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Effect of Hydrogen-Rich Water Intake on the Polyamine Profile of Colostrum and Milk of Goat

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ABSTRACT: The present study aimed to evaluate the effect of hydrogen-rich water (HRW) intake on the polyamine profile of colostrum and milk. Two groups of Gurcu goats were provided *ad libitum* access to normal water (NW) and HRW from the 21st day prepartum until the 21st day postpartum. All polyamines were increased in NW- and HRW-fed colostrum and milk. On the 0- and the 21-days, all polyamines were higher for HRW-fed colostrum/milk samples than NW-fed ones. HRW supplementation to goats before parturition increased the polyamines in colostrum/milk with a positive potential impact on the nutrition and health of goats and newborn kids.

Keyword: Goat; Hydrogen-rich water; Milk; Colostrum; Polyamine; Lactation.

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INTRODUCTION

Recently, increasing interest in naturally occurring polyamines has been observed (Michaelidou, 2008). The polyamines, especially aliphatic di-, tri-, and tetra-amines, i.e. putrescine, spermidine, and spermine, play important role in the gastrointestinal system and in reducing the incidence of food allergy in infants (Dandrifosse et al., 2000; Giorgio et al., 2018). The most common polyamines found in aqueous environments are putrescine, spermidine, and spermine (Larqué et al., 2007). These compounds are ubiquitous in nature and play essential roles in cell growth, proliferation, and differentiation (Pegg, 2016). Polyamines can enter water sources through the decomposition of organic matter, microbial activity, industrial and agricultural runoff, and wastewater effluents (Nishibori et al., 2003).

The cells in the human body need polyamines to function properly (Bardócz et al., 1998). Exogenous sources, such as diet, can provide additional polyamines than endogenous biosynthesis in newborns (Gugliucci, 2004). Milk is the first source of exogenous polyamines for newborn infants and animal kids, and high levels of spermine enhance immune protection (Dandrifosse et al., 2000). Colostrum milk has a high protein content, especially immunoglobulins, with total protein levels at 15-20% (compared to 3-4% in mature milk) (Uruakpa et al., 2002). The immunoglobulins include IgG (85-90%), IgA (5-10%), and IgM (5-10%), along with lactoferrin (0.1-0.3%) and lysozyme (0.1-0.2%) (Stelwagen et al., 2009). Carbohydrates in colostrum differ from mature milk, with lactose at 2-3% and oligosaccharides at 0.05-0.1% (Gopal & Gill, 2000). Colostrum's fat content is lower, at 1-2%, but it is richer in essential fatty acids and fat-soluble vitamins (Contarini et al., 2014). Vitamins A and E in colostrum are 2-5 and 2-3 times higher, respectively, and it contains higher amounts of vitamin B12, folate, and minerals, such as sodium, potassium, and chloride (McGrath et al., 2016). Colostrum also has growth factors, such as Insulin-like Growth Factor-1 (IGF-1), Epidermal Growth Factor (EGF), and Transforming Growth Factor-β (TGF-β) (Pakkanen & Aalto, 1997). It is also rich in nucleotides and cells, containing 1-5 million leukocytes per milliliter and various epithelial cells (Ballard & Morrow, 2013).

The polyamine concentration in infant and follow-up formulas using goat milk powder is lower than that in breast milk (Romain et al., 1992) but slightly higher than that of cow's milk counterparts (Prosser et al., 2008). Recently, special milk formulas have been proposed for infants who cannot take breast milk or who cannot meet the recommended nutritional requirements from breast milk (Polidori et al., 2022). The polyamine levels in milk are also important in the diets of individuals with special needs, and polyamine-rich milk can form a valid natural alternative source to human milk (Giorgio et al., 2018). Goat milk forms a source of polyamines in infant and follow-up formulas (Plakantara et al., 2010; Silanikove et al., 2010).

