Article

Comparison of Cold and Warm Water Intake in Winter on Growth Performance, Thermoregulation, Rumen Fermentation Parameters, and Microflora of Wandong Bulls (Bos taurus)

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Comparison of Cold and Warm Water Intake in Winter on Growth Performance, Thermoregulation, Rumen Fermentation Parameters, and Microflora of Wandong Bulls (*Bos taurus*)

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Abstract: Efficient farm practices are crucial for livestock health and performance, and cold stress is a major challenge for cattle in winter. This study aimed to investigate the effects of cold and warm water in winter on growth performance, thermal stress indicators, serum hormones and metabolites, rumen fermentation parameters, rumen fiber degrading enzyme and rumen microflora of yellow cattle during winter. Eight Wandong Bulls (*Bos taurus*) were divided into two groups: group C, which received cold water (6.36 ± 1.99 °C) and group E, which received warm water (32.00 ± 3.12 °C) for 30 d. Results showed that warm-water intake significantly increased ADG ($p = 0.024$) and DMI ($p = 0.046$), and decreased ($p = 0.047$) the ratio of feed intake to weight gain. Furthermore, the heat production, respiratory rate, surface temperature and rectal temperature of cattle did not alter varying with water temperature, but the heat production value of bulls increased (29.64 vs 25.76 $MJ/W^{0.75}$ h^{-1}) by cold-water intake compared to warm water. The concentrations of thyroxine ($p = 0.021$), serum urea nitrogen ($p = 0.025$), and glucose ($p = 0.011$) increased with bulls drinking cold water compared to warm water. The concentrations of NH_3-N ($p = 0.048$), total VFA ($p = 0.010$), acetate ($p = 0.009$), propionate ($p = 0.009$), cellulase ($p < 0.01$) and xylanase ($p < 0.05$) were lower in group C compared to group E. The total bacterial abundance, as well as specific species including *Ruminococcus flavus*, *Ruminococcus albus*, and *Prevotella ruminicola*, were lower ($p < 0.05$) in group C compared to group E. In conclusion, in comparison with cold water, drinking warm water during the winter enhanced growth performance by influencing energy metabolism, regulating serum hormones and metabolites, and modulating ruminal microflora of bulls.

Keywords: energy metabolism; growth performance; rumen microbiota; wandong bulls; water temperature

1. Introduction

The cattle industry is becoming increasingly important in providing meat and dairy products to meet the demands of a growing global population[1]. However, cattle production is influenced by a variety of factors, including environmental conditions, forage availability, genetics, feeding practices, and disease[2-4]. Among these, temperature is a key factor that impacts animal health, productivity, and gut microbiota composition[5, 6]. Cold stress, in particular, is a temperature-related factor that can lead to a range of health and performance issues. Castellani et al. emphasized that cold exposure adversely affects animal physiology[7]. In mice, cold stress exacerbates lipopolysaccharide-induced intestinal damage, leading to dysbiosis in the gut microbiome [8]. Similarly, in rat offspring, cold stress has been linked to increased anxiety levels [9]. For dairy cows and beef cattle, cold stress not only decreases productivity but also raises their susceptibility to diseases[10]. Wang et al. reported that long-term cold stress decreased growth performance, nutrient digestion and rumen volatile fatty

acids (VFA) production[11]. These findings underscore the need for effective environmental management strategies to protect animals from the harmful effects of cold stress.

