Research Article

Effect of Probiotic Supplementation on Physiological Traits in Pregnant Holstein Cows and Some Studied Characteristics of Newborns During Summer

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Abstract | Pregnant cows in Iraq are exposed to significant heat stress during the summer season, particularly in the months of June, July, and August, when temperatures are extremely high. This heat stress adversely affects the productive performance of cows, largely due to its negative impact on rumen microbiota. Therefore, the present study was conducted to evaluate the role of probiotics in mitigating heat stress and improving the physiological and productive performance of heat-stressed pregnant cows. The experiment was conducted at Al-Khalis Dairy Cow Station on 15 dairy cows of multiparous Holstein cattle. They were divided into 3 treatments, each treatment has 5 replicates. Treatment T1 was a control treatment; T2 was the probiotic treatment at a concentration of 5 g and T3 was the probiotic treatment at a concentration of 10 g. The probiotic was encapsulated in gelatin capsules and administered orally to the cows every 48 hours throughout pregnancy. The study aimed to assess its impact on physiological traits, body measurements, and the birth weight of newborn calves. The results revealed a significant ($P \leq 0.05$) improvement in treatment groups (T2 and T3) compared to the control group (T1) in terms of newborn calf body weight. Additionally, highly significant ($P \leq 0.01$) differences were observed in various body dimension traits of newborn calves, including withers height, back height, overall height, chest depth, abdominal girth, average body girth, thigh length, chest girth, and body length, with T2 and T3 outperforming T1. Statistical analysis of physiological parameters namely internal body temperature, respiratory rate, pulse rate, heat tolerance index, and heat adaptation index demonstrated that both probiotic treatments (T2 and T3) significantly ($P \leq 0.05$) improved these parameters compared to the control group during May and June. In July and August, when the Temperature Humidity Index (THI) reached 74.77, 74.70, 79.60, and 84.10, respectively, the probiotic-treated groups maintained significantly ($P \leq 0.05$) lower respiratory and pulse rates than the control group. In September, both probiotic treatments showed significant ($P \leq 0.05$) superiority over the control in thermal adaptation, respiratory rate, pulse rate, internal body temperature, and heat tolerance coefficient. Similarly, in October, both treatments exhibited highly significant ($P \leq 0.01$) improvements in all physiological traits compared to the control group, except for pulse rate, where no significant difference was observed between treatments. Due to the limited number of studies conducted in Iraq on the effects of probiotics on heat-stressed cows, particularly during the summer season, this research provides important insights into their potential role in improving animal performance and thermal tolerance.

Received | May 17, 2026; **Accepted** | August 09, 2026; **Published** | August 26, 2026
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Citation | Hussin, I.A., M.A. Shwayel, Q.A. Khalaf. 2026. Effect of probiotic supplementation on physiological traits in pregnant Holstein cows and some studied characteristics of newborns during summer. *Advances in Agriculture and Animal Sciences,* 42(2): 73-82.
DOI | https://dx.doi.org/10.17582/journal.aaas/2026/42.2.73.82
Keywords | Heat stress, Dairy cows, Newborn, *Clostridium butyricum*, *Bacillus subtilis*

Introduction

The diet of ruminants primarily consists of complex carbohydrates such as cellulose and hemicellulose, which are difficult to digest and are broken down enzymatically by rumen microorganisms. Therefore, probiotics play an important role in enhancing the digestive process by supporting microbial populations that degrade fibrous feed, thereby improving feed intake and nutrient utilization (Reuben *et al*., 2022). In recent years, the use of dietary supplements, particularly microbial additives, has become widespread to improve animal performance and health. Probiotics, when administered, help maintain a balanced gut microbiota, promote the formation of a beneficial intestinal mucosal layer, and enhance the digestion and absorption of nutrients.

Encapsulation of probiotics is employed to protect beneficial microorganisms from harmful environmental conditions, including exposure to oxygen, stomach acids, and antagonistic microbes, ensuring their safe delivery to the intestines where they can exert their functional effects (Doleyres and Lacroix, 2005; Pandey *et al*., 2021). Furthermore, probiotics are increasingly being used as alternatives to antibiotics to improve growth performance and animal health, particularly as many countries have restricted or banned the use of antibiotics in animal feed (Sharma *et al*., 2012).

Heat stress is one of the most critical environmental factors negatively affecting dairy and pregnant cows, impairing feed intake, digestion, hormonal balance, and fetal development (Hansen, 2007). It also disrupts rumen microbial populations, reducing beneficial bacteria and consequently decreasing fiber digestion efficiency and overall productivity. Studies have shown that heat stress significantly reduces populations of key rumen bacteria such as *Ruminococcaceae* and *Lactobacillaceae*, leading to decreased rumination, saliva production, and microbial activity (Baek et al., 2020). This microbial imbalance can lower feed efficiency and increase susceptibility to pathogenic microorganisms.

