were signatures of the spoilage, however, the general composition was not affected to any great extent.

Propionic acid and Sodium benzoate

Sodium benzoate is an approved additive for use in silage (European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003), however, it's not approved as additive in TMR. Many dairy farmers have wondered why sodium benzoate was not approved in TMR and the present study obtained permission to test the product in a production setting. Propionic acid is approved for treatment of TMR, however, it's corrosive and unless acquired in a mixture, it cannot be used as an on-farm feed additive with a HACCP system. The price in 2018 was approx. 11 DKK/kg of propionic acid (1.5 €/kg). Sodium benzoate is a Sodium salt of benzoic acid and it is not corrosive and does not have the pungent smell, which is characteristic for propionic acid. With a price in the range from 17-25 DKK/kg (2.3-3.4 €/kg), Sodium benzoate is also attractive if 1 kg of Sodium benzoate can replace 3 L propionic acid.

A potential disadvantage of sodium benzoate is that it needs to be solubilized in order to have an effect. In the present study, sodium benzoate was dissolved in water (33% weight/volume) before use. It might be possible simply to add dry sodium benzoate to a soaking mix and let it solubilize during the standing time of the soaking mix. Another potential disadvantage of sodium benzoate is that it comes as a finely ground powder and it will be necessary with protective measures in order to avoid inhalation of dust.

In the present study, a dose of 1 kg sodium benzoate/ton of TMR was not superior to 3 L propionic acid/ton. Without being an exhaustive investigation of the stabilizing effects of sodium benzoate in TMR, the study did not support investing in approval of sodium benzoate for use in TMR. The effect of sodium benzoate was less or equal to the effect of a cost equivalent dose of propionic acid and the advantages in personnel safety can be accomplished with alternative commercially available dry acids.

Salvana TMR and FreshFOSS

Salvana TMR and FreshFOSS were more convenient to use than both propionic acid and sodium benzoate. The dry acid products were handled as standard bagged mineral products and were not corrosive, solubilizing before use was not necessary, and even though the price/kg is higher compared with propionic acid (Salvana TMR 22.5 DKK/kg ≈ 3 €/kg and FreshFOSS 24.5 DKK/kg = 3,3 €/kg), the treatment cost per ton of TMR is less than the treatment cost using propionic acid when 1 kg of dry acid substitutes 3 L of propionic acid.

Salvana TMR in a dose of 1 kg/ton was not superior to 3 L propionic acid with the exception that Salvana TMR treated TMR passed the leftover palpation test in 1 of 3 days. TMR treated with propionic acid failed on 3 out of 3 days. There were several indicators in the study identifying FreshFOSS as a superior product. Leftovers from TMR treated with FreshFOSS passed the palpation test in 3 out of 3 days. Also the growth of aerobic bacteria and yeast tended to be inhibited in samples treated with FreshFOSS when they were incubated at 20 °C. The aerobic stability of TMR treated with FreshFOSS was greater compared with stability of both untreated TMR and TMR treated with propionic acid, sodium benzoate, and Salvana TMR.

One drawback using the dry acids is that recipes are secret and it is not known if there are any special nutritional concerns using the products. If dry cows are fed Calcium restricted rations for the prevention of parturient paresis (milk fever), it should be noticed that both dry acids contain Calcium sources.

Conclusions

The study investigated the effects of using preserving additives in TMR under summer conditions. Comparison of treatments with propionic acid (3 L/ton), sodium benzoate (1 kg/ton), Salvana TMR (1 kg/ton) and FreshFOSS (1 kg/ton) showed the greatest effect of FreshFOSS. Sodium benzoate was used under experimental license. Sodium benzoate was not found to be superior to other products when considering handling, cost or biological effects and the study outcome does not encourage seeking approval of sodium benzoate for treatment of TMR. The study indicated that dairy farmers should consider commercially available dry acid products when they detect a need for prevention of aerobic spoilage of TMR. The best product is convenient to handle, does not require HACCP and has a strong biological effect. The study showed that a simple palpation test where leftovers are examined the day after feeding, a TMR is a powerful test for detecting problems with aerobic spoilage of TMR.

Conflicts of Interest Statement

The present study was conducted without any subsidies or involvement of companies producing or marketing feed additives. The products used in the study were purchased as standard commodities from the following Danish suppliers: R2 Agro A/S, Linds A/S, and Mollerup Mølle A/S.

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