Impact of a feed additive (acidifier and toxin-binder) in milk production in dairy cattle

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Abstract

Dairy cow feeding plays an important role in milk production. The present study was conducted to evaluate the effect of a feed additive (association of acidifier and mycotoxin binder) on milk production in dairy cattle. For this purpose, 22 cows belonging to three breeds (Montbeliard, Holstein, and Flekveih) were used. The cows were divided into two groups; a control group with seven cows and an experimental group with 15 animals. The results showed that the additive had a positive effect on milk production (23.14±5.87 litres for the experimental group *vs* 18.00±6.90 litres for the control). The additive also had a good effect on the percentage of sub-clinical mastitis: the Californian Mastitis Test (CMT) carried out at monthly intervals showed a clear improvement in the udder health of females in the experiment with 13 positive samples in the first test for nine cows (with four affected teats) and 10 positive samples for the second test in three cows (with three affected teats). Moreover, statistical tests revealed a significant difference in the mean fat content (35 g/L *vs* 23.86 g/L, respectively) while the average Faeces Consistency Score and Dornic acidity was lower in the experimental group compared to the control (2.23 *vs* 3.21 and 13.83 *vs* 16.14, respectively). These results show the importance of incorporating the feed additive into the diet of dairy cows and the need to implement an extension programme and zootechnical supervision of all actors in the sector to ensure the quality of milk production and the performance of dairy cows.

Key words: *dairy cow; milk; organic acidifier; sub-clinical mastitis; scores*

Introduction

Algerians consume nearly 4 billion litres of milk each year (MADR, 2017), though domestic farms do not even cover a third of this consumption. The "Milk" issue has never left the Algerian news since independence (Meribai et al., 2016). Algeria has long resorted to imports to ensure the supply of the local market with basic necessities (milk, cereals, sugar, oils). Today, the country is the world's seventh

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[#] These authors have contributed equally in this work largest importer of dairy products and these products represent 24% of African dairy imports by value (Mamine et al., 2021).

Many efforts have been made by the various actors in the sector; a policy of importing high-performance heifers has been initiated, with the essential aim of filling the deficits in dairy production and meeting the growing demand for milk consumption (Meklati et al., 2020; Lamari et al., 2021). However, this restocking has not achieved the yields expected by the public authorities due to a lack of technicality, fodder unavailability (especially green), and the poor acclimatization of these animals to local breeding conditions (Merdaci and Chemmam, 2016).

Almost all milk production comes from dairy cows. These cows cannot produce milk without reproducing due to physiological interactions between lactation and reproduction (Ghozlane, 2003). Milk production and reproductive performance are two major determinants of dairy cow profitability. The goal of each breeder is to have one calf/lactation/year. Nevertheless, the conduct of these functions requires a good mastery of the management of rationing and reproduction, both at the zootechnical level and at the prophylactic and veterinary level (Mimoune et al., 2017).

In this context, and in light of the mediocre performance of domestic farms, an important question arises within dairy cattle breeding. It is essential to look into the conditions of these farms because a global vision of their structures is necessary. In order to improve performance, many studies have focused on the nutritional additives to animal feed, which, when administered in adequate quantities, produce health benefits (Bouchicha et al., 2022). These additives are recognised as alternatives to antibiotics, their use is credible in modern production systems. The intake of these substances in the diet of ruminants has shown variable effects on zootechnical performance and on individual resistance to pathogens (Đuričić et al., 2017; Mimoune et al., 2021).

The objectives of this study were to evaluate the effects of a feed additive composed of a mixture of organic acids and a mycotoxin scavenger on milk production in dairy cows and their performance in the study area based on the recording of milk production, the evaluation of BCS, faeces consistency score, cleanliness score, and the detection of mastitis in the animals included in the study.

Material and methods

Ethical statement

All animal studies were conducted with the utmost regard for animal welfare, and all animal rights issues were appropriately observed. No animal suffered during the course of this research. All experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA. 14).

