Haryana Veterinarian (Dec., 2025) **64(2),** 27-29 ISSN 0033-4359 (P)

Research Article

EFFECT OF BLOOD METABOLITES CONCENTRATION ON GROWTH AND DEVELOPMENT OF OVARIAN FOLLICLES IN SAHIWAL COWS UNDER THERMAL STRESS

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Received: 18.04.2024; finalized 22.07.2024

ABSTRACT

Effect of circulating concentration of blood metabolites *viz*. non-esterified fatty acid (NEFA) and blood urea nitrogen (BUN) on growth and development of ovarian follicles was evaluated in Sahiwal cows from March to June. The presence of a dominant follicle (≥ 10 mm in diameter) was detected on alternate days between two and three months after parturition in these animals (n=16) up to next 21 days through ultrasonography. Four animals (25%) were classified as cyclic whereas, 12 animals (75%) were found acyclic on the basis of presence or absence of dominant follicle. Beginning from day 0 of observation, serum NEFA and BUN concentrations were estimated at weekly interval up to 21 days using commercially available kits. Temperature-humidity index (THI) was assessed by using daily maximum temperature and relative humidity. There was no significant difference between animals of cyclic and acyclic group in mean serum NEFA concentration on days 0, 7 and 14; however, cyclic animals showed significantly ($P < 0.01$) lower serum NEFA concentration on day 21 than acyclic animals. Presence of dominant follicle was observed in cyclic animals on day 21 of observation. Both cyclic and acyclic animals recorded almost similar mean serum BUN concentration on each day of observation with no significant difference. No relationship could be established between mean serum NEFA and BUN level with that of THI values. It was concluded that summer stress can not be predicted based on serum concentrations of NEFA and BUN in Sahiwal cows.

Keywords: BUN, NEFA, Ovarian activity, Sahiwal cows, Summer stress, THI

How to cite: Singh, R.K., Awasthi, M.K., Khan, J.R., Parveen, K. and Bhonsle, D. (2025). Effect of blood metabolites concentration on growth and development of ovarian follicles in Sahiwal cows under thermal stress. *Haryana Veterinarian.* **64(2)**: 27-29.

Domesticated animals are subjected to a variety of abiotic stressors including temperature, nutritional, chemical and physical stress. The most significant among these stresses is thermal or heat stress and higher ambient temperature during summer, which is the most stressful condition for animals in tropical country like India with a greater ambient temperature (Kolli *et al.*, 2014). Thermal stress has negative impacts on productive and reproductive performance of production animals, resulting in significant financial losses to the dairy farmers to the extent that it affects nearly 60% of the world's dairy farms (Behl *et al.*, 2010).

Most large domestic animals perform best in thermo-neutral environments where they can successfully maintain equilibrium between heat production and heat loss. Temperature-humidity index (THI) is a useful tool to assess quantum of thermal stress in dairy animals, where the effects of ambient temperature and relative humidityare utilized to calculate THI. The reproductive efficiency in dairy cows and THI were reported to be negatively correlated (Habeeb *et al.*, 2018). Heat stress had a multifaceted impact on reproductive performance through a variety of mechanisms, including direct impacts on the reproductive system and indirectlyvia metabolic and nutritional pathway (De Rensis *et al.*, 2017).

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Thermal stressed cows display poor appetite and reduced intake of dry matter leading to extension of period of negative energy balance with high circulating level of NEFA (Baumgard and Rhoads, 2013). An increased likelihood of delayed ovarian activity has been considerably demonstrated when a severe negative energy balance appeared (Opsomer *et al.*, 2000). Wheelock *et al.* (2010) demonstrated that the impact of heat stress significantly change the concentration of metabolites *viz*. NEFA and BUN. In the light of these perspectives, the effect of circulating concentrations of NEFA and BUN on growth and development of ovarian follicle was assessed in Sahiwal cows during hot months of April, May and June in present study.

MATERIALS AND METHODS

The present work utilized postpartum acyclic Sahiwal cows at Bull Mother Experimental Farm, College of Veterinary Science and Animal Husbandry Anjora, Durg (C.G.) during April, May and June, 2022. Acyclic Sahiwal cows (n=16) were randomly selected at 60-90 days postpartum. These animals had history of normal parturition and their reproductive systemhad no palpable abnormalities. Their ovaries were screenedthrough ultrasonography on alternate days between 60 and 90 days postpartum for next 21 days through rectal probe of 5-7.5 MHz frequency to detect the presence of dominant follicle

Table 1. Mean serum NEFA(mmol/l)and serum BUN (mg/dL) concentrations in post-parturient Sahiwal cows

Days of blood collection	Mean serum concentration of NEFA		Mean serum concentration of BUN	
	Cyclic (n=4)	Acyclic (n=12)	Cyclic (n=4)	Acyclic (n=12)
0	0.72± 0.02	0.72 ± 0.04	18.93 ± 0.69	20.37 ± 0.59
7	0.74 ± 0.04	0.74 ± 0.05	21.39 ± 1.26	20.22 ± 0.65
14	0.76 ± 0.07	0.76 ± 0.05	19.43 ± 1.06	20.33 ± 0.61
21	0.77 ± 0.06	0.80 ± 0.05**	19.45 ± 0.70	20.61 ± 0.51

** = (P < 0.01)

Table 2. Relationship between THI and mean serum NEFA concentration (mmol/l) during hot months in Sahiwal cows