Molecular hydrogen (H_2) is a non-toxic gas with a food additive code of E949 (Bulut et al., 2022). Hydrogen-rich water (HRW) is a functional water containing dissolved hydrogen at 1.6 mg/L (Alwazeer, 2024a). Different studies revealed many health-benefits of HRW including nutritional (Alwazeer, 2024b), antidiabetic (Kajiyama et al., 2008; Zheng et al., 2021), anti-depression (Zhang et al., 2016), antioxidant activity (Zor et al., 2023), anti-inflammatory (Köktürk et al., 2022b), and antistress (Köktürk et al., 2022a) as well as its positive effects on the blood lipid profile (Kuru et al., 2024; Todorovic et al., 2023). In ruminant nutrition, it has been noted that providing elemental Mg supplements leads to a rise in ruminal dissolved H_2 levels. This elevation restrains the fermentation process within the rumen, stimulates methane generation, and seems to redirect fermentation pathways toward propionate instead of acetate. Moreover, it has been found that elemental Mg supplementation modifies microbial composition by diminishing fungi populations and boosting methanogen numbers (Wang et al., 2017). Additionally, rumen fermentation is influenced by the movement of metabolic hydrogen within and between cells, focusing on its production, transfer between different species, and integration into various competing metabolic pathways (Ungerfeld, 2020).

Few studies investigated the polyamines in goat milk (Galitsopoulou et al., 2015; Tolenaars et al., 2021; Wang et al., 2019). However, no study has evaluated the effect of HRW intake on the polyamine profile and content in goat colostrum and milk.

MATERIALS AND METHODS

Animal materials

The animals were obtained from Kafkas University, Faculty of Veterinary Medicine, Prof. Dr. Ali Rıza AKSOY Education, Research and Application Farm in Kars province. Oestrus synchronization was conducted on 40 clinically healthy Gurcu goats aged between 3 and 4 years, all of which had previously undergone at least one parturition. Goats were exposed to six Gurcu bucks during the mating phase after oestrus synchronization. The presented study obtained the approval of Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK 2022/134), Kars, Türkiye.

Nutrition

Goats were housed under intensive care conditions and were given hay and alfalfa as roughage during the study. Furthermore, 450 g concentrate feed per goat (16% crude protein and 2.700 kcal/kg metabolizable energy) obtained from local suppliers (Hasel Yem, Türkiye) was daily provided. The content of the concentrate feed was not changed to not produce changes in biochemical parameters during the dry and lactation periods.

Oestrus Synchronization

The sponge containing medroxyprogesterone acetate was inserted into the vagina, and 400 IU pregnant mare serum gonadotropin (PMSG, Oviser®, Hipra, Türkiye) + 0.075 mg d-cloprostenol (Gestavet PROST®, Hipra, Türkiye) was injected intramuscularly on the 7th day. Sponges were removed on the 9th day, and bucks were left with goats after 24 hours.

Pregnancy examination

Pregnancy examination was performed by transrectal ultrasonography (5-7.5 MHz, Draminski iScan, Draminski, Poland) 30-32 days and 60 days after goats were mated. Goats with singleton embryos were included in the study (n=21).

Groups

The goats were divided into two groups 21-23 days before the estimated date of parturition after the recorded mating date as follows:

Group 1 (Control, n=10): The goats in this group were fed with normal water (NW) *ad libitum*.

Group 2 (goats, n=11): The goats in this group were fed with HRW *ad libitum* starting from the 21st-23rd day before the parturition until the 21st day after the parturition.

HRW preparation

HRW was prepared using the hydrogen generator (HB-33+ model, Taiwan). Both normal water and HRW were provided to goats every 5-6 hours, with the withdrawal of the old water. About 15 mL of colostrum/milk samples was taken from every goat on the 0-day of parturition and the 7th, 14th, and 21st day postpartum and stored at -80°C until analysis.

Determination of polyamines

Sample preparation

The sample preparation was performed according to the procedures described by (Romain et al., 1992). The frozen colostrum/milk samples were put in the refrigerator (+4°C) overnight to thaw. They were then vortexed for 30 sec. 300 μL of sample and 300 μL of distilled water were mixed with 20 mg of solid sulfosalicylic acid, and the mixtures were left overnight at -20°C to precipitate proteins. After centrifugation (10,000×g, 15 min), a 200 μL supernatant was pipetted into a test tube for dansylation operation. The following ingredients were added and properly mixed by vortexing for 30 seconds: 8 μL of a 20 nmol/mL solution of 1,6-diamino hexane (internal standard), 292 μL of a 0.5 M carbonate buffer (pH 9.2), 100 mg of anhydrous potassium carbonate, and 500 μL of a 2 mg/mL dansyl chloride in acetone. The samples with the reagents were then combined in a single tube and 5 mL of n-heptane was added.

