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EFFECT OF THE SYMBIOTIC ON SUBCLINICAL MASTITIS IN DAIRY CATTLE AND IMPROVEMENT OF MILK COMPOSITION

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ABSTRACT

Mastitis, an inflammation of the mammary gland, can be clinical or subclinical; it is one of the most important multifactorial pathologies. The current research aimed to evaluate the impact of a symbiotic on subclinical mastitis in dairy cattle and its effect on milk quality. Two milk samples were taken from two groups of dairy cows of different breeds, particularly Montbéliard, Flekvi, and Holstein, all fed the same ration. The cows were divided into two groups: an experimental group of 18 cows that received the symbiotic "SYMBIOVEBA®" as an alternative treatment and 10 cows served as a control group. Subclinical mastitis was assessed using the California Mastitis Test (CMT), and milk quality was analyzed with the Lactoscan. The symbiotic's impact on preventing as well as reducing subclinical mastitis in dairy cattle, and on milk quality was evaluated. According to the obtained results, the symbiotic caused a modification in the composition of milk, characterized by a nonsignificant increase in butyrous content. These results suggest that the use of the symbiotic has a positive impact on subclinical mastitis. The change in milk composition, with a nonsignificant increase in butyrate, may indicate an improvement in udder health and a reduction in the symptoms of subclinical mastitis. This research highlights the potential importance of using symbiotics as an alternative treatment for subclinical mastitis and providing a foundation for future studies and interventions to improve dairy cattle health and milk quality

Keywords: Subclinical mastitis, symbiotic, physico-chemical parameters, dairy cattle, CMT.

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INTRODUCTION

Livestock farms are faced with several major challenges, including diseases affecting the reproductive system, and mobility problems such as lameness, and mastitis, which is one of the most worrying conditions in cattle farming. These diseases have considerable economic consequences, require extra labor, and significantly affect animal welfare (Rushen *et al.*, 2001; Seegers *et al.*, 2003; Coulon *et al.*, 2005).

Given its significant global economic impact, mastitis is the subject of considerable investment for its prevention, treatment, and control. Subclinical mastitis, in particular, is the most damaging form of the disease for dairy farms, causing great concern for both the dairy cattle and their owners. Significant financial resources are allocated to this disease, in recognition of its detrimental effects on dairy herds (Rémy; 2010).

Subclinical mastitis is characterized only by increased levels of leukocytes and epithelial cells, detected by various cell count tests. These tests are essential for assessing the health of the udder and guaranteeing the quality of milk intended for consumption. It is therefore essential to carry out systematic and regular monitoring on dairy farms to detect cases of subclinical mastitis.

In Algeria, as in many other countries, mastitis is a concerning disease due to its pathological effects. Although milk production has grown remarkably over the last decade, Algeria has still not achieved a sufficient level of milk production, despite considerable efforts. Consequently, guaranteeing the hygienic quality of milk depends on the health of the mammary glands (Gabli, 2005).

Most Algerian dairy herds do not undergo regular milk testing, which explains the high incidence of subclinical mastitis

(Beroual, 2003). The consequence of this mastitis is a reduction in milk production (Seegers, 2003). However, the losses associated with cases of subclinical mastitis are much more serious. They include a persistent reduction in milk production, impaired lactation performance in infected cows (Wattiaux, M. 2000), problems with raw material processing (Le Maréchal, 2011), premature culling of animals with subclinical mastitis (Seegers, 2003), financial penalties linked to milk quality based on somatic cell count (SC), extra work for the farmer and high treatment costs (products and veterinary expenses) (Barkema, 2006).

The prevention and treatment of these diseases depend mainly on the use of antibiotics, but this has a negative impact on milk quality, and their efficacy is still limited (Bouchard, D. 2013).

The main aim of this experiment is to explore the potential of the symbiotic in terms of reducing the prevalence of subclinical mastitis, as well as its impact on milk quantity and quality. This study aimed to determine and evaluate the possible effects, of SYMBIOVEBA®, as an alternative treatment for subclinical mastitis, on milk production in cattle.

MATERIALS AND METHODS

Area and period of study

The study was carried out in the semi-arid region of the wilaya of M'Sila which lies 256 km to the south-east of the capital of Algeria, at the farm and physicochemical laboratory of the Hodna dairy. The survey was carried out between March and June 2023.

