

The Effect of Iodophor Post-Milking Teat Disinfection on Iodine Content in Goat Milk

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ABSTRACT

Iodine intake is important for thyroid function and human health. Goat milk can be an important source of iodine for human nutrition. However, data regarding the effect of iodophor post-milking teat disinfection on iodine content in goat milk is lacking. Our aim was to assess the iodine concentrations in raw milk of dairy goats and to investigate the effect of post-milking teat-dipping iodophor practice on iodine content in goat milk. Two groups of dairy goats ($n=6$ in each) were treated with different post-milking teat-dipping disinfection: iodine-free solution (iodine-free group) and iodine-based solution (4,000 µg/L) (iodophors group). Treatments were carried out for 19 experimental days, following a 14-day pre-experimental period, in which only iodine-free sanitizer was used for both groups. The results showed that Iodine concentrations in milk of all goats were 49 ± 23 , 49 , 17 - 86 µg/100g (mean \pm SD, median, range) at days -3, -2, -1 and 45 ± 26 , 42 , 14 - 96 µg/100g (mean \pm SD, median, range) at days 17, 18, 19 of treatment. Iodine concentration increased by 7 µg/100g (mean) in the iodophors group while iodine concentration decreased by 15 µg/100g by day 17-19 of treatment in the iodine free group. It was concluded that relatively high iodine concentrations were found in raw milk of dairy goats whose teats were dipped post-milking in disinfectants with or without iodine. Post-milking teat-dipping iodophor practice may increase iodine content in goat milk within an average period of 20 days. This information can help in controlling iodine content in goat milk and iodine intake in the public.

Key words: Iodophors; Iodine; Dairy goats; Post-milking Teat Disinfection; Iodine Intake; Public health.

INTRODUCTION

Iodine intake is important for thyroid function and human health. Either high or low iodine intake may lead to thyroid disease (1). Excessive iodine intake is linked with increased risk of iodine-induced hyperthyroidism and autoimmune thyroid disease (2, 3). Iodine deficiency impairs thyroid hormone production and may lead to damages to the developing

brain *in utero* and during childhood, loss of intellectual potential (4,5), mental retardation (up to cretinism) during pregnancy (6), goiter (enlargement of the thyroid gland), hypothyroidism and iodine induced hyperthyroidism (4). These disorders are afflicting increasing numbers of people as iodine deficiency is still a problem in developing regions and has recently re-emerged in industrialized countries (7,

8). Thus, assessing iodine concentration of dietary iodine contributors in industrialized countries is essential for public health as adequate iodine intake is required for normal thyroid function.

Dairy products could offer a significant source of iodine in both developed and developing regions. For example, milk and dairy products were estimated to contribute approximately 60% of total iodine intake in the Norwegian population (9) and 40% among French children (10). Milk consumption in developing countries almost doubled during 1961-2007, while roots and tubers consumption slightly declined during the same period (11). Additionally, soy-based beverages (sometimes perceived as a milk alternative), which are rarely fortified with iodine, are not considered as replacement to the iodine in ruminants milk, due to negligible iodine concentration (12-15). Therefore, milk of ruminants may represent an important source of iodine for humans.

Milk and dairy products from goats are important for human nutrition, especially in developing countries. Goat milk accounts for 2.4 percent of global production, making it the third contributor after cow milk (83.1 percent) and buffalo milk (12.8 percent) ranking above sheep milk (1.4 percent) (11). Among human, cow, buffalo, sheep and goat milk, the latter is the richest in short and medium chain fatty acids (16) as well as iron (11). Anecdotal evidence, stemming in part from cultural beliefs and in part from research studies, suggests that goat milk has lower allergenicity than cow milk (17,18). All of these data make goat milk of important interest.

With old traditions being preserved, surging population growth and human quest for "healthy" foods worldwide, goat milk and products are increasingly produced to meet increased demand. Goat milk contribution is relatively significant in sub-Saharan Africa and parts of South Asia and of East and Southeast Asia (excluding China) for 10 percent of the total production (19). Field observations in developing countries indicate that goat milk impact on both household food security and poverty alleviation is substantial (11). In developed countries, goat cheese is now holding a perception of both a "healthy" and a culinary food (20). The goat industry within the U.S. is growing, both in inventory and markets for goat milk products (21). During the years 2008-2017, the local Israeli production of goat milk has increased by 38%, while this of cow milk has increased by 19%. Additionally, hard goat cheese export from Israel has increased by 40% during these years (22). These

reports emphasize the significance of investigating possible contribution of goat milk to public health.

