

Effects of Using Calcium Propionate and Trisodium Citrate in Dairy Cows on Daily Milk Yield and Milk Quality

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ABSTRACT

In the current study, 3 groups with similar characteristics were formed in Uşak Cattle Breeders Association Research Farm. A total of 21 cows, which were the third and fourth lactation, were selected and grouped as including 7 Holstein cows in each group. The animals in the first group were given 143 g / kg calcium propionate and the animals in the second group were give 30 mg / kg trisodium citrate orally for two months. The animals in the third group received no additives as a control group. In the farm where the samples were taken, the maintenance and feeding conditions were carried out in optimum conditions. Somatic cell count (SCC), protein, fat, dry matter, lactose, Ph parameters and daily milk yields were examined in the collected raw milk samples. As a result of the quality analysis in the collected milk, in the groups given calcium propionate and trisodium citrate, there was no statistical difference between the groups in all the parameters except for the number of somatic cells ($p>0.05$). However, a very significant difference was observed in the mean number of somatic cells in the cows treated with trisodium citrate ($p<0.001$) and Trisodium citrate administration was found to help reduce the number of somatic cells in milk.

Key words: Trisodium citrate, calcium propionate, milk quality, the number of somatic cells.

Süt İneklerinde Kalsiyum Propiyonat ve Trisodyum Sitrata Kullanımının Günlük Süt Verimi ve Süt Kalitesi Üzerine Etkileri

ÖZ

Bu çalışmada, Uşak Damızlık Süt Sığırcı Yetiştiricileri Birliği Çiftliğinde benzer özellikte 3 grup oluşturulmuştur. Rastgele seçilen üçüncü ve dördüncü laktasyon olan 21 inek belirlenmiş, her bir grupta 7 Holştayn ırkı sığır olacak şekilde gruplandırılmıştır. Birinci gruptaki hayvanlara 143 gr/kg kalsiyum propiyonat, ikinci gruptaki hayvanlara 30mg/kg trisodyum sitrat oral yolla verilmiştir. Üçüncü gruptaki hayvanlar ise kontrol grubu olarak hiçbir ilave verilmemiştir. Preparatlar iki ay boyunca haftada bir kez çirilmiş ve ertesi gün sağımdan sonra çiğ sütteki kalite parametreleri incelenmiştir. Numunelerin alındığı çiftlikte bakım ve besleme koşulları optimum şartlarda uygun olarak gerçekleştirilmiştir. Toplanan çiğ süt numunelerinde somatik hücre sayısı (SHS), protein, yağ, kuru madde, laktoz, Ph parametreleri ve günlük süt verimleri incelenmiştir. Sütte kalite analizlerinde; kalsiyum propiyonat ve trisodyum sitrat verilen gruplarda somatik hücre sayısı hariç tüm parametrelerde grup arasında istatistiksel fark görülmemiştir ($p>0,05$). Ancak Trisodyum sitrat uygulanan ineklerde ortalama somatik hücre sayıları arasında çok önemli farklılık görülmüştür ($p<0,001$) ve Trisodyum sitrat uygulamasının sütlerde somatik hücre sayısının azalmasına yardımcı olduğu tespit edilmiştir.

Anahtar kelimeler: Trisodyum sitrat, kalsiyum propiyonat, süt kalitesi, somatik hücre sayısı.

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INTRODUCTION

The amount and quality of milk produced is affected by many factors. Some of these factors are related to the animal (genetics, age, etc.), others are related to environmental factors (nutrition, climate, hygiene, etc.). It is possible to increase the amount and quality of milk by paying attention to factors that can be taken under control. The amount of milk increases in direct proportion to the age of the dairy cow, but cows in herds that are poorly managed and not adequately cared for can easily get mastitis. Mastitis causes a large amount of somatic cells to pass into the milk. Since the increase in the number of somatic cells in the milk decreases the milk quality, the value of the milk produced decreases and may even cause it to be disposed of before it can be used. Generally, the number of somatic cells in the milk is checked to determine the quality of milk. The increase in the number of somatic cells in milk causes a change in the composition of the milk. While increasing somatic cell count decreases milk lactose, casein and fat ratios, it increases chlorites, sodium, immunoglobins, serum proteins and milk pH (Mbonwanayo, 2013).