Study population

Twenty-eight dairy cows of different breeds (7 Montbeliarde, 19 Holstein, and 2 Fleckvieh) were selected for this study. They were clinically healthy, had 2 lactations, and had not received any



Figure 1: The location of the study area (Merniz 2018)

medication in the period before the experiment. The selected cows were randomly divided into two batches to compare the effect of the symbiotic on the quantity and quality of milk from the two batches:

- A control batch: consisting of 10 dairy cows subjected to the same operations without symbiotic administration.

-An experimental batch of 18 dairy cows received 50 ml of symbiotic feed once a month at fixed times. The symbiotic treatment lasted two months.

All cows are clinically healthy and had not received any medication in the period before experimentation, and they were fed the same ration based on the following components: 20 kg corn silage/day/cow, 10 kg hay/day/cow, and 10 kg VL18/day/cow (VL18: dairy cow concentrate with 18% protein).

Symbiotic solution (SYMBIOVEBA®)

Symbioveba® is a biological nutritional additive intended exclusively for veterinary use; it is given orally. Symbioviba is an organic product consisting of various elements such as probiotics (*Lactobacillus* and *Saccharomyces cerviciae*); enzymes; plant extracts (*Taraxacum officinalis*; *Zingiber officinalis*) and water. This mixture is obtained using a patented exclusive process called MESEN®.

Symbioveba® is a liquid solution intended for oral administration. It is essential to note that it must be diluted in mineralized water. For cattle, the recommended dosage is 50 ml of symbioveba® with 50 ml of mineral water, to be administered once a month.

Sampling

The milk samples were collected during the morning milk-out on the same day as the treatment. Milk was collected aseptically in sterile 50 ml tubes. During the period of the present study, two samples were taken, one for each month, and immediately tested with the CMT test and underwent physicochemical analysis.

Analysis methods

For detecting sub-clinical mastitis in the dairy cattle included in this target population, Californian Mastitis Test (CMT) according to (Quinn *et al.*, 1999), and lactoscan have been used for milk physicochemical analysis, which provides percentages of some milk parameters, including lactose, dry milk extract, protein, and butterfat.

Statistical analysis

Study data were entered into Microsoft Excel to facilitate statistical analysis. To evaluate the differences between the two groups, SPSS software was used and the Student T statistical test was applied, to calculate the mean and standard deviation of each parameter for the two batches. A threshold of significance of $P \leq 0.05$ was used.

RESULTS

In the control batch, we detected subclinical mastitis in just one cow for the two tests and one doubtful case, either a prevalence of the disease estimated at 10%, which indicated that the animals in the untreated batch were affected and that the infection persisted over the two months of this experiment.

Number of cows in Results of CMT		Results of CMT	
control batches	control batches Sampling 1		
8	-	-	
1	+	+	
1	D	D	
- : Negative / + : Post	prevalence =10%		

Table 1: CMT (California mastitis test) results for the two samples of control batches

Table 2: CMT results for the two samples in the experimental batch.

Number of cows in	Results of CMT	Results of CMT
experimental batch	Sampling 1	Sampling 2
14	-	-
1	D	-
1	+	D
1	D	D
1	-	D
		D 1.61

- : Negative / + : Positive / D : Doubtful

According to Tables (1 & 2), subclinical mastitis was not found in experimental cows, but it was detected in control cows with a prevalence of 10%.

According to this experiment, it was clear that the symbiotic can reduce substantially the inflammation of the mammary glands in dairy cattle. To establish the influence of the use of symbiotic the Odds ratio (OR) was calculated between the two batches which gave a value of (0,8) which is statistically not significant.

Table 3: Effect of Symbiotic supplementation on milk protein levels.

Total Protein % –	Control batch n=10	experimental batch n=18	Р	
	3.1850 ± 0.17828	3.1839 ± 0.17037	0,783 NS	

P: significance level; $P \leq 0.05$; NS: not significant.

According to the above prevalence (Table 3), we have noticed that the mean values of protein levels in the two batches are very close to each other, with the control batch having a mean of (3,185) and the

experimental batch having a slightly lower mean of (3,1839). This suggests that the two batches have similar average protein levels, so there is no significant difference.