The microbiota refers to the diverse community of microbes inhabiting the intestinal tract, including bacteria, protozoa, archaea, viruses, and fungi [12]. Often referred to as the "second genome," these microorganisms play vital roles in digestion, metabolism, immunity, and maintaining the gut barrier [13]. The composition of this dynamic microbiota is influenced by a variety of factors, including genetics, nutrition, and environmental conditions[14]. Disruptions to this balance have been linked to a range of diseases, such as inflammatory bowel disease[15], diarrhea[16], and liver disorders[17]. It is reported that water temperature can affect the rumen temperature, and the lower the water temperature, the longer the rumen recovery temperature[18]. In fact, the effect of water temperature on rumen may be more direct due to the direct contact of water with rumen fluid and rumen microorganisms compared with temperature stress. In many regions of China, winters can be harsh, and continuous access to warm water in cowsheds is often unavailable. This is particularly common on smaller farms, where cattle are typically provided with cold water during the winter months. As a result, cattle production and health may be negatively impacted by the cold water. However, there is limited research on how water temperature influences the microbiota of Wandong Bulls during these cold winter months. Therefore, we hypothesized that drinking cold water would reduce growth performance, rumen fermentation, fiber enzyme activity, and microbial function in cattle, while increasing thermoregulation, energy metabolism, and heat production compared to drinking warm water. The objective of this study was to compare the growth performance, thermal stress indicators, serum hormones and metabolites, ruminal fermentation characteristics, fiber enzyme activity, and microbiota of bulls drinking warm versus cold water during the winter months, in order to explore an often overlooked aspect of animal health in response to environmental conditions.

2. Materials and Methods

The animals were treated as approved by the Humane Animal Care and Ethics Committee of the Nanjing Agricultural University, China (SYXK2011-0036).

2.1. Experiment Design

Eight Wandong Bulls (*Bos taurus*) (initial body weight of 365.45 ± 22.90 kg) in this study were kept on a local cattle farm in Chuzhou, China, and were divided into two groups: group C (cold water) and group E (warm water). In December, when the local temperature was averaged 5.74 ± 2.63 °C, bulls in group C received cold water at 6.36 ± 1.99 °C, while bulls in group E were given warm water at 32.00 ± 3.12 °C. The bulls were individually housed in pens with free access to water and were fed ad libitum a total mixed ration (TMR) with a concentrate-to-forage ratio of 1:9. Diet composition and nutrient levels were shown in Table 1. Cold water is the conventional drinking water for bulls exposed to low temperatures in winter, and actual water temperature is 5.74 ± 2.63 °C. Warm water is supplied by a constant temperature water tank equipped with a temperature control and detection system, and the temperature is set to 30 °C (actual water temperature is 32.00 ± 3.12 °C). The study included a 7-d adaptation period, followed by a 30-d data and sample collection period.

Table 1. Ingredients and chemical composition of experimental diets.

Item	Content
Ingredients, %	
Corn silage	85.50
Dry straw	4.50
Ground corn	7.00
Soybean meal	2.50
Calcium carbonate	0.04
Calcium hydrogen phosphate	0.06
Sodium bicarbonate	0.10
Sodium chloride	0.08
Limestone	0.13
Premix[1]	0.09
Chemical composition, % of DM	
Dry matter	87.26
Organic matter	94.47
Crude protein	8.14
Ether extract	6.53
Neutral detergent fiber	61.95
Acid detergent fiber	34.24
Calcium	0.83
Phosphorus	0.27
Metabolic energy (MJ kg^{-1})[2]	7.30

[1] Supplied per kilogram of dietary DM: VA 264 IU, VD 34 IU, VE 2.6 IU, Cu 1.2 mg, Mn 4.8 mg, Fe 6.0 mg, I 0.03 mg, Co 0.012 mg, Se 0.036 mg. [2] Metabolic energy was a calculated value.

2.2. Data and Sample Collection

The initial and final weights of each bull were recorded before morning feeding to calculate the average daily gain (ADG). The dry matter intake (DMI) of each bull was recorded every day and the ratio of feed intake to weight gain (F:G ratio) was calculated according to DMI and weight gain. Heat production in bulls was measured by a 80 × 80 × 136 cm respiratory metabolic apparatus at d 25, 26 and 27 of collection period, and was calculated according to the formula described by McLean et al[19]:

Heat production = -0.2047 × ΔF(O2) × V × 60 / kg$^{0.75}$

Where "ΔF(O2)" is oxygen concentration difference between intake and exhaust, "V" is exhaust volume per unit time of the breathing chamber under standard conditions. The respiratory rate of cattle was recorded through the breathing cage. Bull surface temperature at different ambient temperature (5-9, 9-11 and 14-16 ℃) was analyzed using hand-held infrared thermometer (59 Mini, Fluke®, USA) base on the weighted average of the body surface temperature of different parts of the bull and the proportion of the skin area of the part to the total body area. Rectal temperature was measured using a hand-held bovine rectal thermometer (29110, Jinan Borui agriculture and animal husbandry products Co., Ltd., China) inserted 15 cm into the anus of bull daily.

