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### EFFECT OF ADDING CHOLINE CHIORIDE TO DIETIN MILK COMPONENTS OF HOLSTEINCOWS

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#### Abstract

Aimed of This study to show the effect of adding choline chloride to the feed on milk components during the summer in central Iraq. This study was conducted at Al-Khalis cows station in Divala Governorate during the period from 21/6 to 21/9/2022, where 25 Holstein cows between the ages of 3-5 years used, the cows were divided into five treatments, so that treatment T1 represents the control treatment (without adding Choline chloride), while treatments T2 and T3 add choline chloride untreated and treated with formaldehyde to the concentrated feed with an amount of 35 g /cow /day, T4 and T5 add choline chloride untreated and treated with formaldehyde with an amount of 70 g/cow/day. The addition of choline chloride did not affect in the milk components. Which includes the percentage of protein, ash, non-fat solids, the density of milk and the degree of its freezing, as well as the sensory evaluation characteristics in milk, which include flavor, color, appearance, texture, acidity, and the degree of acceptance, but it affected the percentage of fat and lactose sugar in milk for some periods, as it was found that there was a high Significant ( $P \le 0.05$ ) at the end of the first month of fat in treatment T3, as the percentage of fat was 5.00% compared to 3.07% in treatment T4, and there was a significant decrease (P $\leq 0.05$ ) for lactose sugar in milk at the beginning of the experiment for treatment T5, as it reached 4.10% compared to 4.52% for the control treatment T1.we conclude from the current experiment that adding Choline chloride untreated and treated with formaldehyde It does not significantly affect the components of milk, and does not have any negative effect on the sensory characteristics of milk in during the experimental period in the season Summer when the average Temperature Humidity Index for this season is 79 and cows are under heat stress.

Key word :choline chloride ,milk components , heat stress

#### Introduction

Milk is an essential component of the diet of eight billion people around the world, and it contains nutrients needed by the human body, such as calcium, vitamin D and potassium, in addition to energy, protein and fat, and dairy cows will produce 80% or more of the milk consumed globally (FAO, 2021).Heat stress has a negative effect on productive performance in imported Holstein cows in central Iraq ,In order to overcome this problem the researchers conducted several studies to reduce the negative effects of heat stress on cows, including feed additives(Al-Qudsi, andShwayel 2015 ; Shwayel and Al Mafragi, 2019;Awwad and Shwayel ,2020), including the addition of choline chloride to the feed, which is a multi-functional ingredient. In the blood, thus reducing the risk of developing fatty liver and reducing the level of ketone bodies in the blood. It also works to reduce the level of non-steric fatty acids and increase the content of glycogen in the liver (Davidson et al. 2008; Gutiérrez et al., 2019).Acharya et al. (2020) found that the addition of protected choline led to a highly significant increase in the

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quantity and quality of milk production by increasing the proportion of protein and fat in it. Holdorf and White (2021) stated that the addition of protected choline to the feed during the summer led to a reduction in the effects of heat stress when the temperature and humidity index was 77, as choline effectively prevents oxidative stress and apoptosis caused by heat stress, thus increasingmilk components. The aim of the current study is to demonstrate the effect of adding choline chloride treated and untreated with formaldehyde to diet to reduce its anylysis in the rumen in milk components of Holstein cows.

#### Materials and Methods

The study was conducted at Al-Khalis cows station in Diyala Governorate under conditions of heat stress in central Iraq, as 25 Holstein cows were randomly selected during the period from 6/21 to 9/21/2022 summer between the ages of 3-5 years. The cows were divided into five treatments, each treatment Five cows, treatment T1 represents the control treatment (without adding choline chloride) and treatments T2 and T3 in which untreated and formaldehyde-treated choline chloride were added to the concentrated feed at an amount of 35 g / cow / day, respectively, and treatments T4 and T5 in which untreated choline chloride was added and treated with formaldehyde to concentrated feed with an amount of 70 g / cow / day, respectively.And that the use of formaldehyde treatment can reduce the decomposition of the fodder in the rumen and thus increase the utilization of it, and this method is easy to apply and highly effective in reducing the degree of decomposition (Qasim , 2010).

