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Research Article

The Effect of Supplementation with A Mixture of Organic Acids and A Mycotoxin Scavenger on Milk Composition and Metabolic Disease in Early Lactation Dairy Cows

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Abstract:
In the dairy sector, improving the metabolic health of dairy cows at the start of lactation is crucial to ensure productivity and animal welfare. With this in mind, this study aims to evaluate the effects of supplementation with a mixture of organic acids and a mycotoxin sensor on milk composition and metabolic health in early-lactation Holstein dairy cows. This research was carried out on fifty-six multiparous cows, randomly divided into two groups: a control group (n= 28) with no feed supplementation and an experimental group (n= 28), receiving a daily supplement of 40 g of additive per cow for three months. The additive was mixed by hand into the cows' daily ration. Milk composition was analyzed, and blood samples were taken from all cows for biochemical analysis. The results showed that supplementation had no impact on milk composition nor on aspartate aminotransferase (ASAT) and creatinine levels in either sample ($P > 0.05$). However, alanine aminotransferase (ALAT) and cholesterol levels were initially higher in the treated group ($P < 0.05$), but this difference was no longer observed in the second sampling. In conclusion, this study indicates that supplementation with organic acids and mycotoxin scavengers has no positive effect on dairy cows milk composition or metabolic health.

Keywords: Organic acids; Dairy cows; Early lactation; Calcium propionate; Malic acid; Metabolic diseases; Mycotoxin sensor.

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Introduction:

The dairy industry occupies a central position in global agriculture, providing a vital source of nutrition and making a significant contribution to the world's economies. In modern dairy farming, maximizing the health and production of dairy cows is crucial. However, the transition period, encompassing the three weeks before and after calving, represents a critical time for the health and production performance of dairy cows (Kong et al., 2024; Rissanen et al., 2023) and represents a major challenge for farmers and their animals.

During this critical phase, cows undergo significant physiological changes in their metabolism, exposing them to a high risk of metabolic disorders linked to energy deficits (Kour et al., 2024) due to reduced feed intake and increased energy requirements for milk production (Orellana Rivas et al., 2021; Rearte et al., 2023). These disorders include ketosis, ruminal acidosis and fatty liver syndrome, compromising animal health and longevity while reducing milk production and increasing veterinary costs.

To guarantee animal welfare, farm sustainability and the quality of the milk produced, it is essential to understand and prevent the diseases that affect them. New approaches are being researched, including dietary supplementation with probiotics, prebiotics, phytochemicals, enzymes and organic acids (Pearlin et al., 2020). According to several studies, organic acid supplementation from various sources, such as feed and microbial fermentation, offers a promising alternative for improving the health and performance of dairy cows (Chahardoli et al., 2020; da Silva Dias et al., 2021; Kennedy et al., 2020; Stokes and Goff, 2001). These contribute to maintaining rumen pH (Carro and Ungerfeld, 2015), promote nutrient absorption (Haque et al., 2009; Nguyen et al., 2020) and have antimicrobial properties (Seppälä et al., 2013), thus strengthening the animal's natural defences during this critical period.

However, feeding dairy cows entails risks, including contamination of mycotoxin (Penagos-Tabares et al., 2024). This can adversely affect the performance, milk quality and health of cows, causing problems such as liver, neurological and kidney disorders, as well as reduced appetite, nutrient absorption and the reproductive and productive capacity of cows (Chen et al., 2023; Fink-Gremmels, 2008; Gallo et al., 2020). Therefore, in addition to ensuring that cows are fed a diet adapted to their needs, it is essential to take measures to prevent this mycotoxin contamination and limit their transfer into the milk in order to ensure the safety of dairy products, such as the use of special binders (Gallo et al., 2020; Kiyothong et al., 2012; McGuffey, 2017).

This article examines the use of a mixture of organic acids and a mycotoxin scavenger, whose trade name is (Rumitox; Tecnoaditivos espanoles avanzados sl, Navarre, Spain), as a supplement for dairy cows in early lactation. This experiment tests whether supplementation with organic acids and mycotoxin improves dairy cows' metabolic health and milk quality.