Dairy cows, particularly Holstein breeds in Iraq, are highly susceptible to heat stress during the summer months, where the Temperature Humidity Index (THI) can reach critical levels (e.g., 82.96 in August and 78.14 in September), further compromising productivity (Al-Khafaji *et al*., 2023). Supplementation with probiotics helps replenish beneficial gut bacteria, thereby improving rumen health, feed utilization, and animal resilience under heat stress conditions. In addition, previous studies have reported that probiotic supplementation can significantly improve calf birth weight. For example, the use of *Enterococcus faecium* at a concentration of 1.3×10^{12} g/kg resulted in higher average birth weights (44.7 kg) compared to control groups (44.1 kg). Based on these findings, the present study aims to evaluate the effect of administering encapsulated probiotics containing beneficial microorganisms (including bacteria, fungi, and yeasts) on the productive performance, milk composition, and heat stress tolerance of Holstein cows during the summer season.

Materials and Methods

Animals

The experiment was conducted at Al-Khalis Cows Station, located in Hebheb Subdistrict, Al-Khalis District, Diyala Governorate, Iraq, during the period from 01/05/2025 to 24/10/2025. The study involved 30 Holstein cows, including 15 pregnant cows at different stages of gestation (ranging from the third to the seventh month) and 15 lactating cows at various stages of milk production. The animals were housed in semi-open pens measuring 75 m × 25 m, equipped with iron-grill feeders that allowed cows to feed comfortably. The facility also included a designated restraining area used for administering treatments and conducting experimental procedures. Water was provided through self-filling, rust-resistant troughs, and the flooring of the pens was constructed of concrete. Probiotic treatment was administered to the cows every 48 hours in the form of gelatin capsules. The capsules were given orally after restraining the animals in the designated handling area, which was also used for mechanical milking. Throughout the experimental period, the health status of all cows was closely monitored by a veterinarian, and all necessary vaccinations were administered. Calving of the pregnant cows occurred between late September and early October.

Cows feeding

The cows were fed a mixture of concentrated feed consisting of feed materials in the proportions shown in Table 1. Green fodder, consisting of alfalfa and clover, was provided in two meals daily at a rate of

20-25 kg per cow per day. Dry roughage, consisting of hay, was also provided in two meals, morning and evening. The concentrated feed was provided at a rate of 4-5 kg per cow in the herd. The pens were equipped with salt blocks during the trial period and these were provided freely.

Table 1: *Percentages of the components of concentrated feed.*

Percentage	Ingredients
50	Barley
37	Wheat Bran
10	Soybean Meal
2	Limestone
1	Salt
100	Total
16.116%	Protein*
5.059 mcg/kg	Energy*
70.89%	Total Digestible Nutrients (TDN)

**Crude protein and total energy were calculated according to (NRC, 2001).*

Weighing of newborn calves

The weight of each newborn calf was measured immediately after birth using a digital scale. In addition, body measurements including body length, chest circumference, abdominal circumference, lumbar height, sacral height, and diagonal body length were recorded using a measuring tape and measuring rod following parturition (Al-Qudsi and Elia, 2010).

Probiotic and its method of administration

An imported Chinese-made probiotic was used in this study, manufactured by Hebei Weierli Animal Pharmaceutical Group Co., Ltd. The probiotic used in the study contained *Clostridium butyricum* at a concentration of 1.3×10^7 CFU/g and *Bacillus subtilis* at 1.2×10^9 CFU/g. The probiotic was stored at 21°C in accordance with the manufacturer's instructions. Doses of 5 g and 10 g of probiotics were administered to the cows based on previous studies that reported positive outcomes using similar levels, although those studies delivered probiotics through feed or water. In the present study, the probiotic was administered orally in gelatin capsules to ensure its protection from ruminal degradation and to facilitate its delivery to the intestines, thereby maximizing the beneficial effects of the viable bacterial population.

The experiment was conducted at Al-Khalis cows Station, located in Hebheb Subdistrict, Al-Khalis District, Diyala Governorate, Iraq, from 01/05/2025 to 24/10/2025. A total of 30 Holstein cows were included, comprising 15 pregnant cows at different stages of gestation (third to seventh month) and 15 lactating cows at various stages of milk production. The animals were housed in semi-open pens measuring 75 m × 25 m, equipped with iron-grill feeders that allowed easy access to feed. Probiotic doses were administered every 48 hours after restraining the cows in the designated handling area, where capsules were given orally. Calving of the pregnant cows occurred during August and September.

The treatments included: T1, the control treatment without adding probiotics; T2, adding 5 gm of probiotics and T3, adding 10 gm of probiotics. Then it is placed on the device, and the measurement result appears on the device screen after a minute and a half.

