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Comparative Antimicrobial Effectiveness of Camel, Buffalo, and Goat Milk Marinades Against *E. coli* in Refrigerated Beef

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Abstract

Background: Traditional meat-improvement techniques like marination have evolved to improve meat quality, safety, and meet the need for "clean label" products. This study evaluated the antimicrobial effects of camel, buffalo, and goat milk marinades on *Escherichia coli (E. coli*) in raw beef ribeye meat **Methods:** Forty meat cuts were divided into four groups and inoculated at 5 log CFU/mL of freshly prepared *E.coli* culture strain then marinated the groups using camel, buffalo, or goat milk at a 1.5:1 (v/w) ratio, with distilled water serving as the control and stored at 4°C for 48 hours. Microbiological analysis involved serial dilution and plating on Hekton Enteric Agar to enumerate *E. coli,* while pH values were measured using a calibrated pH meter at 0, 6, 12, 18, 24, and 48 hours. **Results:** Significant reductions in *E. coli* counts were observed in all milk-marinated groups compared to the control ($p < 0.001$). After 48 hours, goat milk achieved the greatest reduction to 4.19 ± 0.02 log CFU/g (−40%), followed by buffalo milk (5.38 ± 0.03 log CFU/g, −27.5%) and camel milk (5.43 ± 1.78 log CFU/g, −25%). In contrast, the control group showed a consistent increase, reaching 8.69 ± 0.2 log CFU/g at 48 h. A significant decrease in pH was also recorded for all milk treatments ($p < 0.001$), with goat milk-treated meat reaching the lowest pH (5.16 ± 1.01), compared to the control (6.03 ± 0.01). **Conclusions:** These findings confirm that goat milk possesses the most potent antimicrobial and acidifying effects, likely due to its higher concentration of bioactive compounds. Milk-based marinades, particularly goat milk, offer a viable natural method to improve microbial safety and extend meat shelf life. Further research should assess sensory characteristics, broader pathogen targets, and efficacy under extended storage conditions.

Keywords: Food safety, Natural Antimicrobial, Dairy marinades, Food preservation, Foodborne Pathogens, Food Quality.

1. Introduction

Foodborne pathogens, particularly *Escherichia coli* (*E. coli*), pose a significant public health risk, contributing to numerous foodborne illnesses outbreaks worldwide. *E. coli*O_{157}:H_7 alone is responsible for an estimated 63,000 cases of hemorrhagic colitis annually[1]. Meat is especially susceptible to microbial contamination due to its high moisture content and rich nutrient profile, which creates an ideal environment for bacterial growth [2]. Although traditional meat preservation methods often rely on chemical additives, concerns over food safety and consumer preference for natural preservatives have driven research into alternative antimicrobial solutions[3]. Marination, a process commonly used to enhance meat quality, has been explored as a potential method to improve food safety by reducing microbial contamination [4].

Milk from various species, including camels, buffaloes, and goats, is known to contain bioactive compounds with antimicrobial properties, such as lactoferrin, lysozyme, lactoperoxidase, and immunoglobulins, which can inhibit the growth of foodborne pathogens, including Escherichia coli[5]In particular, camel milk has been reported to exhibit strong antimicrobial activity owing to its high lactoferrin and lysozyme contents[6]. Similarly, buffalo and goat milk contain bioactive peptides and fatty acids that contribute to their antibacterial efficacy[7, 8]. The acidic nature of these milk types, combined with their bioactive components, may help lower meat pH and create unfavorable conditions for bacterial proliferation[9].

Despite the well-documented antimicrobial properties of these milk types, their direct application as meat marinades has largely been underexplored. Understanding the potential of camel, buffalo, and goat milk marinades to reduce E. coli contamination in meat could provide valuable insights into natural preservation strategies. This study aimed to evaluate the comparative antimicrobial effectiveness of these milk-based marinades in controlling E. coli contamination and assesses their impact on the pH of refrigerated ribeye meat samples. Findings from this study aim to provide valuable insights into the practical application of milk marinades, contributing to safer meat products and sustainable natural preservation strategies.

