India is bestowed with a diverse genetic group of buffaloes. According to the 19th Livestock Census (BAHS 2015), buffalo population in the country is 108.7 million. India is the global leader in milk production and buffalo is the chief milk producer in India sharing 49% of national milk production (DAHDF 2016–17). In developing countries, the dietary energy intake is 1900–2600 Kcal/person/day which is 2900–3400 kcal/person/day in developed countries and the share of total dietary energy coming from dairy products is 2–4% in developing countries which is low in comparison to 14–15% in developed countries. High thermal stress affects negatively milk production traits like milk yield, fat and protein percentage. Buffaloes due to their dark skin, sparse hair coat and poor heat dissipation capacity are much prone to heat stress. Temperature humidity index (THI) that combines dry bulb and wet bulb temperature along with relative humidity is one of the efficient ways to measure the heat load (Thom 1959). Till today, no study has been conducted to define the thermal comfort and critical heat stress period in a year in relation to the Energy Corrected Milk Yield (ECMY) of Murrah buffaloes by using THI. Determination of heat stress zone and critical heat stress zone will help in undertaking managemental interventions at farm to enhance productivity.

MATERIALS AND METHODS

Source of data: Data on 9,864 monthly test day fat percentage data on 734 Murrah buffaloes spread over 20 years (March 1994 to 2013) were collected from ICAR-National Dairy Research Institute, Karnal and climatic parameters, viz. dry bulb temperature, wet bulb temperature and relative humidity for corresponding periods were collected from ICAR-CSSRI, Karnal. The overall least-squares means for monthly test day fat % ranged from 7.58±0.04 in TD2 to 8.02±0.04 in TD9. The monthly test day fat % data was adjusted for the significant non-genetic factors and was used to estimate ECMY (Kcal/kg). The non-heat stress zone (October–March) and heat stress zone (April–September) were identified based on the trend of monthly average test day ECMY in relation to monthly average THI. Within the heat stress zone, July to September exhibited maximum decline in the trait and was empirically identified as the CHSZ. Regression of monthly average ECMY (Kcal/kg) on monthly average THI in the empirically identified CHSZ was carried out to identify the most CHSZ. During July and August, maximum decrease (–8.18) in monthly average ECMY (Kcal/kg) was observed per unit rise in THI. Therefore, July and August months were identified as CHSZ for ECMY in Murrah buffaloes under subtropical climatic condition. Hence, heat stress amelioration programmes should be undertaken during the period to improve ECMY in Murrah buffaloes.
The effects of test day on adjusted monthly test day fat % for non-genetic factors (age group, parity and year) was estimated by using least-square analysis for non orthogonal data as suggested by Harvey (1990).

The data on monthly test day fat % were further adjusted for the significant effect of test days. Based on the adjusted monthly test day fat %, energy corrected milk yield of Murrah buffaloes was estimated by using standard practices as suggested by Overmann and Sammann (1926) as follows:

\[ F = 113.7334 (A + 2.4404) \]

where, A is the adjusted monthly test day fat %.

Based on the trend of monthly average ECMY in relation to monthly average THI values under each heat stress model, two zones like Non heat stress zone (NHSZ) and heat Stress Zone (HSZ) were identified empirically. Within the heat stress zone, the months where maximum depression in the monthly average ECMY was observed, were considered empirically as the critical heat stress zone. The most critical heat stress zone (CHSZ) within the empirically identified critical heat stress zone of the year was identified by assessing the decline in monthly test day ECMY in relation to unit change of monthly average THI values using regression analysis (Draper and Smith 1982).

The regression model used was

\[ Y_i = a + b x + e_i \]

where, \( Y_i \), monthly average energy corrected milk yield (Kcal/kg); \( a \), intercept; \( b \), regression coefficient; \( x \), monthly average THI; \( e_i \), random residual, NID (0, \( \sigma^2_e \)).

The zone within the empirically identified critical heat stress zone where maximum decrease in monthly test day ECMY was observed with per unit rise in monthly average THI (highest negative partial regression coefficient), was confirmed as the critical heat stress zone affecting monthly test day ECMY of Murrah buffaloes.

**RESULTS AND DISCUSSION**

Among the non-genetic factors, year had significant effect (P<0.01) on monthly test day fat % in all ten test days while age group at first calving had significant effect only on test day 6 (P<0.05). Chitra et al. (2015) reported non-significant impact of age group on monthly test day fat % in all the test days. Pawar et al. (2012) reported non-significant effect of parity and significant effect of year of calving on yearly average fat % (P<0.05). Test day had significant effect (P<0.01) on monthly test day fat %. The overall least-squares means for monthly test day fat % ranged from 7.58±0.04 in TD2 to 8.02±0.04 in TD9. The least square means along with standard errors for monthly average test day fat % is presented in Table 2.

The lowest monthly average THI (62.01) was found in January and the highest mean THI (82.17) was in the month of May followed by June (81.57) over 20 years period. Similar trend of THI values at Karnal was observed by Dash (2013). Upadhyay et al. (2012) earlier reported THI value 75 in February which increased to 85 in May and 95 in July and August in Karnal. Based on the adjusted monthly test day fat % data over 20 years, energy corrected milk yield

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**Table 1. Edited data structure for monthly test day fat % in Murrah buffaloes**

| Edited data of buffaloes | Monthly test day fat % |
|--------------------------|------------------------|
|                         | No of records | No of lactational | TDMFR |
|                         | lactational | buffaloes |
| Total no of records     | 1405         | 734            | 9864  |
| Total no of records excluded | 298      | 268            | 1196  |
| No of records included for study | 1107    | 466            | 8668  |

TDMFR, Test day fat % records.

