

CITRIC AND FORMIC ACID FOR STABILIZATION OF TOTAL MIXED RATIONS

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ABSTRACT

Total mixed rations (TMR) are exposed to aerobic deterioration after mixing, critically accelerated by elevated temperatures. This study evaluates the efficacy of CitroFor stabilizer (citric and formic acid, 95:5 diluted 1:4 with water) across three TMR types (dry cows, lactating cows, post-partum cows) at two temperatures (20 and 30 degrees C) using continuous temperature monitoring (84 h, n = 3). The dry cow TMR showed the highest benefit: at 30 degrees C, CitroFor at 4 l/t fresh matter (FM) reduced the temperature increase from +12.5 to +7.7 degrees C (38%), with a statistically significant ANOVA result ($F = 6.39$; $p = 0.033$) and complete elimination of mould and yeast. For lactating cow TMR, even 4 l/t was insufficient to prevent mould and yeast growth. The 2 l/t dose was counterproductive for post-partum TMR (critical time shortened by 10%). Results highlight a fundamental interaction between TMR type, stabilizer dose and temperature regime with practical implications for seasonal feeding management.

Keywords: aerobic spoilage; dairy cattle; yeast inhibition; temperature monitoring; dose-response interaction

INTRODUCTION

Aerobic spoilage of TMR is a major economic and nutritional challenge in dairy farming. The warming of TMR above ambient temperature signals uncontrolled microbial activity driven primarily by yeasts and moulds that oxidise residual fermentation products. At temperatures above 40 °C, Maillard reactions reduce amino acid bioavailability (Borreani *et al.*, 2018). Organic acids – particularly propionic, formic and citric acid – are known to suppress aerobic deterioration in silage and TMR (Gheller *et al.*, 2021; Seppälä *et al.*, 2013). However, the interaction between TMR nutritional composition, stabilizer dose and ambient temperature has not been systematically addressed. The present study tested CitroFor, a novel combination of citric and formic acid, across contrasting TMR types and temperature regimes.

MATERIAL AND METHODS

Three TMR types prepared on a commercial dairy farm (Netluky, Czech Republic) were tested in November 2025 at the Institute of Animal Science. TMR composition is summarised in Table 1. Treatments were: Control (A) – no additive; CitroFor 2 l/t FM (B); CitroFor 4 l/t FM (C). The stabilizer (95 parts of citric acid diluted 1:4 with water + 5 parts formic acid) was applied by surface spraying. Samples of each treatment were distributed into

labelled polyethylene bags (3 replicates × 3 treatments × 3 TMR types × 2 temperatures = 54 bags). Bags were stored at constant 20 °C or 30 °C in climate chambers for 84 hours. Temperature was recorded every hour using DS1922L data loggers. Visual assessment (mould, yeast, heating, sliminess) was performed at 72 h on a 0–3 scale (0 = none, 3 = severe). Statistical analysis used one-way ANOVA ($\alpha = 0.05$) with the final temperature at 84 h as the dependent variable.

RESULTS AND DISCUSSION

ANOVA results and key temperature data are presented in Table 2. A statistically significant treatment effect was found only for dry cow TMR at 30 °C ($F = 6.39$; $p = 0.033$). CitroFor at 4 l/t reduced the final temperature from 44.4 ± 1.2 °C to 37.1 ± 4.1 °C, a reduction of 7.3 °C, keeping TMR safely below the critical 40 °C threshold for Maillard reactions. The temperature increase above ambient was reduced from +12.5 °C (control) to +7.7 °C (38% reduction). Visual assessment confirmed complete suppression of mould and yeast growth at 4 l/t for dry cow TMR even after 72 h at 30 °C (score 0/3; Table 3).