2. Materials and Methods

2.1. Ethical approval

The study proposal was approved by the Care and Use Committee Research Ethics, Faculty of Veterinary Medicine, Banha University BUFVTM 13-10-24

2.2. Meat samples

Approximately 2000 g of raw meat from beef rib-eye was procured from Benha City, Egypt, 24 h postslaughter, and immediately transported to the laboratory by maintaining the cold chain in a cool box at 4°C.

2.3. The preparation of pathogens and inoculums

E. coli ATCC strain (25922) was obtained from the Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt.A fresh working culture of *E. coli* was produced. *E. coli* was plated on Hekton Enteric Agar (Merck, Darmstadt, Germany) for 48 h at 37 °C. After 48 h, five colonies from the plate were cultured in trypticase soy broth (Oxoid) at 37 °C. Two milliliters of grown cells were centrifuged at 13,000 × g at 4 °C for fifteen minutes using a 0.85% w/v sodium chloride solution. After the cells were suspended in 2 mL saline, a cell solution containing approximately 5 log CFU/mL was produced.

2.4. Meat inoculation and marination

The experiment was carried out with 40 ribeye meat cuts, each averaging 50 g, categorized into four groups (10 cuts per group) across six inspection points (0, 6, 12, 18, 24, and 48 h).*E. coli* was injected into each of the four groups at a rate of 2 mL/100 g from an overnight culture at 37 °C, and serially adjusted to 5 log CFU/mL[2]. The inoculated samples were stored at 20 °C for 30 min to allow for cell attachment to the meat surface. Marination of the ribeye cuts was conducted using Camel, Buffalo, and Goat milk, and samples treated with distilled water were used as controls. Marination was performed at a concentration of 1.5/1 (v/w) (marinade milk/meat cut) overnight (18 h) at 4 °C.

2.5. pH determination

Approximately 5 g of meat was homogenized for 30 s using a Stomacher400R (Seward, UK) in 45 mL sterile distilled water. pH was measured using a calibrated pH meter (Jenway 3510; Cole-Parmer, Stone, UK) [10]. The marinated and control groups were maintained at 4 °C, pH were recorded at 0, 6, 12, 18, 24, and 48 h.

2.6. Microbiological Analysis

Microbiological analyses were conducted during storage to determine the cell density of inoculated *E. coli*. Each group's ribeye, weighing approximately 25 g, was deposited in sterile stomacher bags and mixed with a 225 ml of sterile physiological solution. The mixture was then homogenized in a Stomacher 400R (Seward, UK) for 2 min, followed by serial dilution with physiological solutions. An appropriate inoculum from serially diluted *E. coli-inoculated* beef ribeye cuts was plated on the surface of sterile petri dishes containing Hekton Enteric Agar (Merck, Darmstadt, Germany).The plates were subsequently incubated at 37̊ C for 48 h[11].

2.7. Statistical analysis

SPSS Version 22 (SPSS Inc., Chicago, IL, USA) was used to analyze the data using one-way ANOVA. The statistical model compared the means using Tukey's multiple comparison test. A *P-value* of less than 0.05 was considered to indicate a significant difference.

3. Results

The comparative effectiveness of camel, buffalo, and goat milk marinades on the growth and reduction of *E. coli* in experimentally inoculated ribeyemeat samples stored at 4°C for 48 h was illustrated (Figure 1).