Blood samples were collected from the jugular vein of each bull using vacutainer tubes (gel & clot activator) before morning feeding on day 30 of the collection period. The collected samples were then centrifuged at 3,000 rpm for 15 minutes at 4°C to separate the serum, which was stored at -20°C until further analysis.

Rumen fluid samples were collected using a rumen catheter through the mouth on d 28, 29 and 30 of collection period. Fresh rumen fluid samples were measured pH value using pH meter (PHS-3E, INESA Scientific Instrument Co., Ltd., Shanghai, China). The rumen fluid samples were divided into 3 fractions; two portions were mixed with either 25% HPO_3 (v/v) or 1% H_2SO_4 (v/v) and stored at -20 ℃ until laboratory analysis; and the 3rd portion was collected into cryotubes, immediately frozen in liquid nitrogen, and stored at -80 °C for DNA extraction.

2.3. Laboratory analysis

2.3.1. Nutritional Composition

The samples of TMR were analyzed for dry matter (method 930.15), crude protein (method 990.03), ether extract (method 920.39) and ash (method 942.05) as outlined by AOAC (2005)[20]. The content of organic matter in the TMR is calculated by the difference between 100 % and ash. The contents of NDF (heat-stable α-amylase-treated) and ADF in TMR were determined according to the method described by Van Soest et al. (1991)[21].

2.3.2. Serum Indices

The concentration of triiodothyronine, growth hormone, blood urea nitrogen, glucose and total protein in serum were determined using commercial ELISA kits (Feiya Biology Co., Ltd., Jiangsu, China), and the serum thyroxine concentration was analyzed using commercial ELISA kit (Beijing Rigor Bioscience Development Co., Ltd., Beijing, China). The specific operation steps are performed according to the manufacturer's instructions. The absorbance of the reaction was measured by a microplate reader (Multiskan Go, Thermo Fisher Scientific Oy, Vantaa, Finland) and the serum hormones and metabolites concentrations were calculated according to the formula on the instructions.

2.3.3. Ruminal Fermentation Characteristics and Cellulases

VFAs in rumen fluid samples from both group C and group E were analyzed using gas chromatography (Shimadzu GC-4800A, Kyoto, Japan) equipped with a 30 m × 0.32 mm × 0.25 mm capillary column and flame ionization detection. The temperatures of injector oven, column oven and detector were set at 230, 160 and 240 ℃, respectively. The NH_3-N concentration in the rumen fluid was measured according to the indophenol colorimetric method described by Broderick and Kang (1980)[22]. The rumen fluid was centrifuged at 12000 ×g for 20 min, and 40 μL of the supernatant was mixed with 2.5 mL phenol chromogenic agent and 2 mL hypochlorite solution in turn, and the color was developed in a water bath at 37 ℃ for 30 min. The NH_3-N concentration was calculated according to the absorbance of the solution at the wavelength of 550 nm and the standard curve of NH_3-N.

The activity of rumen cellulases were measured using bovine-specific cellulase and xylanase ELISA kits provided by Feiya Biology Co., Ltd. and Lvye Biology Co., Ltd. The rumen fluid samples were preliminary treated by ultrasonic disruption to ensure the optimal decomposition of the microbial cell wall to enhance the extraction of enzymes, and then follow the manufacturer's instructions for subsequent operations.

2.3.4. Ruminal Flora Quantitative Analysis

Total bacterial DNA was extracted from rumen fluid samples collected from group C and group E using the APINeasy DNA Kit for feces (Beijing ZEPING Bioscience & Technology Co., Ltd, China). The purity of the extracted DNA was assessed through agarose gel electrophoresis (1.5%), as recommended in a previous study[23]. The concentration of the DNA products was measured using a UV spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). All samples were adjusted to a standardized concentration of 50 ng/μL for subsequent analyses.

