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Iodine concentration in milk evaluated by iodized agents during milking

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Abstract

This research article describes the effect of different concentrations of iodine in the teat-dipping before milking and post-dipping solution on milk, urine, thyroid hormone, chemical composition and somatic cell count. Four multiparous cows were distributed in a 4 x 4 Latin square experimental design in a tie-stall system. The treatments were 0.5%, 1%, and 2% iodine and the control was treated with chlorhexidine. The iodine concentration of milk, food and urine was quantified. Thyroid hormones were analyzed by chemiluminescence microparticle immunoassay. Milk composition by differential absorption of infrared waves and somatic cell countby (SCC) flow cytometry. There was no significant effect of iodine concentrations in teats soaking solution before milking on urine iodine concentration and thyroid hormones (T3 and T4). Likewise, no effect of treatments was observed for the contents of protein, fat, lactose, total solids, Milk urea nitrogen and SCC. However, increasing iodine concentrations in the teat soaking solution before milking linearly increased the iodine concentration in milk. Therefore, the iodine concentration in the pre-milking and post-dipping solutions can change the iodine concentration in the milk.

Keywords: milking milk; composition; teat-dipping; thyroid hormones; urine.

Practical Application: This research contributes to investigate the effects of iodine concentrations on pre-milking teats immersion and post-dipping solution on milk, urine, thyroid hormone, chemical composition and somatic cell count.

1 Introduction

The increasing animal productivity and meeting sanitary requirements in Brazil have demanded milk producers to adopt strict control in the production system and animals, especially with the health of the mammary gland (Izquierdo et al., 2017; Silva et al., 2022). Milk that does not meet sanitary standards has been associated with poor hygienic practices in the milking process (Picinin et al., 2019). One of the practices that directly improves milk quality and udder health is the use of disinfectants before and after milking. In this sense, asepsis of the cow teat with iodine-based solutions is quite common and has been pointed out as a practice that reduces the occurrence of mastitis (Martins et al., 2017; Bobbo et al., 2017). However, O'Brien et al. (2013), Flachowsky et al. (2014) reported that disinfecting teats with solutions with different concentrations of iodine and iodine supplementation in a diet of dairy cows influences the concentration of this mineral in milk.

Iodine plays an important role in metabolism since it is directly linked to the synthesis of hormones T3 and T4 by the thyroid, which has a fundamental role in the metabolism of macronutrients, as well as in the development of various organs and growth (Norouzian, 2011; O'Kane et al., 2018). In urine, iodine has been used as an indicator of iodine intake (Boasquevisque et al., 2013; Arns-Glaser et al., 2022).

However, iodine can be a problem if it is present in high concentrations (van der Reijden et al., 2018; Shen et al., 2019). The human body can accept exposure or ingestion of iodine over the limits; however, some individuals are less tolerant. This group includes those who have some thyroid disease, patients with risk factors, the elderly, children, fetuses and newborns, that could potentially develop iodine-induced dysfunction, such as hypothyroidism or hyperthyroidism (van der Reijden et al., 2019).Therefore, we sought to evaluate the use of iodine solutions in pre-dipping and post-dipping on the concentration of iodine in milk, urine, and serum levels of thyroid hormones and the composition of milk and somatic cell count (SCC).

2 Materials and methods

The experiment was carried out in the dairy cattle sector at the Experimental Farm of Iguatemi of the State University of Maringá, located at 550 m altitude at Latitude 23º 25 '0.0 "S and longitude 51 º 57' 0.0", with annual average rainfall 1297 mm. The experimental proposal was submitted to the Ethics Committee

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on the Use of Animals at the State University of Maringá (CEUA/UEM) and was approved and filed under CEUA nº 5899220217.

The animals used in the experimental protocol were four multiparous Holstein cows weighing 566 ± 64 kg, with 120 ± 20 days of lactation and a daily average production of 18 kg/day of milk. The animals were housed in a tie-stall system. Milking was performed twice a day, with the first milking at 6 am and the second at 15 pm.