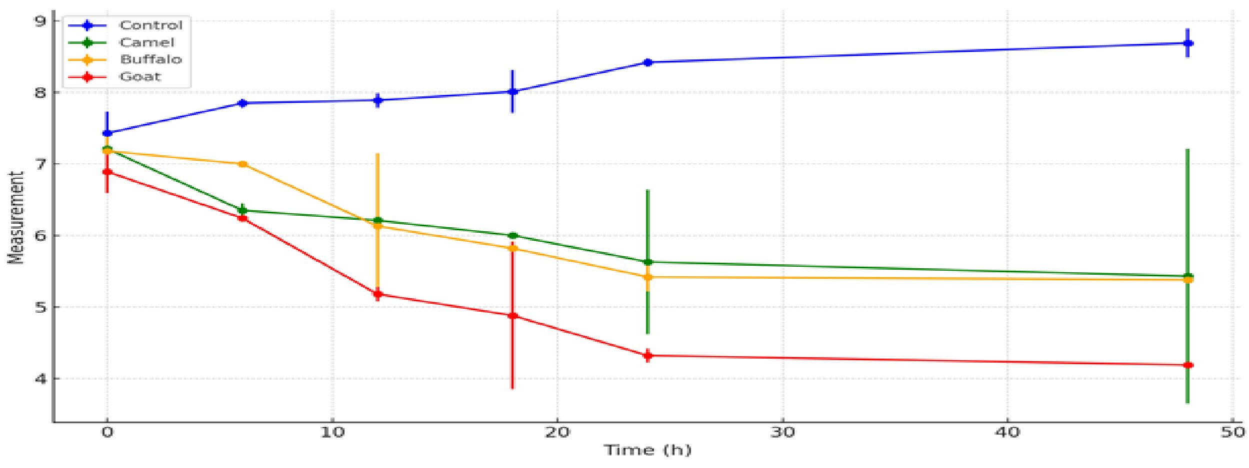

Fig. (1) Impact of different milk marinades on *E. coli* counts (log CFU/g) in meat over time.

At the beginning (0 hours), bacterial levels were similar across all treatments, ranging between approximately 6.89±0.3 and 7.43±0.3 log CFU/g. Over time, the control group exhibited a steady increase in *E. coli* levels, which reached the highest count at 48 h.In contrast, all the milk-marinated samples demonstrated a consistent decline in bacterial numbers, indicating their antimicrobial properties(P < 0.001).Among the treatments, goat milk was the most effective, reducing *E. coli* levels to approximately 4.19±0.02 log CFU/g by the end of the storage period(P < 0.001). Buffalo and camel milk also contributed to bacterial reduction (P < 0.001), lowering counts to approximately 5.38±0.03 and 5.43±1.78 log CFU/g, respectively (Table 1).

Table (1) Impact of different milk marinades on *E. coli* counts (log CFU/g) in meat over time.

Times(hrs.)	Treatment groups				
	Control	Camel milk-marinated meat	Buffalo milk-marinated meat	Goat milk-marinated meat	*p*-value
0	7.43±0.3 [a]	7.21±0.05 [a]	7.18±0.2 [a]	6.89±0.3 [b]	0.0157
6	7.85±0.06 [a]	6.35±0.1 [c]	7.0±0.01 [b]	6.24±0.02 [c]	<0.001
12	7.89±0.1 [a]	6.21±0.1 [b]	6.13±1.02 [b]	5.18±0.1 [c]	<0.001
18	8.01±0.3 [a]	6.0±0.02 [b]	5.82±0.1 [c]	4.88±1.03 [d]	<0.001
24	8.42±0.06 [a]	5.63±1.01 [b]	5.42±0.2 [b]	4.32±0.1 [c]	<0.001
48	8.69±0.2 [a]	5.43±1.78 [b]	5.38±0.03 [b]	4.19±0.02 [c]	<0.001

[a-d] means within a row not sharing a common superscript differ significantly ($p < 0.05$).

Figure 2 illustrates the reduction in *E. coli* (%) in meat samples marinated with different types of milk over a 48-hour period. At the initial point (0 h), bacterial reduction was minimal across all the treatments. However, as marination time increased, a progressive decline in E. coli counts was observed, with goat milk exhibiting the highest antimicrobial effect. After 12 h, *E. coli* reduction reached approximately 10–25%, with goat milk showing the most significant effect, followed by buffalo milk and camel milk. This trend continued over time, with bacterial reductions reaching around 30% for goat milk at 18 h, while camel and buffalo milk demonstrated reductions of approximately 15-20%. At 24 and 48 h, goat milk marination resulted in a substantial *E. coli* reduction of nearly 40%, whereas buffalo and camel milk treatments achieved an approximately 25–30% reduction. These findings suggest that goat milk possesses the strongest antibacterial properties, possibly because of its unique composition of antimicrobial peptides, organic acids, and bioactive compounds.

