International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; SP-9(1): 346-349

Received: 24-10-2020
Accepted: 30-12-2020

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Neutrophil lymphocyte ratio of Karan Fries heifers in natural and artificial climatic condition

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DOI: https://doi.org/10.22271/chemi.2021.v9.i1f.11837

Abstract
A study was carried out to find out the effect of temperature humidity index on neutrophil lymphocyte ratio of Karan-Fries heifers in five different periods based on maximum ambient temperature/minimum ambient temperature (Tmax/Tmin) *viz.* P1: <20 ºC/<10 ºC; P2: >20 ºC/<10 ºC, P3: >25 ºC/<15 ºC; P4: >35 ºC/<20 ºC and P5: >35 ºC/>20 ºC. The study investigated the effect of acute thermal exposure on neutrophil lymphocyte ratio of Karan-Fries heifers. Exposures were done at 40 ºC and 50% RH and 45 ºC and 50% RH in climatic chamber for 4 consecutive hours. Neutrophil leukocyte ratio was calculated from differential leukocyte count of blood smears stained with Leishman's stain. Neutrophil lymphocyte ratio of Karan-Fries was stable throughout the year in natural climatic condition. Exposure I and Exposure II in climatic chamber caused no significant change in neutrophil lymphocyte ratio of Karan-Fries. The study revealed good thermotolerance of Karan-Fries cattle both in natural climatic condition and thermal stress in climatic chamber.

Keywords: Cold, heat, Karan-Fries, neutrophil lymphocyte ratio, stress

Introduction
The haematological parameters have been a measure for detection and diagnosis of various diseases in animals. Factors contributing to variation in haematological parameters include age, sex, stress, diet, body condition, reproductive status, recent activity, hydration, ambient temperature, and altitude (Roland *et al.* 2014) [1]. Animals continuously experience some degree of physiological stress due to their exposure to inevitable stressors like extremes of temperature that may be in the form of heat during summer or cold during winter. A study of 2010 on comparison of reference intervals of healthy North American cows from 1957 to 2006 indicated significant increase in neutrophil count whereas reference intervals for lymphocyte, monocyte, and eosinophil counts as well as hemoglobin concentration had decreased (George *et al.*, 2006) [2].
The neutrophil lymphocyte ratio in adult cattle of different breeds and origin is approximately 1:2 (Kinkaid 1999; Tornquist and Rigas, 2010; Wood *et al.*, 2010) [3-5]. In presence of stress, the leukogram of cattle indicated neutrophilia (Tornquist and Rigas, 2010) [3] and lymphopenia (Jones and Allison, 2007) [6]. Neutrophil lymphocyte ratio had been indicated to be a marker of acute (Swan and Hickman, 2014) [7] and chronic (Hickman, 2017) [8] stress in laboratory animals, several other types of stress in cattle (Lynch *et al.*, 2010) [9] and long-term stress in pigs (Sanchez *et al.*, 2019) [10]. However, there is no report so far on neutrophil lymphocyte ratio of Karan-Fries cattle both in natural climatic condition and in response to thermal exposures.
The present study was carried out to find out the effect of temperature humidity index on neutrophil lymphocyte ratio of Karan-Fries heifers. The study further investigated the effect of acute thermal exposure on neutrophil lymphocyte ratio of Karan-Fries heifers.

Materials and Methods
Rearing and maintenance of animals
A total of 30 Karan-Fries heifers were selected from the herd maintained at National Dairy Research Institute, Karnal (NDRI), Karnal. The animals were in the age group of 2-2.5 years and average body weight was 301.3±6.91kg. The animals were fed an ad lib roughage and water as per Kearl standard (Kearl, 1982) [11].

Maintenance ration of concentrate mixture (12% CP and 60%TDN) consisting of mustard cake, maize, wheat bran, rice bran, mineral mixture and salt was fed @1kg/animal.

Study in natural climatic condition

Five different combinations of maximum ambient temperature (Tmax) and minimum ambient temperature (Tmin) were selected based on the climatograph prepared from the records of past 10 years. The conditions for five different temperature conditions (Tmax/Tmin) were P1: <20 ºC/<10 ºC; P2: >20 ºC/<10 ºC, P3: >25 ºC/<15 ºC; P4: >35 ºC/<20 ºC and P5: >35 ºC/>20 ºC.