Information regarding factors influencing iodine content in goat milk is rather scarce. The iodine contents were found to vary by season with higher concentration in the summer, probably due to lower thyroid activity (23, 24) and increased content by iodine supplementation to the lactating goat (25). However, lactating goats seem to have relatively higher dietary iodine needs than lactating cows, probably due to higher iodine deficiency sensitivity. The iodine requirements of lactating goats are normally set for 0.8 mg/kg dry matter (DM) (unlike 0.5 mg/kg DM for lactating cows) (26). Iodine content of cow milk, unlike goat milk, is also affected by drinking water and dairy practice. Nevertheless, commonly used non-supplemented feeds and water do not meet the iodine requirements of food-producing animals, leaving the dairy practice with greater impact (26).

The effect of iodophor practice of teat-dipping on milk iodine content has been described in many dairy cows studies (27) and showed correlation of milk iodine content with increasing iodine content of iodophors (26, 28). However, data regarding such influence in goat milk is lacking. The effect of post-milking iodophor practice on iodine content in goat dairy products is likely to possess a global importance, given the recent re-emergence of iodine deficiency in varied areas (7) and the apparent rise in goat dairy products demand (21, 22). Therefore, our aims were: (1) to assess the iodine concentrations in raw milk of dairy goats and (2) to investigate the effect of post-milking teat-dipping iodophor practice on iodine content in goat milk.

MATERIALS AND METHODS

Experimental Design and Conditions

The trial was carried out at the modern experiment pen at the Department of Animal Sciences, the Robert H. Smith Faculty of Agricultural, Food and Environment, the Hebrew University of Jerusalem, Rehovot, Israel. All procedures involving the goats used in this study were approved by the Hebrew University Institutional Animal Care and Use Committee (Israel). A total number of 12 multiparous dairy goats (six Israeli Saanen, three Nubian "Shami" and three mixed of both of these two breeds) in the early stages of lactation were divided into two groups, considering the milk yield, age, and days in milk (DIM). At the beginning of the

trial (14 days before treatment), all goats were 103 ± 27 DIM, had 2.6 ± 1.0 previous lactations and were with a daily milk yield of 2.1 ± 1.4 L (mean \pm SD).

Feed, milking, trial duration and treatments

The goats were group fed with an average of 2.2 kg total mixed ration (TMR) daily that was formulated as follows: ration composed of (36:64, on DM basis) a commercial pelleted dairy concentrate (mix #5094; Ambar Feed Mill co-operative association Ltd., Hadera, Israel) and roughage TMR (Newmix; International Feeding Center, Massuot Itzhak, Israel). The pellets were comprised of (DM basis) 18% crude protein (CP) ($N \times 6.25$), 20.4% neutral detergent fiber (NDF), 6.3% acid detergent lignin (ADL), 1.79 Mcal of NE_L (calculated)/kg of DM and a vitamin and mineral premix (Zemach Feed Mill co-operative association Ltd., Zemach, Israel), including estimated 88.1 µg iodine per 100g (0.88 mg/kg) premix (DM basis, an estimated daily feeding with of 704.8 µg iodine). The TMR was composed (on DM basis) of wheat silage (33.3%), wheat straw (23.3%), wheat bran (4%), ground wheat grains (4%), wet corn gluten feed (10.0%), distillers dried grains with solubles (4.8%) and molassed soybean hull (19.2%). The DM ratio of the TMR was 58.6% and it had 10.4% CP, 51% NDF, 28% ADF, and 1.39 Mcal of NE_L/kg of DM. All goats had free access to drinking water at all times. All goats were housed in group pens with earth/dirt bedding.

Milking took place once a day from 07:30 h to 08:30, at which time milk yield was recorded with automatic milk meters. A 14-day pre-experimental period, in which all goats were fed to requirements and no iodine sanitizer was used, was included to eliminate the effects of previous iodine intake and iodophor usage. During the 19 experimental days that followed, the two groups were assigned randomly by using a random number table to one of two treatments. Teats of the iodine-free group were dipped with an iodine-free teat dip after each milking (Klorwet, troclosene sodium: 53%, Conzept, USA;). Teats of iodophor group were dipped with 4,000 µg/L concentration solution of iodine-based teat dip after each milking (Comoseptil V: Glycerol 5%, Iodine 2.2%, Johnson Diversey, Israel Ltd., Israel).