Study area

The municipality of Guellal in the Setif region is the area chosen for our study, as it represents an important dairy basin (Figure 1). It is characterised by a semi-arid climate, with hot, dry summers and harsh winters. The rains are insufficient and irregular both in time and space. The Babor Mountains receive the most precipitation, about 700 mm per year, and this decreases appreciably to only 400 mm on average per year on the high plains. On the other hand, the South-South-East zone is the least watered, with rainfall not exceeding 300 mm. The average temperature varies by season, from 6.1°C in January, the coldest month, and 26.8°C in July, the hottest month.

The farm is characterised by dairy breeding, it has hard livestock buildings of 225 m^2 and a park of 450 m^2, the floor is concrete and ventilation is respected. The stall is of the semi-shackled type. Feed (concentrated) is stored in a container near the building and hygienic conditions are relatively good.

Figure 1. Geolocation of the Guellal zone in Algeria

Animals

This study included 22 dairy cows, two of the Holstein, 19 Montbeliard, and one Fleckvieh breeds. Cows were divided into two groups; an experimental group (15 cows) and a control group (seven cows). These animals were selected ensuring that they were as homogeneous as possible with regard to average age (5 years), average weight (650 kg), average milk production level (around 22 kg), and average parity (3 parturitions) per cow.

They received the same feed (concentrated livestock feed dairy cow SIM composed of: wheat bran, soy hull, corn, soybean meal, calcium carbonate, rapeseed, soybean oil, dairy cow CMV 1%). Each cow received 10 kg/day as well as 2 kg of wheat bran/ day and 6 kg of straw in the evening.

Milk sampling

Milk samples were taken during each visit. They were collected individually at 5 p.m., after cleaning the udder and eliminating the first jets. An equal amount of milk was sampled from each quarter, stored in clean 60 mL bottles until physico-chemical analysis (each bottle was labelled with the identification number of the cow sampled). Once the milk was collected, it was transported to the analysis laboratory within 2 hours of collection, under isothermal conditions at 4°C.

Physico-chemical analysis of milk

In this study, fat content (Butyrometer), pH (pH meter), and Dornic acidity were assessed (Figure 2).

Feed additive

The feed additive (Rumitox) is composed of sodium salt of malic acid, malic acid, calcium propionate, calcium formate, Yeast cell wall extract MOS ET 1.3 1.6 beta glucans, sepiolite bentonite and Kieselgur, propyl gallate and calcium citrate, and mineral salt.

It was administered in the diet of dairy cows in the form of four scoops, either the equivalent of 40 g per day in a single dose, or in two doses of 20 g (one in the morning and the second in the evening mixed into the feed).

Experimental design

During the day, cows were at pasture and grazed on grass. They were milked twice daily, at 7 am and 5 pm, when they received the concentrated feed.

Cows were subjected to a clinical examination (general examination) (temper-

Fat analysis equipment used | PH meter (Jenway model 3305 pH meter) | Dornic acidity equipment

Figure 2. Material for physico-chemical analysis of milk

ature, respiratory examination, and pulse) and a thorough examination of the udder.

All cows were evaluated twice at a monthly interval for the following scores: BCS (Body Condition Score), faeces consistency score, cleanliness score.

The CMT test was carried out to detect infected quarters very early, thus limiting the risks associated with sub-clinical mastitis. It gives an indication of the quantity of somatic cells present in milk. This test will only react visibly from a rate of 400,000+ cells (Durel et al., 2004).

The recording of milk production was also carried out twice during the experiment.

The distribution of the cows was carried out according to the stage of lactation:

Stage I: Beginning (Start) of lactation (1-4 months of lactation).

Stage II: Mid-lactation (4-7 months of lactation).

Stage III: End of lactation (more than 7 months of lactation).

The BCS of dairy cows was assessed at each visit, according to the method developed by Edmondson et al. (1989). The scale varies from 1 (extremely lean cow) to 5 (extremely fat cow).