The diet of the cows was adjusted according to Nutrient Requirements of Dairy Cattle NRC (National Research Council, 2001) in a total mixed ration (TMR) with the proportion 60:40, composed of corn silage and concentrate based on corn, soybean meal, wheat, vitamin and mineral premix (5%) (Table 1).

The animals were randomly distributed according to the treatments (iodine concentration in the pre-dipping and post-dipping periods) in a 4 x 4 Latin square with four periods of 21 days with 14 days of adaptation. The treatments were 0.5%, 1%, and 2% iodine, respectively, and the control (without iodine) was treated with chlorhexidine (Prima Contact®; G3 Química. Lajeado / RS - Brazil). The concentrations of 0.5% and 1% iodine were based on commercial products sold on the market and 2%, an extrapolated value.

The sanitizers of 0.5%, 1% and 2% were prepared from a solution of 10% iodine Vansil® (Vansil Saúde Animal; Descalvado - SP) with the following composition: iodine + potassium iodide + bidistilled glycerin (Zafalon et al., 2008).

During milking, cows' teats were washed with running water and dried with paper towels only when excessive dirt was present. The clinical mastitis test was performed using a black-bottomed mug, pre-dipping performed with the respective experimental treatments with the aid of a cup-type applicator, in which the teat was completely immersed in the disinfectant solution. After 30 seconds, the excess product was removed using paper towels (Zafalon et al., 2008). After handling the hygiene of the teats, the milking unit attached for the complete and uninterrupted withdrawal of milk. After milking, post-dipping was carried out with experimental solutions with the same concentrations.

Table 1. Diet ingredients and chemical composition.

Ingredients	(g/kg of DM)
Corn Silage	600.0
Ground corn	202.8
Soyabean meal	165.2
Molasses	5.0
Vitamin and mineral premix	22.0
Limestone	5.0
Nutrients (g/kg of DM)	
Dry matter (DM)	453.0
Organic matter (OM)	934.0
Crude protein (CP)	145.0
Ether extract (EE)	27.80
Neutral detergente fiber (NDF)	332.0
Non fiber carbohydrates (NFC)	427.0
Total digestible nutrients (TDN)	694.0
Iodine in Corn silage iodine (ICS)	30.88
Iodine Concentrate (IC)	39.01

The samples of silage and concentrated feed were collected on the 17th day of the experimental period in a homogeneous collection, resulting in a single sample per animal/treatment/period. This sample was frozen in an ultra-freezer at -80 ºC for further bromatological and iodine analysis (Association of Official Analytical Chemists, 2000). The samples of concentrates, corn silage and leftovers were analyzed for dry matter, inorganic matter, total nitrogen, crude protein, ether extract (Association of Official Analytical Chemists, 2000), neutral detergent fiber and acid detergent fiber (van Soest et al., 1991)

The iodine concentration of the feed samples was determined based on the technique of Sandell & Kolthoff (1937) by means of acid extraction followed by an oxidation reaction between Ce^{4+} and AS^{3+} based on the methodology of Hedayati et al. (2007).

Blood was collected in duplicate in 10 mL Vacutainer® tubes containing heparin (Vaculplast® Cotia/SP - Brazil) on the 19th day of the experimental period. For plasma separation, blood was centrifuged at 3000 rpm for 10 minutes at 15 ºC, stored in an Eppendorf tube and frozen at -80 ºC for analysis of total T3 and T4 (Schöne & Rajendram, 2009). The T3 and T4 analyses were performed using a commercial kit (Architect Total T3 and Architect Total T4, Abbott Laboratories® (Abbott Laboratórios Do Brasil LTDA; Cidade Monções - São Paulo / SP- Brazil) for immunoassay analysis of microparticles by chemiluminescence (Refsal et al., 1984; Schöne et al., 2009).