To quantify bacterial concentrations, real-time PCR was conducted using specific primers targeting total bacteria, *Ruminococcus albus* (*R. albus*), *Ruminococcus flavus* (*R. flavus*), *Butyrivibrio fibrisolvens* (*B. fibrisolvens*), *Fibrobacter succinogenes* (*F. succinogenes*), *Methanogens*, *Succinimonas amylolytica* (*S. amylolytica*), *Streptococcus bovis* (*S. bovis*), and *Prevotella ruminicola* (*P. ruminicola*), as listed in Table 2. Initially, PCR amplification was performed, and the resulting products were purified using the GenElute Gel Recovery Kit (Sigma-Aldrich, USA). Following purification, the concentrations of the PCR products were assessed, and gradual dilutions were prepared to generate standard curves for real-time PCR quantification. Finally, bacterial concentrations were calculated based on the quantitative analysis methods described Li et al. (2014)[24] to provide details of the microbial community and its response to varying water temperatures in the feed of Eastern Anhui Yellow cattle.

Table 2. Specific primers used for real-time PCR in this study.

Target gene	Primers	Primer sequence（5′→3′）	Size(bp)
Total bacteria	F	CGGCAACGAGCGCAACCC	130
	R	CCATTGTAGCACGTGTGTAGCC	
Ruminococcus albus	F	CCCTAAAAGCAGTCTTAGTTCG	176
	R	CCTCCTTGCGGTTAGAACA	
Ruminococcus flavus	F	CGAACGGAGATAATTTGAGTTTACTTAGG	132
	R	CGGTCTCTGTATGTTATGAGGTATTACC	
Butyrivibrio fibrisolvens	F	GCCTCAGCGTCAGTAATCG	121
	R	GGAGCGTAGGCGGTTTTAC	
Fibrobacter succinogenes	F	GTTCGGAATTACTGGGCGTAAA	121
	R	CGCCTGCCCCTGAACTATC	
Methanogens	F	TTCGGTGGATCDCARAGRGC	232
	R	GBARGTCGWAWCCGTAGAATCC	
Succinimonas amylolytica	F	CGTTGGGCGGTCATTTGAAAC	139
	R	CCTGAGCGTCAGTTACTATCCAGA	
Streptococcus bovis	F	ATGTTAGATGCTTGAAAGGAGCAA	127
	R	CGCCTTGGTGAGCCGTTA	
Prevotella ruminicola	F	GAAAGTCGGATTAATGCTCTATGTTG	102
	R	CATCCTATAGCGGTAAACCTTTGG	

2.4. Statistical Analysis

The valid difference analysis between bulls drinking warm and cold water was performed using t-test in SPSS (version 27.0). All results were presented as means ± standard deviation (SD), with statistical significance recognized at $p < 0.05$, whereas a tendency was considered at $0.05 \leq p < 0.10$.

3. Results

3.1. Growth Performance

The initial body weight (BW) and final BW did not differ between group C and E (Table 3). However, drinking warm water increased ADG (p = 0.024) and DMI (p = 0.046), and decreased (p = 0.047) the F:G ratio of bulls.

Table 3. Growth performance in bulls provided with cold water versus warm water.

Item[1]	Group[2]		P-value
	C	E	
IBW, kg	359.34±10.62	371.57±14.56	0.523
FBW, kg	399.98±8.54	420.08±16.11	0.313
ADG, kg/d	0.95±0.05	1.15±0.03	0.024
DMI, kg/d	6.45±0.92	6.76±0.08	0.046
F:G, ratio	6.71±0.26	5.89±0.22	0.047

[1] IBW = initial body weight, FBW = final body weight, ADG = average daily gain, DMI = dry matter intake, F:G ratio = the ratio of feed intake and weight gain. [2] C, bulls drinking cold water; E, bulls drinking cold water.

Table 4. Thermal stress indicators in bulls provided with cold water versus warm water.