Materials and methods:

Ethical approval:

The experiment was carried out in accordance with the guidelines laid down by the Directive 2010/63/EU of the European Parliament for Animal Ethics Committee for the use of animal experimentation.

Animals and experimental design:

The study ran from December 2022 to February 2023 and involved a herd of dairy cows in the Boussaâda region, located south of the Wilaya of M'sila, Algeria, some 234 km southeast of the capital. This area is renowned for its arid, temperate climate, characterized by hot, dry summers and mild winters, sometimes cold at night.The cows selected for the study were in good clinical health and had not received any drug treatment prior to the start of the experiment. A total of fifty-six Holstein dairy cows (n=56) were included in our study. They were randomly divided into two groups of twenty-eight cows: a control group and an experimental group, and the cows in both groups were kept under identical management conditions. Cows in the experimental group received a daily supplement of 40 g of the organic acid/mycotoxin scavenger mixture incorporated into their feed from the postpartum period until 60 days after calving. In contrast, no supplementation was given to the control group. All cows were milked twice daily, with a 12-hour interval between milkings. The owner uses a mechanical milking system provided by a mobile milking wagon.

Additive composition:

Rumitox is a purely biological feed additive for veterinary use. It comes in the form of a micronized powder designed to improve cow health. Its composition mainly comprises:

A mixture of organic acids and their salts, including sodium salt, malic acid, calcium propionate, calcium formate and calcium citrate.

Mycotoxin scavengers, such as yeast cell wall extracts, sepiolite and bentonite.

This product also contains propyl gallate and mineral salts.

Blood sampling and biochemical analysis:

Two blood samples were taken from each cow, one month apart. Blood was collected from the coccygeal vein using Vacuette® vacuum heparin tubes and immediately centrifuged at 3,000 rpm for 10 minutes. Plasma was separated and transported in a cooler to the analysis laboratory, where it was stored in a freezer until analysis. Biochemical analysis was performed at the medical biochemistry laboratory of Higher National Veterinary School, Algiers, Algeria. Standard plasma biochemical parameters, namely aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), cholesterol and creatinine, were measured using standard commercial kits (Spinreact, Sant Esteve De Bas (GI), Spain). Absorbances were measured using a spectrophotometer (Biochrom WPA Lightwave II, UK).

Milk sampling and analysis:
Milk samples were taken aseptically every month, after evening milking, at the same time as blood samples. Milk was collected individually in 50 ml sterile tubes. Immediately after harvesting, physicochemical analysis was carried out using a "LACTOSCAN SP Ultrasonic Milk Analyzer". Fat, protein, lactose, mineral, non-fat solids and total solids contents were measured. In addition, the fat: protein ratio (F: P) was calculated.

Statistical analysis of data:
Data entry and statistical analysis were carried out by comparing means using software (IBM SPSS Statistics 26). Data were not normally distributed and analyzed with the Mann-Whitney U test to assess group differences. In contrast, the Kruskal-Wallis test assessed differences between the two measurement times. Data are expressed as mean ± standard error of the mean; P <0.05 was considered statistically significant.

Results :

- **Blood metabolites and animal health:**

The mean values of ASAT, ALAT, creatinine, and cholesterol in dairy cows' blood plasma are shown in Table (1) each time. The addition of the acidifier showed no significant variation between treatment and control groups regarding ASAT concentration and creatinine either at time 1 (89.32 vs 80.78, p=0.193 for ASAT and 12.67 vs 12.38, p=0.533 for creatinine) or time 2 (56.25 vs. 43.21%, p=0.634 for ASAT and 12.33 vs. 11.72%, p=0.554 for creatinine). In contrast, ALAT and cholesterol concentrations were significantly higher in the treated group at time 1 (31.26 vs 26.56, p=0.009 for ALAT and 2.28 vs 1.84%, p=0.001 for cholesterol). However, this difference was no longer observed at time 2 (28.06 vs 27.46, p=0.857 for ALAT and 2.68 vs 2.85%, p=0.993 for cholesterol).
The cows included in the trial showed no cases of ketosis or milk fever during the study period. The incidence of acidosis was similar in both groups, with three cases observed in the control cows and three in those receiving the feed supplement.