The cleanliness score and the faeces consistency score were assessed at each visit for all cows. They were carried out according to the Scoring methods (Delaval, 2006; Aytekin et al., 2020).

Statistical analysis

Statistical analysis of the results obtained from the experimental and control groups regarding the scores and the milk parameters was performed using the Student test (and Wilcoxon-Mann-Whitney test for the pH and the milk production) with the SPSP statistics software, at a significance level P at 5%. Data were represented by mean ± standard deviation.

Results

Our data are presented as follows:

- A descriptive study of the variables used on the farm during the implementation of the experimental protocol, with two visits at a one-month interval, specifying the physiological stages of the cows: notations of health scores (BCS, faecal matter consistency score, cleanliness score), milk production, and CMT test.
- A statistical study highlighting the effect of organic acids and mycotoxin sensor on the different performances studied, by comparisons between the

results of the variables obtained from the two groups (experimental and control), during the 1st and 2nd visits.

Descriptive study of the variables

The scores enable an evaluation of the digestive function, in particular the BCS, and the faeces consistency score, as well as cleanliness score were assessed on a grid ranging from a low score of 1 to a high score of 5.

Average scores

During the first visit, the difference between scores was not significant except for milk production, which was higher in the experimental group than in the control. During the 2nd visit (after 4 weeks), the BCS the cleanliness score and the milk production of the experimental group were higher than that of the control animals. The faeces consistency score of the experimental group decreased in comparison to that of the control (Table 1).

BSC and faeces score by stage of lactation

During the 1st visit, the BCS of the control group was 2.7 at the start of lactation and 3.5 in the middle of lactation, and decreased to 2.8 at the end of lactation. In the experimental group, the average values were of 2.8, 3.0, and 2.8 according to the stage of lactation, respectively. In parallel, for the control group, the faeces score was 2.5 at the beginning of lactation, 3.0 in the middle, and 3.5 at the end of lactation. In comparison, in the experimental group, the faeces score was of 2.0, 2.7 and 2.3 following the stage of lactation, respectively.

During the 2nd visit, the BCS of the control group at the start of lactation was 2.5, increasing to 3.5 in mid-lactation, and then decreasing to 2.7 at the end of lactation. On the other hand, for the experimental group, it was 2.8 at the beginning of lactation, , increasing to 3.1 in mid-lactation and remained at the same level at the end of lactation (3.0). In addition, the faeces score of the control group remained the same in early and mid-lactation at 3.0 and increased to 3.5 at the end of lactation. For the experimental group, this score was the same at the beginning and at the end of lactation with a score of 2.0 and increased to 2.5 in the middle of lactation (Figure 3).

CMT test according to stage of lactation

During the first visit, the CMT test showed that at the start of lactation, three cows presented sub-clinical mastitis with

Table 1. Mean scores (BCS, faeces consistency, cleanliness) and milk production during the 1st and 2nd visits

	Mean	BCS	Faeces consistency score	Cleanliness score	Milk production (L)
1st visit	Control	2.82 ± 0.59	3.00 ± 0.91	2.07 ± 1.06	18.43 ± 7.18
	Experimental	2.9 ± 0.71	2.43 ± 0.84	2.57 ± 1.10	22.93 ± 5.75
	P value	0.78	0.19	0.33	0.17
2nd visit	Control	2.71 ± 0.64	3.21 ± 0.64	2.21 ± 0.95	18.00 ± 6.90
	Experimental	2.98 ± 0.76	2.23 ± 0.68	2.43 ± 1.12	23.14 ± 5.87
	P value	0.4	0.006	0.64	0.14