Record of temperature and temperature humidity index

Prevailing temperature in Karnal as recorded at Central Soil Salinity Research Institute (CSSRI), Karnal were obtained. Everyday records of minimum ambient temperature, maximum ambient temperature, dry bulb and wet bulb temperature recorded at I: 0722\ 0830 and II: 1422 h IST were obtained for a period of 1 year. The temperature humidity index (THI) as proposed by National Research Council (National Research Council, 1971) [12] was calculated by using dry bulb (db) and wet bulb (wb) temperatures in the following formula.

$$\mathrm{THI} = 0.72(\mathrm{Tdb} + \mathrm{Twb}) + 40.6$$

Thermal exposure in climatic chamber

Karan-Fries heifers were kept in a climatic chamber (22’6’’x10’10’’x8’) which was insulated and thermostatically fitted with heat convector for thermal exposure. Prior to initiation of thermal exposure animals were kept in the climatic chamber at the prevailing temperature for 4h every day for 10 days to accustom with the chamber environment. Thermal exposure was done at two intervals. Exposure I was done at 40±1ºC, 50% relative humidity (RH) during P3. Exposure II was done at 45±1ºC, 50% RH during P4. Thermal exposure was carried out for 4h continuously.

The THI during the thermal exposures were calculated (Johnson *et al.*, 1962) [13] by using ambient temperature (T) and relative humidity (H) in the the following formula.

$$\mathrm{THI} = 0.08\mathrm{T} + \mathrm{RH}\frac{\mathrm{T}-14.4}{100} + 46.4$$

Blood sampling

Blood samples were collected from each of animals in natural climatic condition in 5 different periods. Blood samples were also collected from the animals in the thermal exposure at 0h, 1h, 2h, 3h and 4h of thermal exposure. A few drops of blood samples were collected from jugular vein by using Di-sodium salt of EDTA coated vacutainer tubes with needles.

Calculation of neutrophil lymphocyte ratio

A small drop of freshly collected blood was placed in the central line of a slide about 1-2 cm, from one end. A spreader slide with a smooth edge was placed at an angle of 45° onto the slide and then moved back to the blood drop. The drop was spread out along the line of contact of the spreader slide. The blood film was dried by waving the slide once in the air. Leishman stain (0.15% in methyl alcohol) was poured enough on the smear to cover it fully and allowed to act for 2 min. Distilled water was added twice the amount of stain and allowed to mix thoroughly by blowing air onto the mixture. Staining was done for 10 min. The smear was washed in the running tap water until colour stops draining. It was allowed to dry in air by keeping the slide in inverted position with the broad end of the film up.

Differential leukocyte count (DLC) was done by using oil emersion objective at 100x of an inverted binocular light microscope in a strip running the whole length of the film. The lateral edges of the film were avoided. The film was inspected from head to the tail. Approximately more than 200 cells were counted from one slide. DLC were expressed in %. From the result of DLC neutrophil lymphocyte ratio was calculated.

Statistical Analysis

The data were analyzed by using SPSS 16 version. One way analysis of variance was carried to find out the difference between different periods and between different hours of thermal exposure (Snedecor and Cochran, 1989) [14].

Ethical Approval for Animal Experimentation

Ethical approval was obtained from Institutional Animal Ethical Committee for thermal exposure and blood sampling.

Results ad Discussion

Average ambient temperatures and THI during different temperature conditions and thermal exposures in climatic chamber have been presented in Table 1 and 2 respectively. The neutrophil lymphocyte ratio of Karan-Fries heifers in natural climatic condition and thermal exposure have been presented in Table 3 and 4 respectively.

Ambient temperature and THI

The lowest Tmin and lowest average ambient temperature (Tav) were recorded in P1. The highest Tmax and the highest Tav were recorded in P5. Heat stress in dairy cattle starts at THI of 72 which corresponds to 22ºC at 100% humidity, 25ºC at 50% humidity, or 28ºC at 20% humidity (Johnson *et al.*, 1963) [15]. In the present study, average THI recorded in P4 and P5 were higher than 72 indicating presence of heat stress. THI >72 was found to cause thermal stress in Sahiwal and Karan-Fries in the form of changes in physiological indices (Mayengbamm *et al.*, 2015; Mayengbam *et al.*, 2016) [16-17]. Experience of thermal stress was also evident in Sahiwal and Karan -Fries cattle during THI >72 by presence of increase in expression of heat stress markers (HSP70) in Sahiwal and Karan-Fries cattle (Mayengbam *et al.*, 2016) [17] and antioxidant enzyme genes (Cu-SOD and Mn-SOD) in Sahiwal and Karan-Fries cattle (Mayengbamm *et al.*, 2015) [16].

