



## Comparison of estrone and 17 $\beta$ -estradiol levels in commercial goat and cow milk<sup>1</sup>

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### ABSTRACT

Increased levels of estrogen metabolites are believed to be associated with cancers of the reproductive system. One potential dietary source of these metabolites that is commonly consumed worldwide is milk. In North America, dairy cows are the most common source of milk; however, goats are the primary source of milk worldwide. In this study, the absolute concentrations of unconjugated and total (unconjugated plus conjugated) estrone (E<sub>1</sub>) and 17 $\beta$ -estradiol (E<sub>2</sub>) were compared in a variety of commercial cow milks (regular and organic) and goat milk. A lower combined concentration of E<sub>1</sub> and E<sub>2</sub> was found in goat milk than in any of the cow milk products tested. The differences in E<sub>1</sub> and E<sub>2</sub> levels between regular and organic cow milks were not as significant as the differences between goat milk and any of the cow milk products. Goat milk represents a better dietary choice for individuals concerned with limiting their estrogen intake.

**Key words:** goat milk, cow milk, estrone, 17 $\beta$ -estradiol

### INTRODUCTION

Although cow milk is the most consumed milk in North America, several studies indicate that goat milk is the most ingested milk globally (Haenlein, 2001). The nutritional and medical benefits of goat milk have been widely acknowledged, but little unbiased medical research has been conducted and the physiological and biochemical properties of goat milk are barely known (Haenlein, 2004). Given the expressed need for further research regarding milk consumption and increased cancer risk, it is imperative to further explore milk products that are consumed on a regular basis, particularly goat milk, given its worldwide prevalence (Willett,

2003; Larsson et al., 2004; Courant et al., 2008). Moreover, the need to directly compare goat and cow milk to better understand the benefits and limitations of each has been expressed by the Dairy Research and Information Center (<http://drinc.ucdavis.edu/goat1.htm>).

The popularity of using goats as a dairy source has risen in recent years because they require minimal land use. In addition, goats are often maintained on pasture that would otherwise be inaccessible to other dairy animals with no decrease in the nutritive value of their milk (Larsson et al., 2004). Even though there are obvious benefits to their use as dairy animals, the goat milk industry has not flourished in the United States, a truth made evident by the fact that goat milk is largely sold in specialty stores or purchased directly from the farmer. Part of the reason lies in the fact that most small dairymen have great difficulty with meeting government sanitation standards for commercial products. In spite of these difficulties, the top producers of commercially available goat milk in the United States have increased production more than 30% to keep up with demand. This increased demand is likely due to the growing ethnic diversity in the United States today; consequently, it is expected that the goat industry will continue to expand as long as the ethnic population continues to grow.

The literature suggests that goat milk has higher nutritional value than cow milk. Goat milk has higher concentrations of phosphorous, potassium, vitamin A, and calcium; cow milk does, however, have a higher concentration of folate (Willett et al., 2003; Qin et al., 2004; Courant et al., 2008). Although conventional thought suggests that cow milk is the best source of calcium, goat milk actually provides more calcium per serving (Willett, 2003; Courant et al., 2008). Cow milk supplies approximately 276 mg of calcium per cup compared with 327 mg per cup for goat milk (Qin et al., 2004). Although this 19% increase in calcium content may be small, it may be an important factor for growing children and individuals suffering from osteoporosis.

Allergic reactions to cow milk proteins have become increasingly common (Dias et al., 2010). Many allergies are related to the protein casein  $\alpha$ S1, which is found in higher concentration in cow milk than in goat milk

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(Savilahti et al., 2010). Lactalbumin, which can also provoke allergic reactions, specifically in small children, is not present in goat milk (Solinas et al., 2010). The presence of simpler, smaller proteins and fat molecules is considered to be one of the primary reasons why goat milk is easier to digest than cow milk (Coila, 2010). Another possible reason for easier digestion may be that the fat globules in goat milk do not cluster due to the lack of the protein agglutinin, which is found in cow milk (<http://fiascofarm.com/dairy/rawmilk.htm>).

The largest health concern for consumers of goat milk is likely to be its elevated fat content compared with cow milk. Whereas goat milk has 10.1 g of fat per a single cup serving, cow milk contains 7.9 g (Liehr, 2000; Ganmaa and Sato, 2005). More troubling for consumers, perhaps, is how much of the fat content in goat milk is composed of saturated fat. Goat milk has 6.5 g of saturated fat per cup compared with 5.0 g in cow milk. Despite its higher content, the fat in goat milk is easier to digest than that found in cow milk due to the fact that more of the fat is made of short- and medium-chain fatty acids. However, because low-fat and non-fat varieties of goat milk are hard to find commercially, if one is looking to have a heart-healthy diet that includes dairy, the literature suggests goat milk may not be the best alternative to cow milk.