Reverse-phase high-performance liquid chromatography (HPLC) analysis

RP- HPLC (Agilent 1260, USA) instrument and Nucleosil C18 wide pore analytical column (5 mm, 30 nm, 250×4 mm) (Macherey Nagel, Germany) were used to identify the polyamines. The eluent A contained a 20 mM solution of acetic acid and sodium 1- heptane-sulphonate in water. Acetonitrile was used as eluent B. Dansylated polyamines were eluted using a gradient of 0-9 min, 57% B; 9-16 min, 57-100% B; 16-20 min, 100% B; 20-21 min, 100-57% B; and 21-25 min, 57% B at a flow rate of 1 mL/min.

Statistical analysis

The repeated measures at five different sampling times postpartum were analyzed using the ANOVA method following the linear model, which involves one factor between treatments (NW and HRW) and one factor within experimental units (Gomez and Gomez, 1984). This analysis is equivalent to the analysis of a split-plot design. Before performing the analysis of variance, the assumptions of normality and homogeneity of variance were checked. The SPSS ver. 17.0 statistics program was used for all statistical analyses. All hypothesis testing techniques had $\alpha = 0.05$ set as the significance level. The animal number included in the study was 10 for the control group and 11 for the HRW group. Three milk samples were taken from each animal on every planned day. Duncan's post hoc comparisons were performed to further analyze the significant differences observed between groups, as shown in Table 1 and Figure 1.

RESULTS

Table 1 shows the effect of both the water type intake and the lactation period on the polyamine profile and content of colostrum/milk. Putrescine is one of the primary polyamines found in milk products. It is formed from the decarboxylation of ornithine and arginine by lactic acid bacteria. Putrescine levels in milk products can increase during fermentation and storage and may contribute to the flavor and aroma of aged cheeses. Spermidine and spermine are derived from the amino acid ornithine. These polyamines are involved in cell proliferation, differentiation, and apoptosis. They have been studied for their potential health benefits, including antioxidant and anti-inflammatory properties. Spermidine and spermine levels in milk products are influenced by factors such as the presence of specific bacterial strains and the duration of fermentation (Komprda et al., 2008; Minois, 2014).

Statistical analysis showed that the type of water supplemented before the parturition (21 days) had a significant effect on the profile and content of polyamines of colostrum/milk of goats ($P<0.01$) (Table 1 and Fig. 1). The results showed that the interaction between the treatment and lactation day factors was statistically significant ($P<0.001$). However, the difference between the treatments is not constant for all days and should be further investigated.

Table 1 shows the mean concentrations of polyamines of goat colostrum/milk during the lactation period. The interaction between water type and lactation days showed a significant effect on the polyamine levels of colostrum/milk ($P<0.001$).

At 0- day (parturition day), the levels of putres-

Table 1. Effect of water type and lactation day on polyamine concentration (µmol/L) expressed as (mean+SEM) in goats fed with normal- and hydrogen-rich waters

Water type	Lactation Days	Putrescine (µmol/L)	Spermidine (µmol/L)	Spermine (µmol/L)	Total Polyamines (µmol/L)
Normal water (n=10)	0	0.38 ±0.016^{b,B}	0.20 ±0.002^{b,B}	0.33 ±0.013^{b,B}	0.91 ±0.035^{b,B}
	3	0.24 ±0.008^{c,B}	0.12 ±0.004^{c,B}	0.25 ±0.009^{c,B}	0.61 ±0.025^{d,B}
	7	0.36 ±0.017^{b,B}	0.19 ±0.007^{b,B}	0.32 ±0.007^{b,B}	0.87 ±0.036^{c,B}
	14	0.39 ±0.015^{b,B}	0.20 ±0.009^{b,B}	0.33 ±0.014^{b,B}	0.92 ±0.021^{b,B}
	21	0.86 ±0.036^{a,B}	0.47 ±0.019^{a,B}	0.64 ±0.025^{a,B}	1.97 ±0.063^{a,B}
Hydrogen-rich water (n=10)	0	0.56 ±0.020^{b,A}	0.38 ±0.017^{b,A}	0.66 ±0.023^{b,A}	1.60 ±0.045 ^{b,A}
	3	0.43±0.016^{c,A}	0.23 ±0.014^{c,A}	0.50 ±0.018^{c,A}	1.16 ±0.044^{c,A}
	7	0.54 ±0.014^{b,A}	0.36 ±0.016^{b,A}	0.63 ±0.020^{b,A}	1.54 ±0.056^{b,A}
	14	0.57 ±0.020^{b,A}	0.38 ±0.019^{b,A}	0.67 ±0.023^{b,A}	1.62 ±0.061^{b,A}
	21	1.07 ±0.044^{a,A}	0.85 ±0.025^{a,A}	1.24 ±0.040^{a,A}	3.16 ±0.114^{a,A}
Analysis of variance (*P*-value) of 3 measures traits of colostrum/milk samples of goats fed with normal- and hydrogen-rich waters during the lactation period					
Treatment (Normal water/ Hydrogen-rich water)		<0.001	<0.001	<0.001	<0.001
Lactation Days		<0.001	<0.001	<0.001	<0.001
Treatment×Lactation Day		<0.001	<0.001	<0.001	<0.001