Collection and Sampling

The collection of all raw goat milk samples was made during February and March 2016. A total of 108 samples were col-

lected on days -3, -2, -1, 7, 8, 9, 17, 18 and 19 relative to the start of the treatment period. Every three replicates of each period (at days -3 to -1, 7-9 and 17-19) from each raw goat milk were equally pooled by volume and then homogenized and weighed to prepare equal samples. Additionally, three samples of the goats' drinking water for each period (at days -3 to -1, 7-9 and 17-19) were collected. All-in-all, the collected 108 raw goat milk replicates were pooled into 36 different samples, along with three drinking water samples.

The 39 samples were frozen at -20°C in 5ml screw-capped polypropylene centrifuge tubes until analysis and were shipped in this frozen condition to the National Institute of Nutrition and Seafood Research, Bergen, Norway. Before analysis, each sample was left to thaw at room temperature.

Laboratory Analysis

The determination of iodine concentration in the raw goat milk was carried out using Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) after digestion in tetra methyl ammonium hydroxide (TMAH) as previously described (29). A test sample of 1ml milk was added to 5ml deionized water and 1ml TMAH. Extraction was carried out in a dry oven at $90^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 3 hours. After cooling, 1 mg/L Tellurium was added as an internal standard to the sample solution and diluted to a final volume of 25 ml with deionized water. Prior to analysis, all samples were filtrated through a 0.45 µm filter using a syringe. Certified reference material was selected and used to measure the accuracy and precision of the method.

Statistical Analysis

Descriptive statistics and statistical analyses were performed with JMP Pro software (version 13). Goodness-of-fit for the distribution of iodine concentrations of complete study sample and each group was determined by both Shapiro-Wilk W and Cramer-von Mises W tests. Tests of the equality of variance for each group were determined by both Levene and Bartlett tests. The non-parametric test of Kruskal-Wallis (Rank Sums) was used to compare iodine concentrations by group. Multivariate correlation technique was utilized to explore relations between all continuous variables (age, number of previous lactations, lactation stage and milk yield). Comparison of groups' iodine concentration least square means versus time was determined with MANOVA followed by Univariate adjustment Epsilon test. A two tailed P-value < 0.05 was considered statistically significant.

RESULTS

Descriptive characteristics and milk yield of the participating goats

The descriptive characteristics of the two groups are shown in Table 1. Groups did not differ significantly with respect to breed distribution, lactation and milk yield. Age and number of previous lactations were significantly higher among iodophors group. However, there was no significant integrated difference in iodine concentration after multivariate analysis. Milk yield was similar in both groups and did not differ by treatment (at days 7-9 and 17-19).

Iodine content determination

The iodine concentration values in milk of all goats participating in this study were 49 ± 23 , 12-108 $\mu\text{g}/100\text{g}$ (mean \pm SD, range) at days -3 to -1 of treatment and 45 ± 26 , 14-96 $\mu\text{g}/100\text{g}$ (mean \pm SD, range) at days 17 to 19 of treatment. The iodine concentration values by group for the entire study period are given in Table 2. Throughout all collection periods, none of the sample results was below the suggested range of raw milk iodine concentration indicating iodine deficiency among dairy goats: 0.8-2.5 $\mu\text{g}/100\text{g}$ (30, 31). All three drinking water samples contained no iodine.

Post-milking teat-dipping iodophor practice effect on goat milk iodine content

Average values of iodine concentrations at days 7, 8, 9 and days 17, 18, 19 among iodophors group were higher by 11.6 $\mu\text{g}/100\text{g}$ and 17.7 $\mu\text{g}/100\text{g}$ (respectively) than those of

Table 1: Descriptive characteristics, milk yield by treatment days and lactation duration by teat dip treatment groups