In contrast, lactating cow TMR – characterised by a high proportion of maize silage (33.3% FM) and concentrate (17.6% FM), and high starch content (27.2% DM) – showed mould (score 0.67/3)

Tab. 1: Composition of tested TMR (kg/head/day; % of total fresh matter)

Component	TMR 1 Post-partum		TMR 2 Lactation		TMR 4 Dry cows	
	kg/head/d	%	kg/head/d	%	kg/head/d	%
Alfalfa-grass silage	13.0	24.8	21.0	38.9	20.0	68.3
Maize silage (35% DM)	22.0	41.9	18.0	33.3	6.0	20.5
Wheat straw	—	—	—	—	3.0	10.2
Wet maize grain	4.0	7.6	3.5	6.5	—	—
Liquid feed MGP	2.0	3.8	2.0	3.7	—	—
Concentrate mix	11.5	21.9	9.5	17.6	0.3	1.0
TOTAL	52.5	100.0	54.0	100.0	29.3	100.0
DM (%)	49.3	—	47.5	—	40.6	—
NEL (MJ/kg DM)	7.17	—	6.75	—	4.79	—
NDF (% DM)	15.4	—	16.6	—	33.4	—
Starch (% DM)	28.6	—	27.2	—	—	—

Tab. 2: ANOVA results and temperature data at 84 h for all TMR × temperature combinations (ns = not significant; *p < 0.05; n.a. = not applicable)

TMR type	Temp. (°C)	F value	p value	Control (°C)	CF 4 l/t (°C)	Reduction
Post-partum	20	1.09	0.394 ns	n.a.	n.a.	n.s.
Post-partum	30	0.98	0.428 ns	n.a.	n.a.	n.s.
Lactation	20	0.24	0.792 ns	n.a.	n.a.	n.s.
Lactation	30	0.39	0.693 ns	n.a.	n.a.	n.s.
Dry cows	20	1.40	0.316 ns	n.a.	n.a.	n.s.
Dry cows	30	6.39	0.033*	44.4 ± 1.2	37.1 ± 4.1	−7.3 °C (38%)

Tab. 3: Visual assessment scores at 72 h / 30 °C and temperature increase above ambient (scale 0–3; CF = CitroFor; n.d. = not determined)

TMR type	Mould	Yeast	Heating (°C above ambient)	Note
Dry cows — Control	0/3	0/3	+12.5	Maillard risk
Dry cows — CF 4 l/t	0/3	0/3	+7.7	Safe < 40 °C
Lactation — CF 4 l/t	0.67/3	1.33/3	n.d.	Insufficiently protected
Post-partum — CF 2 l/t	n.d.	n.d.	n.d.	Critical time –10%

and yeast (score 1.33/3) contamination despite 4l/t application (Table 3). The abundant water-soluble carbohydrates provide an ideal substrate for rapid yeast proliferation that cannot be controlled by the doses tested. For post-partum TMR (highest energy density, 7.17 MJ NEL/kg DM), the 2l/t dose was counter-productive: the critical time to visible spoilage was shortened by approximately 10%, likely because a sub-inhibitory acid concentration stimulates rather than suppresses yeast metabolism.

The selective effect of CitroFor across TMR types can be explained by differences in buffering capacity and substrate availability. Dry cow TMR contains high structural NDF (33.4% DM) which physically limits oxygen diffusion into the mass and slows microbial activity to a level manageable by the applied acid mixture. The mechanism of action of citric acid – a polyprotic acid (pKa 3.13, 4.76, 6.40) – involves

chelation of metal ions essential for microbial growth and direct antimicrobial activity of undissociated molecules penetrating cell membranes (Li *et al.*, 2024). Formic acid (pKa 3.75) acts synergistically by rapid pH depression. The combination thus addresses both the metabolic and physical dimensions of spoilage (Rinne *et al.*, 2018).

These findings are consistent with Seppälä *et al.* (2013), who reported that TMR stabilizer response depends strongly on silage quality, and with Gheller *et al.* (2021), who found that 4l/t organic acid blends were optimal for maize silage-based TMR. The novel contribution of this study is the direct comparison across physiologically contrasting TMR types in a single experiment, revealing that dose recommendations cannot be universal.

CONCLUSION

CitroFor at 4l/t FM significantly improved aerobic stability of dry cow TMR at 30 °C, reducing temperature increase by 38% and eliminating visible mould and yeast growth. For lactating and post-partum TMR, the tested doses were insufficient or counter-productive due to high fermentable substrate content. The 4l/t dose is essential during high summer temperatures (>30 °C) for dry cow TMR; 2l/t may be appropriate under moderate barn conditions for less susceptible TMR types. TMR composition must be considered when selecting stabilizer dose. Repeated trials with larger replication and chemical analysis of water-soluble carbohydrate content are needed to refine dose recommendations.

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