Trisodium Citrate; It is used as flavouring, stabilizing agent and acidity regulating component in the food industry. It is highly soluble in water and has a positive effect on flavour. Citric acid loses its protons of three carboxy groups in solution, which makes citric acid an excellent pH control tool with a buffering effect in acid solutions. Available in the form of white granular crystal or white crystalline powder, trisodium citrate dihydrate is odourless and has a pleasant salty taste. Trisodium citrate, which tends to form large crystals in humid air, dissolves easily in water. Biologically fully degradable trisodium citrate is used to maintain pH levels in soft drinks (Anonymous 2020a).

Calcium Propionate; It is an organic salt formed by the reaction of Calcium Hydroxide with propionic acid. Calcium propionate can be produced in both crystal and powder form. Calcium propionate is easily soluble in water. Calcium propionate is very effective in preventing the growth and reproduction of mould, yeast and other microorganisms in bakery products (Anonymous 2020b).

With the onset of lactation in dairy cows, it is necessary to create care and feeding conditions that allow dry matter consumption to reach a sufficient level in a short time. In addition, a metabolic adaptation should be created that enables them to meet their increased energy and calcium needs from body reserves when necessary (Goff, 1999, Goff 2001). Calcium propionate is used to reduce the incidence of hypocalcemia and ketosis in transition periods of dairy cows, since it is both a source of calcium and energy (Defrain et al., 2005; Mandebvu et al., 2003; Stokes and Goff, 2001; Goff et al., 1996, Melendez et al., 2002).

The study aimed to determine effects of using calcium propionate and trisodium citrate in dairy cows on daily milk yield and milk quality traits in Holstein cows.

Properties of Quality Analysed in Milk

In terms of milk quality, the ratios of milk fat, milk protein and non-fat dry matter vary with the quality of the feed given to animals. Generally, the ratios of essential fatty acids from the feed are as follows: 65% acetic acid, 20% propionic acid, 12% butyric acid, 3% other fatty acids. If cows are fed with high density concentrate, low level of roughage and pelleted feeds, the acetic acid decreases, the propionic acid ratio increases and the fat ratio in milk decreases accordingly (Bhoite and Padekar, 2002).

Milk protein and non-fat dry matter (NFDM), protein and NFDM levels in milk are affected by environmental factors at a low level. Protein and NFDM rates in milk decrease by 0.2% in case of malnutrition. However, NFDM and protein ratios increase by 0.2% in feeding with 25% more than the requirement (Anonymous 2019).

The protein found in milk is in the form of casein and serum proteins (immunoglobulins, α -lactalbumin, β -lactoglobulin, serum albumin and proteose peptones). While caseins are synthesized from amino acids in the blood, immunoglobulins come from the immune system. In addition, 80% of the total protein in milk is in the form of casein. While the rate of immunoglobulins is high at the beginning of lactation, it decreases to a low amount after the 5th day of lactation (Bhoite and Padekar, 2002).

Lactose; Acetic acid, butyric acid and propionic acid are taken from rumen feeds. Through the blood, propionic acid converts into glucose in the liver and reaches the mammary glands through the blood. Glucose reaching the udder is used in the synthesis of lactose. Since the lactose concentration is high in the alveoli, it increases the amount of milk by coming into the water (Anonymous 2019). 99% of the carbohydrates in milk are in the form of lactose. The amount of lactose in cow's milk is between 48 and 50 g / litre (Hoden and Coulon, 1991).