Characteristics	iodophor ^I	iodine-free
<i>n</i>	6	6
Breed (Saanen, Shami, mixed) ^B	2,2,2	4,1,1
Age (y)		
Mean \pm SD	3.9 \pm 1.2	2.5 \pm 0.5
Median	3.5	2.6
Range	3.1-6.1	2.0-3.0
Previous lactation		
Mean \pm SD	3.2 \pm 1.0	1.8 \pm 0.4
Median	3.0	2.0
Range	2.0-5.0	1.0-2.0
DIM (d) ^D		
Mean \pm SD	105.0 \pm 5.6	100.8 \pm 47.6
Median	105.7	112.0
Range	98.7-111.3	11.2-154.0
Milk yield (L/d) ^E		
Mean \pm SD	2.6 \pm 1.6	2.4 \pm 1.5
Median	2.4	2.4
Range	0.7-5.1	0.8-4.2
Milk yield (L/d) ^F		
Mean \pm SD	2.7 \pm 1.7	2.5 \pm 1.7
Median	2.3	2.5
Range	0.7-5.1	0.8-4.2

^I Dipped with 4,000 $\mu\text{g}/\text{L}$ concentration solution of iodine-based teat dip.

^B Saanen = Israel Saanen breed; Shami = Cyprus Shami breed; Mixed = mixed of both Saanen and Shami.

^D At the beginning of the trial (14 d before treatment).

^E During all trial period (33 d).

^F During treatment period (19 d)

Abbreviations: y = years; d = days; SD = standard deviation; L/d = liters per day; DIM = days in milk; $\mu\text{g}/\text{L}$ = microgram per liter.

Table 2: Average milk iodine concentration for teat dip groups by treatment days.

Characteristics	iodophor ^I				iodine-free			
	Treatment days	-3 to -1	7 to 9	17 to 19	Δ	-3 to -1	7 to 9	17 to 19
Iodine content ($\mu\text{g}/100\text{g}$)								
Mean \pm SD	46.8 \pm 21.3	55.3 \pm 27.1	54.0 \pm 31.6	7.2	50.9 \pm 36.3	43.7 \pm 19.8	36.3 \pm 18.3	-14.6
Median	50.6	60.4	53.1	2.5	41.7	40.8	34.7	-7.0
Range	12.1-69.5	16.6-85.6	14.2-96.2		15.9-108.2	21.3-67.8	15.1-61.8	

^I Dipped with 4,000 $\mu\text{g}/\text{L}$ concentration solution of iodine-based teat dip.

Δ Total mean difference in milk iodine concentration between treatment days from (-1 to -3) to (17 to 19) for each group.

^a The difference with the iodine-free group alternate mean was non-significant ($P=0.82$, *t*-test).

^b The difference with the iodine-free group alternate mean was non-significant ($P=0.42$, *t*-test).

^c The difference with the iodine-free group alternate mean was non-significant ($P=0.26$, *t*-test).

Abbreviations: $\mu\text{g}/100\text{g}$ = microgram per one hundred grams; SD = standard deviation; $\mu\text{g}/\text{L}$ = microgram per liter

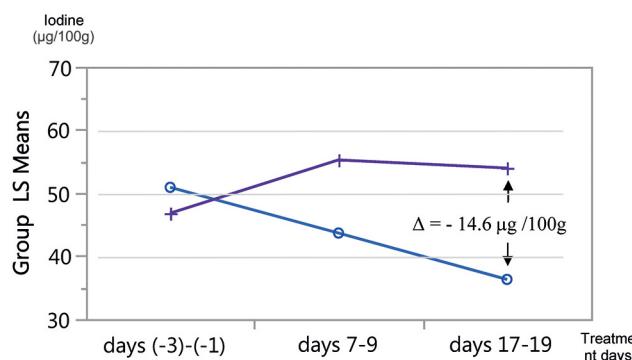


Figure 1: Iodine content changes over time in goats' milk by teat-dip treatment.

Averages of iodine content ($\mu\text{g}/100\text{g}$) time changes* in goats' unprocessed milk by teat dip group.

—○— iodine-free solution ($n=6$).

—+— 4,000 $\mu\text{g}/\text{L}$ iodine concentration iodophor solution ($n=6$).

Δ Total means difference of milk iodine concentration between groups in days 17 to 19.

* Mutual effect of time and treatment ($P=0.0766$, Manova, Univariate adjustment Epsilon test).