A volume of 150 mL of urine was collected from each cow on the 20th day of each period for iodine analysis. The samples were frozen in a freezer at -18 ºC for later analysis (Moxon & Dixon, 1980; Hedayati et al., 2007). For analysis, urine samples were thawed in a water bath at a temperature between 36 °C and 38 °C. The iodine content was determined by means of acid extraction following an oxidation reaction between Ce4+ and AS3+ based on the methodology of Ohashi et al. (2000).

Milk samples were collected using a composed sample of milk collected from 3 days (19 to 21 days of the period) for analysis of iodine, chemical composition, and SCC.

Milk samples for iodine analysis were collected from each animal/treatment/period in a composed sample, making 2/3 of the bottle in the morning milking and 1/3 in the afternoon milking totaling 100 mL of milk. The samples were frozen in an ultra-freezer at -80 ºC for further analysis.

For analysis, the milk samples were thawed in a water bath at a temperature between 36 °C and 38 °C. The iodine was determined by means of acid extraction followed by an oxidation reaction between Ce^{4+} and AS^{3+} based on the methodology of Hedayati et al. (2007) with modifications, where the digestion solution was replaced by nitric acid and the maximum temperature of the block was 150 ºC.

A 50 µL aliquot of raw milk sample was added to digestion tubes, with dimensions of 25 x 250 mm plus 2000 µL of HNO_3. After the tubes were covered, they were placed in a digester block, and the temperature was raised every 50 °C and remained for 30 minutes in heating until reaching 150 °C, thus continuing

for 1 hour in digestion. At the end of the digestion process, for safety, the samples were cooled in the chapel. Then, for the redox reaction, 50 µL of the digested solution was added to the microtiter wells in polystyrene plates with 96 wells in quadruplicate. Another 100 µL of arsenic acid was added, and finally, 50 µL of cerium ammonium solution was added with the aid of a multichannel pipette. The samples were read at 405 nm after 20 min of incubation in a microplate reader (Molecular devices - Versa max, tunnable, LabCommerce, INC. ™; San Jose, CA- USA).

The calibration curve was prepared from a stock solution of 1000 µg/mL potassium iodide (IK) with concentrations of 0, 0.05, 0.1, 0.25, 0.5, 0.75 and 1 µg/mL, and the blank was ultrapure Milli-Q water (Benkhedda et al., 2009; Hedayati et al., 2007; Ohashi et al., 2000). The linearity coefficient for the regression of the logarithmic absorbance averages between 0 and 1 µg/mL was $R^2 = 0.9941$. The detection limit calculated by the equation DL = SD * 3/slope of the curve was 0.12 µg/mL (Ohashi et al., 2000; Peixoto et al., 2008).

Milk collection for chemical analysis and somatic cell count was performed in 40 mL bottles containing Bronopol preservative forming a composite sample representative of daily production 2/3 in the morning milking and 1/3 in the afternoon milking. The fat, protein, lactose, total solids (TS), and (mg/dL) milk urea nitrogen (MUN) contents in milk were determined by a spectrophotometer; Bentley Instrument, Inc., Chaska, MN, USA), and the SCC (SC/mL) was obtained using an electronic counter (Somacount 500®, Chaska, MN, USA) (Voltolini et al., 2001), carried out by the dairy control laboratory of the *Associação Paranaense dos Criadores de Rebanho Bovino da Raça Holandesa,* located in Curitiba, PR.

The experimental design used was a Latin square 4 x 4, composed of 4 cows, 4 periods and 4 levels of iodine in pre-dipping and post-dipping solutions. The evaluated variables were submitted to analysis of variance according to the statistical model (Equation 1):

$$Yijk = \mu + \alpha i + Tj + \beta j + eijk \quad (1)$$

Where: Yijk = observed variables, µ = overall average; αi = animal effect, ranging from 1 to 4; Tj = period effect j, ranging from 1 to 4; βj = effect of iodine levels (product) k, ranging from 1 to 4; eijk = random effect.