Somatic Cell Count; In mastitis, after the macrophage cells in the mammary gland stimulate the cow's defence system, the defence system sends the neutrophil cells to the place where the bacteria occupy and the neutrophil cells try to kill the bacteria. For this reason, more than 90% of the somatic cells found in mastitis milk are neutrophil cells (Pamela and Douglas 2002). In other words, we can list the types of leukocytes found in milk with mastitis in a descending order as follows; neutrophils, macrophages and lymphocytes. If there is no intervention for mastitis occurring in the herd, mastitis decreases milk yield and milk quality, causes breast blindness, decreases the value of dairy animals and causes them to be sent to slaughter. In addition, the milk of the mastitis-infected animal harms human

health and the duration of mastitis-infected cows in breeding is shortened.

Generally, the number of somatic cells in milk is used to determine the status of breast inflammation. If the number of somatic cells in the milk is below 200,000 SHS / ml, it means that the milk is not with mastitis, and if it exceeds this limit, the presence of mastitis is considered. However, it is accepted that drinking milk and products made from this milk with the number of somatic cells lower than 400,000 SHS / ml according to the standards of the European Union and lower than 500,000 SHS / ml according to the Turkish Food Codex are not harmful to human health (Çoban et al., 2006).

MATERIAL and METHOD

The research was conducted at the Uşak Cattle Breeders Association Research Farm, following the approval of the Uşak University Animal Experiments Local Ethics Committee (USAKHADYEK 2018 / 1-03). A total of 21 cows, which were the third and fourth lactation, were selected and grouped as

including 7 Holstein cows in each group thus a total of 3 groups were formed.

1. 1 litre of aqueous solution containing 680 g of calcium propionate was given orally to each cow for two months, once a week after morning milking.

2. 500 ml of aqueous solution containing 18 g of trisodium citrate was given orally to the cows in the group, once a week after morning milking for two months. Milk samples were taken the next day.

3. No calcium propionate or trisodium citrate was given to the cows in the group. Feeding continued with the same ration as it was before. No intervention was made to the cows in the control group.

The preparations were given once a week between 1 November 2019 and 30 December 2019 for two months, and the quality parameters in raw milk were examined the next day after milking

The cows selected for the study were randomly divided into experimental and control groups. The animals were housed under the existing farm conditions without any special feeding and housing treatments. In addition to the ration in Table 1, vitamin premix, yeast and toxin binders were given to animals milked by machine twice a day, in the morning and in the evening.

Table 1. Example of Ration Given to Cows

Feed Ingredients	Example of ration given to dairy cows (kg)
Corn Silage, 30-35% KM	15,0
Alfalfa Hay	5
Cattle Milk Feed, 21HP, 2750 ME	5
Wheat Straw	1
Sugar Beet Pulp	5
Wheat Bran	2
Rations ME Mcal / kg KM	2,36

Business records were used for daily milk yields. Milk samples were taken into 200 ml sterile containers during milking and divided into two every Monday, the day after calcium propionate and trisodium citrate were given for two months. Somatic cell counts were made by microscopic method in the laboratory of the Uşak Dairy Cattle Breeders Association. After the milk was spread on two areas of 5x20 mm² on the slide, it was kept in an oven at 37 °C for 1 hour, and leukocytes and epithelial cell nuclei were stained by dripping a dye solution containing methylene blue. Using a 100 immersion objective, 20 fields were counted in each preparation and their averages were calculated. SHS in 1 ml of milk was calculated by multiplying these obtained averages by the microscope factor. In other samples, they were sent to Uşak University Scientific Analysis and Technological Application and Research Center by cold chain, and protein, fat, dry matter, lactose, pH parameters were examined by using Milkotester brand

(Master Classic LM2 P1 Model) Milk Quality Analyzer.

Analysis of the data; The data obtained in terms of the properties emphasized were evaluated with the variance analysis technique. Duncan test was used to determine different groups. Variance analysis was conducted in IBM SPSS statistics 20 program package.

RESULTS

As can be seen in Table 2, as a result of the analysis of variance in terms of mean milk yields, the differences in the sampling months and between the groups were found to be insignificant ($p>0.05$).