Table 4: Effect of S	ymbiotic supp	lementation on	milk butter content.
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	Control batch n=10	Experimental batch n=18	Р
Butyrate %	1.6110 ± 0.81677	1.7594 ± 1.06710	0.575 NS

P: significance level; $P \leq 0.05$; NS: not significant.

The average values of milk fat of the two batches are distinct, with the control batch having an average of (1.611) and the experimental batch having a higher average of (1.7594) (Table 4). Statistical analysis revealed that there was no significant difference.

 Table 5: Impact of Symbiotic on lactose content in milk.

Lactose %	Control batch n=10	experimental batch n=18	Р	
Lactose 76	4.5300 ± 0.24993	4.5244 ± 0.24718	0.850 NS	
D ' 'C' 1	1 D (0.05)10	· C'		

P: significance level; $P \leq 0.05$; NS: not significant.

The average values for the lactose continent of the two batches were very close to each other, with the control batch having an average of (4.53) and the experimental batch having a slightly lower average of (4.5244) (Table 5). However, there was no significant difference between the two batches.

Table 6: Effect of Symbiotic supplementation on dried milk extract.

Dry milk	Control batch n=10	experimental batch n=18	Р	
extract	10.0570 ± 1.14125	10.2183 ± 1.29427	0.778 NS	

P: significance level; $P \leq 0.05$; NS: not significant.

The mean values of the two batches of dried milk extract are distinct, with the control batch having a mean of (10.057) and the experimental batch having a higher mean of (10.2183) (Table 6), but this difference between the means is not statistical.

Breed-specific changes in milk physicochemical values

The different modifications in milk chemical values according to breed are illustrated in the following table (Table 7).

Table 7: Effect of symbiotic on milk composition of the different dairy cattle breeds

	Physicochemical values							
Breed	Protein content (%)		Butyrate (%)		Lactose (%)		Dry milk extract (%)	
	Control batch	Experimental batch	Control batch	Experimental batch	Control batch	Experimental batch	Control batch	Experimental batch
Montbeliard (MTB)	3.17	3.15	9.96	10.10	4.50	4.47	9.96	10.10
Flekvih (FKV)	-	3.15	-	11.35	-	4.43	-	11.35
Holstein (HL)	3.12	3.18	9.59	9.42	4.44	4.54	9.59	9.42

According to the above data and when comparing the two batches, it was found that the addition of symbiotics may change the values of the milk's chemical components. Indeed, the butyrate in the MTB breed was increased by the use of symbiotics in the experimental batch (1.75) compared with the control batch (1.61); unlike the HL breed, where the butyrate decreased in the cows in the experimental batch (1.32) as compared with the control batch (1.70); while the FKV had the highest TB value (2.74) among the other two breeds.

As a result, the protein rate in the MTB breed was reduced when symbiotic was used in the experimental batch (3.15) relative to the control batch (3.17); unlike the HL breed where the protein rate increased in the experimental batch (3.18) as compared to the control batch (3.12).

The dry extract in the MTB breed increased during the use of symbiotics in the experimental batch (10.10) compared with the control batch (9.96); unlike the HL breed in which the dry extract decreased in the experimental batch (9.42) compared with the control batch (9.59); whereas the FKV had the highest dry extract (11.35) compared with the other two breeds.

However, the lactose in the MTB breed decreased when the symbiotic was used in the experimental batch (4.47) compared with the control batch (4.50); unlike the HL breed, the lactose increased in the experimental batch (4.54) by comparison with the control batch (4.44); whereas the FKV showed the lowest percentage of lactose (4.43) compared with the other two breeds.

DISCUSSION

It was noted that the use of symbiotics as a feed additive in dairy cows during the lactation period has a positive effect on mammary gland health and reduces subclinical mastitis. Regarding the fight against infectious diseases, probiotics play an important role in the defence process against pathogens by improving the barrier function of the digestive tract, thereby preventing infections (Lucey et al., 2021). Some studies have indicated that oral administration of probiotics enhances immunity and protects animals against mastitis (Li et al., 2021). Furthermore, feeding cows with Saccharomyces cerevisiae and Lactobacillus lactis has been shown to reduce inflammation

in the mammary gland (Gao *et al.*, 2020). The addition of symbiotics to the diet also reduced the prevalence of mastitis (Lamari *et al.*, 2021).