Abbreviations: $\mu\text{g}/100\text{g}$ = microgram per one hundred grams; LS = least square; $\mu\text{g}/\text{L}$ = microgram per liter.

iodine-free group, as reported in Table 2 and shown in Figure 1 and Figure 2, but did not differ significantly ($P=0.42$ and $P=0.26$, t -test, respectively).

Time and treatment mutual impact on goat's milk iodine content

After an average period of 19 days, mean iodine concentration increased by 7 $\mu\text{g}/100\text{g}$ (15% increase) in the iodophors group while decreasing in the iodine-free group by 15 $\mu\text{g}/100\text{g}$ (29% decrease). The mutual effect of time and treatment on goats' milk iodine content in this study was almost significant ($P=0.0766$, Manova, Univariate adjustment Epsilon test). Time and treatment mutual impact of the treatment are shown in Table 2 and described in Figure 1 and Figure 2.

DISCUSSION

The overall iodine concentration in the raw milk of this study sample ($N=12$) at the end of the treatment with two of the most locally frequently used solutions (based of iodophors and chloral-based solution) for post-milking teat-dip disinfection (32), is indicative of iodine adequacy. First,

none of the samples results (before and during treatment) was below the suggested interval of raw milk iodine concentrations, indicating iodine deficiency among dairy goats: 0.8–2.5 $\mu\text{g}/100\text{g}$ (30,31). Second, the overall iodine concentration at the end of the treatments ($45\pm26 \mu\text{g}/100\text{g}$, mean \pm SD) was higher than in a similar study (30). These data suggest that the iodine exposure of the current studied goats was adequate.

As the raw milk iodine content of the goats in this study demonstrated iodine adequacy, the contribution of the iodine supplements should be investigated. These goats were fed with pellets containing 88 $\mu\text{g}/100\text{g}$ (of DM) iodine (as reported by the manufacturer). This level is higher than the recommended 80 $\mu\text{g}/100\text{g}$ (33). There are three reasons to focus on the iodine supplementation of the pellets as the primary contributor to the goats' adequate iodine intake. First, other study showed an effect of dietary iodine supplementation on raw dairy goats iodine content (25). Second, the goats in the current study were supplied with iodine-free drinking water. Third, these goats had no other probable dietary source of iodine except for the supplementation (within the pellets). In Israel, the majority of crops, including the component of the TMR in this study, are irrigated with iodine-depleted desalinated water (34–36). In other words, our study results suggest exploring the contribution of iodine supplementation to iodine sufficiency among lactating goats in regions with access mainly to iodine-depleted desalinated water.

This study revealed relatively high iodine concentrations in raw milk of lactating goats who were treated with the maximal local recommended concentration (Israeli Dairy Board) level of post-milking teat-dip iodophor solution (4,000 $\mu\text{g}/\text{L}$) for 19 experimental days. Studies from Western Czech Republic reported lower iodine concentrations in goats milk in comparison to the current study: $14\pm10 \mu\text{g}/100\text{g}$ (mean \pm SD, $n=17$, estimated feed iodine supplementation: 70 $\mu\text{g}/100\text{g}$ of DM) (30). Other studies investigating iodine concentrations in goat milk reported lower concentrations: $7\pm3 \mu\text{g}/100\text{g}$ (mean \pm SD, $n=10$), East Slovakia (37), 6 $\mu\text{g}/100\text{g}$ (mean, $n=10$, estimated feed iodine supplementation: 63 $\mu\text{g}/100\text{g}$ of DM) in Italy (25) and $39\pm11 \mu\text{g}/\text{L}$ (mean \pm SD, $n=24$, estimated feed iodine supplementation: 110 $\mu\text{g}/100\text{g}$ of DM) south-east of the Czech Republic (38). The lower milk iodine concentrations among the Czech and Italian dairy goats (25, 30) may be explained, in part, by the lower