As a result of the variance analysis in terms of milk fat, sampling time and cow group interaction were not found to be significant ($p>0.05$). Similarly, sampling times and differences between groups were

not found to be significant ($p>0.05$). Although the difference is not significant, the average milk fat ratios (Calcium Propionate-Trisodium Citrate) measured in the experimental group as a result of the application ($4.08 \pm 0.77\%$, $4.63 \pm 0.40\%$) were found to be lower than the average milk fat ratio measured in the control group ($4.75 \pm 0.31\%$) (Table 2)

As can be seen in Table 3, as a result of the analysis of variance in terms of milk protein, the differences in the sampling months and between groups were not found to be significant ($p>0.05$). Although the difference is not significant, the average milk fat ratios (Calcium Propionate-Trisodium Citrate) measured in the experimental group ($3.54 \pm 0.44\%$, $3.84 \pm 0.36\%$) as a result of the applications were found to be lower than the average milk fat ratio measured in the control group ($3.99 \pm 0.22\%$). In addition as a result of the analysis of variance in terms of dry matter in milk, the differences in the sampling months and between the groups were found to be insignificant ($p>0.05$). As a result of the application, the mean dry matter ratios (Calcium Propionate-Trisodium Citrate-Control) measured in the experimental group ($9.49 \pm 0.48\%$ - $9.67 \pm 0.34\%$) were found to be similar to the

mean dry matter ratio ($9.96 \pm 0.22\%$) measured in the control group.

As can be seen in Table 4, as a result of the analysis of variance in terms of lactose in milk, the differences in the sampling months and between the groups were found to be insignificant ($p>0.05$). As a result of the application, the mean lactose ratios (Trisodium Citrate) measured in the experimental group ($5.16 \pm 0.44\%$) were found to be lower than the lactose ratio measured in the control group ($5.31 \pm 0.18\%$). Also as a result of the analysis of variance in terms of Ph in milk, the differences in the sampling months and between the groups were found to be insignificant ($p>0.05$).

As seen in Table 5, a very significant difference was observed between the mean numbers of somatic cells before and after trisodium citrate was given to the cows ($p<0.001$). The difference between the mean numbers of somatic cells of the control and experimental groups was significant before trisodium citrate was given ($P < 0.05$), while the difference between the two groups was very significant after trisodium citrate was given ($p<0.001$). It was found that trisodium citrate administration helped reduce the number of somatic cells in milk.

Table 2. Mean Milk Yields (gr/kg) and Fat (%)

Groups	N	Parameters	Yield (gr/kg)			Fat (%)		
			November	December	P	November	December	P
Calcium Propionate	7	Min.	15.70	16.38	-	2.90	3.09	-
		Max.	23.00	23.75		4.78	5.22	
		$\bar{x} \pm S\bar{x}$	20.14 ± 2.87	20.88 ± 2.74		3.73 ± 0.69	4.08 ± 0.77	
Trisodium Citrate	7	Min.	15.50	11.50		3.36	4.05	-
		Max.	22.30	23.38		4.47	4.99	
		$\bar{x} \pm S\bar{x}$	18.90 ± 2.74	19.45 ± 4.66	-	3.97 ± 0.51	4.63 ± 0.40	
Control	7	Min.	13.80	14.63		3.88	4.33	-
		Max.	19.40	19.50		4.51	5.17	
		$\bar{x} \pm S\bar{x}$	16.86 ± 2.24	16.85 ± 2.41	-	4.12 ± 0.24	4.75 ± 0.31	
Total	21	Min.	11.68	11.85		3.26	3.26	
		Max.	15.05	14.40		3.87	3.87	
		$\bar{x} \pm S\bar{x}$	12.85 ± 1.90	13.35 ± 1.33		3.94 ± 0.32	3.6590 ± 0.34	
			(A)	(A)		(A)	(A)	

$p > 0.05$; -: n.s; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ Capital letters are used to compare the means in the sampling months.