Studied traits

- Rectal body temperature of cows: Rectal temperature was measured using a thermometer.
- Respiration and pulse rate of cows per minute: The number of breaths was measured from the waists and the pulse from the tail.
- Heat tolerance coefficient of cows: Heat Tolerance Coefficient (HTC) was calculated using the (Rhoad, 1944) method according to the following equation:

$$HTC = 100 - 10\,(ART - 38.3)$$

Where; HTC= Heat Tolerance Coefficient, ART= Average Rectal Temperature, 38.3= Normal Rectal Temperature in degrees °C, while 10 and 100 are constants.

Benezra thermal adaptation coefficient (BCA): It was calculated according to method of Benezra (1954) using following equation:

$$HTC = RT/38.3 + RR/23$$

Where; HTC= thermal tolerance coefficient, RT= rectal temperature, RR= number of respirations.

THI (Temperature Humidity Index): The temperature and humidity index (THI) was calculated using the method of (Al-Qudsi and Elia,2010) which depends on the dry-bulb temperature in °C and relative humidity, according to the following equation:

$$THI= (0.8 \times DBT) + (RH/100) \times (DBT - 14.4) + 46.4$$

Where; THI= Temperature Humidity Index, DBT= Dry-Bulb Temp, RH= Relative Humidity 0.8 Constants, 14.4, 46.4

Statistical analysis

A randomized complete block design (RCBD) was employed to eliminate the effect of parity (number of births) and its interaction with treatment effects. Data were analyzed using SAS Software (2011) at a significance level of $P \leq 0.05$. Differences among treatment means were determined using Duncan's multiple range test (Duncan, 1955), according to the following mathematical model.

$$Yij = \mu + Pj + Ti + eij$$

Yij= Observation effect, μ= Overall mean of the studied trait, Pj= Sector effect (number of births), Ti = Treatment effect, eij= Random error that is normally distributed.

Results and Discussion

Table 2 presents the mean body weight and body measurements of the newborn calves. The results showed that the treatment with 10 g of probiotic (T3) resulted in a significant increase ($P \leq 0.05$) in newborn body weight compared with the control treatment (T1), while the 5 g treatment (T2) did not differ significantly from either T1 or T3. The mean body weights were 25.20, 35.60, and 43.90 kg for T1, T2, and T3, respectively.

Regarding body measurements, both probiotic treatments (T2 and T3) significantly ($P \leq 0.01$) outperformed the control in back height and newborn height. The mean values for back height were 72.40, 86.20, and 89.20 cm, while newborn height averaged 74.60, 87.00, and 90.20 cm for T1, T2, and T3, respectively.

In addition, probiotic treatments showed a significant improvement ($P \leq 0.05$) in withers height (referred to as "cotton height"), with mean values of 73.20, 83.60, and 84.20 cm for T1, T2, and T3, respectively. Furthermore, T3 exhibited a highly significant superiority ($P \leq 0.01$) over both T1 and T2 in sacral (hip) height, with mean values of 70.00, 78.80, and 86.80 cm, respectively.

The present results regarding birth weight and body dimensions are in agreement with Williams (2010), who reported the superiority of probiotic-treated groups over the control in terms of newborn body weight. In that study, the average birth weights were 44.1 and 44.7 kg for the control and probiotic treatments, respectively, using *Enterococcus faecium* at a concentration of 1.3×10^{12} CFU/g of feed per day in pregnant Holstein cows.

Table 2: *Effect of probiotic dosing in pregnant Holstein cows on newborn body weight and body measurements during the summer season (Mean ± Standard Error).*

Trait	T1 (Control)	T2 (5 g)	T3 (10 g)	General Mean	Significance
Newborn weight (kg)	25.20 ± 3.02^{b}	35.60 ± 3.63^{ab}	43.90 ± 4.34^{a}	34.90 ± 9.09	*
Back height (cm)	70.00 ± 2.70^{c}	78.80 ± 1.82^{b}	86.80 ± 1.88^{a}	78.53 ± 4.20	**
Lower back height (cm)	73.20 ± 2.72^{b}	83.60 ± 2.11^{a}	84.20 ± 2.69^{a}	80.33 ± 5.83	*
Sacrum height (cm)	72.40 ± 3.31^{b}	86.20 ± 2.95^{a}	89.20 ± 1.35^{a}	82.60 ± 5.99	**
Newborn height (cm)	74.60 ± 2.74^{b}	87.00 ± 2.56^{a}	90.20 ± 2.08^{a}	83.93 ± 6.36	**
Body length (cm)	65.40 ± 3.07^{b}	78.00 ± 2.93^{a}	82.60 ± 1.86^{a}	75.33 ± 5.60	**
Chest circumference (cm)	73.20 ± 3.76^{b}	86.40 ± 1.46^{a}	87.20 ± 2.70^{a}	83.26 ± 6.82	*
Thigh length (cm)	33.60 ± 2.18^{b}	43.00 ± 0.89^{a}	45.00 ± 1.09^{a}	40.53 ± 3.57	**
Abdominal circumference (cm)	75.80 ± 3.05^{b}	87.00 ± 1.81^{a}	89.00 ± 2.68^{a}	83.93 ± 5.85	**
Chest depth (cm)	37.80 ± 1.15^{b}	43.60 ± 1.28^{a}	48.60 ± 2.50^{a}	43.33 ± 3.47	**

Means within the same row bearing different superscript letters differ significantly at P ≤ 0.05 (*) and P ≤ 0.01 (**), while NS indicates no significant differences among treatments within the same column. T1 = control (no probiotic); T2 = probiotic at 5 g/cow; T3 = probiotic at 10 g/cow administered every 48 hours.