All effects were considered fixed, except the animal effect, which was random. The differences between the treatment means were determined by the Tukey test considering (α = 0.05) significance.

The concentrations of iodine in milk, urine, and thyroid hormones T3 and T4, chemical composition, and SCC were assessed using the mixed model (proc MIXED) and linear regression analysis (α = 0.05). The relation of feed iodine and blood, milk and urine parameters were correlated with the package (proc CORR), and both analyses were performed with the aid of the SAS program (Statistical Analysis System, version 9.2. - 2013) (SAS/STAT software, 2013).

3 Results

The use of iodine-based disinfectant in the antisepsis of teats at different concentrations showed a positive linear effect for the pre- and post-dipping treatment with the iodine concentration of milk (P = 0.002) (Table 2).

This effect can be visualized by the linear regression model (Equation 2):

$$\left[Iodine\right] = 0.11991 * Content + 0.34068 \quad (2)$$

As the iodine concentration in the disinfectant of the pre- and post-dipping treatment increased (0, 0.5, 1 and 2%), the same effect of 368.9, 400.5, 444.4, and 604.2 µg/L (P < 0.05) occurred in milk (Table 2).

The iodine concentration in milk was 35% higher when using a 2% disinfectant solution compared to 1%, 604.2 ug/and 444.4 of iodine, respectively (Table 2).

The concentration of iodine in the urine was not affected by pre- or post-dipping with different concentrations of iodine (P > 0.05) (Table 2).

The synthesis of thyroid hormones T3 and T4 is dependent on iodine. The studied concentrations and the absorption of iodine through the skin did not influence the blood concentrations of T3 and T4 (P = 0.727, P = 0.349), respectively.

There was a positive correlation between food iodine and 0.636 x 0.008 milk (Table 3). The excretion of iodine in milk increased with the increase in iodine in the diet, but not linearly, as the mammary gland acts as a biological regulator. The amount of total iodine in the experimental diet was 69 mg/kg DM.

Based on the above, the results of this work contribute to reinforcing that measures to prevent milk contamination at the time of milking must be taken.

Table 2. Concentration of iodine in milk and urine and concentration of hormones T3 and T4 of Holstein cows pre- and post-dipping with sanitizers containing different concentrations of iodine pre- and post-dipping.

Samples	Iodine concentrations				SEM	P effect		
	0%	0.5%	1%	2%		Level	Linear	Quadratic
Milk (µg/L)	368.9	400.5	444.4	604.2	0.06	0.013	0.002	0.353
Urine (µg/L)	593.4	634.4	660.1	572.5	0.11	0.842	0.793	0.425
[1]T3 (ng/dL)	69.93	66.65	71.75	64.31	4.99	0.727	0.522	0.642
[2]T4 (µg/dL)	3.60	3.40	3.64	3.20	0.27	0.349	0.651	0.423

[1]Total triiodothyronine; [2]Total thyroxine. P > 0.05 shows no significant difference. SEM: Standard error mean.

Table 3. Correlation between dietary iodine and milk, urine, T3 and T4.

	N	Mean	SD	Min	Max	Pearson Correlation	
Feed (mg/kg MS)	16	36.97	8.13	30.85	50.39		
Milk (µg/mL)	16	0.5	0.14	0.25	0.88	F x M	0.636 x 0.008
Urine (µg/mL)	16	0.61	0.2	0.39	1.1	F x U	-0.100 x 0.711
[1]T3 (ng/dL)	16	3.46	0.53	2.58	4.66	F x T3	-0.273 x 0.305
[2]T4 (µg/dL)	16	68.15	9.42	51.41	81.38	F x T4	-0.218 x 0.415

[1]Total triiodothyronine; [2]Total thyroxine. Min: Minimum; Max: Maximum.

Table 4. Chemical composition, MUN, SCC in milk from Holstein cows, after subjected to pre- and post-dipping with a sanitizer with different levels of iodine.