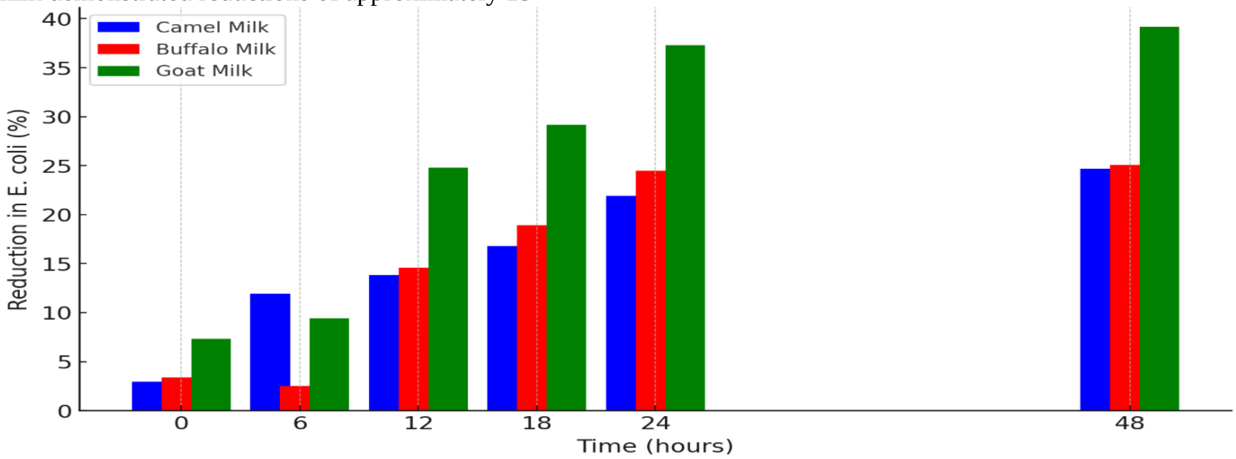

Fig. (2) Reduction of *E. coli* by different milk marinades over 48 hrs.

Table 2 and Fig. (3) illustrate the effect of different milk marinades on the pH of meat over time. At the initial measurement (0 h), the pH values for all treatments were relatively similar, with slight variations between the control (5.64±0.01), camel milk (5.6±1.21), buffalo milk (5.6±1.1), and goat milk (5.74±1.01).Over time, a noticeable decline in pH was observed for all milk-marinated samples, whereas the control group exhibited an increasing trend .After 6 h, camel milk showed the most pronounced decrease (5.38±1.01), followed by buffalo (5.50±0.02) and goat milk (5.65±1.01), indicating the acidifying effect of these marinades. As the marination period progressed, the pH of the control group steadily increased, reaching 6.03±0.01 at 48 h, whereas all milk treatments exhibited a continued decline.

Table 2 Effect of different Milk marination on the pH of ribeye meat

Time (hrs.)	Control	Camel milk -marinated meat	Buffalo milk -marinated meat	Goat milk -marinated meat
0	5.64±0.01	5.6±1.21	5.6±1.1	5.74±1.01
6	5.52±0.02	5.38±1.01	5.50±0.02	5.65±1.01

12	5.65±1.01	5.38±0.01	5.45±0.02	5.61±0.02
18	5.74±0.12^{a}	5.4±0.1^{b}	5.45±0.1^{b}	5.49±1.01^{b}
24	5.82±0.02^{a}	5.32±0.02^{b}	5.34±0.01^{b}	5.43±0.23^{b}

$^{a, b}$ means within a row not sharing a common superscript differ significantly when ($p < 0.05$).

4. Discussion

This study demonstrated that marination with camel, buffalo, and goat milk significantly inhibited *Escherichia coli* (*E. coli*) growth in inoculated beef ribeye samples stored at 4°C over 48 hours, with goat milk showing the highest antimicrobial activity. The starting bacterial counts were comparable across all treatment groups (6.89 ± 0.3 to 7.43 ± 0.3 log CFU/g), allowing for a valid comparison of antimicrobial effects over time. Notably, by the end of the storage period, the *E. coli* count in goat milk-marinated samples was reduced to 4.19 ± 0.02 log CFU/g—a 40% decrease from baseline—whereas buffalo and camel milk treatments achieved reductions to 5.38 ± 0.03 log CFU/g and 5.43 ± 1.78 log CFU/g, respectively. In stark contrast, the control group exhibited an upward trend, peaking at 8.69 ± 0.2 log CFU/g at 48 hours.