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**Statistical analysis:** The data were adjusted for significant non-genetic factors for parity (1–5), buffaloes calved in different years (1–20) and age group at first calving (1–3) using a fixed least-squares model (Harvey 1990). The model considered was

\[ Y_{ijkl} = \mu + Y_r + P_a + A_G_k + e_{ijkl} \]

where, \( Y_{ijkl} \), observation of \( j^\text{th} \) animal \( k^\text{th} \) age group, \( j^\text{th} \) parity and \( i^\text{th} \) year of calving; \( \mu \), overall mean; \( Y_r \), fixed effects of \( i^\text{th} \) year of calving (1 to 20); \( P_a \), fixed effects of \( j^\text{th} \) parity (1 to 5); \( (A_G)_k \), fixed effects of \( k^\text{th} \) age group (1 to 3); \( e_{ijkl} \), random residual, NID (0, \( \sigma^2_e \)).

The least-square means and standard errors monthly test day fat % was estimated. Difference of least-squares means between sub-classes for each effect was tested by modified Duncan’s Multiple Range Test (Kramer 1957). The monthly test day fat % trait was adjusted for significant non-genetic factors.
Empirical identification of heat stress, critical heat stress and non heat stress zones: Based on the trend of monthly average ECMY (Kcal/kg) and monthly average THI values, it was observed that the ECMY (Kcal/kg) started declining from the month April, remained declined till September and gradually increased from October onwards. Therefore, the whole year was empirically classified into two zones like Non heat stress zone (NHSZ) and Heat stress zone (HSZ). NHSZ included months such as January, February, March, October, November and December whereas, HSZ included months from April to September. Within the heat stress zone, July to September exhibited maximum decline in the trait, thus, empirically considered as the critical heat stress zone. The trend of monthly average ECMY (Kcal/kg) in relation to monthly average THI is presented in Fig. 1.

Identification of critical heat stress zone by regression analysis: Regression of monthly average ECMY (Kcal/kg) on monthly average THI in the empirically identified critical heat stress zone was carried out to confirm identification of the most critical heat stress zone and the result is presented in Table 4. It was observed that during the month of July and August, maximum decrease in monthly average test day ECMY (–8.18 Kcal/kg) was observed per unit change in THI values. Therefore, in the present study it was confirmed that July and August months were the critical heat stress zone for monthly average ECMY in Murrah buffaloes. Till today there is no literature on determining the thermal comfort and critical heat stress zone for monthly average ECMY in Murrah buffaloes.

The result revealed that the months April to September were the heat stress zone and July to August months was the critical heat stress zone for energy corrected milk yield (Kcal/kg) in Murrah buffaloes. A decline of 8.18 Kcal/kg in ECMY per unit rise in THI value was observed during the critical heat stress zone. Since, climatic factors seem to

**Table 2. Least squares means along with standard error for monthly average test day fat %**

| Monthly test day | Fat %     |
|------------------|----------|
| Overall µ        | 7.84±0.01|
| TD1              | 7.74±0.04|
| TD2              | 7.58±0.04|
| TD3              | 7.72±0.04|
| TD4              | 7.84±0.04|
| TD5              | 7.92±0.04|
| TD6              | 8.01±0.04|
| TD7              | 7.97±0.04|
| TD8              | 7.90±0.04|
| TD9              | 8.02±0.04|
| TD10             | 7.69±0.05|

Similar superscripts indicate non-significant difference and dissimilar superscripts indicate significant differences (P<0.01).

**Table 3. Monthly average energy corrected milk yield (Kcal/kg) of Murrah buffaloes**

| Month     | ECMY (Kcal/kg) |
|-----------|----------------|
| January   | 1180.61        |
| February  | 1166.45        |
| March     | 1171.67        |
| April     | 1164.68        |
| May       | 1162.94        |
| June      | 1162.68        |
| July      | 1159.10        |
| August    | 1164.65        |
| September | 1163.19        |
| October   | 1165.70        |
| November  | 1170.09        |
| December  | 1165.14        |

**Table 4. Linear regression ECMY (Kcal/kg) on THI in empirically identified critical heat stress zone**

| Empirically identified CHSZ | \(a\) | \(b\) | \(R^2\) (%) |
|-----------------------------|------|------|-------------|
| July–August                 | 1807.34 | -8.18 | 100.00      |
| August–September            | 1147.89 | -0.04 | 9.77        |
| July–September              | 1333.49 | -2.18 | 40.00       |

a, Intercept; b, regression coefficient; \(R^2\), coefficient of determination, CHSZ, critical heat stress zone.

Fig. 1. The trend of monthly average energy corrected milk yield (Kcal/kg) in relation to monthly average THI (March 1994–2013).
affect ECMY (Kcal/kg), to enhance the energy content of milk and enhance the productivity in Murrah buffaloes, heat stress amelioration managemental programmes should be undertaken during the critical heat stress zone.

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