In contrast, the current findings disagree with Zabransky *et al.* (2022), who reported no significant differences in birth weight among treatments when using a probiotic mixture containing *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* at a concentration of 4.1 × 10^7 CFU. Their reported mean body weights were 47.81, 47.63, and 47.76 kg for probiotic and control groups, respectively.

The present study also demonstrated a significant ($P \leq 0.05$) improvement in body length and chest girth in probiotic-treated groups compared to the control, with mean values of 65.40, 78.00, and 82.60 cm for body length and 73.20, 86.40, and 87.20 cm for chest girth in T1, T2, and T3, respectively. Moreover, highly significant ($P \leq 0.01$) improvements were observed in thigh length, abdominal circumference, and chest depth. The corresponding means were 33.60, 43.00, and 45.00 cm for thigh length; 75.80, 87.00, and 89.00 cm for abdominal circumference; and 37.80, 43.60, and 48.60 cm for chest depth in the control and probiotic treatments, respectively.

However, these results are not consistent with Guo *et al.* (2022), who found no significant differences in body weight, body length, body height, or chest circumference of newborn calves at one day of age when probiotics (2, 4, and 6 g) were added to milk. Similarly, Wang *et al.* (2021) reported no significant differences in body weight at one day of age among treatments using probiotics containing *Lactobacillus* and *Bacillus subtilis* at concentrations of 0.12 and 1.2 × 10^8 CFU/g, with mean values of 41.9, 40.9, and 41.0 kg for control and probiotic groups, respectively. However, they observed a significant increase in calf height at one month of age for the higher probiotic dose (1.2 g), while no significant differences were found in body length.

Table 3: *Effect of probiotic dosing on rectal temperature, pulse rate, respiration rate, heat tolerance coefficient, and Benezra thermal adaptation coefficient in pregnant cows before calving during the summer months (Mean ± SE).*

Rectal temperature °C	Pulse rate beats/ minute	Respiration breath/ minute	Heat tolerance coefficient	BCA	Treatments
THI(74.77) May					
0.15 ^{b} ± 38.72	0.50 ^{b} ± 53.10	0.355± ^{b} 2.08	1.19 ^{b} ± 95.72	0.02 ^{b} ± 3.27	T1
0.16 ^{a} ± 38.18	0.45 ^{a} ± 48.92	0.39 ^{a} ± 47.42	1.09^a ± 100.08	3.04 ± 0.02 ^{a}	T2
0.12 ^{a} ± 38.06	0.39 ^{a} ± 48.66	0.20 ^{a} ± 47.50	1.19 ^{a} ± 101.86	0.01 ^{a} ± 3.05	T3
0.28 ± 38.32	0.80± 50.22	0.84± 49.00	1.89 ± 99.22	0.01 ± 3.12	General average
**	**	**	**	**	Significant
June THI(74.70)					
0.22 ^{b}± 39.12	0.47 ^{b} ± 60.32	0.29 ^{b} ± 56.36	1.28 ^{b} ± 92.44	3.47 ±0.01 ^{b}	T1
0.09 ^{a} ± 38.22	0.69 ^{a} ± 55.44	0.70 ^{a} ± 51.32	0.76 ^{a} ±100.32	3.20 ±0.04 ^{a}	T2
0.11^a± 37.92	0.66 ^{a} ± 54.88	0.93 ^{a} ± 50.76	1.67 ^{a} ± 102.92	3.16 ±0.06 ^{a}	T3
0.40± 38.42	56.88 ± 1.65	1.30 ± 52.81	3.29 ± 98.56	3.27±0.09	General average
**	**	**	**	**	Significant
July THI(79.60)					
0.24 ^{b} ± 39.62	0.71 ^{b} ± 59.70	0.84 ^{b} ± 55.42	2.30 ^{b} ± 86.02	3.44 ±0.03 ^{b}	T1
0.22 ^{ab} ± 39.02	0.84 ^{a} ± 56.04	0.83 ^{a} ± 52.48	1.81 ^{a} ± 92.82	3.23 ±0.07 ^{a}	T2
38.82 ±0.19 ^{a}	0.47 ^{a} ± 55.06	0.57 ^{a} ± 51.98	2.24 ^{a} ± 93.68	3.27 ±0.02 ^{a}	T3
39.15±0.43	56.93 ± 1.85	1.58 ± 53.29	4.52 ± 90.84	3.31±0.11	General average
*	**	**	*	*	Significant
THI(84.10) August					
0.07 ^{b} ± 39.84	0.85 ^{b} ± 63.60	0.89 ^{c} ± 71.48	0.80 ^{b} ± 84.18	4.16 ±0.04 ^{c}	T1
0.11 ^{a} ± 39.32	1.04 ^{a} ± 59.38	1.01 ^{b} ± 67.06	1.20 ^{a} ± 89.52	3.96 ±0.03 ^{b}	T2
0.23 ^{a} ± 39.20	0.66 ^{a} ± 59.44	0.68 ^{a} ± 64.22	2.12 ^{a} ± 90.46	3.83 ±0.02 ^{a}	T3
0.36 ± 39.45	2.06± 60.80	1.53 ± 67.58	3.64 ± 88.05	±0.08 3.98	General average
*	**	**	*	**	Significant