Days of observation	APRIL			MAY			JUNE		
	THI	Mean serum NEFA level	Regression P Value	THI	Mean serum NEFA level	Regression P Value	THI	Mean serum NEFA level	Regression P Value
0	90.28	0.73	0.7763	93.16	0.72	0.9827	91.72	0.73	0.0930
7	93.16	0.75		91.0	0.75		91.72	0.76	
14	91.72	0.79		94.6	0.77		89.56	0.78	
21	91.72	0.81		92.44	0.83		88.84	0.80	

Table 3. Relationship between THI and mean serum BUN concentration (mg/dL) during hot months in Sahiwal cows

Days of observation	APRIL			MAY			JUNE		
	THI	Mean serum BUN level	Regression P Value	THI	Mean serum BUN level	Regression P Value	THI	Mean serum BUN level	Regression P Value
0	90.28	0.73	0.7763	93.16	0.72	0.9827	91.72	0.73	0.0930
7	93.16	0.75		91.0	0.75		91.72	0.76	
14	91.72	0.79		94.6	0.77		89.56	0.78	
21	91.72	0.81		92.44	0.83		88.84	0.80	

(≥ 10 mm in diameter). Follicle was defined as non-echogenic spherical structures with a clear demarcation between the wall and antrum. The maximum diameter of each follicle was measured by in-built electronic caliper. The animals were classified in to cyclic (follicle diameter ≥ 10 mm) and acyclic (follicle diameter ≤ 10 mm)group during experimental period. Blood samples were collected on days 0, 7, 14 and 21 of ovarian scanning to determine concentrations of NEFA and BUN in blood circulation using commercial kits (Clementia Biotech-Cat no. E-BC-K013-S and Delta Lab, Product Code DL2502) through semi auto-analyzer.

Data on daily maximum temperatures and relative humidity were collected from local Automatic Weather Station of Indian Meteorology Department and these data were utilized to calculate THI for each day by following equation (National Research Council, 1971).

$$THI = 0.72 (Cdb + Cwb) + 40.6$$

Where, Cdb = Dry bulb temperature (°C)

Cwb = Wet bulb temperature (°C)

Comparison in mean serum levels of NEFA and BUN was made using independent 't' test between cyclic and acyclic animals. The association between mean serum levels of NEFA and BUN with THI values on days of observation was determined by adopting linear regression model through computerized statistical programme.

RESULTS AND DISCUSSION

Mean serum NEFA and BUN concentrationsin cyclic and acyclic animals are presented in Table1. Four animals (25%) were found cyclic that showed presence of dominant follicle between days 18 and 21 of the ultrasound scanning, while 12 animals (75 %) were found acyclic. Two cyclic cows showed behavioural signs of estrus during experimental period.

There was no significant difference in mean serum NEFA concentration of on days 0, 7 and 14 between cyclic and acyclic animals; however, cyclic animals recorded significantly lower (P<0.01) serum NEFA level on day 21

than that of acyclic animals. On the contrary, there was no significant difference in mean serum BUN concentration on days 0, 7, 14 and 21 between cyclic and acyclic animals.

Cyclic animals had significantly lower (P<0.01) serum NEFA values on day 21 of observation than that of acyclic animals suggesting that cyclic animals returned to energy equilibrium, thereby ovarian follicle reached to pre-ovulatory stage (> 10 mm in diameter). Present finding approximates with the report of Ali *et al.* (2012) who documented significantly lower (P<0.01) serum NEFA levelin cyclic than in acyclic Sahiwal cows during 50 and 60 days postpartum. Grimard *et al.* (1995) reported that concentration of plasma NEFA is negatively correlated with LH pulse frequency, while the size of the ovarian follicle is inversely proportional to NEFA concentration. Concentrations of NEFA in follicular fluid and serum are almost similar while, follicular concentration of NEFA is inversely proportional to that of estradiol (Comin *et al.*, 2002; Jorritsma *et al.*, 2004).

In the present study, non-significant difference in mean BUN concentrations on different days of observation between animals of cyclic and acyclic group indicated that animals of both groups had similar level of BUN and follicular activity was not influenced by its level.

Mean serum concentrations of NEFA and BUN in relation to THI value during hot months is presented in Table 2 and 3, respectively. Average serum values of NEFA and BUN on various observation days were put as dependent variable into a regression analysisin relation to THI value as independent variable on days 0, 7, 14 and 21 during months of April, May and June in post-partum animals. However, no statistical association (P > 0.05) could be found between serum NEFA and BUN concentrations with that of THI value. The evidence at hand suggests that THI values did not influence the circulating level of NEFA and BUN in postpartum Sahiwal cows during hot months. Serum NEFA and BUN concentrations were estimated with the hypothesis that heat stress would suppress the appetite of post-partum animals and thereby circulating concentration of these metabolites would be increased. However, this hypothesis was not supported by results of present study.

Contrary to present finding, many earlier research reports have established that heat stress resulted in higher circulating concentration of NEFA and BUN in exotic breeds of cows (Baumgard and Rhoads, 2013). The possible explanation of present finding on relationship ofserum concentrations of NEFA and BUN with that of THI values in postpartum Sahiwal cows might be the good heat tolerance of this breed of cow by virtue of presence of comparatively higher density and volume of sweat glands in per unit of skin surface areaand greater total body surface area owing to presence of hump and a dewlap in comparison to exotic breeds of cow and their hybrids (Wang *et al.*, 2014).

Inference may be drawn from the results of present study that circulating concentrations of NEFA and BUN were not influenced by higher THI values, which potentiates the fact that Sahiwal cows possess good heat tolerance property. Therefore, it may be concluded that assessment of heat stress cannot be made on the basis of circulatory concentrations of serum NEFA and BUN in Sahiwal cows.

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