a-d Different lowercase letters (in the same column) indicate the presence of significant differences ($P< 0.05$) between samples for each water type in the different lactation days.

A, B Different uppercase letters (in the same column) indicate the presence of significant differences ($P< 0.05$) between the two water-type samples on the same lactation days ($P<0.05$).

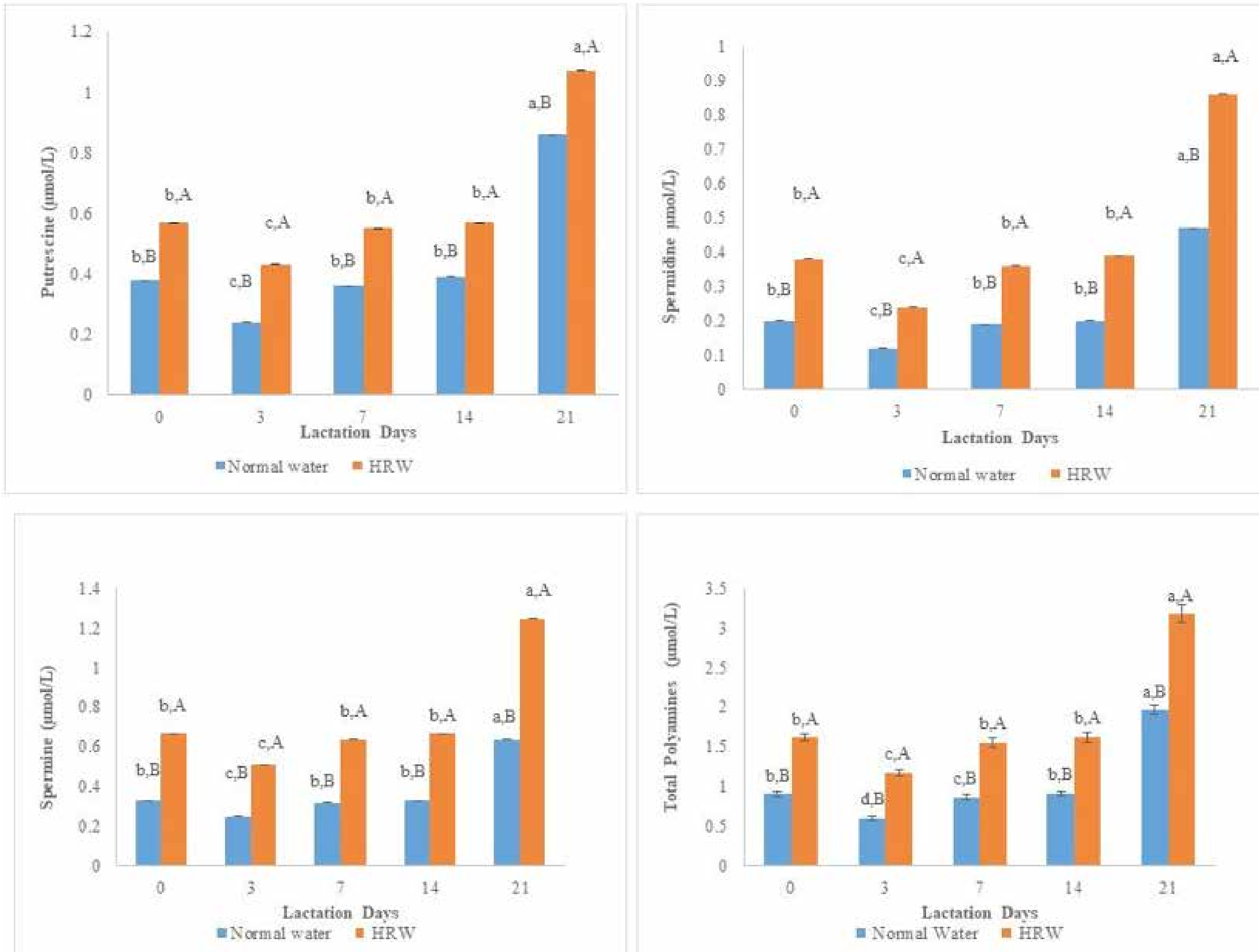

Figure 1. Evaluation of polyamines in colostrum and milk of Gurcu goats during the lactation period in relation to the type of water (normal and hydrogen-rich waters). a-d Different lowercase letters indicate the presence of significant differences (P< 0.05) between samples for each water type in the different lactation days. A, B Different uppercase letters indicate the presence of significant differences (P<0.05) between the two water-type samples on the same lactation days (P<0.05).

cine, spermidine, and spermine were (µmol/L): 0.38, 0.20, and 0.33 for NW- and 0.56, 0.38, and 0.66 for HRW- fed goat colostrum samples, respectively (P<0.001). The total polyamine content (µmol/L) was 0.91 and 1.60 for NW- and HRW-fed goat colostrum (P <0.001).

These results show that all polyamines were increased when HRW was provided to goats instead of NW. The amounts of all polyamines increased during the lactation period (Fig. 1). This increase was non-significant during the first two weeks, while it was great during the 3rd week of lactation. This notice was observed for both NW- and HRW-fed goat colostrum samples. At the end of the third week, the amounts of putrescine, spermidine, and spermine, were (µmol/L): 0.86, 0.47, and 0.64 for NW- and 1.07, 0.85, and 1.24 for HRW-fed goat milk samples, respectively (P <0.001).

DISCUSSION

The polyamine content of goat milk showed the highest level on the 0-day (parturition day). Among different polyamines, putrescine was the highest for NW-fed goat colostrum/milk, while spermine showed the highest levels for HRW-samples.

One of the possible reasons for the change in the concentration of polyamines may be because H_2 might affect polyamine synthesis, change the signal type in the mammary gland during lactation, affect hormonal levels (Płoszaj et al., 1997), change intestinal activities, and modify the regulation of ornithine decarboxylase (Larqué et al., 2007).