Means within the same column bearing different superscript letters differ significantly at P ≤ 0.05 (*) and P ≤ 0.01 (**), while NS indicates no significant differences among treatments within the same column. T1 = control (no probiotic); T2 = probiotic at 5 g/cow; T3 = probiotic at 10 g/cow administered every 48 hours.

The positive effects observed in the present study may be attributed to the role of probiotics in improving gut health, enhancing nutrient absorption, and promoting growth performance. Probiotics have been shown to reduce digestive disorders, improve growth rates, and limit the proliferation of pathogenic microorganisms in the gastrointestinal tract (Orman *et al*., 2023).

Table 3 presents the results of the statistical analysis of physiological traits of pregnant cows during the summer months. The findings revealed significant differences among treatments, indicating a clear effect of probiotic supplementation on these physiological characteristics. Probiotic treatments showed a significant reduction in rectal temperature compared with the control treatment during May, June, and July. However, treatment T2 did not differ significantly from T1 during June and July.

Furthermore, both probiotic treatments (T2 and T3) significantly improved heat tolerance compared with the control across all summer months. Nevertheless, T2 did not differ significantly from the control treatment in June for this trait. In addition, the probiotic treatments significantly reduced pulse rate (beats per minute) compared with the control during May, June, July, and August. This reduction indicates improved physiological stability under heat stress conditions.

It is important to note that a lower heat tolerance coefficient (HTC) is associated with a better thermal adaptation, as reflected by a lower Benezra's Coefficient of Adaptability (BCA). These results are consistent with the findings of Kumar *et al*. (2023), who reported that the BCA of dairy cows during summer was 2.51, while the HTC was 90.95, under temperature–humidity index (THI) values of 76.4, 80.2, 81.8, and 80.1 during June weeks. The elevated temperature–humidity index (THI) recorded during the summer months indicates the presence of heat stress in pregnant cows. This is supported by the increased respiration and pulse rates observed in the control group compared with probiotic-treated groups. Overall, the improvements observed in BCA and HTC values suggest a higher level of adaptation and heat stress resistance in cows supplemented with probiotics compared with those in the control group. These findings highlight the beneficial role of probiotics in alleviating heat stress in pregnant cows.

Table 4 shows that probiotic treatments had a highly significant effect ($P \leq 0.01$) on rectal temperature (RT) and heat tolerance coefficient (HTC) during both September and October compared with the control treatment (T1). In September, the mean RT values were 39.44, 38.72, and 38.84 °C for T1, T2, and T3, respectively. Similarly, in October, RT values decreased to 38.92, 38.10, and 37.90 °C for T1, T2, and T3,respectively, indicating improved thermoregulation in probiotic-treated cows. The HTC values in September

Table 4: *Effect of probiotic dosing on rectal temperature (RT), pulse rate, respiration rate, heat tolerance coefficient (HTC), and Benezra's coefficient of adaptability (BCA) of dairy cows during September and October (Mean ± standard error).*

RT (°C)	PR pulse/minute	Respiration breath/minute	HTC	BCA	Treatments
September THI (73.4)					
39.44 ±0.10 ^{b}	63.46 ±0.65 ^{b}	67.22 ±0.77 ^{b}	88.38 ±1.03 ^{b}	3.94 ±0.03 ^{b}	T1
38.72 ±0.05 ^{a}	59.38 ±0.58 ^{ab}	62.56 ±0.73 ^{a}	95.08 ±0.39 ^{a}	3.79 ±0.07 ^{a}	T2
38.84 ±0.10 ^{a}	59.64 ±0.70 ^{a}	62.58 ±0.49 ^{a}	93.48 ±0.70 ^{a}	3.73 ±0.02 ^{a}	T3
39.00 ± 0.22	60.82 ± 1.58	49.26 ± 4.62	92.31 ± 1.96	3.82 ± 0.08	General average
**	**	**	*	**	Significant
October THI (68.67)					
38.92 ±0.10 ^{b}	56.68±3.14	52.02 ±0.43 ^{b}	93.48 ±1.09 ^{b}	3.27 ±0.02 ^{b}	T1
38.10 ±0.17 ^{a}	48.02±1.17	47.04 ±0.36 ^{a}	102.02 ±1.72 ^{a}	3.01 ±0.01 ^{a}	T2
37.90 ±0.13 ^{a}	49.88±2.49	45.84 ±0.79 ^{a}	103.14 ±1.54 ^{a}	2.98 ±0.03 ^{a}	T3
38.30 ±0.33	51.52±6.03	48.30 ±1.21	99.54 ±3.36	3.09 ± 0.05	General average
**	NS	**	**	**	Significant