Table 1: Effects of supplementation with a mixture of OAs and mycotoxin sensors on blood metabolites in dairy cows

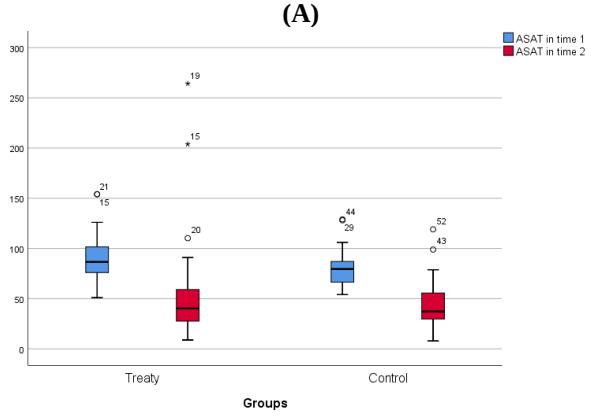
Parameters [1]	Treatment*		p value
	Control	Treaty	
ASAT (Ul/l)			
Time 1	80,78±3,60 [a]	89,32±4,89 [a]	0,193
Time 2	43,21±4,77 [a]	56,25±10,52 [a]	0,634
ALAT (Ul/l)			
Time 1	26.56 ± 1.30	31,26±1,09	0,009
Time 2	27,46±3,05	28,06±3,21	0,857
Cholesterol (g/l)			
Time 1	1,84±0,11 [a]	2,28±0,09 [a]	0,001
Time 2	2,85±0,20 [a]	2,68±0,14 [a]	0,993
Creatinine (mg/l)			
Time 1	12,38±0,38	12,67±0,28	0,533
Time 2	11,72±0,57	12,33±0,61	0,554

* Control (without additive), treated (with 40 g/cow/day of additive).

[1] ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; Time 1: represents the first sample; time 2: represents the second sample.

Values represent mean ± standard error.
Means in a column with a common exponent differ significantly (p<0.05)

(A)