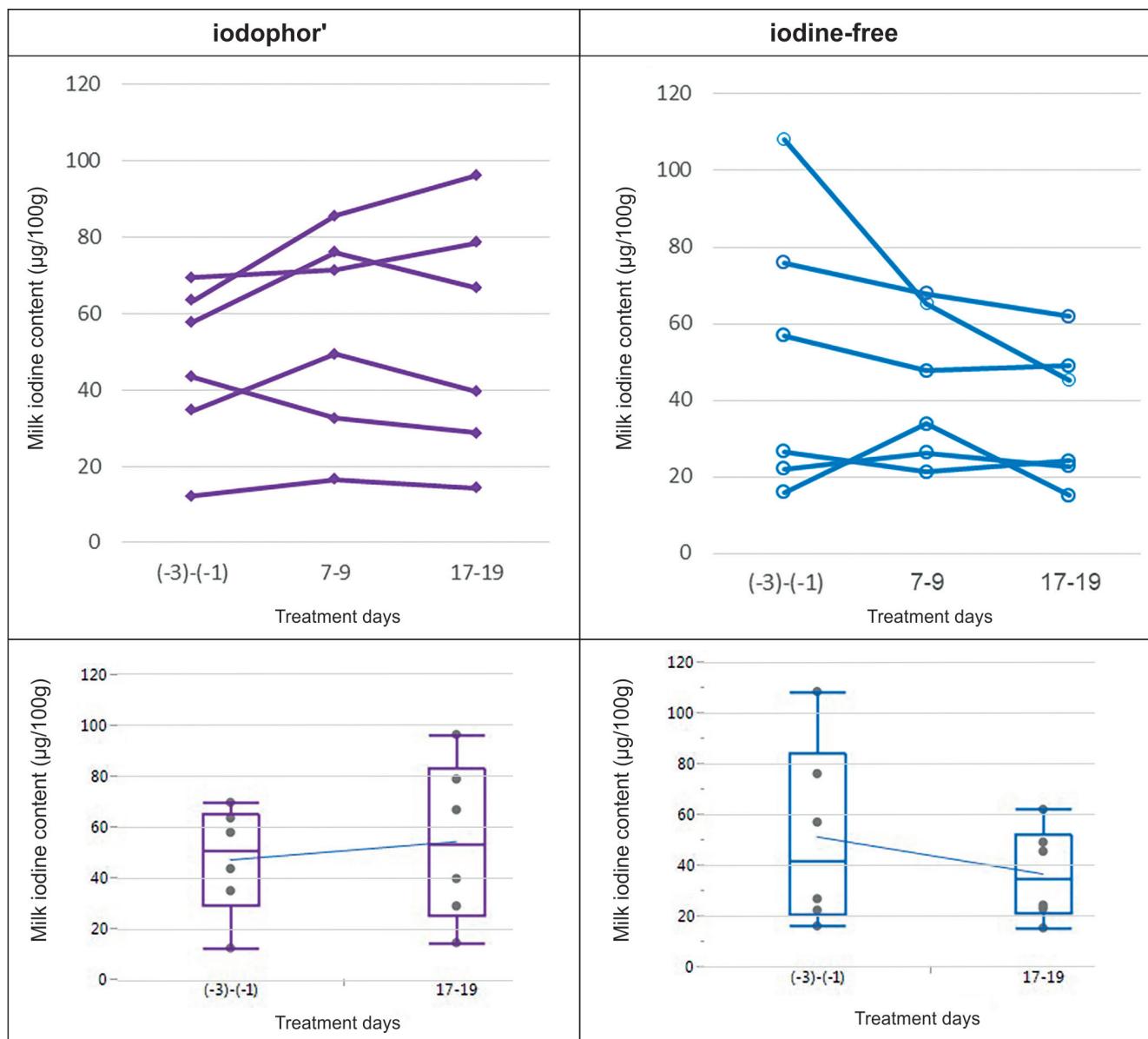


Figure 2: Iodine content means by each goat (above) and box and whisker plots of median and IQR (below) of iodine content changes in goats' milk over time by teat dip treatment for iodophor^a and iodine-free groups^b.

Box minimum and maximum values are 25th and 75th percentiles \pm 1.5*IQR. Total range is between upper and lower Whisker extreme lines.
dipped with 4,000 µg/L concentration solution of iodine-based teat dip.

^a Mean of iodine concentration within iodophors group increased during the treatment from 47 to 54 µg/100g with no significant difference ($P=0.65$, t -test).

^b Mean iodine concentration within iodine-free group decreased during the treatment from 51 to 36 µg/100g with no significant difference ($P=0.39$, t -test).

Abbreviations: µg/100g = microgram per one hundred grams; µg/L = microgram per liter

— iodine-free solution.