Means within the same column bearing different superscript letters differ significantly at P ≤ 0.05 (*) and P ≤ 0.01 (**), while NS indicates no significant differences among treatments within the same column. T1 = control (no probiotic); T2 = probiotic at 5 g/cow; T3 = probiotic at 10 g/cow administered every 48 hours.

were 88.38, 95.08, and 93.48 for T1, T2, and T3, respectively, while in October they increased to 93.48, 102.02, and 103.14 for the same treatments. This improvement reflects enhanced heat tolerance in cows receiving probiotics. Respiration rate (RR) was also significantly affected by probiotic supplementation in both months. In September, RR values were 67.22, 62.56, and 62.58 breaths/min for T1, T2, and T3, respectively, while in October they were reduced to 52.02, 47.04, and 45.84 breaths/min. These reductions indicate alleviation of heat stress in probiotic-treated groups. Likewise, Benezra's coefficient of adaptability (BCA) was significantly improved (lower values) in probiotic treatments compared with the control in both months, suggesting better physiological adaptation to heat stress conditions.

Regarding pulse rate (PR), T3 differed significantly from T1 in September, while T2 did not differ significantly from either T1 or T3. The mean PR values in September were 63.46, 59.38, and 59.64 beats/min for T1, T2, and T3, respectively. However, in October, no significant differences were observed among treatments, with values of 56.68, 48.02, and 49.88 beats/min for T1, T2, and T3, respectively. The temperature–humidity index (THI) values recorded were 73.40 in September and 68.67 in October. These values indicate that cows were exposed to heat stress conditions, particularly in September. The elevated respiratory and pulse rates observed in the control group compared with probiotic-treated groups confirm this stress. The results demonstrate the important role of probiotics in reducing heat stress in pregnant cows. Improvements in RT, RR, HTC, and BCA indicate enhanced thermal adaptation and physiological stability in treated animals. Heat stress occurs when the animal's internal body temperature cannot be maintained within normal limits due to high environmental temperatures. The temperature–humidity index (THI) is commonly used to assess heat stress levels; values below 72 indicate no heat stress, while higher values reflect increasing stress severity. These findings are consistent with Hamdi *et al.* (2021) who reported that increasing THI negatively affects milk production and overall performance in dairy cows.

The results of the statistical analysis highlight the important role of probiotics in improving the physiological status of dairy and pregnant cows under heat stress conditions. Heat stress is considered one of the most critical environmental factors negatively affecting the productive performance of dairy cows, which ultimately reduces the economic return of milk production. Heat stress leads to several physiological and metabolic disturbances, including reduced rumination activity and dry matter intake, alterations in hormonal balance, and increased heart and respiration rates. It is generally more pronounced in high-producing cows compared with low-producing ones, as higher metabolic rates result in greater internal heat production. Consequently, this stress can also negatively influence reproductive performance, including reduced birth weight of newborn calves, as reported by Atrian and Shahryar (2012). Benezra's Coefficient of Adaptability (BCA) is an important indicator used to evaluate the ability of pregnant cows to adapt to environmental stressors. Heat stress adversely affects feed intake, production efficiency, and vital physiological functions. Therefore, measuring BCA provides a useful assessment of the animal's adaptive capacity under such conditions, as indicated by Rachmawati *et al.* (2024). There is an inverse relationship between BCA and heat tolerance. Lower BCA values indicate better adaptability and greater resistance to heat stress. For example, Mandal *et al.* (2023) reported that when BCA was 1.65, the heat tolerance coefficient (HTC) was 101.55, whereas when BCA increased to 2.16, HTC decreased to 93.21. This demonstrates that improved adaptability is associated with better heat tolerance responses. Probiotic bacteria, particularly *Clostridium butyricum*, play a significant role in mitigating the adverse effects of heat stress in animals. These microorganisms have been shown to reduce rectal temperature during hot seasons, thereby helping maintain thermal balance in cows. Rectal temperature is a key physiological indicator of heat stress and thermoregulation. The beneficial effects of this probiotic strain were confirmed by Cai *et al.* (2021). Overall, the use of probiotics can be considered an effective nutritional strategy to alleviate heat stress, enhance physiological stability, and improve the adaptive capacity of pregnant and lactating dairy cows under high environmental temperatures.

