



NEGATIVE ENERGY BALANCE INDICATORS AS PREDICTORS FOR MILK PRODUCTION IN HIGH YIELDING MURRAH BUFFALOES

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ABSTRACT

This study has developed algorithms for predicting the amount of peak milk yield by using negative energy balance indicators (NEBI) as predictors. Equations using four selected independent variables (Body weight (BW), Body Condition Score (BCS), Serum Glucose (SG) and Serum Non esterified fatty acid (SNEFA)) gave 'R' values ranging from 0.459 to 0.803. Further, the preciseness of the equations was increased by using the four parameters, instead of serum metabolites (SG and SNEFA) or physical indicators (BW and BCS) alone. The Pearson correlation (R) between the predicted peak milk yield and actual peak yield values was highest in the equation obtained by plotting the NEBI at 90 d postpartum indicating that 90 d serum metabolites are better predictors of milk yield. The patterns followed by the predictors during the experimental study were normal without any deviation. Both BW (kg) and BCS (5 score points scale) showed an increased (P<0.01) trend from the day of drying to calving, followed by a decreased pattern from calving to 90 d postpartum. The SNEFA (μ moles/l) and SG (mg/dl) concentrations followed completely opposite pattern, during the entire study, with the respective peak values at 60 days postpartum and drying. It could be concluded that the NEBI could be used as indicators to predict total milk yield, and high correlations among the indicators and 6% fat corrected milk yield (FCMY) aids in developing better prediction equations which helps in prevention of various metabolic disorders apart having other management advantages.

KEY WORDS: Body weight, Body condition score, Serum Glucose, Serum Non esterified fatty acids, Milk yield prediction, Predictors pattern, Murrah Buffalo

INTRODUCTION

Cows or Buffaloes those fail to transition successfully into lactation are vulnerable to a host number of problems which occur just after the calving, include lowered milk production, Immunodepression and compromised reproductive performance. Dairy animals have the ability to compensate for deficits of food energy through the mobilization of adipose reserves. This situation leads to the -oxidation of body fat reserves, declining the fat depots and so the body weight (BW) along with Body Condition Score (BCS). Since, BW and BCS are more readily affected by body energy reserves, and because of their easily calculated complexion, they can be considered as physical indicators of negative energy balance. Further, a few adaptations occur in the animal's body including increased hepatic gluconeogenesis, reduced use of glucose by peripheral tissues, increased mobilization of non-esterified fatty acids (NEFA) from adipose and increased use of NEFAs by peripheral tissues (Bell, 1995). Excessive usage of glucose for milk production leads to a reduced SG levels with increased milk yield. The imbalance in energy status results in most dairy animals experiencing a period of negative energy balance (NEB) after calving, which increases the risk of both metabolic and infectious diseases (Duffield, 2000). To facilitate efficient and sustainable dairy management and reduce losses to dairy farmers, there is a need for precise

assessment of milk production based on the extent of negative energy balance in the dairy animals. Further, much of the works in this context are geared towards high yielding cows, neglecting other species, especially buffaloes. The regions with highly humid climatic conditions and the resourceful paddy growing areas of India are dominated by Buffaloes (Raju *et al.*, 2017), owing to a total 57% of the world's buffalo population. So, a work was carried out to develop prediction equations for 6% FCMY in high yielding Murrah Buffaloes by using indicators of negative energy balance as independent variables.

MATERIALS & METHODS

Forty eight multiparous Murrah buffaloes in different dry period (DP) lengths were selected from organized private buffalo farms located at Veeravalli village, Krishna District, Andhra Pradesh state. The buffaloes were fed individually with concentrate mixture (1 kg for maintenance, 1 kg for 2 kg milk production, and 1.5 kg in last 30 days pregnancy to meet the fetus requirements) along with *ad libitum* Hybrid Napier, and 4 kg Paddy straw. The ingredients and nutrient composition of the diet fed are presented in table 1 and 2, respectively. Proximate analysis was done as per the AOAC (2007), and forage fibre fractions according to Vansoest *et al.* (1991).
Determination of Physical indicators:

Milk production in high yielding Murrah buffaloes

Body weights of Murrah buffaloes were determined at DP, calving, and 30, 60, and 90 d postpartum by using Shaeffer's formula (Sastry *et al.*, 1983).

$$\text{Body weight (Pounds)} = LG^2/300$$

Where, L = Length of the animal in inches, and G = Heart girth of the animal in inches.

(1 inch = 2.5 cms and 1 kg = 2.5 pounds)

The body length of Murrah buffaloes was measured using a measuring tape from the point of shoulder to the point of pin bone. The heart girth was measured by drawing the measuring tape around the area behind front legs, a point slightly behind the shoulder blade, down the fore-ribs.