Item	Group[1]		P-value
	C	E	
Heat production, MJ/W$^{0.75}$ h^{-1}	29.64±6.95	25.76±4.52	0.385
Respiratory rate, min^{-1}	8.94±2.38	10.44±2.25	0.395
Body surface temperature, ℃			
Spatial temperature 5-9℃	15.97±1.49	16.19±1.83	0.746
Spatial temperature 9-11℃	20.67±1.64	21.06±0.43	0.673
Spatial temperature 14-16℃	23.00±1.89	23.96±1.12	0.416
Rectal temperature, ℃	38.00±0.50	38.24±0.23	0.417

[1] C, bulls drinking cold water; E, bulls drinking cold water.

3.2. Thermal Stress Indicators

The heat production, respiratory rate, surface temperature and rectal temperature of cattle were not different with varying water temperature.

3.3. Serum Hormones and Metabolites

The concentrations of triiodothyronine (trend, p = 0.058), thyroxine (p = 0.021), blood urea nitrogen (p = 0.025), glucose (p = 0.011) and total protein (trend, p = 0.063) increased in bulls drinking cold water compared to warm water (Table 5).

Table 5. Serum hormones and metabolites in bulls provided with cold water versus warm water.

Item	Group[1]		P-value
	C	E	
Triiodothyronine, ng/mL	1.53±0.09	1.22±0.09	0.058
Thyroxine, ng/mL	134.28±1.04	128.99±1.35	0.021
Growth hormone, ng/mL	5.26±0.15	5.38±0.26	0.688

Serum urea nitrogen, mmol/L	6.79±0.40	5.55±0.13	0.025
Glucose, mmol/L	4.85±0.09	4.40±0.08	0.011
Total protein, g/L	58.82±3.61	54.07±2.45	0.063

[1] C, bulls drinking cold water; E, bulls drinking cold water.

3.4. Ruminal Fermentation Characteristics and Fiber Enzyme Activity

The rumen pH of bulls decreased (trend, $p = 0.061$) in group E than group C. The concentrations of NH_3-N ($p = 0.048$), total VFA ($p = 0.010$), acetate ($p = 0.009$), propionate ($p = 0.009$), butyrate (trend, $p = 0.066$), isobutyrate (trend, $p = 0.074$) and valerate (trend, $p = 0.091$) concentrations were lower in group C compared to group E, whereas the A:P ratio was not changed (Table 6).

Table 6. Rumen pH and fermentation parameters in bulls provided with cold water versus warm water.

Item	Group[2]		*P*-value
	C	E	
Rumen pH	7.27±0.04	7.19±0.03	0.061
NH_3-N, mg/dL	4.67±0.19	5.23±0.11	0.048
Total VFA, mM	60.52±2.19	78.61±4.34	0.010
Acetate, mM	41.92±1.62	55.14±3.04	0.009
Propionate, mM	10.26±0.40	13.49±0.75	0.009
Butyrate, mM	4.53±0.18	5.30±0.29	0.066
Iso-butyrate, mM	0.88±0.08	1.09±0.06	0.074
Valerate, mM	1.19±0.11	1.47±0.08	0.091
Iso-valerate, mM	1.25±0.17	2.11±0.21	0.139
A:P, ratio[1]	4.36±0.08	4.48±0.17	0.539

[1] A:P = the ratio of acetate and propionate. [2] C, bulls drinking cold water; E, bulls drinking cold water.