The superior antimicrobial performance of goat milk can be attributed to its unique composition, including low-molecular-weight antimicrobial peptides, organic acids, and enzymes such as lactoperoxidase. Singh et al. [12]reported that goat milk-derived peptides (<3 kDa) effectively inhibited both *E. coli* and *Bacillus cereus* by disrupting bacterial membranes and inhibiting cellular processes. In addition, Ningsih et al.[13] identified a goat milk-derived peptide (P3) that exerted significant antimicrobial activity against *E. coli* and *Staphylococcus aureus*, supporting the results of the current study.

Buffalo milk also demonstrated notable efficacy, reducing E. coli counts by 27.5%. This result aligns with Kalhoro et al.[8], who found that buffalo milk-derived lactic acid bacteria exhibited potent antibacterial properties against Gram-negative pathogens. The antimicrobial activity of buffalo milk is likely linked to bioactive molecules such as lactoferrin and casein-derived peptides that interfere with bacterial iron uptake and cellular metabolism[14].

Camel milk showed the least reduction in *E. coli* (25%) among the three yet still performed significantly better than the control ($p < 0.001$). The antimicrobial potential of camel milk has been previously attributed to high levels of lactoferrin, lysozyme, and immunoglobulins[5, 6]. However, the relatively lower efficacy observed here may reflect differences in enzyme concentrations or buffering capacity compared to goat and buffalo milk.

The trends in pH change further support the antimicrobial outcomes. Initially, all treatments exhibited similar pH values (~5.6–5.74). By 48 hours, the goat milk group had the lowest pH (5.16 ± 1.01), followed closely by camel (5.19 ± 1.03) and buffalo (5.21 ± 0.1) treatments. The control group's pH increased to 6.03 ± 0.01, consistent with bacterial proliferation and proteolysis. Lower pH is known to inhibit E. coli growth by impairing membrane function and enzyme activity [9]. The ability of milk marinades to lower pH likely stems from their acidogenic microbial flora and organic acid content, contributing to the observed antimicrobial effects.

Comparatively, Lopes et al. [4]reviewed natural marination strategies and concluded that acidic marinades consistently reduce microbial load in meat products. The present findings reinforce this conclusion, with goat milk providing not only a strong acidifying effect but also bioactive compounds that act synergistically to suppress *E. coli.*

The standard deviation of *E. coli* counts and pH values across time points was generally low for goat and buffalo milk, indicating consistent effects, whereas camel milk exhibited higher variability, suggesting possible inconsistencies in bioactive content or distribution within samples.

Taken together, the current study supports the use of goat milk as a highly effective natural marinade against *E. coli* in meat. While all three milk types provided measurable antimicrobial effects, goat milk outperformed the others quantitatively and consistently. The statistical differences observed throughout the experiment ($p < 0.001$) underscore the reliability and reproducibility of these results under refrigerated storage conditions.

5. Conclusions

This study clearly demonstrates that camel, buffalo, and goat milk marinades exert significant antimicrobial effects against *Escherichia coli* in inoculated ribeye beef samples stored at 4°C. Among the treatments, the goat milk marinade achieved the highest reduction in *E. coli* counts, approximately 40% after 48 hours—followed by buffalo (27.5%) and camel milk (25%), confirming statistically significant differences between treatments ($p < 0.05$). These reductions strongly correlated with a consistent decline in pH, with goat milk producing the lowest final pH (5.16 ± 1.01), suggesting enhanced microbial inhibition through acidification and bioactive milk components. The findings establish goat milk as a superior natural marination agent for microbial control in meat, compared to buffalo and camel milk. These results are directly supported by the microbiological and pH data collected during the study. No synthetic preservatives were required to achieve these effects, indicating the practical value of this approach in clean-label meat preservation strategies. Goat milk marinade applied at a

1.5:1 (v/w) ratio for 18 hours at 4°C is an effective, natural method to reduce *E. coli* contamination and lower pH in refrigerated beef, contributing to improved food safety and extended shelf life. Future research should evaluate the sensory attributes of milk-marinated meat, explore broader microbial targets, and assess efficacy under different storage durations and commercial-scale conditions.

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