Polyamines are organic compounds found in breast milk, playing essential roles in cell growth and physiological processes (Soda, 2022). In kids, they support growth and development by aiding gastrointestinal maturation and immune function. While research on polyamines in goat milk and human health is limited, studies suggest potential benefits such as promoting gut health and possessing antioxidant properties (Sánchez-Pérez A. et al., 2018). Breast milk's spermine and spermidine content was reported to be severalfold higher than formula milk's. Additionally, the intake of polyamine (spermine and spermidine) for infants of 0-3 months was estimated at about 3.5 µM/day (Bardócz, 1995). This shows that the HRW-fed goat milk of the 21st lactation day may provide 107, 25, and 36% of the daily requirement of putrescine, spermidine, and spermine, while normal water-fed goat milk provides only 86, 13, and 18%, respectively.

It was reported that there was a relationship between both polyamine-rich foods (Nishimura et al., 2006) and gut microbiota (Matsumoto et al., 2012; Sugiyama et al., 2017), and polyamine biosynthesis (Hirano et al., 2021), especially during lactation, and polyamine biosynthesis rate in the mammary gland and the secretory activity of milk components. Considering that the feeds provided to animals in the present study were the same, one can assume that the water type might affect the polyamine profile and content of colostrum/milk. Moreover, the effect of HRW on the change of polyamine profile of colostrum/milk may be related to its potential effect on the growth of the gut microbiota of goats or the biosynthesis of polyamines.

In newborns, milk serves as the primary source of exogenous polyamines, with elevated levels of spermine boosting immune defense mechanisms (Dandrifosse et al., 2000). The presence of polyamines in milk plays a pivotal role in meeting the dietary requirements of individuals with specific needs, offering a natural alternative to human milk (Giorgio et al., 2018). This study demonstrates that supplementing goats with HRW during the peripartum period increases polyamine levels in both colostrum and milk. Consequently, it is conceivable that HRW consumption by animals could bolster immune responses during the neonatal phase by augmenting polyamine levels. Moreover, formulas derived from HRW-enhanced goat milk may yield beneficial effects for neonates or those with specialized nutritional requirements due to their enriched polyamine concentrations.