Conclusion

In summary, the results of this study demonstrate that supplementation with the probiotic strains *Bacillus subtilis* and *Clostridium butyricum* had a positive effect on the physiological performance of pregnant dairy cows under heat stress conditions. The findings indicate that probiotic treatments improved the Benezra's Coefficient of Adaptability (BCA), reflecting enhanced thermal adaptation in treated cows compared with the control group. In addition, probiotic supplementation increased the heat tolerance coefficient (HTC), indicating better resistance to heat stress. Significant improvements were also observed in key physiological traits, including rectal temperature (RT), respiration rate (RR), and pulse rate (PR), where probiotic-treated groups showed lower values compared with the control in several cases. These effects were particularly evident during July and August, which represent the peak heat stress months. The reduction in body temperature, respiration rate, and pulse rate suggests that probiotics play an important role in alleviating heat stress and improving physiological stability. Heat stress is known to negatively affect rumen function by reducing the population of beneficial microorganisms. This disruption can lead to decreased feed intake, reduced production efficiency, and increased ruminal acidity, ultimately impairing fiber digestion. Furthermore, heat stress reduces rumination activity and saliva production, which are essential for maintaining a stable rumen environment and a healthy microbial ecosystem. The use of probiotics helps to mitigate these negative effects by supporting rumen microbial balance and improving overall physiological responses. Therefore, it can be concluded that both probiotic concentrations used in this study were effective in enhancing heat tolerance, improving adaptation, and supporting the physiological performance of cows under heat stress conditions. Probiotic supplementation improved calf body weight, body dimensions, and physiological adaptation of cows under heat stress. Since both 5 g and 10 g levels were effective, the 5 g dose is recommended as a more economical option for practical use.

Acknowledgments

The researchers extend their thanks and appreciation to the staff at the cow station in Diyala for providing the related support to compile this work.

Novelty Statement

The Novelty of this study lies in its evaluation of the impact of a multi-strain bacterial probiotic administered to heat-stressed pregnant cows during the Iraqi summer. Research on the use of probiotics for heat-stressed pregnant cows remains limited, particularly under Iraq›s specific climatic conditions; therefore, this study aims to bridge this knowledge gap by assessing the probiotic›s ability to improve the productive performance of cows in hot environments.

Author's Contribution

This study was conducted through the collaboration of all the researchers Hussin, I.A and M.A. Shwayel, who collected and statistically analyzed the data, and the researchers Khalaf, Q.A how manager of the dairy farm where the study took place contributed by supporting the practical aspects of the research.

Ethics approval and consent to participate

The experiment was approved by the Ethical Committee for Animal Research at the College of Agriculture, University of Diyala, Iraq (Approval No. 3rd). All procedures were conducted in accordance with international, national, and institutional guidelines for the humane treatment of animals.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

References

Al-Khafaji, M.A.Q., Shwayel, M.A. and Al-Azzawi, A.M., 2023. Effect of spraying water on the body of holstein cows under heat stress conditions in milk production and components. In: Conference series: Earth and Environmental Science. 1225(1): 012044. IOP Publishing. https://doi.org/10.1088/1755-1315/1225/1/012044

Al-Qudsi, N.H. and J.V. Elia. 2010. Dairy cattle production. Department of animal wealth. College of Agriculture – University of Baghdad.

Atrian, P. and Shahryar, H.A., 2012. Heat stress in dairy cows (a review). Res. Zool., 2(4): 31-37.

Baek,Y.C., H.Choi,.J. Jeong., S.D. Lee,. M. J.Kim,. S.Lee,. S. Y. Ji and M. Kim. (2020).The impact of short-term acute heat stress on the rumen microbiome of hanwoo steers. J Anim Sci Technol; 62(2):208-217.

Benezra, M.V., 1954. A new index measuring the adaptability of cattle to tropical conditions. J. Anim. Sci., 13(4).

Cai, L.Y., Hartanto, R., Xu, Q.B., Zhang, J. and Qi, D.S., 2021. *Saccharomyces cerevisiae* and *Clostridium butyricum* could improve B-vitamin production in the rumen and growth performance of heat-stressed goats. Metabolites, 12(8): 766. https://doi.org/10.3390/metabo12080766

Doleyres, Y. and Lacroix, C., 2005. Technologies with free and cells immobilised for probiotic bifidobacteria production and protection. Int. Dairy J., 15(10): 973–988. https://doi.org/10.1016/j.idairyj.2004.11.014

Duncan, D.B., 1955. Multiple range and multiple F test. Biometrics, pp. 11-19. https://doi.org/10.2307/3001478

Guo, Y., Li, Z., Deng, M., Li, Y., Liu, G., Liu, D., Liu, Q. and Sun, B., 2022. Effects of a multi-strain probiotic on growth, health, and fecal bacterial flora of neonatal dairy newborn. Anim. Biosci., 35(2): 204-216. https://doi.org/10.5713/ab.21.0084