Indeed, many probiotic bacteria (e.g., Lactobacillus) produce chemical substances such as hydrogen peroxide and bacteriocins, which inhibit the growth of pathogenic bacteria (Ladha and Jeevaratnam, 2018). Similar effects have been observed against parasitic infections (Ramirez *et al.*, 2021). In the mammary gland, positive effects have been observed in relation to mastitis, either through improved digestive function, reduced germ loads, enhanced local immunity, or strengthened mucosal barrier functions (Steinberg *et al.*, 2021).

There was no significant difference in major milk constituents, including protein, fat, and dry matter, which may be attributed to diet composition, the dairy animal's genotype, and increased milk production due to symbiotic supplementation. The slight increase in dry milk extract in treated dairy animals aligns with the findings of Nocek et al. (2003) and Kembabazi et al. (2021). This increase may be due to the growth of ruminal cellulolytic bacteria and the ability of probiotics to prevent ruminal acidosis, which negatively affects feed intake and overall rumen function (Reuben et al., 2022).

Similarly, results obtained on milk quality with the use of probiotics (Suntara *et al.*, 2021) have shown an improvement in milk protein levels following probiotic supplementation. Improvements in milk production and butyrate levels were also reported by Sun *et al.* (2022).

Many studies have demonstrated the impact of a balanced digestive flora, assisted by probiotics, on the production of neuromodulators such as tryptophan or neuropeptides. This balance reduces stress and improves the overall well-being of animals (McFarland *et al.*, 2021).

CONCLUSION

The study demonstrates that the use of symbiotics as a feed additive in dairy cows during the lactation period positively influences milk composition, notably through increased butyric acid and protein content. These findings highlight enhanced mammary gland health and a significant reduction in subclinical mastitis. Considering the pressing challenge of antibiotic resistance, probiotics present a promising alternative for disease management. To fully realize and quantify the potential of symbiotics as a feed additive, further research with larger sample sizes and optimized study conditions is imperative. Such efforts could contribute to preventing infectious diseases during lactation while simultaneously improving the chemical quality and overall value of milk.

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تأثير السيمبيوتيك على التهاب الضرع تحت السريري لدى الأبقار الحلوب وتحسين تركيبة الحليب

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يعد التهاب الضرع أحد أهم الأمراض متعددة العوامل، وهو عبارة عن التهاب الغدة الثديية، ويمكن أن يكون سريريا مصحوبا بأعراض أو تحت سريريا أي بدون أعراض. يهدف هذا البحث إلى تقييم تأثير منتج سيمبيوتيك على التهاب الضرع تحت السريري لدى الأبقار الحلوب وتأثيره على جودة اللبن، من أجل ذلك قمنا بتقسيم الأبقار من سلالات مختلفة - مونبيليارد، فليكفي و هولشتاين- إلى مجموعتين: الأولى تجريبية وتضم ١٨ بقرة والثانية ضابطة وتضم ١٠ بقرات، تمت تغذية المجموعتين بنفس العليقة مع اضافة السيمبيوتيك "SYMBIOVEBA" إلى المجموعة الأولى، كما تم أخذ عينتين من اللبن من كل مجموعة. تم فحص العينات باستخدام اختبار التهاب الضرع (CMT) وجهاز اللاكتوسكان. وفقا للنتائج المتحصل عليها تسبب السامبيوتيك في تعديل تركيب اللبن من خلال زيادة غير معنوية في محتوى المواد الدسمة بالإضافة إلى التأثير الإيجابي على التهاب الضرع تحت السريري مما يشير إلى تحسن في صحة الضرع. تسلط هذه الدراسة الصوء على الأهمية المحتملة لاستخدام المتبيوتيك كعلاج بديل الالتهاب الضرع تحت السريري ما يسلم وتوفر أساسًا للدراسات المامبيوتيك في تعديل تركيب اللبن من خلال زيادة غير معنوية في محتوى المواد الدسمة بالإضافة إلى التأثير الإيجابي على التهاب الضرع تحت السريري مما يشير إلى المو تحسن في صحة الضرع. تسلط هذه الدراسة الصوء على الأهمية المحتملة لاستخدام السيمبيوتيك كعلاج بديل اللبن.