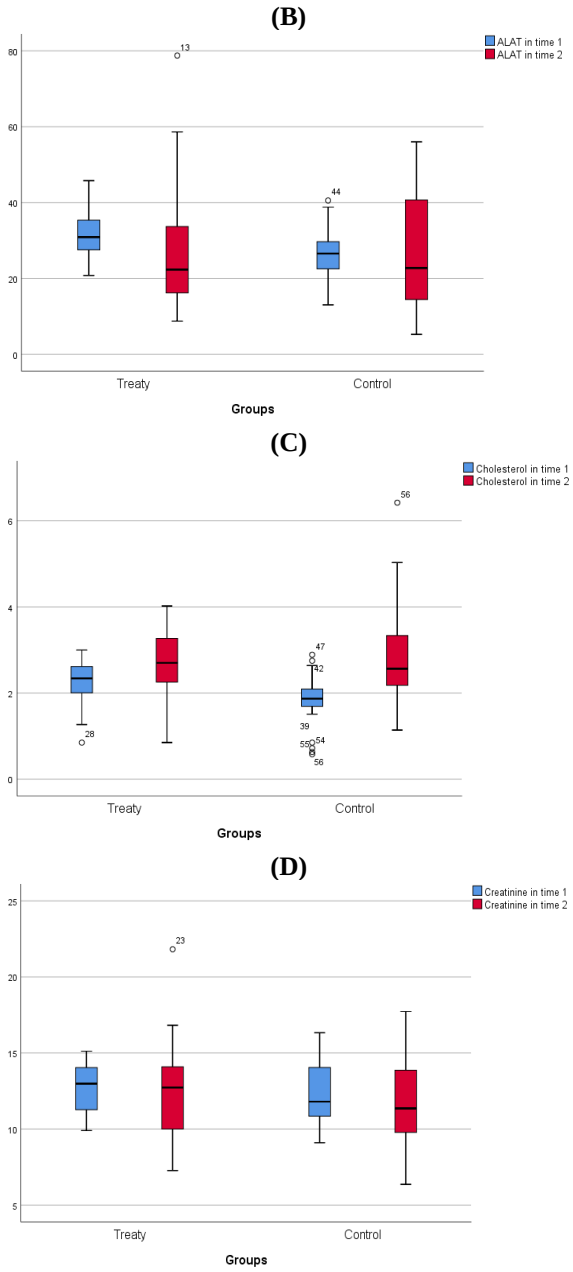


Figure 1: Box plot illustrating the effect of organic acid supplementation on levels of AST (A), ALT (B), cholesterol (C) and creatinine (D) in treated and control groups at times 1 and 2

• Milk composition :

Table (2) shows the acidifier and mycotoxin sensor supplementation results on milk composition. The results for acidifier supplementation show no significant variation between the treated and control groups for all milk components.

The data indicate that, for milk fat levels, no statistically significant difference was observed between the treated and control groups, either at time 1 (3.32% vs. 3.27%, p=0.83) or at time 2 (3.43% vs. 3.46%, p=0.83). However, for protein levels, although the difference between groups was not significant at time 1 (3.33% vs 3.36%, p=0.42), a downward trend was observed in the treated group at time 2 compared with the control group (3.31% vs 3.24%, p=0.12).

Lactose concentrations in milk tended to be higher for cows in the control group at each measurement, at time 1 (5.05% vs 5.13%, p=0.35) and at time 2 (4.99% vs 5.05%, p=0.99).

With the addition of the acidifier at time 2, the percentages of fat-free and total dry matter and the mineral content of the milk appeared to increase slightly. However, these differences were not significant (8.99% vs 8.87%, p=0.17), (12.49% vs 12.33%, p=0.56), and (0.74% vs 0.72%, p=0.10), respectively. Similarly, at time 1, there was no significant difference between the two batches for these parameters (9.19% vs 9.20%, p=0.68) and (12.43% vs 12.45%, p=0.99) (0.74% vs 0.75%, p=0.44) respectively.

Table 2: Effects of supplementation with a mixture of OAs and mycotoxin sensors on milk components in dairy cows

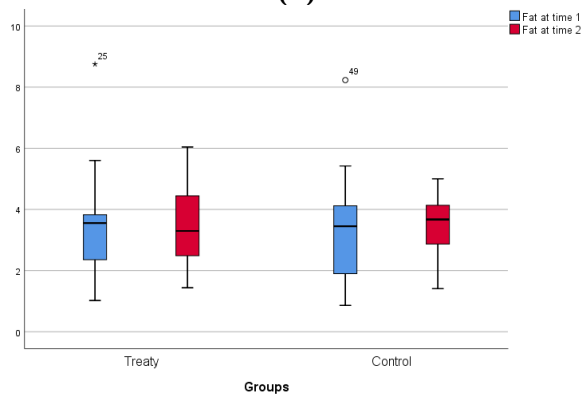
Parameters[1]	Treatment		p value
	Control	Treaty	
Fat (%)			
Time 1	3,27±0,30	3,32±0,30	0,83
Time 2	3,46±0,18	3,43±0,24	0,83
Protein (%)			
Time 1	3,36±0,03	3,33±0,02[a]	0,42
Time 2	3,24±0,02	3,31±0,03[a]	0,12
Fat:protein ratio			
Time 1	0,97±0,08	1,00±0,08	0,83
Time 2	1,07±0,06	1,04±0,07	0,65
Lactose (%)			
Time 1	5,13±0,07	5,05±0,05	0,35
Time 2	5,05±0,06	4,99±0,09	0,99
Non-fat solids (%)			
Time 1	9,20±0,09	9,19±0,09[a]	0,68
Time 2	8,87±0,06	8,99±0,12[a]	0,17
Total solids (%)			
Time 1	12,45±0,32	12,43±0,31	0,99
Time 2	12,33±0,17	12,49±0,22	0,56
Mineral matter (%)			
Time 1	0,75±0,01	0,74±0,01[a]	0,44
Time 2	0,72±0,00	0,74±0,01[a]	0,10

[1] Time 1: represents the first sampling; time 2: represents the second sampling.