TABLE 1.0. Ingredient composition of the Concentrate mixture prepared

Ingredient	Percentage
Maize	33.25
Deoiled Rice Bran	34.00
Soybean meal	18.75
Sunflower cake	10.00
Urea	1.00
Mineral mixture	2.00
Salt	1.00
Sub Total	100.00

TABLE 2.0. Nutrient composition of the diets fed throughout the experimental period

Nutrient*	Hybrid Napier	Paddy straw	Concentrate mixture
DM	25.30	90.85	90.2
CP	6.23	3.54	21.3
EE	1.84	1.65	9.14
CF	23.11	42.58	5.98
NDF	69.09	69.80	14.88
ADF	39.60	46.30	6.47
TA	13.54	19.45	5.03
AIA	3.28	2.54	0.65

*DM – Dry matter, CP – Crude Protein, EE – Ether extract, CF – Crude fiber, NDF – Neutral detergent fiber, ADF – Acid detergent fiber, TA – Total Ash, AIA – Acid insoluble ash.

BCS of the buffaloes was determined at DP, calving, and 30, 60, and 90 d postpartum using BCS scale developed by Anitha *et al.* (2010). The score card was designed based on the amount of fat reserves at eight skeletal check points by vision and palpation. The skeletal check points were identified based on the anatomical features and carcass fat reserves. Based on the amount of fat reserves the scores were prioritized on a 1 to 5 scale of the new BCS proposed. The new BCS chart for condition scoring on a 1 to 5 scale using 0.5 increments was prepared. These check points include:

- (I) Tail head to pin bones, (II) Spinous processes of the lumbar vertebrae, (III) Depression between the spinous and transverse processes, (IV) Transverse processes of lumbar vertebrae, (V) Point between 12th and 13th ribs, (VI) Sacral crest, (VII) Depression between sacral crest and hooks, and (VIII) Depression between hooks and pins.

Estimation of Serum metabolites :

Estimation of SNEFA and SG concentrations at DP, calving, and 30, 60, and 90 d postpartum were done as per Falholt *et al.* (1973) and Trinder's method (By using Erba Mannheim Glucose kits), respectively.

The 6% FCM was calculated as per Rice *et al.* (1970), by using the formula;

$$6\% \text{ FCM} = 0.308 \times \text{Total Milk Yield} + 11.54 \times \text{Total Fat Yield (kg)}.$$

Development of Prediction equations :

Equations for 6% FCMY prediction were developed by using the linear Regression equation;

$$Y \text{ predicted} = B_0 + B_1X_1 + B_2X_2 + \dots + B_pX_p + E_p$$

Where,

Y predicted: Predicted score of dependent variable

B_0 : Intercept

P : Number of predictors

B_1 - B_p : Weights or partial regression coefficients for predictors / slope

X_1 - X_p : Scores of predictors

E_p : Errors of prediction

Statistical Analysis :

The estimated data of BW, BCS, ABWP, and ABCSP levels was subjected to ANOVA (Snedecor and Cochran, 1994) using software package SPSS version 17.0, and differences in mean were assessed by using Duncan's multiple range test (Duncan, 1955). Data was analyzed for statistical correlation (two tailed test of significant) of BW, BCS and 6% FCM at 30, 60 and 90 d postpartum using Pearson coefficient.

RESULTS & DISCUSSION

The mean values of negative balance indicators and 6% FCMY at DP, calving, 30, 60, and 90 d postcalving are presented in Table 3. The BW (kg) showed an increased ($P < 0.01$) trend from the day of drying to calving, followed by a decreased trend from calving to 90 d postpartum (fig 1). BCS also followed a similar trend from drying to 90 d postpartum (fig 2). The increased fetal growth due to advanced pregnancy at the time of drying might be responsible for the increased BW and BCS up to calving. Even though the feed intake increases along with the increased milk production during postpartum period, the intake cannot meet the required energy for lactation (NE_L), leading to the fastened mobilization of body reserves and decreased BCS as the postpartum days advance (Reddy *et al.*, 2016).

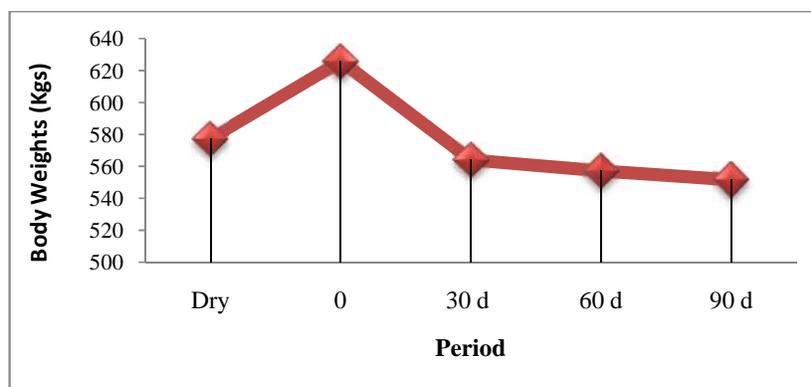


FIGURE 1. Pattern of Body weights (BW) during the experimental period

TABLE 3.0. Mean values of negative balance indicators and 6% FCMY at DP, calving, and 30, 60 and 90 days postpartum periods