CONCLUSION

The study reveals significant findings regarding the impact of water type on polyamine content in goat colostrum and milk. Hydrogen-rich water (HRW) supplementation consistently resulted in higher levels of polyamines than normal water (NW) throughout the lactation period. The main outcomes of the present study are the following:

1. At parturition (day 0), HRW-fed goats produced colostrum with significantly higher total polyamine content (1.60 µmol/L) compared to NW-fed goats (0.91 µmol/L).
2. Polyamine levels increased throughout the lactation period, with a marked increase during the third week for both groups.
3. At the end of the third week, HRW-fed goats' milk contained substantially higher levels of all three polyamines (putrescine, spermidine, and spermine) than NW-fed goats.
4. The interaction between water type and lactation days significantly affected polyamine levels ($P<0.001$), indicating that the impact of water supplementation varies across the lactation period.

These results suggest that HRW supplementation could be a potential strategy to enhance the polyamine content of goat milk. This finding may have implications for the nutritional value and potential health benefits of goat milk products. However, further research is needed to fully understand the mechanisms behind this effect and explore its long-term impacts on animal health and milk quality. Additionally, investigations into the potential benefits for milk consumers with enhanced polyamine content would be valuable for determining the practical applications of these findings.

The study also highlights the need for further investigation into the non-constant differences between treatments across all days of lactation. This variability suggests that the relationship between water supplementation and polyamine production is complex and may be influenced by additional factors not yet identified in this study.

In conclusion, this research provides valuable insights into the potential for manipulating polyamine content in goat milk through water supplementation, opening up new avenues for enhancing the nutritional profile of dairy products.

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Authorship contribution

MB: Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Visualization; MK: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Project administration; YÇ: Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Visualization; BBK: Methodology, Investigation; MM: Methodology, Resources, Project administration; DA: Conceptualization, Resources, Writing - Review & Editing, Supervision.

REFERENCES

Alwazeer, D., 2024a. Use of hydrogen extraction in the food industry, in: Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. Elsevier. https://doi.org/10.1016/b978-0-443-15978-7.00010-2

Alwazeer, D., 2024b. Consumption of Hydrogen-Treated Foods Provides Nutritional and Health Benefits, in: Slezak, J., Kura, B. (Eds.), Molecular Hydrogen in Health and Disease. Springer Nature Switzerland, Cham, pp. 319–337. https://doi.org/10.1007/978-3-031-47375-3_19

Ballard, O., & Morrow, A. L. (2013). Human milk composition: nutrients and bioactive factors. Pediatric Clinics, 60(1), 49-74.

Bardócz, S., 1995. Polyamines in food and their consequences for food quality and human health. Trends Food Sci Technol 6, 341–346. https://doi.org/10.1016/S0924-2244(00)89169-4

Bardócz, S., Hughes, E.L., Grant, G., Brown, D.S., Duguid, T.J., Pusztai, A., 1998. Uptake, inter-organ distribution and metabolism of dietary putrescine in the rat. J Nutr Biochem 9, 332–338.

Bulut, M., Çelebi Sezer, Y., Ceylan, M.M., Alwazeer, D., Koyuncu, M., 2022. Hydrogen-rich water can reduce the formation of biogenic amines in butter. Food Chem 384, 132613. https://doi.org/10.1016/j.foodchem.2022.132613

Contarini, G., Povolo, M., Pelizzola, V., Monti, L., Bruni, A., Passolungo, L., ... & Degano, L. (2014). Bovine colostrum: Changes in lipid constituents in the first 5 days after parturition. Journal of Dairy Science, 97(8), 5065-5072.

Dandrifosse, G., Peulen, O., El Khefif, N., Deloyer, P., Dandrifosse, A.C., Grandfils, C., 2000. Are milk polyamines preventive agents against food allergy? Proceedings of the Nutrition Society 59, 81–86. https://doi.org/10.1017/S0029665100000100

Galitsopoulou, A., Michaelidou, A.-M., Menexes, G., Alichanidis, E., 2015. Polyamine profile in ovine and caprine colostrum and milk. Food Chem 173, 80–85. https://doi.org/10.1016/j.foodchem.2014.09.168

Giorgio, D., Di Trana, A., Claps, S., 2018. Oligosaccharides, polyamines and sphingolipids in ruminant milk. Small Ruminant Research 160, 23–30.

Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agricultural research. John wiley & sons.

Gopal, P. K., & Gill, H. S. (2000). Oligosaccharides and glycoconjugates in bovine milk and colostrum. British Journal of Nutrition, 84(S1), 69-74.