Table 3. Protein (%) and Dry Matter in Milk (%)

Groups	N	Parameters	Protein (%)		P	Dry Matter in Milk (%)		
			November	December		November	December	P
Calcium Propionate	7	Min.	3.03	3.08	-	8.81	9.02	-
		Max.	4.29	4.24		9.98	10.17	
		$\bar{x} \pm S\bar{x}$	3.58±0.46	3.54±0.44		9.26±0.47	9.49±0.48	
Trisodium Citrate	7	Min.	3.61	3.29		8.72	9.15	-
		Max.	4.13	4.17		9.87	10.08	
		$\bar{x} \pm S\bar{x}$	3.85±0.22	3.84±0.36	-	9.44±0.46	9.67±0.34	
Control	7	Min.	3.81	3.80		8.21	9.72	-
		Max.	4.43	4.36		10.05	10.31	
		$\bar{x} \pm S\bar{x}$	4.11±0.23	3.99±0.22	-	9.53±0.75	9.96±0.22	
Total	21	Min.	3.37	3.01		10.99	11.56	
		Max.	3.96	3.99		11.99	11.93	
		$\bar{x} \pm S\bar{x}$	3.57±0.33	3.59±0.51		11.48±0.49	11.74±0.18	
			(A)	(A)		(A)	(A)	

p > 0.05; -: n.s; *: P < 0.05; **: P < 0.01; ***: P < 0.001 Capital letters are used to compare the means in the sampling months.

Table 4. Lactose in Milk (%) and Ph in Milk (%)

Groups	N	Parameters	Lactose in Milk (%)		P	Ph in Milk (%)		
			November	December		November	December	P
Calcium Propionate	7	Min.	5.21	5.29	-	6.58	6.63	-
		Max.	5.54	5.49		6.72	6.77	
		$\bar{x} \pm S\bar{x}$	5.36±0.15	5.38±0.08		6.65±0.05	6.71±0.05	
Trisodium Citrate	7	Min.	3.81	4.37		6.58	6.70	-
		Max.	5.40	5.39		6.85	7.21	
		$\bar{x} \pm S\bar{x}$	5.03±0.69	5.16±0.44	-	6.66	6.82	
Control	7	Min.	5.06	5.02		6.56	6.70	-
		Max.	5.46	5.45		6.68	6.77	
		$\bar{x} \pm S\bar{x}$	5.32±0.16	5.31±0.18	-	6.62±0.05	6.74±0.02	
Total	21	Min.	5.32	5.31		6.61	6.64	
		Max.	5.48	5.50		6.64	6.74	
		$\bar{x} \pm S\bar{x}$	5.39±0.07	5.40±0.09		6.62±0.01	6.67±0.05	
			(A)	(A)		(A)	(A)	

p > 0.05; -: n.s; *: P < 0.05; **: P < 0.01; ***: P < 0.001 Capital letters are used to compare the means in the sampling months..

Table 5. The Number of Somatic Cells in Milk

Group	N	Parameters	November	December	P
Calcium Propionate	7	Min.	163384.60	134182.50	-
		Max.	201949.60	189780.00	
		$\bar{x} \pm S\bar{x}$	180466.92 \pm 16157.57 (A)	165675.05 \pm 20950.29 (A)	
Trisodium Citrate	7	Min.	130528.80	78922.50	***
		Max.	180360.40	101969.25	
		$\bar{x} \pm S\bar{x}$	154579.96 \pm 20410.17 (B)	92204.90 \pm 9057.03 (B)	
Control	7	Min.	176313.80	165345.50	-
		Max.	208935.00	200780.00	
		$\bar{x} \pm S\bar{x}$	189107.32 \pm 13463.28 (A)	184633.90 \pm 15067.61 (A)	
Total	21	Min.	130528.80	134182.50	
		Max.	208935.00	200780.00	
		$\bar{x} \pm S\bar{x}$	174.718 \pm 57 *	147.504 \pm 21 ***	
			P<0.05	p<0.001	

p > 0.05; -: n.s.; *: P < 0.05; **: P < 0.01; ***: P < 0.001 Capital letters are used to compare the means in the sampling months.