The chemical composition of concentrated feed for cows contained 20.21% crude protein, 11.5% crude fiber, 6.20% fat and 47.0% carbohydrates in addition to hay, alfalfa and alfalfa as rough feed. Water was Always available at all times, the daily milk production of dairy cows was recorded during the experiment in the morning (5 am) and evening (4 pm) for each cow, and the cows were milked with automatic milking machines inside the station, the percent of fat, protein , lactose, ash, density and freezing point in the milk It was measured by a German milk component analyzer, As for the analysis of the sensory characteristics of milk, it was measured by residents within the specialty of animal production, using a special form(Sheikh Zahir,1999).

A randomized complete block design (RCBD) was used using the ready-made program (SPSS) (2011) for the calculator in analyzing the results for the purpose of excluding the effect of the milk season and its overlap with the effect of the coefficients.

#### **Results and Discussion**

The following table (Table 1) shows the effect of adding choline chloride to the feed In the percentage of milk fat for Holstein cows during the summer season, it is noted from the table at the beginning of the experiment that there are no significant differences between the treatments, but after a month of the experiment, significant differences were observed if the treatments T3, T5, T1 increased if they were 5.00, 4.40, 4.28%, respectively, over the treatment. T4 which was 3.07%. After two months of the experiment, it was observed that there were no significant differences, but an arithmetic increase was observed for the two treatments T2 and T3, they were 6.05 and 6.20%, respectively. There are also not significant differences between the treatments in the studied trait to another months in the percentage of milk fat. This study did not agree with other studies conducted by researchers Acharya and others (2020), which found that adding choline to milk cows in an amount of 55 g per day / cow led to a significant improvement in the percentage of fat in milk, as it reached In the control treatment, 3.95% compared to 4.09% in the choline treatment, while Mečionytė et al. (2022) found that eating cows 10 g / cow / day of protected herbal choline led to a significant increase in the percentage of milk fat, reaching 4.705.06% in the control treatment and choline treatment respectively. The researchers indicated

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that choline supplementation leads to an increase in fat percentage and prevents metabolic disorders such as ketosis and fatty liver (Zenobi et al. 2018; Gutiérrez et al., 2019), and agreed with a previous study that indicated that there were no significant differences between the treatments when protected choline was added to the feed for cows during the transition period. With an amount of 12.9 g/day/cow, the fat percentage in the control treatment was 4.43% and choline 4.62% (Pinotti et al., 2003; Potts et al., 2020).

Treatments	The beginning of the experiment 2022/6/21	after a month 2022/7/21	After two months 2022/8/21	Three months later 2022/9/21
T1Control treatment without addition	0.40±5.48	0.15±4.28 A	0.61 ±5.98	0.52±5.96
T2Add 35 g/cow/day choline chloride	0.99±4.80	0.43 ±4.05 AB	$0.67 \pm 6.05$	1.10±4.90
T3Add 35 g/cow/day of formaldehyde- treated choline chloride	0.20±5.80	0.54 ±5.00 A	0.57 ±6.20	0.53±6.00
T4Add70g/cow/daycholine chloride	0.81±4.87	0.34 ±3.07 B	0.61 ±5.67	0.73±4.65
T5Add70g/cow/dayofformaldehyde-treatedcholinechloride	0.78±5.08	0.94 ±4.40 A	0.25 ±5.56	0.38±5.70
Sig	NS	*	NS	NS

#### Table (1) Effect of adding choline chloride to the feed in the percentage of fat in the milk of Holstein cows during the summer season (SE ± mean)

NS= indicates no significant differences

The different letters within the same column indicate that there are significant differences between the treatments at a significant level of P<0.05

In the second table, there were no significant differences between the treatments n protein percent in the milk, but we notice an arithmetic increase in During the experiment periods, if at the beginning of the experiment the protein percentage was 2.80, 2.87, 2.92, 2.92, 2.72%, and after a month of the experiment, the protein percentage was 3.06, 3.00, 2.95, 3.05, 2.84%, and after two months of the experiment, the protein percentage was 3.08, 3.30, 2.90, 3.07, 2.92%, and after three months of the experiment, the protein percentage was 3.08, 3.30, 2.90, 3.07, 2.92%, and after three months of the experiment, the protein percentage was 3.20, 3.17, 3.07, 3.40, 3.02% within the experiment for the treatments T1,T2, T3, T4 and T5 respectively, This study agreed with previous studies conducted by Bollatti et al. (2020) who indicated that the addition of protected