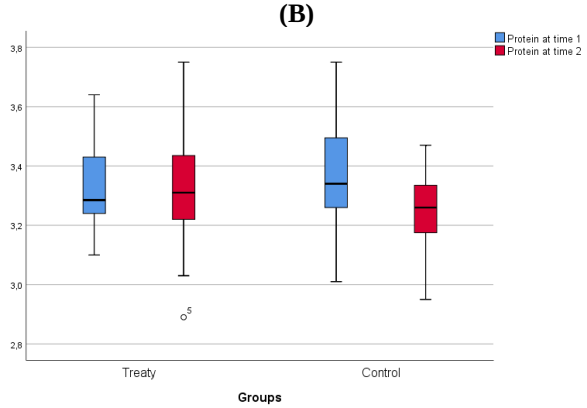
* Control (without additive), treated (with 40 g/cow/day of additive).
Values represent mean ± standard error.

Means in a column with a common exponent differ significantly ($p<0.05$)

(A)



(B)



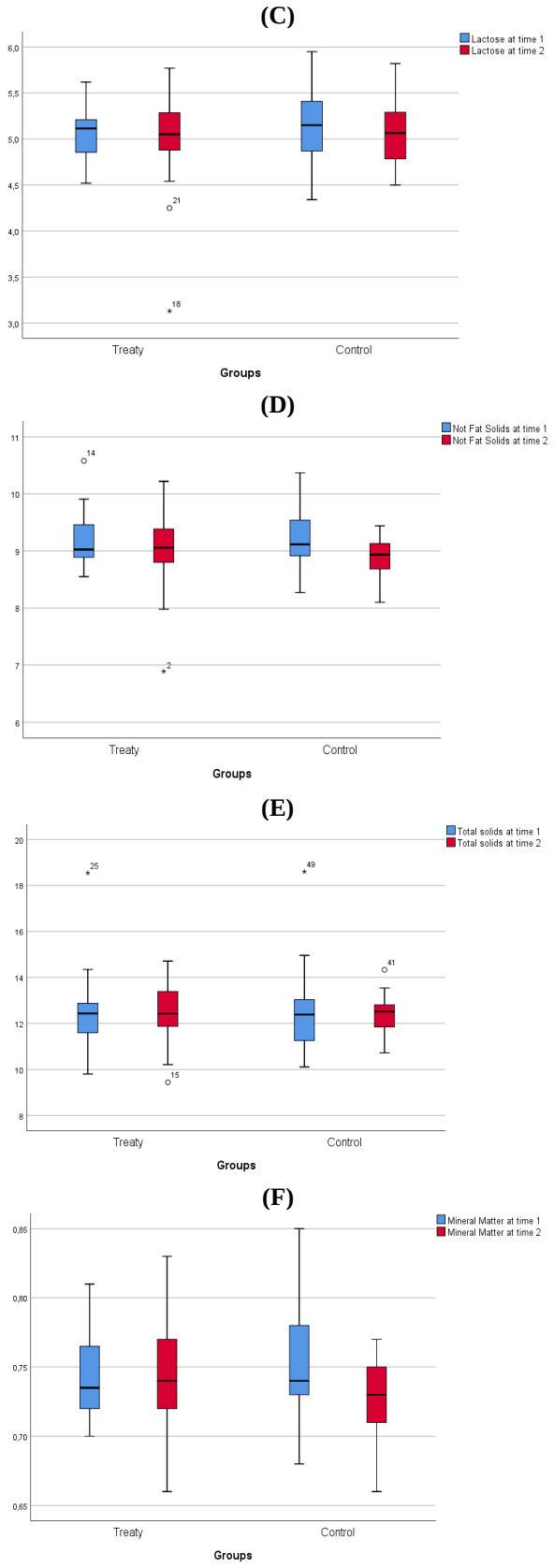


Figure 2: Box pot illustrating the effect of organic acid supplementation on levels of fat (A), protein (B), lactose (C), Non-fat solids (D), total solids (E), and mineral matter (F) in treated and control groups at times 1 and 2