Bulls drinking warm water significantly increased the concentrations of cellulase ($p < 0.01$) and xylanase ($p < 0.05$) in rumen fluid compared to cold water (Figure 1).
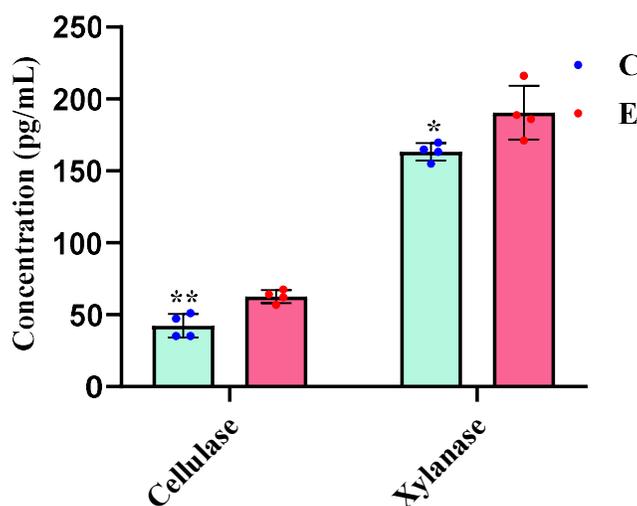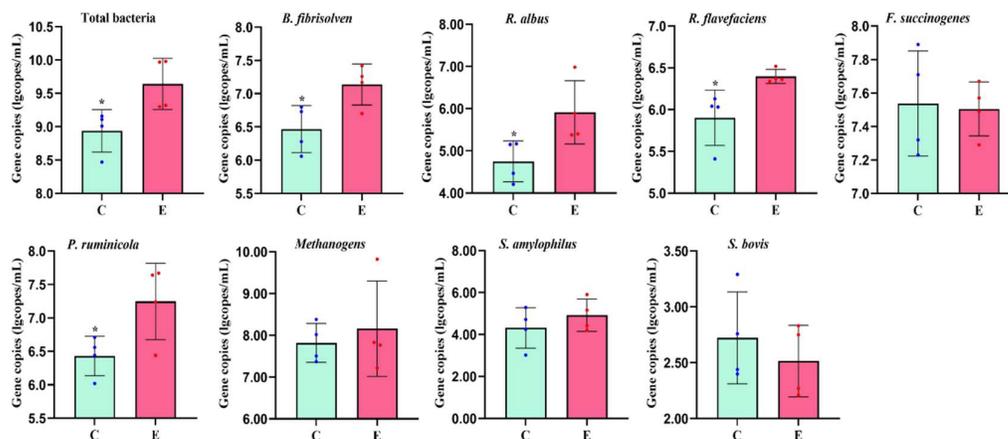

Figure 1. Comparative analysis of rumen cellulase levels in bulls provided with cold versus warm water (*, $p < 0.05$; **, $p < 0.01$). C, bulls drinking cold water; E, bulls drinking cold water.

3.5. Rumen Bacterial Abundance

Rumen bacterial analysis revealed that the total bacterial abundance, as well as specific species including *Ruminococcus flavus* (*R. flavus*), *Ruminococcus albus* (*R. albus*), and *Prevotella ruminicola* (*P. ruminicola*) were significantly lower ($p < 0.05$) in group C compared to group E. Conversely, there was no difference between the two groups in the abundances of *Fibrobacter succinogenes* (*F. succinogenes*), *Methanogens*,

Succinimonas amylolytica (*S. amylophilus*), and *Streptococcus bovis* (*S. bovis*) (Figure 2).

Figure 2. Comparative analysis of rumen bacterial abundance in bulls provided with cold versus warm water (*, $p < 0.05$; **, $p < 0.01$). C, bulls drinking cold water; E, bulls drinking cold water.

4. Discussion

4.1. Growth performance

Cold temperature is a well-documented stressor that can impair antioxidant activity, immune function, and overall growth performance in animals [25, 26]. In this study, we observed that bulls provided with cold water had lower weight gain compared to those given warm water. This finding is consistent with previous studies that show similar effects in beef cattle receiving warmer water during cold seasons[25], as well as in weaned piglets[27] and yaks[28].Together, these finding reinforce the conclusion that cold water have negatively impact on the animal performance, likely due to the increased metabolic demands associated with maintaining body temperature. Moreover, the digestion and absorption of feed in the digestive tract determines feed efficiency, and low feed digestibility may reduce feed efficiency and thus affect weight gain[29]. In our study, bulls drinking warm water during the winter showed enhanced cellulase and xylanase activity, suggesting that warm water may promote feed digestion by boosting cellulase activity in the rumen, thereby improving weight gain and feed efficiency. Additionally, cold stress is known to increase feed intake as the animal works to generate more heat [30]. For example, Kennedy et al. reported that cold exposure increased the DMI of sheep but decreased the digestibility of dry matter and organic matter[31]. In our study, bulls drinking warm water during the winter showed enhanced cellulase and xylanase activity, suggesting that warm water may promote feed digestion by boosting cellulase activity in the rumen, thereby improving weight gain and feed efficiency. In addition, cattle reduced feed intake to reduce heat production to adapt to heat stress[30], and on the contrary, cold stress increased feed intake to increase heat production. Kennedy et al. reported that cold exposure increased the DMI of sheep, but decreased the digestibility of dry matter and organic matter[31]. However, in the current study, dulls drinking cold water reduced the feed intake, which may be due to the fact that cold water reduced the activity of microorganisms, cellulase and xylanase in the rumen