Table 1: Ambient temperature and temperature humidity index in different periods

Parameters		Periods				
		P1	P2	P3	P4	P5
T	Max	16.70	20.40	30.80	36.00	37.79
	Min	2.50	9.00	13.60	17.80	23.16
	Average	9.60	14.70	22.20	26.90	30.48
T_{db} (ºC)	Max	18.40	19.80	30.50	36.20	35.73
	Min	3.60	10.40	14.90	20.50	27.00
	Average	11.00	15.10	22.70	28.35	31.37
T_{wb} (ºC)	Max	11.40	14.80	20.10	20.70	24.45
	Min	2.80	9.50	14.30	17.40	22.98
	Average	7.10	12.15	17.20	19.05	23.72
THI	Max	62.05	65.51	77.01	81.51	83.93
	Min	45.21	54.98	61.62	67.86	76.58
	Average	53.63	60.25	69.32	74.69	80.26

The THI recorded inside the climatic chamber during thermal Exposure I and II were below the stress level as compared to previous records (Johnson *et al.*, 1963) [15]. However, acute thermal exposure at such THI levels were found to cause thermal stress in Sahiwal and Karan-Fries cattle and the same was detected by presence of higher expression of heat stress marker HSP70 mRNA (Mayengbam and Upadhyay, 2014) [18] and HSP70 protein (Mayengbam *et al.*, 2016) [17].

Table 2: Ambient temperature and temperature humidity index during thermal exposures

Exposure	Climatic chamber			Environment					
	T (ºC)	RH (%)	THI	Tmax	Tmin	Tav (ºC)	Tdb (ºC)	Twb (ºC)	THI
Exposure I	40±1	50	62.4	30.80	13.60	22.20	22.70	17.20	69.32
Exposure II	45±1	50	65.3	36.00	17.80	26.90	28.35	19.05	74.69

Neutrophil lymphocyte ratio

During different periods (P1-P5) neutrophil lymphocyte ratio of Karan-Fries ranged from 0.41±0.02 to 0.48±0.02 which was well within the normal range (Kinkaid 1999; Tornquist and Rigas, 2010; Wood *et al.*, 2010) [3-5]. There was no significant change in neutrophil lymphocyte ratio of Karan-Fries during different periods. In Holando Argentino cows, neutrophil lymphocyte ratio was found to increase in summer (Tolini *et al.*, 1917) [19]. During P4 and P5 of present study, THI as high as 74.69 and 80.26 respectively were recorded in Karnal. The present study found no effects of high THI on neutrophil lymphocyte ratio. There was however increase in expression of heat stress marker HSP70 (Mayengbam *et al.*, 2016) [20] and HSP70 mRNA in response to THI>72 in Karan-Fries (Mayengbam *et al.*, 2016) [17]. Karan-Fries maintained stable neutrophil lymphocyte ratio throughout the year even during the periods of extremes of temperature in winter and summer. The present finding indicated better adaptability of Karan-Fries to extremes of climatic conditions.

Table 3: Neutrophil lymphocyte ratio (mean± SEM) of Karan-Fries heifer during different periods

P1	P2	P3	P4	P5
0.44	0.43	0.42	0.41	0.48
±0.02	±0.03	±0.03	±0.02	±0.02

When Karan-Fries heifers were subjected to acute thermal stress in Exposure I with 40ºC and 50% RH and Exposure II with 45ºC and 50% RH for 4 consecutive hours and neutrophil lymphocyte ratio was estimated there was no significant change in neutrophil lymphocyte ratio. In cattle, several types of stress *viz.* post parturient stress (Kulberg *et al.*, 2002) [21], exercise stress (Garcia-Belenquer *et al.*, 1996) [22] abrupt weaning (Lynch *et al.*, 2010) [9] and sole ulcer (O'Driscoll *et al.*, 2015) [23} were found to increase neutrophil lymphocyte ratio. The present finding indicated that Karan-Fries maintained stable neutrophil lymphocyte ratio in presence of acute thermal stress. There were however reports that Karan-Fries cattle exhibited higher expression of heat stress marker (HSP70) in response to acute thermal stress (Mayengbam and Upadhyay, 2014 16) and antioxidant stress marker *viz.* Mn-SOD and Cu-SOD (Mayengbam *et al.*, 2015 15). Presence of stable neutrophil lymphocyte ratio in response to acute thermal stress indicated high adaptability of Karan-Fries to acute thermal stress.

Table 4: Neutrophil lymphocyte ratio (mean± SEM) of Karan-Fries heifer during thermal exposure

Exposure	0h	1h	2h	3h	4h
I	0.41 ±0.05	0.41 ±0.00	0.45 ±0.02	0.45 ±0.01	0.47 ±0.01
II	0.43 ±0.03	0.47 ±0.01	0.40 ±0.02	0.42 ±0.02	0.46 ±0.01

Conclusion

The study found good thermotolerance of Karan-Fries cattle in winter as well as summer. Karan-Fries heifers maintained a stable neutrophil lymphocyte ratio in natural climatic condition even in the extremes of cold and hot in winter and summer. Acute thermal exposure caused no significant change in neutrophil lymphocyte ratio of Karan-Fries heifers.

Acknowledgement

Authors are grateful to ICAR Network Project on 'Impact, Adaptation of Vulnerability of Indian Agriculture to Climate Change' for financial support.

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