Hamdi, M.N., Darej, C., Attia, K., El-Akram, Z.I., Khattab, R., Djelailia, H., Bouraoui, R., Taboubi, R., Marzouki, L. and Ayadi, M., 2021. Modelling THI effects on milk production and lactation curve parameters of Holstein dairy cows. J. Therm. Biol., 99: 102917. https://doi.org/10.1016/j.jtherbio.2021.102917

Hansen, P.J., 2007. Effects of environment on bovine reproduction. Curr. Ther. Large Anim. Theriogenol., 9(347): 431-442. https://doi.org/10.1016/B978-072169323-1.50059-3

Kumar, K., Sinha, R.R.K., Singh, P.K., Kishor, A., Kumar, P., Chauhan, S.S. and Kumar, R., 2023. Effects of organic selenium and probiotic supplementation on the performance of indigenous and crossbred dairy cows during summer in the tropics. 3(2). 158–171. https://doi.org/10.3390/ruminants3020015

Mader, T.L., Davis, M.S. and Brandl, T.B., 2006. Environmental factors influencing heat stress in feedlot cattle. 1(2): 712–719. https://doi.org/10.2527/2006.843712x

Mandal, D.K., Rai, S., Chatterjee, A., Bhakat, C., Dutta, T.K. and Ghosh, M.K., 2023. Assessment of physiological responses and milk production in Jersey crossbred cows at different stratum of THI inside the cow barn. Indian J. Anim. Sci., 93(9): 916–922. https://doi.org/10.56093/ijans.v93i9.119779

NRC, 2001. Nutrient requirements of dairy cattle. 7th Rev. ed. National Academies Press, Washington, DC.

Orman, M.E., Fatima, S., Cetingul, I.S. and Bayram, I., 2023. Application of probiotics in the dairy.

Pandey, P., Mettu, S., Mishra, H.N., Ashokkumar, M. and Martin, G.J.O., 2021. Multi layer co-encapsulation of probiotics and γ-amino butyric acid (GABA) using ultrasound for functional food applications. LWT, 146: 111432. https://doi.org/10.1016/j.lwt.2021.111432

Rachmawati, A., Taufiq, M.N. and Arif, A.A., 2024. The correlation of heat tolerance coefficient (HTC) and temperature humidity index (THI) with milk production of dairy cows in Bangun Lestari farm, Tulungagung J. Agripecis. Soc. Impact, V1(2): 147-156. https://doi.org/10.62793/japsi.v1i2.22

Ravagnolo, O., Misztal, I. and Hoogenboom, G., 2000. Genetic component of heat stress in dairy cattle, development of heat index function. J. Dairy Sci., 83(9): 2120-2125. https://doi.org/10.3168/jds.S0022-0302(00)75094-6

Reuben, R.C., Elghandour, M.M., Alqaisi, O., Cone, J.W., Márquez, O. and Salem, A.Z., 2022. Influence of microbial probiotics on ruminant health and nutrition: Sources, mode of action and implications. J. Sci. Food Agric., 102(4): 1319-1340. https://doi.org/10.1002/jsfa.11643

Rhoad, A.O., 1944. The Iberia heat tolerance test for cattle. Trop. Agric. Sci. Technol., 21(9): 162-164.

SAS, 2011. Statistical analysis system, users guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Sharma, D.K., Arya, M., Dua, G.S., Chhatwal and Johri, A.K., 2012. Nano-technology for targeted drug delivery to combat antibiotic resistance. 9(11): 1325-1332. https://doi.org/10.1517/17425247.2012.717927

Wang, H., Yu, Z., Gao, Z., Li, Q., Qiu, X., Wu, F., Guan, T., Cao, B. and Su, H., 2021. Effects of compound probiotics on growth performance, rumen fermentation, blood parameters, and health status of neonatal Holstein newborn. J. Dairy Sci., 105(3): 2190–2200. https://doi.org/10.3168/jds.2021-20721

Williams, N.T., 2010. Probiotics. Am. J. Health Syst.

Pharma., 67(6): 449-458. Baek, Y.C., Choi, H., Jeong, J., Lee, S.D., Kim, M.J., Lee, S., Ji, S.Y. and Kim, M., 2020. The impact of short -term acute heat stress on the rumen microbiome of Hanwoo steers. J. Anim. Sci. Technol., 62(2): 208-217. https://doi.org/10.5187/jast.2020.62.2.208

Zabransky, L., Poborska, A., Galik, B., Soch, M., Broz, P., Kantor, M., Kernerova, N., Rezac, I., Rolinec, M., Hanusovsky, O., Strnad, L. and Havrdova, N., 2022. Influence of probiotic strains *Bifidobacterium*, *Lactobacillus* and *Enterococcus* on the health status and weight gain of newborn, and the utilization of nitrogenous compounds. Antibiotics, 11(9): 1273. https://doi.org/10.3390/antibiotics11091273