Due to the lower lactose quantities of goat milk (4.1%) compared with cow milk (4.7%), research indicates that goat milk may be more easily digested and tolerated by individuals who are slightly or mildly lactose intolerant. Even the slightly lower lactose levels, however, are not enough to make goat milk consumable by individuals who are fully lactose intolerant (Bernstein and Ross, 1993).

One group of functional molecules that have not been widely compared within the dairy industry is steroid hormones, in particular estrogens. According to numerous epidemiological studies in recent decades, estrogens are now considered to be risk factors for cancer, particularly in the breasts, ovaries, and prostate (Chen et al., 2006; Yager and Davidson, 2006). Given that milk and dairy intake account for 60 to 70% of total estrogen consumption, it is important to investigate commercially available milk products to better understand whether they pose a risk factor for cancer (Ganmaa and Sato, 2005; Farlow et al., 2009).

## MATERIALS AND METHODS

### Reagents and Materials

Estrone (**E<sub>1</sub>**) and 17 $\beta$ -estradiol (**E<sub>2</sub>**) standards were obtained from Steraloids Inc. (Newport, RI). The stable

isotope-labeled estrogens, estradiol-13,14,15,16,17,18-<sup>13</sup>C<sub>6</sub> (<sup>13</sup>C<sub>6</sub>-**E<sub>2</sub>**) and estrone-13,14,15,16,17,18-<sup>13</sup>C<sub>6</sub> (<sup>13</sup>C<sub>6</sub>-**E<sub>1</sub>**), were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA). Both **E<sub>1</sub>** and **E<sub>2</sub>** were used without further purification and have reported chemical and isotopic purities  $\geq 98\%$ . Dichloromethane, methanol, and formic acid were obtained from EM Science (Gibbstown, NJ). Glacial acetic acid, sodium bicarbonate, and L-ascorbic acid were purchased from J. T. Baker Inc. (Phillipsburg, NJ). Sodium hydroxide and sodium acetate were purchased from Fisher Scientific Co. LLC (Fair Lawn, NJ).  $\beta$ -Glucuronidase/sulfatase (*Helix pomatia*, Type HP-2) was obtained from Sigma Chemical Co. (St. Louis, MO). Dansyl chloride and acetone were purchased from Aldrich Chemical Co. (Milwaukee, WI). All chemicals and solvents used in this study were HPLC or reagent grade.

### Milk Samples

Seven milk samples were collected for this study: whole milk, 2% milk, nonfat milk, organic whole milk, organic 2% milk, organic nonfat milk, and regular goat milk. All milk samples are assumed to be from cows unless otherwise indicated. The cow milks were produced by Bloom (Salisbury, NC) and purchased at a local grocery store (Bloom, Frederick, MD). The goat milks were purchased at MOM's Organic Market (Frederick, MD). Milk was aliquoted and stored at  $-40^{\circ}\text{C}$  until analyzed. Each sample was analyzed in triplicate in 4 separate batches, yielding a total of 12 analyses for each milk product.

### Preparation of Stock and Working Standard Solutions

Stock solutions of **E<sub>1</sub>** and **E<sub>2</sub>** were prepared at a concentration of 80  $\mu\text{g/mL}$  by dissolving 2 mg of each steroid hormone in 25 mL of methanol containing 0.1% (wt/vol) L-ascorbic acid. Time-dependent degradation of the standards within the stock solutions was monitored by measuring the absolute peak height of **E<sub>1</sub>** and **E<sub>2</sub>** using capillary liquid chromatography-tandem mass spectrometry (**LC-MS/MS**). No degradation was observed for these solutions stored at least 2 mo at  $-20^{\circ}\text{C}$ . Working standard solutions of **E<sub>1</sub>** and **E<sub>2</sub>** (as well as the stable isotope-labeled versions of these steroid hormones) having a concentration of 8 ng/mL were prepared by diluting the stock solutions with methanol containing 0.1% (wt/vol) L-ascorbic acid.

### Calibration Standards

Milk fortified with 0.1% (wt/vol) L-ascorbic acid and having no detectable levels of estrogen metabolites

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