of bulls, resulting in a decrease in the digestion rate of feed and increasing the retention time of feed in the rumen, thus reducing the feed intake[32]. Furthermore, Serviento et al. reported the water intake of dairy cows reduced when the ambient temperature was reduced from 17.25 ℃ to 8.27 ℃[33]. In fact, water intake of cattle is positively correlated with DMI, the decrease of water temperature in this study may reduce the water intake, thereby reducing the DMI[34].

4.2. Thermal stress indicators

Cold-stimulated ruminants adapt to temperature reduction by increasing heat production and reducing heat dissipation[35]. In this study, cold water intake increased heat production (29.64 vs 25.76 MJ/W0.75 h-1), likely as a result of the body's efforts to maintain thermal balance. In fact, when temperatures rise, cattle can increase the frequency of their breathing to facilitate heat exchange and help regulate body temperature[36]. Conversely, in response to colder temperatures, cattle reduce their respiratory rate to conserve heat. However, in this study, water temperature did not alter respiratory rate of bulls, which may be due to the limited change in energy exchange between bulls and the environment at the same temperature. Furthermore, the study found that cold water intake did not affect the bulls' body surface or rectal temperature, which may reflect the final outcome of the body's heat balance regulation. Although heat production, as well as levels of triiodothyronine, thyroxine, and glucose, increased, these changes likely helped elevate body temperature. However, the heat produced was counterbalanced by the cold water intake, which may have prevented any observable changes in the bulls's body surface or rectal temperature.

4.3. Serum Hormones and Metabolites

Cold-stimulated cattle can trigger changes in the concentrations of corticosterone, triiodothyronine, and thyroxine in the bloodstream through the regulation of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes. These hormonal changes help maintain body temperature stability and enable the cattle to adapt to environmental temperature fluctuations [37]. In this study, the higher concentrations of triiodothyronine and thyroxine in the serum of bulls drinking cold water suggest that cold water exposure imposed additional cold stress, prompting a physiological response to maintain thermal balance during the winter months.

Glucose, as a vital energy source, plays a crucial role in providing heat through energy metabolism[38]. In this study, bulls drinking cold water in winter had elevated serum glucose concentration. This likely indicates that the cold water stimulated the bulls to release more heat in order to maintain body temperature, which in turn resulted in increased glucose concentration. Wang et al. also reported a significant increase in serum glucose concentrations in beef cattle subjected to long-term cold stress [11].

Serum urea nitrogen and total protein levels reflect the protein balance in cattle[39]. It has been reported that long-term cold stress can increase the secretion of glucocorticoids in cattle, thereby enhancing the decomposition of proteins to obtain energy and leading to an increase in serum urea nitrogen concentration[11, 40]. Moreover, the synthesis of cold shock proteins in organisms increased to protect and maintain the normal physiological functions of the body when the ambient temperature decreases[41]. Therefore, the serum concentrations of urea nitrogen and total protein were both increased in bulls drinking cold water compared to warm water.

4.4. Ruminal Fermentation Characteristics and Cellulase

Warm water intake during winter may promote the production of beneficial VFAs such as acetate and propionate, which are microbial byproducts that support energy metabolism and gut health [42, 43]. The increase in total VFA concentration in the rumen of bulls drinking warm water could be due to the enhanced activity of rumen microorganisms and feed digestion, which in turn boosts VFA production. In addition, VFA as an important energy substance of the body, may be rapidly consumed to maintain body temperature balance, while the intake of cold water consumes the body's heat to maintain body temperature, resulting in a decrease in total volatile acid in the rumen fluid of bulls fed with cold water. Acetate is primarily produced by anaerobic bacteria such as *Bacteroides spp* and *Akkermansia muciniphila*[44], which contributes to energy and lipid metabolism, immunity, and stress resilience[45]. Propionate is mostly generated through carbohydrate metabolism, supports lipid metabolism, mitochondrial function, and immune health[46, 47]. The higher concentrations of acetate and propionate in bulls drinking warm water further indicate that