Gugliucci, A., 2004. Polyamines as clinical laboratory tools. Clinica chimica acta 344, 23–35.

Hirano, R., Shirasawa, H., Kurihara, S., 2021. Health-Promoting Effects of Dietary Polyamines. Med Sci (Basel) 9, 1–8. https://doi.org/10.3390/medsci9010008

Kajiyama, S., Hasegawa, G., Asano, M., Hosoda, H., Fukui, M., Nakamura, N., Kitawaki, J., Imai, S., Nakano, K., Ohta, M., 2008. Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Nutrition Research 28, 137–143.

Köktürk, M., Atalar, M.N., Odunkıran, A., Bulut, M., Alwazeer, D., 2022a. Evaluation of the hydrogen-rich water alleviation potential on mercury toxicity in earthworms using ATR-FTIR and LC–ESI–MS/MS spectroscopy. Environmental Science and Pollution Research 29. https://doi.org/10.1007/s11356-021-17230-x

Köktürk, M., Yıldırım, S., Eser, G., Bulut, M., Alwazeer, D., 2022b. Hydrogen-Rich Water Alleviates the Nickel-Induced Toxic Responses (Inflammatory Responses, Oxidative Stress, DNA Damage) and Ameliorates Cocoon Production in Earthworm. Biol Trace Elem Res 200, 3442–3452. https://doi.org/10.1007/S12011-021-02908-7

Komprda, T., Burdychová, R., Dohnal, V., Cwiková, O., Sládková, P., 2008. Some factors influencing biogenic amines and polyamines content in Dutch-type semi-hard cheese. European Food Research and Technology 227, 29–36. https://doi.org/10.1007/s00217-007-0688-7

Kuru, M., Makav, M., Boğa Kuru, B., Bektaşoğlu, F., Demir, M.C., Bulut, M., Alwazeer, D., 2024. Hydrogen-rich water supplementation improves metabolic profile during peripartum period in Gurcu goats and enhances the health and survival of kids. Res Vet Sci 171, 105208. https://doi.org/10.1016/j.rvsc.2024.105208

Larqué, E., Sabater-Molina, M., Zamora, S., 2007. Biological significance of dietary polyamines. Nutrition 23, 87–95. https://doi.org/10.1016/j.nut.2006.09.006

Matsumoto, M., Kibe, R., Ooga, T., Aiba, Y., Kurihara, S., Sawaki, E., Koga, Y., Benno, Y., 2012. Impact of intestinal microbiota on intestinal luminal metabolome. Sci Rep 2, 233.

McGrath, B. A., Fox, P. F., McSweeney, P. L., & Kelly, A. L. (2016). Composition and properties of bovine colostrum: a review. Dairy Science & Technology, 96(2), 133-158.

Michaelidou, A.M., 2008. Factors influencing nutritional and health profile of milk and milk products. Small Ruminant Research 79, 42–50.

Minois, N., 2014. Molecular Basis of the 'Anti-Aging'' Effect of Spermidine and Other Natural Polyamines - A Mini-Review.' Gerontology 60, 319–326. https://doi.org/10.1159/000356748

Nishibori, N., Fujihara, S., & Akatsu, T. (2003). Amounts of polyamines in foods in Japan and intake by Japanese. Food Chemistry, 81(1), 107-116.

Nishimura, K., Shiina, R., Kashiwagi, K., Igarashi, K., 2006. Decrease in polyamines with aging and their ingestion from food and drink. J Biochem 139, 81–90. https://doi.org/10.1093/jb/mvj003

Pakkanen, R., & Aalto, J. (1997). Growth factors and antimicrobial factors of bovine colostrum. International Dairy Journal, 7(5), 285-297.

Pegg, A. E. (2016). Functions of polyamines in mammals. Journal of Biological Chemistry, 291(29), 14904-14912.

Plakantara, S., Michaelidou, A.-M., Polychroniadou, A., Menexes, G., Alichanidis, E., 2010. Nucleotides and nucleosides in ovine and caprine milk during lactation. J Dairy Sci 93, 2330–2337.

Płoszaj, T., Ryniewicz, Z., Motyl, T., 1997. Polyamines in goat's colostrum and milk. Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology 118, 45–52. https://doi.org/10.1016/S0305-0491(97)00018-7

Polidori, P., Rapaccetti, R., Klimanova, Y., Zhang, J.-J., Santini, G., Vincenzetti, S., 2022. Nutritional parameters in colostrum of different mammalian species. Beverages 8, 54.