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choline to cows during the transitional period in the amountof 12.9 g / cow / day led to did not significantly affect the protein ratio, as it reached 3.42 and 3.38% in the control and choline treatments, respectively. It also agreed with the other study conducted by Potts et al. (2020), which found that giving protected choline to milk cows after calving in an amount of 60 g / cow / day led to protein percentage was not affected significantly by giving choline to cows, as it was 2.88 and 2.66% in the control and choline treatments respectively, while it did not agree with other studies conducted by Acharya et al. (2020) that giving choline to milk cows in an amount of 55 g / cow / day led to an improvement in milk production in quantity and quality The protein percentage was 3.15 and 3.25% in the control and choline treatments, respectively.

Treatments	The beginning of the experiment 2022/6/21	after a month 2022/7/21	After two months 2022/8/21	Three months later 2022/9/21
T1Control treatment without addition	0.16±2.80	0.86±3.06	0.86±3.08	0.22±.320
T2Add 35 g/cow/day choline chloride	0.07±2.78	0.08±3.00	0.33±3.30	0.21±3.17
T3Add 35 g/cow/day of formaldehyde- treated choline chloride	0.06±2.92	0.02±2.95	0.04±2.90	0.07±3.07
T4Add70g/cow/daycholine chloride	0.04±2.92	0.05±3.05	0.14±3.07	0.20±3.40
T5Add70g/cow/dayofformaldehyde-treatedcholinechloride	0.10±2.72	0.11±2.84	0.06±2.92	0.09±3.02
Sig	NS	NS	NS	NS

# Table (2)Effect of adding choline chloride to the feed in the percentage of protein in the milk of Holstein cows during the summer (SE ± mean)

NS= indicates no significant differences

It is noted in table 3 that there are significant differences between the treatments at the beginning of the experiment in the percentage of lactose sugar, where the treatment T1 is higher than T5 if it is 4.52 and 4.10%, respectively. But after that, it is noticed that there are no significant differences between the treatments, as after a month of the experiment, the percentage of lactose sugar reached 4.58, 4.55, 4.42, 4.57, 4.28%, and after two months of the experiment, the percentage of lactose sugar also reached 4.66, 5.00, 4.42, 4.70, 4.38%. and After three months of the experiment, the lactose sugar level reached 4.98, 4.80, 4.62, 5.10, 4.54% within the experiment period for treatments T1, T2, T3, T4 and T5 respectively. This study agreed with previous studies, as the percentage of lactose sugar in milk was not affected by the addition of choline chloride To the feed in an amount of 12.9 g / cow / day, as the percentage of lactose sugar

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in the control treatment was 4.67%, and in the addition treatment, the percentage of lactose was 4.66% (Arshad et al.,2020).

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Treatments	The beginning of the experiment 2022/6/21	after a month 2022/7/21	After two months 2022/8/21	Three months later 2022/9/21
T1Control treatment without addition	0.12 ±4.52 A	0.11±4.58	0.11±4.66	0.33±4.98
T2Add 35 g/cow/day choline chloride	0.10 ±4.20 AB	0.14±4.55	0.50±5.00	0.33±4.80
T3Add 35 g/cow/day of formaldehyde- treated choline chloride	0.62 ±4.42 AB	0.04±4.42	0.08±4.42	0.13±4.62
T4Add70g/cow/daycholine chloride	0.12 ±4.30 AB	0.06±4.57	0.23±4.70	0.31±5.10
T5Add 70 g/cow/day of formaldehyde- treated choline chloride	0.14 ±4.10 B	0.17±4.28	0.10±4.38	0.13±4.54
Sig	*	NS	NS	NS
NS=	indicates	no	significant	differences

Table (3) Effect of adding choline chloride to the feed in the percentage of lactose in the
milk of Holstein cows during the summer (SE ± mean)