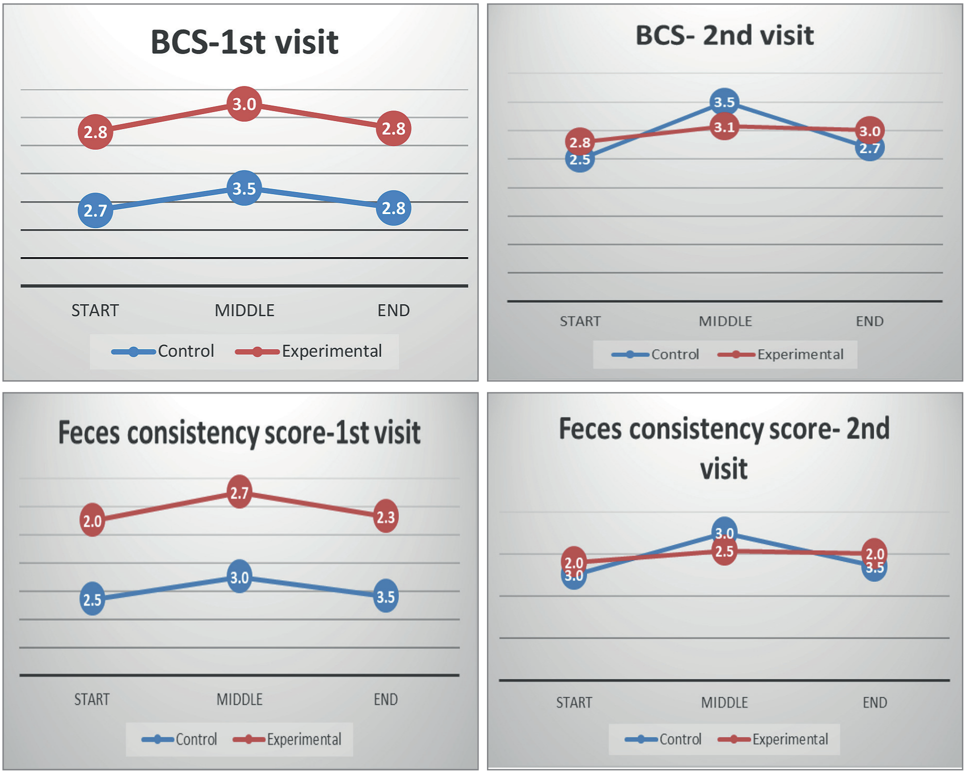

Figure 3. Evolution of the BCS and the faeces consistency score in the two groups according to the stage of lactation during the 1st and 2nd visit.

four teats affected. In the middle of lactation, one cow had sub-clinical damage in all four teats, and at the end of lactation, three cows had sub-clinical mastitis (two cows with four affected teats and one with one affected teat). On the other hand, in the experimental group, at the start of lactation, three cows showed sub-clinical mastitis with four affected teats; in mid-lactation, five cows were affected by sub-clinical mastitis, two cows with four teats affected, and three cows with one teat affected. At the end of lactation, five cows were affected by sub-clinical mastitis, four cows with four affected teats and one cow with three affected teats.

During the second visit (after one month), the CMT test showed the following: at the start of lactation, three cows showed sub-clinical mastitis, one with four affected teats, one with three affected teats, and one with one affected teat. In the middle of lactation, one cow was affected by sub-clinical mastitis of all four teats, and at the end of lactation, three cows had sub-clinical mastitis (two with four affected teats and one with one affected teat). On the other hand, in the experimental group, at the start of lactation, two cows presented sub-clinical mastitis each with one affected teat. In the middle of lactation, three cows showed sub-clinical mastitis, two with one affected teat, and one with two affected teats. At the end of lactation, five cows presented sub-clinical mastitis, three with three affected teats and two with one affected teat (Table 2).

Milk physico-chemical parameters, milk production, and cleanliness score comparison

During this study, we compared the effect of sub-clinical mastitis and cleanliness score on milk production and the physico-chemical quality of milk (Fat content, Dornic acidity, and PH) according to the stage of lactation (performed only during the 2nd visit).