DISCUSSION

Kara et al. (2009) conducted a study to determine the effects of calcium propionate in cattle at the time of retention secundinarum, first oestrus and first insemination, and concluded that there is no difference in terms of milk yield and composition and this finding concurs with the finding of the current study.

Dhillon et al. (1995) reported that when mastitis is detected in water buffaloes, continuous administration of tri-sodium citrate reduces the bacterial content of milk. Prakash et al. (2010) observed that injection of tri-sodium citrate (30 mg/kg) with trimethoprim venously for 7 days once a day effectively cured mastitis in dairy cows. Trisodium citrate reduces the bacterial content in milk, in parallel with decreasing the number of somatic cells, which is consistent with the study. In addition, in the same study by Parakash et al. (2010), the increase in the fat ratio of trisodium citrate in milk differs from the study.

Eyduran et al. (2005) found that lactation order and seasonal factors had a negative effect on the number of somatic cells in a study conducted in August and November in 27 Holstein cows. In the study, the decrease in the number of somatic cells in cattle is due to drinking trisodium citrate, not different factors.

Mbonwanayo et al. (2016) investigated the effect of Trisodium Citrate on somatic cells in cattle and they determined that there was no difference between the two groups in terms of daily milk yield, fat, protein, milk dry matter and milk ORP values and that the number of somatic cells decreased in the group containing Trisodium Citrate, which concurs with the finding of the current study.

A decrease was observed in the number of somatic cells in the calcium propionate group, but the difference was found to be insignificant. Çağdaş et al. (2009) examined the effects of calcium propionate on hypocalcemia, dry matter intake, body condition score, milk production and reproductive disorders in dairy cows and reported that calcium propionate drunk twice was beneficial in the treatment of milk fever and had a preventive effect for metritis when drunk three times. This shows that there are other benefits of drinking Calcium Propionate to cows.

No significant effect of calcium propionate on milk quality was determined in the study. Kara et al. (2010) in his study by dividing 24 dairy cattle into 3 groups in Bursa; calving time, 24 hours after calving and 7 days after calving, three times calcium propionate (0.68 kg calcium propionate was given) was administered. It was determined that the group administered 3 times calcium propionate tended to decrease the serum BHBA concentration and the incidence of subclinical ketosis during the first four

weeks of lactation. This can be counted among the benefits of giving calcium propionate to dairy cattle.

CONCLUSION

In the current study, a decrease in the number of somatic cells was observed in the group that contained calcium propionate, but the difference was found to be insignificant. It was found that calcium propionate did not cause a change in the protein, fat, dry matter, lactose, Ph parameters and daily milk yields in milk samples.

In the study, the ratio of citrate in the alveoli was increased by drinking trisodium citrate to dairy cows. A lower somatic cell count was detected in the experimental group that was given trisodium citrate compared to the control group. In lactating older cows, using trisodium citrate can be an effective and useful way to counter the increasing trend of somatic cell count in milk.

It can be said that the use of trisodium citrate, which is very easy and cheap to supply, can be used in many fields, especially in the preservation of human food, and has no known harmful effects, in lactating dairy animals can be particularly beneficial in keeping the number of somatic cells in milk at a certain level. On the other hand, more detailed studies are needed to demonstrate the possible effects of longer-term use of trisodium citrate on milk quality, milk components and the general physiological state of the animal. The fact that trisodium citrate is easily available, low-cost and has no adverse health effects will facilitate the transfer of this application to the field.

Ethics Committee Information: This study was carried out after obtaining permission from Uşak University Animal Experiments Local Ethics Committee (USAKHADYEK 2018/01-03).

Conflict of interest: The authors declare that there is no conflict of interest.

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