Samples	Iodine concentrations				SEM	P effect		
	0%	0.5%	1%	2%		Level	Linear	Quadratic
Protein (%)	3.50	3.40	3.64	3.21	0.15	0.24	0.23	0.23
Fat (%)	3.34	4.07	4.09	3.02	0.44	0.21	0.38	0.06
Lactose (%)	4.25	4.09	3.95	3.82	0.19	0.47	0.14	0.72
[1]TS (%)	11.09	11.57	12.13	10.06	0.67	0.17	0.20	0.07
[2]MUN (mg/dL)	12.09	13.35	12.15	13.20	1.87	0.74	0.62	0.99
[3]SCC - Log (SC/mL)	2.05	2.22	2.64	2.25	0.15	0.03	0.50	0.01

$P > 0.05$ shows no significant difference. [1]Total solids; [2]Milk urea nitrogen; [3]Somatic cell count.

For the variables protein, fat, lactose, TS, and MUN, no significant effect was found in the experimental treatments ($P > 0.05$). After using the iodized solutions, a difference was found in the SCC in the treatments ($P < 0.05$) (Table 4).

4 Discussion

The practice of teat disinfection with iodine solution has been identified as a factor that increases the concentration of iodine in milk, both by direct contamination and by absorption by the epithelium of the teats (Flachowsky et al., 2007; O'Brien et al., 2013; van der Reijden et al., 2019). What may have occurred in the present study, since this effect was visible by the dissipation of the yellow color in the skin of the cow's teats. The same evidence was observed by Flachowsky et al. (2014), who concluded that the primary mode of iodine absorption appears to be through the skin. Nevertheless, Nesvadbova et al. (2015) reported that the absorption of iodine by the skin occurs at 12% through direct contact. Likewise, Abraham (2008) also observed that part of the iodine evaporates and the rest that remains in contact with the skin gradually is absorbed.

The physiological explanation for the absorption of iodine by the skin is the transfer of iodine by the Symporter NIS to the mammary gland and then translocated to milk to play a role in the synthesis of thyroid hormone in the young (Ravera et al., 2017; van der Reijden et al., 2019). This same tendency to adhere to the skin was observed by Castro et al. (2012) when they evaluated the use of different concentrations of iodine applied dipping and spraying. When the teat was immersed in the 1% solution in a cup (dip), the levels of iodine were 360 µg/kg, and when using the spray solution, the concentration of iodine in milk was higher (409 µg/kg). These authors concluded that the spray solution covers a larger area of the udder and predisposes to direct contamination of milk when compared to the cup that has contact only with the cow´s teat skin.

However, Rasmussen et al. (1991) also suggested that the iodine residue in milk is due to contamination in the surface of the teats and not skin absorption because when the teats were cleaned with a towel, the increase in iodine in the milk was negligible. Nonetheless, pre-dipping management, including the use of towels, has proven to be efficient both for milk quality and for avoiding direct iodine contamination.

Similar results were observed by French et al. (2016) who evaluated different types of applicators (dip, barrier, and spray) together with the use of disinfectants with different concentrations of iodine (0, 0.25% and 0.50%) and concluded that there was an increase of 20 ± 7 µg/L of iodine in the concentration of milk when using the formulas. In addition, the form of application of the solution on the teats influenced the quantity of iodine in the milk, and the spray application increased the concentration compared to the procedure using immersion.

The pre-dipping iodine concentration influenced the milk iodine concentration in this study, and the same was observed by Flachowsky et al. (2007) who concluded that the use of iodine in pre-dipping essentially contributes to the concentration of this element in milk. In particular, the transfer of iodine from the disinfectant to teats and milk depends on the content of the disinfectant, milk production, and teat immersion frequency. Ahvanooei et al. (2021) corroborated that the concentration of iodine in the post-dipping period increased the levels of iodine in the milk, whereas the pre-dipping period did not influence the levels.

Contamination of milk by iodine related to food management practices has been studied in other countries, such as Canada

and Switzerland. Castro et al. (2010) and van der Reijden et al. (2018) observed that the iodine concentrations in the samples ranged from 54 to 1,902 µg/kg of milk.