According to our data, in the control group at the start of lactation, the cows had an average of 23.0 L/day with a fat content of 17.33 g/L and a Dornic acidity of 17.67°D, and a cleanliness score of 2.50. In the middle of lactation, the average milk production was 18 L/day with a fat rate of 17.00 g/L and a Dornic acidity of 14.0°D with a cleanliness score of 2. At the end of lactation, the average milk production was 10.50 L/day, fat content of 32.67 g/L, Dornic acidity at 15.33°D and cleanliness score of 2 (Table 3).

In the experimental group, at the start of lactation, the cows had an average milk production of 26.67 L/day with

Table 2. Number of cows positive by the CMT test according to stage of lactation during the 1st and 2nd visit.

CMT		Beginning	Middle	End
Control	1st visit	3/3	1/1	3/3
	2nd visit	3/3	1/1	3/3
Experimental	1st visit	3/3	5/7	5/5
	2nd visit	2/3	3/7	5/5

Table 3. Mean values of milk production, fat, CMT test, and cleanliness score of cows in the control group (2nd visit).

Stage of lactation	Milk production (L)	CMT (+)	Cleanliness	Fat g/L	Acidity °D	PH
Beginning	23.00	3/3	2.50	17.33	17.67	6.70
Middle	18.00	1/1	2.00	17.00	14.00	7.08
End	10.50	3/3	2.00	32.67	15.33	6.71

Table 4. Mean values of milk production, fat, CMT, and cleanliness score of cows in the experimental group (2nd visit)

Stage of lactation	Milk production (L)	CMT (+)	Cleanliness	Fat g/L	Acidity °D	pH
Beginning	26.67	2/3	2.50	30.00	15.00	6.79
Middle	26.67	3/7	2.50	32.17	14.17	6.79
End	16.80	5/5	1.50	45.67	12.00	6.81

Table 5. Comparison of milk parameters between the control and the experimental groups.

Group	Fat (g/L)	Acidity	PH
Control	23.86 ± 14.43	16.14 ± 2.54	6.83 ± 0.15
Experimental	35±12.9	13.83 ± 2.12	6.79 ± 0.08
P value	0.01	0.02	0.26

a fat content of 30.0 g/L, a Dornic acidity at 15.0°D and a cleanliness score of 2.5. In the middle of lactation, the average milk production was 26.67 L/day with a fat level of 32.17g/L, a Dornic acidity at 14.17°D and a cleanliness score of 2.5. At the end of lactation, the average milk production was 16.80 L/day with a fat rate of 45.67 g/L, a Dornic acidity of 12.0°D and a cleanliness score of 1.50 (Table 4).

According to the results presented in Table 5, and after the comparison of the milk parameters in the two groups, statistical analysis revealed a significant difference in fat content (higher in the group of animals receiving the additive) and a lower acidity compared to the control group.

Discussion

The main objective of this study was to evaluate the mean scores (BCS, faeces, cleanliness), milk production, and detection of mastitis by CMT test in dairy cows receiving a feed additive. It also aimed to establish the relationships between the variable parameters and the physiological stage concerned.

Our results about the BCS evolution showed that the cows of the experimental group had a relatively good average BCS compared to the control cows. At the time of parturition, the weight was higher in the first month compared to the 2nd and 3rd months of lactation. This may be due to the fact that at the start of lactation and heading towards the peak at the 1st, 2nd and 3rd month, the cow, especially if a high producer, generally does not manage to consume enough dry matter (DM) because milk exports exceed its ingestion capacity and the peak of milk production comes before the peak of DM voluntary consumption. Therefore, the cow needs to draw on the fat reserves to meet this need, losing weight and therefore entering a negative energy balance, resulting in a decrease in BCS and this lasts as long as the cow exports fat reserves (Mammouri and Bensalem, 2021).

At the milk level, we first observed a high butyric rate, which then decreased towards the peak and during the plateau phase (by the dilution effect with the increase in milk production and by the decrease in intraruminal PH which promotes propionic fermentation, more favourable to enhance microbial protein synthesis) (Kononoff and Heinrichs, 2003), and was finally raised as milk production decreased and the consumption became more and more important, exceeding the needs at the end. This allowed the cow to regain condition, returning the energy balance to a positive state, and the BCS improved to again reach that of the moment of parturition before the beginning of the dry period.