The different letters within the same column indicate that there are significant differences between the treatments at a significant level of P<0.05

It is also noted in Table 4 that there are no significant differences between the treatments At the beginning of the experiment in the ash percentage, which amounted to 0.60% for all treatments, and after a month of the experiment, the ash percentage reached 0.62, 0.62, 0.60, 0.62, 0.58%, and after two months of the experiment, the ash percentage also reached 0.62, 0.67, 0.60, 0.65, 0.62%. After three months of the experiment, the ash percentage was 0.64, 0.65, 0.62, 0.70, 0.64% within the experimental period for treatments T1, T2, T3, T4 and T5 respectively. It is clear from the above that there were no significant differences for all months of the experiment between the treatments in the percentage of ash in milk when choline chloride was added to the feed, and there was no study on the percentage of ash in milk when choline chloride was added to the feed for milk cows, as far as we know.

 Table (4) Effect of adding choline chloride to the feed in the percentage of ash in the milk of Holstein cows during the summer (SE± mean)

Treatments th	The beginning of the experiment 2022/6/21	after a		After two months 2022/8/21	Three months later 2022/9/21
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T1Control treatment	0.00±0.60	0.02±0.62	0.02±0.62	0.02±0.64
without addition T2Add 35				
g/cow/day	0.00±0.60	0.02±0.62	0.07±0.67	0.05±0.65
choline chloride				
T3Add 35				
g/cow/day of formaldehyde-	$0.00{\pm}0.60$	$0.00{\pm}0.60$	$0.00\pm0.60$	$0.02{\pm}0.62$
treated choline	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.02
chloride				
T4Add 70			0.05.0.55	0.05.0 50
g/cow/day choline chloride	$0.00\pm0.60$	$0.02 \pm 0.62$	$0.05 \pm 0.65$	$0.05 \pm 0.70$
T5Add 70				
g/cow/day of				
formaldehyde-	$0.00\pm0.60$	$0.02 \pm 0.58$	0.01±0.62	$0.02 \pm 0.64$
treated choline				
chloride				
Sig	NS NS	NS	NS	NS

NS=indicates no significant differences

It is also noted in Table 5 that there are no significant differences between the treatments In the percentage of non-fat solids in the milk of Holstein cows during the summer season, in all months of the experiment in the percentage of non-fat solids, which reached at the beginning of the experiment 8.28, 7.70, 8.12, 8.12, 7.66%, and after a month From the experiment, the percentage of non-fat solidswas 8.38, 8.37, 8.12, 8.40, 7.80%. After two months of the experiment, it was 8.50, 9.15, 8.07, 8.57, 8.02%. After three months of the experiment, the percentage of non-fat solids was 9.14, 8.77, 8.45, 8.45, 9.35, 8.32% within the experimental period for treatmentsT1, T2, T3, T4 and T5 respectively. These results agreed with the study of researchers Acharya et al. (2020) who indicated that adding choline to milk cows at an amount of 55 g/day/cow did not affect the percentage of non-fat solids in milk, which was 8.59, 8.64% in the control and choline treatments, respectively.

 Table (5) Effect of adding choline chloride to the feed in the percentage of non-fat solids in the milk of Holstein cows during the summer season (SE ± mean)

Treatments	The beginning of the experiment 2022/6/21	after a month 2022/7/21	After two months 2022/8/21	Three months later 2022/9/21
T1Control treatment without addition	0.21±8.28	0.20±8.38	0.21±8.50	0.59±9.14
T2Add 35 g/cow/day choline chloride	0.25±7.70	0.17±8.37	0.88±9.15	0.62±8.77
T3Add 35 g/cow/day of formaldehyde-	0.14±8.12	0.11±8.12	0.13±8.07	0.24±8.45

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treated choline chloride				
T4Add 70 g/cow/day choline chloride	0.15±8.12	0.10±8.40	0.41±8.57	0.58±9.35
T5Add 70 g/cow/day of formaldehyde- treated choline chloride	0.29±7.66	0.30±7.80	0.18±8.02	0.23±8.32
Sig	NS	NS	NS	NS