Discussion:

Propionate, resulting from ruminal fermentation of starch and other organic matter, plays a vital role as the primary precursor of glucose, especially in the peripartum period (Larsen and Kristensen, 2013). Decreased appetite in the run-up to parturition can reduce the availability of propionate (Baird et al., 1980). The increasing use of non-metabolizable sources of

propionate that can be efficiently utilized in the rumen, such as calcium propionate, has been observed in recent years.

Feeding organic acids to dairy cows has already been described as an alternative way of improving their performance, particularly milk production and the percentage of fat, protein and lactose, even under stressful conditions (Ali et al., 2013; Fontoura et al., 2022). In this study, an acidifier as a glycogenic supplement was administered to dairy cows in early lactation to provide additional glycogenic substrates. Variations in milk component concentrations were anticipated. However, no significant differences were observed in protein and fat concentrations following treatment. These results are in line with previous studies conducted on calcium propionate supplementation of dairy cows (Liu et al., 2010; McNamara and Valdez, 2005; Zhang et al., 2022a, 2022b) as well as propionic acid supplementation (da Silva Dias et al., 2021; DeFrain et al., 2005) or malic acid (Devant et al., 2007; Kung et al., 1982; Vicini et al., 2003; Wang et al., 2009) which had no significant impact on milk components.

However, these results differ from those of some studies (Martins et al., 2019) and (Gheller et al., 2020) supplemented with calcium propionate and propionic acid, respectively, which reported significant differences. Other studies (El-Zaiat et al., 2019; Zhao et al., 2023), noted numerical but non-significant increases in protein and fat concentrations in milk. These inconsistencies could be linked to the composition of the diet adopted and the dose of organic acid used.

The post-partum period is associated with increased disease incidence, particularly in highly productive cows that have difficulty meeting their high energy requirements. This difficulty is exacerbated by a reduction in their feed intake, resulting in a negative energy imbalance. Studies have shown that organic acid supplementation improves energy status (Mandebvu et al., 2003; Wang et al., 2009). This translates into a reduction in health problems such as retained placenta, hypocalcemia, displaced abomasum and metritis, as observed in a study by (Stokes and Goff, 2001) where cows were given calcium propionate 4 hours before and 4 hours after calving. In practice, monitoring the energy balance of dairy cows is a crucial element in optimal herd management.

According to the literature, the evaluation of dairy parameters such as fat, protein and lactose offers a non-invasive and informative method for monitoring the health and productivity of dairy herds (Antanaitis et al., 2023; Atasever and Stádník, 2015; Rocchetti and O'Callaghan, 2021). This method is also a valuable indicator of the energy balance of dairy cows. Reduced protein and lactose levels in milk and a high fat : protein ratio are signs of an energy deficit (Sakowski et al., 2012). On average, the butterfat content varies between 3.5 and 5%, while the protein content varies between 3.0 and 3.5 (Cauty and Perreau, 2009).

Analysis of our study revealed that milk fat content, in both groups and at both measurement times, was below the recommended optimum values, suggesting possible rumen dysfunction or metabolic disease. Furthermore, it was observed that feed type influences milk composition. Furthermore, the decrease in fat content could be attributed to a low intake of forages due to their low fibre content (Garamu, 2019). Protein levels, on the other hand, are optimal.

In addition, the ratio of milk fat to protein also provides information on health status and the onset of certain metabolic disorders and can be used to detect cows suffering from an energy deficit (Antanaitis et al., 2023; Bunevski et al., 2020; Buttchereit et al., 2010; Jenkins et al., 2015). Ideally, a fat : protein ratio (F : P) should be between 1.2 and 1.4 (Čejna and Chládek, 2006), while ratios exceeding 1.5 increase the risk of ketosis, displaced abomasum, lameness and mastitis (Heuer et al., 1999; Klein et al., 2019) and those below 1.1 signal subacute rumen acidosis (Đuričić et al., 2020).