In general, the decrease in BCS is accompanied by the production of ketone bodies leading to a state of sub-clinical acetonemia, which results in an immunosuppression leading to the cases of sub-clinical mastitis revealed in our data (all seven cows with a positive CMT test for the control group and 10 of 15 cows tested positive for CMT in the experimental group).

The comparison of the milk from the experimental group supplemented with the feed additive to that of the non-supplemented control group living under the same conditions and consuming the same food, showed a better evolution of the BCS, which decreased less and for a shorter period. This can be explained by the action mechanism of the various components including in the additive. Indeed, this additive contains a mixture of acidifiers and mycotoxin sensors which, although acting at different levels, can have a synergistic effect.

It is well known that the organic acids possess a crucial role in enhancing the immune system (Pearlin et al., 2020). In the current study, the acidifier contains propionic and malic acid. Propionate is one of the most important organic acids (Antone et al., 2023) and it constitutes the primary glucose precursor for ruminants (Maldini et al., 2019), which is transformed into glucose in the liver. Once in the udder, this glucose is transformed into lactose and therefore directly impacts the quantity of milk (Nielsen and Ingvartsen, 2004). Similarly, dietary lipids are primarily composed of triglycerides. They are first hydrolysed by microorganisms to give fatty acids and glycerol. This is then metabolised into propionic acid. After hydrolysis, part of the fatty acids is adsorbed on food particles and on microbial membranes. Bacteria can then either store them as free fatty acids or incorporate them into cellular structures as phospholipids. Unsaturated fatty acids are hydrogenated. Due to the anaerobic conditions of the rumen, fatty acids are practically not used as an energy source. A small amount is metabolised into ketone bodies by the rumen epithelium. The absorption of long fatty acids is very low in the rumen (Jouany, 1994).

Malic acid plays a significant role in the succinic acid-propionic acid metabolic pathways in the rumen, and it has an antibiotic-like role that inhibits the growth of harmful microorganisms (Feng et al., 2022). It exerts an effect on the main bacterium of the rumen, in this case by consuming malic acid, which stimulates the use of lactate and its transformation on propionic acid (propionate precursor); this in turn has an effect on milk production and on lactic acidosis (Cao et al., 2021).

The improved ruminal environment allows for better use of the cellulosic part of the food with butyric rate improvement (Nisbet and Martin, 1990). Specifically, it increases total volatile fatty acids, and the propionate to acetate ratio, and can reduce methane emissions by competing with hydrogen (Abdelrahman et al., 2019; Sun et al., 2023). In this context, Vyas et al. (2020) reported that cows with subclinical acidosis receiving malic acid supplementation improved voluntary dry matter intake, feed efficiency, milk production, and milk fat content, as also revealed in the present study.

The better feed efficiency results in a reduction in faeces emissions with a positive impact on the immediate environment of the animals. A cleaner environment leads to a better cleanliness score and therefore less of sub-clinical mastitis and an improvement in the CMT results with more milk and more useful matter, where we noticed the link between the cleanliness score, CMT, Dornic acidity (Dornic degree), and consequently the milk production and the fat content of the milk and pH.

Indeed, the litter constitutes an important reservoir of microorganisms responsible for mammary infections. The main source of environmental mastitis is the milieu in which cows live: mammary infection occurs between milkings from bacteria in the floor or litter, while the teat canal is still open (Mimoune et al., 2021; Saidi et al., 2021).

It was also found that despite the high heat that cows were subjected to while at pasture during part of the experimental period, the cows did not decrease their milk production (although this production decreased in earlier years during this period), and that the persistence of production proved to be significantly better in the experimental group compared to the control group. On the other hand, according to Hristov et al. (2007), the negative effect of climate can cause a decrease in milk yield of 3 to 10%. Furthermore, West (2003) reported that milk production decreases by 33 to 50% when the ambient temperature exceeds 35 to 40°C and, when such increased temperatures are accompanied by high humidity in dairy cattle barns, this results in lower forage consumption and milk production quantity and quality.