NS=indicates no significant differences

It is also noted in Table 6 that there are no significant differences between the treatmentsAt the beginning of the experiment in the milk density was reached 1.026, 1.025, 1.025, 1.026, 1.023 g/cm3. After a month of the experiment the milk density reached 1.025, 1.027, 1.024, 1.028, 1.027 g/cm3. After two months of the experiment the milk density also reached 1.025. And 1.029, 1.023, 1.027, 1.024 g/cm3, and after three months of the experiment, the milk density was 1.029, 1.028, 1.026, 1.030, 1.025 g/cm3 within the experiment period for treatments T1, T2, T3, T4 and T5, respectively. The presence of significant differences in the percentage of fat in milk for most of the experiment periods as indicated previously, was reflected in the absence of significant differences in the density of milk, as there is a correlation coefficient between the percentage of fat in milk and the density of milk. Parmar et al. (2020)

Table (6)Effect of adding choline chloride to the feed in the milk density (g/cm3) of Holstein
cows during the summer (SE $\pm$ mean)

	comb addi	ing the summer (b)	= mean)	
Treatments	The beginning of the experiment 2022/6/21	after a month 2022/7/21	After two months 2022/8/21	Three months later 2022/9/21
T1Control				
treatment	$0.001 \pm 1.026$	0.002±1.025	$0.001 \pm 1.025$	0.002±1.029
without addition				
T2Add 35				
g/cow/day	$0.000 \pm 1.025$	$0.001 \pm 1.027$	$0.004 \pm 1.029$	$0.003 \pm 1.028$
choline chloride				
T3Add 35				
g/cow/day of				
formaldehyde-	$0.000 \pm 1.025$	$0.001 \pm 1.024$	$0.000 \pm 1.023$	$0.001 \pm 1.026$
treated choline				
chloride				
T4Add 70				
g/cow/day	$0.001 \pm 1.026$	$0.000 \pm 1.028$	$0.002 \pm 1.027$	$0.002 \pm 1.030$
choline chloride				
T5Add 70				
g/cow/day of				
formaldehyde-	$0.000 \pm 1.023$	$0.001 \pm 1.027$	$0.001 \pm 1.024$	$0.000 \pm 1.025$
treated choline				
chloride				

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Sig	NS	NS	NS	NS	
NS=indicates no significant differences					

It is also noted in Table 7 that there are nosignificant differences between the treatments the beginning of the experiment in the degree of freezing, which amounted to -0.53, -0.49, -0.53, -0.52, -0.50 °C, and after a month of the experiment, the freezing point reached -0.54, -0.54, -0.51, -0.51, -0.50 °C, and after two months of the experiment, the freezing point was -0.54, -0.52, -0.51, -0.53, -0.50 °C, and after three months of the experiment, the freezing point was -0.54, -0.52, -0.51, -0.53, -0.50 °C, and after three months of the experiment, the freezing point was -0.54, -0.54, -0.54, -0.51, -0.51, -0.52, -0.50 °C within the trial period for the treatments T1, T2, T3, T4 and T5respectively. There is no study showing the effect of adding choline chloride to the feed in milk cows on measuring the degree of freezing of milk, but in general, there were no significant differences between the treatments in non-fat solids in milk, as previously referred to, which was reflected in the degree of freezing of milk, as well as Hanuš et al. (2010)

Table (7)Effect of adding choline chloride to the feed in the freezing point of milk for Holstein cows during the summer season(SE ± mean)