None of the cows in the trial developed ketosis during the study period. Our results concur with those of (Mandebvu et al., 2003), who observed similar results using an energy supplement containing propionic acid. In contrast, the study by (Stokes and Goff, 2001) reported one case of ketosis in the control group and two cases among animals receiving calcium propionate. However, three cases of acidosis were recorded in both the treated and control groups. These observations are corroborated by the F :P ratio of the two groups, which, according to the literature, is not in the ideal range. The presence of acidosis could confirm the decrease in fat levels, as it could result in less efficient digestion of rumen fibres when pH falls. However, it is from the end products of fibre digestion that the cow synthesizes milk fat.

This is in contrast to the studies carried out by Zhang et al. (2022a, 2022b) and Zhao et al. (2023), which reported that calcium propionate supplementation improved milk fat/protein ratios in the supplemented groups compared with the control group, but not significantly. These results indicate the effect of calcium propionate in reducing energy deficiency.

Malic acid supplementation influences lactate absorption by acting on the primary rumen bacterium, *Selenomonas ruminatum*. Once glucose has been depleted from the medium, this bacterium uses lactate as a source of carbon and energy (Sahoo and Jena, 2014), which converts into propionic acid. This reduces lactic acid levels in the rumen, preventing the severe pH drops responsible for acidosis (Carro and Ungerfeld, 2015; Sahoo and Jena, 2014). In addition, this improvement in the rumen environment promotes better utilization of the cellulosic portion of the ration and an increase in the butyrous rate. The emergence of cases of acidosis in the treated group could be attributed to an insufficiently adjusted supplement dose to obtain measurable effects on disease reduction.

Transformed into propionic acid in the rumen, calcium propionate is absorbed into the bloodstream and transported to the liver, which converts it into glucose. The latter is then taken up by mammary epithelial cells via a passive process that facilitates the synthesis of lactose, a crucial component of milk, and helps maintain the osmotic balance between the alveolar lumen and blood (Güner et al., 2022; Zhao, 2014). Our study

revealed lactose levels within physiological ranges for dairy cattle, averaging around 5% (4.8-5.2%) (Roca-Fernández, 2014). Thus, the presence of lactose in milk could be an indicator of healthy mammary glands.

In addition to its role in glucose production, calcium propionate may also contribute to the regulation of calcium balance and the overall health of dairy cows (Zhang et al., 2020). This contribution could explain the absence of milk fever cases observed during our study.

Liver function is vital in maintaining dairy cows production performance in early lactation. This function can be assessed by measuring various enzymes, notably ALAT and ASAT. Elevated serum levels of these enzymes often indicate hepatocyte damage and impaired liver function (Puppel and Kuczyńska, 2016; Sakowski et al., 2012).

Our study showed ALAT activities above the usual mean values (15.58-23.24 U/L) in both groups, suggesting hepatic steatosis. This finding aligns with the study conducted by (Du et al., 2018), which revealed elevated serum ALAT levels in cows with hepatic steatosis (24.7-32.96 U/L).

According to Tainturier et al. (1984), ASAT activity can show irregular and small variations during gestation and early lactation. Maximum activity is observed at the start of lactation, followed by a gradual decrease as lactation progresses (Sakowski et al., 2012; Stojević et al., 2005). This is consistent with the results of our study, which showed a significant decrease in ASAT activity between time 1 and time 2.

Conclusion:

This mixture of organic acids and mycotoxin scavengers had no effect on milk composition, particularly fat, protein, and lactose levels, or creatinine and liver enzymes like ASAT in dairy cow plasma at the start of lactation. The therapy group started with slightly increased ALAT and cholesterol concentrations, but these changes did not last. These findings recommend more investigation with other supplement doses or a larger animal population. This would help us evaluate this approach's potential impact and make more exact dairy breeding and feeding recommendations.

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