higher water temperature may facilitate lipid metabolism to enhance resilience and growth performance during cold period. NH_3-N is the product of protein degradation in the rumen, which is affected by many factors such as dietary nitrogen content, protein degradation rate, microbial synthesis efficiency, rumen wall absorption and energy level. The intake of warm water in this study increased the NH_3-N concentration in rumen fluid, which may be due to the fact that warm water enhanced the activity of proteolytic bacteria such as *P. ruminicola* in the rumen and promoted the digestion of protein in feed [48].

Cellulase and xylanase, two key enzymes produced by microbes, play crucial roles in converting lignocellulose into animal feed or fermentable sugars for ethanol production, with widespread applications in food, medicine, and agriculture[49, 50]. In this study, bulls provided with warm water showed significantly higher activity of cellulase and xylanase, consistent with findings in beef cattle[25]. In fact, cellulase and xylanase in the rumen are metabolism products of bacteria such as *F. succinogenes*, *R. albus*, and *R. flavefaciens*, and their concentrations are closely related to the activity of rumen microorganisms[51]. He et al. reported that feeding cold water to beef cattle in winter reduced the activity of cellulase and xylanase in the rumen, which was consistent with this study[25]. Current study showed that bulls provided with warm water exhibited a significantly higher abundance of total bacteria, *B. fibrisolvens*, *R. albus*, *R. flavefaciens*, and *P. ruminicola*, which may contribute to the observed increase in rumen cellulase and xylanase activity.

4.5. Rumen Bacterial Abundance

Microorganisms play a crucial role in feed digestion and overall animal health [52]. B. *fibrisolvens*, *R. albus*, *R. flavefaciens*, and *P. ruminicola* are known ruminal fiber-degrading bacteria that produce digestive enzymes to break down fiber and contribute to feed digestion in the rumen. Brod et al. reported that the lower (30 to 0 ℃) the intake water temperature of sheep led to a longer time (72 to 108 min) for the rumen to return to normal temperature[18]. Similar studies reported that cold water intake significantly reduced rumen temperature compared to warm water intake[53]. In the present study, cold water intake decreased the abundance of rumen microorganisms, likely due to reduced microbial activity caused by lower rumen temperature[25]. In addition, *B. fibrisolvens* is a prominent rumen bacterium that ferments polysaccharides, assisting in cellulose breakdown [47], and also acts as a probiotic that promotes the production of beneficial metabolites such as butyrate and lactate[54]. *R. albus*, another probiotic and cellulolytic bacterium, enhances fiber digestion and oxidative resistance[55]. Similarly, *R. flavefaciens* is a key fiber-degrading anaerobe associated with improved weight gain, feed digestibility, and fermentation efficiency[56, 57]. *P. ruminicola* (cellulolytic species) has been found in higher abundance in cows supplemented with riboflavin[58] and in high-yielding dairy cattle[59]. These findings suggest that warm water intake during the cold period may enhance cellulose metabolism by promoting the colonization of these beneficial probiotic bacteria in the rumen, ultimately supporting better digestion and improved growth performance.

5. Conclusion

Bulls drinking warm water in winter improved the growth performance of by promoting ADG, DMI, and reducing F:G ratio. This improvement may be attributed to reduced energy loss and increased ruminal VFA concentrations. Additionally, warm water intake alleviated the stress associated with maintaining thermal balance from cold water, as evidenced by lower concentrations of thyroxine, serum urea nitrogen, and glucose in the blood. Furthermore, warm water intake enhanced rumen fermentation by increasing the abundance of total bacteria, fiber-digesting bacteria, and the activity of cellulase and xylanase in the rumen. Therefore, providing warm water to bulls is crucial for improving their growth during the winter months.

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