Treatmentsthe experiment $2022/6/21$ $2022/7/21$ $2022/8/21$ later $2022/9/21$ T1Control treatment $0.01\pm0.53^{\circ}$ $0.01\pm0.54^{\circ}$ $0.01\pm0.54^{\circ}$ $0.01\pm0.54^{\circ}$ T2Add35 g/cow/day $0.01\pm0.49^{\circ}$ $0.01\pm0.54^{\circ}$ $0.00\pm0.52^{\circ}$ $0.03\pm0.49^{\circ}$ T3Add35 g/cow/day $0.00\pm0.53^{\circ}$ $0.00\pm0.51^{\circ}$ $0.01\pm0.51^{\circ}$ $0.01\pm0.51^{\circ}$ T4Add70 g/cow/day $0.01\pm0.52^{\circ}$ $0.00\pm0.53^{\circ}$ $0.00\pm0.53^{\circ}$ $0.00\pm0.53^{\circ}$ T5Add70 g/cow/day $0.01\pm0.52^{\circ}$ $0.02\pm0.50^{\circ}$ $0.02\pm0.50^{\circ}$ $0.02\pm0.50^{\circ}$		Hoistein cows uu	ing the summer se	$ason(DL \pm mcan)$	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Treatments	the experiment			
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	treatment	0.01±0.53 <sup>-</sup>	$0.01{\pm}0.54^{-}$	$0.01 \pm 0.54^{-1}$	$0.01 \pm 0.54^{-1}$
$ \begin{array}{ c c c c c c c c } g/cow/day & of formaldehyde- & 0.00\pm 0.53^{-} & 0.00\pm 0.51^{-} & 0.01\pm 0.51^{-} & 0.00\pm 0.51^{-} & 0.00\pm 0.53^{-} & 0.00\pm 0.53^{-} & 0.00\pm 0.52^{-} & 0.00\pm 0.52^{-} & 0.00\pm 0.53^{-} & 0.00\pm 0.52^{-} & 0.00\pm 0.52^{-} & 0.00\pm 0.52^{-} & 0.00\pm 0.52^{-} & 0.02\pm 0.50^{-} & 0.$	T2Add 35 g/cow/day	0.01±0.49 <sup>-</sup>	0.01±0.54-	0.00±0.52-	0.03±0.49 <sup>-</sup>
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	g/cow/day of formaldehyde- treated choline	0.00±0.53 <sup>-</sup>	0.00±0.51 <sup>-</sup>	0.01±0.51-	0.01±0.51-
g/cow/day of formaldehyde- 0.02±0.50 <sup>-</sup> 0.02±0.50 <sup>-</sup> 0.02±0.50 <sup>-</sup>	g/cow/day	$0.01 \pm 0.52^{-1}$	0.00±0.53 <sup>-</sup>	0.00±0.53-	0.00±0.52-
treated choline chloride	g/cow/day of formaldehyde- treated choline	0.02±0.50 <sup>-</sup>	0.02±0.50 <sup>-</sup>	0.02±0.50 <sup>-</sup>	0.02±0.50 <sup>-</sup>
	Sig			NS	NS

NS=indicates no significant differences

It is noted from Table 8 that there is no significant effect of the treatments when adding choline chloride to the feed in the sensory milk evaluation characteristics, which include taste, flavor, color, texture, acidity, and the degree of acceptance, which gives a general indication of the absence of any negative effects of choline chloride on the qualitative characteristics. For milk, the milk was within the natural characteristics, and there were no significant differences between the treatments and the control, as the addition of choline chloride, treated and untreated with formaldehyde, did not affect the evaluation of the milk that was evaluated by experts in the dairy department at the station.

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# Table (8) The effect of adding choline chloride to the feed on the sensory evaluation characteristics of pasteurized milk of Holstein cows during the summer season (SE± mean)

Treatments	Admissions 10	Acidity 10	The color 15	Texture 30	Taste 35
T1Control treatment without addition	0.00±10.00	0.00±9.00	0.00±15.00	0.00±30.00	0.00±35.00
T2Add 35 g/cow/day choline chloride	0.00±10.00	0.25±9.25	0.00±15.00	0.00±30.00	0.25±34.75
T3Add 35 g/cow/day of formaldehyde- treated choline chloride	0.00±10.00	0.00±9.00	0.00±15.00	0.00±30.00	0.50±34.50
T4Add70g/cow/daycholine chloride	0.00±10.00	0.00±9.00	0.00±15.00	0.00±30.00	0.50±34.50
T5Add 70 g/cow/day of formaldehyde- treated choline chloride	0.40±9.60	0.40±8.60	1.00±14.00	0.00±30.00	0.37±34.20
Sig	NS	NS	NS	NS	NS

NS=indicates no significant differences

We conclude from the current study that the addition of choline chloride treated or not treated with formaldehyde to the feed in the amount of 35 or 70 g / cow / day did not significantly affect the components of milk and the sensory characteristics of milk.

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