Iodine in urine in humans has been used as an indicator to monitor the status of dietary iodine (Boasquevisque et al., 2013). In cows, the use of potassium iodide in the diet increased the concentration of iodine in the urine 327.4 for 408.2, milk 284.3 for 1,501.4 and serum 38.3 for 74.2 on day 2 of sampling, while removing the addition of iodine in the diet, the levels decreased instantly (Ahvanooei et al., 2021).

The absence of a relationship between pre- and post-dipping T3 and T4 in the present study was observed by other authors who reported no effect of pre- and post-dipping treatment on the levels of thyroxine (T4) on days 1 and 19 of treatment (Castro et al., 2012; Ahvanooei et al., 2021). According to National Research Council (2001), the feed had 13.8% iodine above the requirements. There was no correlation with the other variables studied.

Likewise, the results observed by Weiss et al. (2015) when feeding cows canola meal showed that there was a reduction in the concentration of iodine in milk, even though the serum iodine concentration increased linearly. The authors concluded that an increase in serum was probably caused by a reduced transfer of iodine to milk and other organs, such as the thyroid. Some foods cause this reduced iodine transfer effect, as they contain goitrogenic substances that inhibit the binding of iodine in the hormone precursor protein (thyroglobulin), reducing the hormonal and iodine concentrations in milk (European Food Safety Authority, 2014).

Similar to what was exposed in this work, O'Brien et al. (2013) reported a considerable increase in the concentration of iodine in milk from 217 µg/kg in the group of animals that had no supplementation and disinfection of the teats to more than 1,000 µg/kg in the group of animals supplemented and teat disinfection with iodine solution.

Higher tolerance limits have been proposed by the Institute of Medicine for Children and Adolescents, between 200 and 600 µg day, adults and pregnant women 1,100 µg/day (European Food Safety Authority, 2014; Flachowsky et al., 2007). Thus, considering milk consumption in normal diets, they would hardly provide more than 1 mg of iodine per day. Therefore, one of the recommendations is to maintain 0.5% iodine levels post-dipping for both effective disinfection and prevention of contamination (Castro et al., 2012). Additionally, supplementation should be maintained according to the recommended daily doses (50 mg/kg DM) so that it has less effect on milk (National Research Council, 2001).

The values of fat, protein, lactose, and SCC showed results within the standard required by current Normative Instruction no. 76 of the Ministry of Agriculture, Livestock and Supply of Brazil (Brasil, 2018). According to Silva et al. (2019), MUN values in milk between 11 mg/dL and 18 mg/dL remain adequate.

The SCC had a treatment effect ($P > 0.05$), the chlorhexidine and 2% iodine decreased the somatic cell count unlike the 0.5 and 1% iodine treatments (Table 4). In addition, these sanitizing agents, chlorhexidine and 2% iodine are effective in reducing the microbial load responsible for mastitis (Gleeson et al., 2009), which, consequently, has a direct relationship with SCC (Cabral et al., 2016).

The use of iodine post-dipping reduces the SCC in individual milk and the bulk, in addition to contributing to the reduction of intramammary infection when compared to animals that have not been submitted to post-dipping (Williamson & Lacy-Hulbert, 2013).

The results obtained by Castro et al. (2012), who found that there was no effect of the use of iodine from the pre- and post-dipping treatments on the levels of fat, protein ($P = 0.76$ and 0.99) and milk SCC ($P = 0.53$). The same result was obtained by Grace & Waghorn (2005), who also did not observe any difference ($P > 0.05$) in the composition of milk, fat, and protein with injectable iodine supplementation.

5 Conclusion

The use of iodine in the disinfectant solution in the pre- and post-dipping of Holstein cows can influence the concentration of iodine in milk and SCC. However, it does not affect the level of hormones T3 and T4, the excretion of iodine in the urine, or the chemical composition of milk.

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