† 4,000 µg/L iodine concentration iodophor solution. — Means connected by line.

Statement: There are no potential conflicts of interest for each author relevant to this article.

Abbreviations: dry matter (DM), days in milk (DIM), total mixed ration (TMR), neutral detergent fiber (NDF), acid detergent lignin (ADL), Inductively Coupled Plasma – Mass Spectrometer (ICP-MS), tetra methyl ammonium hydroxide (TMAH), limit of detection (LOD), Recommended Daily Allowance (RDA).

Statements: (1) All procedures involving the goats used in this study were approved by the Hebrew University Institutional Animal Care and Use Committee (Israel); (2) This manuscript have not been published previously and have not been submitted for publication elsewhere.

estimated feed iodine supplementation in comparison to the current study; however, the lower milk iodine concentrations among the other Czech dairy goats (38) were lower than the concentrations of the current study (36 vs. 54 µg/100g, respectively). Additionally, the estimated feed iodine supplementation of our iodophor group was lower than that of the recent Czech study (88 vs. 110 µg/100g of DM, respectively). Hence, these differences support the hypothesis regarding dairy goat milk iodine contamination by iodophors sanitizers use during milking.

Studies showed that teat-dipping iodophor practice in dairy cows increases iodine content in raw milk (27,28). In this study, we report an apparent effect of post-milking teat-dipping iodophor practice on iodine content in goat milk of a 15% increase. However, this effect was not significant. These results are meaningful for two main reasons. First, the iodine-free group showed decreased iodine content pattern in the absence of iodophors practice during the treatment. Second, this effect occurred after a short period relative to other similar studies in cows (17–19 days vs. 70–140 weeks) (39, 40). As milking period of dairy goats can last more than 10 months (41,42) and our goats were fed with apparently adequate iodine intake, this pattern indicates that further investigation is required in order to understand the long-term effect of iodophors use on raw milk iodine content on dairy goats exposed to adequate iodine diet.

The overall goat milk iodine content before and at the end of this experiment (mean of 49 and 45 µg/100g, respectively) are lower than the levels in commercial Israeli goat milk: the average iodine concentration of two commercial local goat milk products collected during summer and winter of 2015 was 63 µg/100g (15). This might be as a result of iodine ration content differences or milking practice (e.g. milking once daily vs. twice; measured amount of solution and its concentration of teat dips). This indicate that consuming a single cup (240 ml) of goat milk may be enough to achieve the Recommended Daily Allowance (RDA) for iodine for children and to cover 90%, 72%, 49% and 37% of the RDA for adolescents, adults, pregnant women and lactating women, respectively (43). These results are of importance to public health as milk and dairy products are becoming an increasingly significant source of iodine in the diet of humans in industrialized countries (44) and can be helpful for policymakers in the light of the apparent growing consumption of dairy products from goat milk (21, 22).

The current study has several limitations. First, this study was carried out in a single experiment pen and is not representative of all the types of pens in the country. Second, its design did not allow us to show longer effect of iodophors throughout the whole milking period. However, to the best of our knowledge, there is no available published data about the effect of post-milking teat-dipping iodophor practice on iodine content in goat milk. Additionally, other similar studies in cows reported such effect following a long period in comparison to the present study (39, 40). Third, the number of goats participating in the study (or the sample size in this study) was relatively small ($n=12$) with inherent heterogeneity which might have limited the statistical power. Nevertheless, the study was controlled, including water and feed to decrease related bias effects. Above all, the pattern of increased iodine concentration in goat milk due to iodophors used in our goats was similar to published studies in dairy cows.

In conclusion, relatively high iodine concentrations were found in raw milk of dairy goats whose teats were dipped post-milking in disinfectants with or without iodine. Post-milking teat-dipping iodophor practice may increase iodine content in goat milk within an average period of 20 days. Further investigations, including longer periods of iodophors treatments, are needed. Such studies can be used for controlling iodine content in goat milk and iodine intake in the public.

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Authors' contribution

YSO, AMT and SJM conceived of and designed the study; YSO, SJM drafted the study protocol; CS administered

the raw goat milk samples; YSO collected and handled all samples; LD performed the analysis of iodine content and determination in all samples; YSO analyzed the data and carried out the statistical analysis; YSO and SJM drafted the manuscript; all authors approved the final manuscript.

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