The observation of the faecal score in order to evaluate the quality of digestion, showed an improvement in the consistency of faeces (fewer undigested particles in these materials), and the scores were close to the standards in all the stages. At the beginning of lactation, the faeces were rather liquid in the control group, and less liquid in the experimental one. This is explained by the fact that in the rumen, lactic acid is consumed by the bacteria in the experimental group, and osmotic pressure is reduced n the rumen. Consequently, there is less liquid faeces, with slower transit, allowing better absorption with better production and less faeces emission (less liquid). Thus, it provides a cleaner environment for cows (with a better cleanliness score, less mastitis and consequently more milk production).

Conclusions

In view of the results obtained, it is clear that the effect of the additive was positive. Indeed, overall milk production increased for the entire herd, but particularly for the experimental group, regardless of the physiological status of the cows, whether they were at the start, middle or end of lactation. The sanitary state of the milk showed a clear improvement between the beginning and end of the experiment. The additive improved the efficiency of feed digestion, and improved the immune status of cows on the one hand (more energy and amino acids available for the cow, as well as minerals, vitamins and others), and less waste emission on the other, therefore making the environment cleaner and less hostile, with the consequence of more protection and less aggression and mastitis. This improved health status obviously is positively passed on to production. Therefore, we can consider for this preliminary trial that the experimented additive made up of natural products had the expected effect on milk production and cow health. Therefore, it is important to incorporate it into the feed of dairy cows to improve both production and performance.

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Učinak dodatka hrani (zakiseljivača i sredstva za vezivanje toksina) u proizvodnji mlijeka u mliječnih krava

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Hranidba mliječnih krava je vrlo važna u njihovoj proizvodnji. Ova studija je provedena za procjenu učinka dodatka hrani (kombinacije zakiseljivača i sredstva za vezivanje mikotoksina) na proizvodnju mlijeka u mliječnih krava. U tu su svrhu praćeme 22 krave: 3 pasmine (montbeliard, holstein i flekveih). Krave su podijeljene u 2 skupine: kontrolnu skupinu sa 7 krava i eksperimentalnu skupinu s 15 životinja. Dobiveni rezultati pokazali su da organska kiselina ima pozitivan učinak na proizvodnju mlijeka (23,14±5,87 litara za eksperimentalnu skupinu u usporedbi s 18,00±6,90 litara za kontrolnu skupinu). Uz to, aditiv je imao i pozitivan učinak na postotak subkliničkog mastitisa. Kalifornijski mastitis test (CMT) proveden u razmaku od 1 mjeseca za životinje pokazao je jasno poboljšanje zdravlja vimena krava podvrgnutih istraživanju s 13 pozitivnih uzoraka u prvom testu za 9 krava (s 4 zahvaćene sise) i 10 drugih pozitivnih uzoraka za drugi test u 3 krave (s 3 zahvaćene sise). Statistički testovi su otkrili značajnu razliku između prosječnog udjela masnoća (35 g/L u usporedbi s 23,86 g/L) dok je prosječno bodovanje konzistencije izmeta i kiselost po Dornicu bilo niže u istoj skupini u usporedbi s kontrolnom skupinom (2,23 u usporedbi s 3,21 i 13,83 u usporedbi s 16,14). Podatci su pokazali važnost i potrebu dodatka hrani u hranidbu mliječnih krava i potrebu za provedbom programa proširenja i zootehničkog nadzora svih aktera u sektoru da bi se osigurala kvaliteta proizvodnje mlijeka i učinkovitost mliječnih krava.

Ključne riječi: *mliječna krava, mlijeko, organski zakiseljivač, subklinički mastitis, bodovanje*