Prosser, C.G., McLaren, R.D., Frost, D., Agnew, M., Lowry, D.J., 2008. Composition of the non-protein nitrogen fraction of goat whole milk powder and goat milk-based infant and follow-on formulae. Int J Food Sci Nutr 59, 123–133.

Romain, N., Dandrifosse, G., Jeusette, F., Forget, P., 1992. Polyamine concentration in rat milk and food, human milk, and infant formulas. Pediatr Res 32, 58–63. https://doi.org/10.1203/00006450-199207000-00011

Silanikove, N., Leitner, G., Merin, U., Prosser, C.G., 2010. Recent advances in exploiting goat's milk: quality, safety and production aspects. Small Ruminant Research 89, 110–124.

Soda, K., 2022. Overview of Polyamines as Nutrients for Human Healthy Long Life and Effect of Increased Polyamine Intake on DNA Methylation. Cells 11, 164. https://doi.org/10.3390/cells11010164

Stelwagen, K., Carpenter, E., Haigh, B., Hodgkinson, A., & Wheeler, T. T. (2009). Immune components of bovine colostrum and milk. Journal of Animal Science, 87(suppl_13), 3-9.

Sugiyama, Y., Nara, M., Sakanaka, M., Gotoh, A., Kitakata, A., Okuda, S., Kurihara, S., 2017. Comprehensive analysis of polyamine transport and biosynthesis in the dominant human gut bacteria: Potential presence of novel polyamine metabolism and transport genes. International Journal of Biochemistry and Cell Biology 93, 52–61. https://doi.org/10.1016/j.biocel.2017.10.015

Todorovic, N., Fernández-Landa, J., Santibañez, A., Kura, B., Stajer, V., Korovljev, D., Ostojic, S.M., 2023. The Effects of Hydrogen-Rich Water on Blood Lipid Profiles in Clinical Populations: A Systematic Review and Meta-Analysis. Pharmaceuticals 16, 142.

Tolenaars, L., Romanazzi, D., Carpenter, E., Gallier, S., Prosser, C.G., 2021. Minor dietary components intrinsic to goat milk and goat milk formulas. Int Dairy J 117, 105012. https://doi.org/10.1016/j.idairyj.2021.105012

Ungerfeld, E.M., 2020. Metabolic Hydrogen Flows in Rumen Fermentation: Principles and Possibilities of Interventions. Front Microbiol 11. https://doi.org/10.3389/fmicb.2020.00589

Uruakpa, F. O., Ismond, M. A. H., & Akobundu, E. N. T. (2002). Colostrum and its benefits: a review. Nutrition Research, 22(6), 755-767.

Wang, M., Wang, R., Zhang, X., Ungerfeld, E.M., Long, D., Mao, H., Jiao, J., Beauchemin, K.A., Tan, Z., 2017. Molecular hydrogen generated by elemental magnesium supplementation alters rumen fermentation and microbiota in goats. British Journal of Nutrition 118, 401–410. https://doi.org/10.1017/S0007114517002161

Wang, Z., Wang, R., Meng, C., Ji, Y., Sun, L., Nie, H., Mao, D., Wang, F., 2019. Effects of dietary supplementation of N-carbamylglutamate on lactation performance of lactating goats and growth performance of their suckling kidlets. Small Ruminant Research 175, 142–148. https://doi.org/10.1016/j.smallrumres.2019.01.008

Zhang, Y., Su, W.-J., Chen, Y., Wu, T.-Y., Gong, H., Shen, X.-L., Wang, Y.-X., Sun, X.-J., Jiang, C.-L., 2016. Effects of hydrogen-rich water on depressive-like behavior in mice. Sci Rep 6, 23742.

Zheng, M., Yu, H., Xue, Y., Yang, T., Tu, Q., Xiong, K., Deng, D., Lu, L., Huang, N., 2021. The protective effect of hydrogen-rich water on rats with type 2 diabetes mellitus. Mol Cell Biochem 476, 3089–3097. https://doi.org/10.1007/s11010-021-04145-x

Zor, M., Bulut, M., Göksu Karagöz, S., Çetintaş, Y., Alwazeer, D., 2023. Use of Hydrogen-Rich water in rice milk preparation improves the nutritional and sensory properties of product. Food Chem 137821. https://doi.org/https://doi.org/10.1016/j.foodchem.2023.137821