Period	Physical Indicators		Serum Metabolites		6% FCMY
	BW	BCS	SG	SNEFA	
At Drying	577.31 ^b ± 4.07	3.76 ^a ± 0.024	62.56 ^d ± 0.34	395.21 ^a ± 4.03	-
At Calving	625.7 ^c ± 3.67	4.33 ^c ± 0.019	53.84 ^c ± 0.36	634.39 ^b ± 4.18	-
At 30 d postpartum	564.12 ^{ab} ± 3.86	4.00 ^b ± 0.029	49.76 ^b ± 0.45	732.73 ^c ± 5.83	14.08 ^a ± 0.17
At 60 d postpartum	557.25 ^a ± 4.14	3.83 ^a ± 0.034	45.55 ^a ± 0.43	774.92 ^d ± 6.28	22.19 ^c ± 0.32
At 90 d postpartum	551.55 ^a ± 4.18	3.74 ^a ± 0.037	50.18 ^b ± 0.38	756.73 ^d ± 6.51	20.00 ^b ± 0.34

*Each value is a mean of 48 observations (n=48)

^{abcd}Values bearing different superscripts in columns differ significantly (P<0.05).

TABLE 4. Developed prediction equations of peak milk yield by using negative energy balance indicators as independent variables

Equation	R	R ²	'F' Value
By using Serum Metabolites			
Z = 18.134 + 0.375X ₅ - 0.020Y ₅	0.793	0.628	37.99
Z = 40.070 + 0.128X ₄ - 0.031Y ₄	0.742	0.550	27.53
Z = 39.022 + 0.115X ₃ - 0.031Y ₃	0.697	0.486	21.30
Z = 19.673 + 0.291X ₂ - 0.021Y ₂	0.509	0.259	07.87
Z = 23.741 + 0.170X ₁ - 0.031Y ₁	0.459	0.210	05.99
By using Physical Indicators			
Z = -0.820 - 0.003A ₅ + 6.646B ₅	0.742	0.550	27.54
Z = -3.452 - 0.001A ₄ + 6.883B ₄	0.735	0.541	26.50
Z = -9.227 + 0.003A ₃ + 7.494B ₃	0.702	0.492	21.83
Z = -9.444 + 0.21A ₁ + 5.223B ₁	0.594	0.352	12.23
Z = -12.151 + 0.31A ₂ + 3.460B ₂	0.484	0.234	06.88
By using Serum Metabolites and Physical Indicators			
Z = 12.037 - 0.005A ₅ + 2.385B ₅ + 0.296X ₅ - 0.014Y ₅	0.803	0.644	19.49
Z = 20.782 - 0.002A ₄ + 3.908 B ₄ + 0.053X ₄ - 0.019Y ₄	0.784	0.614	17.13
Z = 17.477 + 0.002A ₃ + 4.609B ₃ + 0.014X ₃ - 0.021Y ₃	0.765	0.586	15.19
Z = -11.471 + 0.013A ₁ + 5.266B ₁ + 0.198X ₁ - 0.15Y ₁	0.661	0.436	8.33
Z = 0.124 + 0.025A ₂ + 3.284B ₂ + 0.145X ₂ - 0.025Y ₂	0.638	0.408	7.40

Z - 6% FCMY at 60 d postpartum (peak milk yield stage); R – Pearson's correlation; R² – Multiple correlation coefficient; X₁, X₂, X₃, X₄, X₅, and Y₁, Y₂, Y₃, Y₄, Y₅ are SG and SNEFA concentrations at drying, calving, 30, 60, and 90 d post calving periods, respectively. A₁, A₂, A₃, A₄, A₅, B₁, B₂, B₃, B₄, B₅ are BW and BCS at drying, calving, 30, 60, and 90 d post calving periods, respectively.

The SNEFA concentration (μ moles/lit) showed an increased trend from the time of drying to 60 days postpartum; and thereafter, a decreased trend from 60 to 90 days postpartum (fig 3). Similarly, Khan *et al.* (2011) reported a peak plasma NEFA concentration at 60 days postpartum in high yielding Holstein cows. During transition period, reduced dry matter intake (DMI) occurs, thereby increasing the mobilization of adipose reserves which leads to the -oxidation of body fat reserves and increased SNEFA levels (Grummer, 1995). The DMI will regain around peak milk yield stage of lactation (60 d in

the present study), thus decreasing the SNEFA levels. The SG concentration (mg/dl) showed a decreased trend from the time of drying to 60 days postpartum in all the groups; and thereafter, an increased trend from 60 to 90 days postpartum (fig 4). In the present study, peak glucose concentration was observed at drying followed by calving and 90 d postpartum. The heavy glucose demand by the mammary gland for lactose synthesis might have decreased the serum glucose concentrations, until peak lactation period, followed by trend of decreased milk production and so, increased serum glucose levels. Weber

et al., (2015) reported that the plasma glucose concentration decreases after calving irrespective of the

dry period allotted, as glucose is primarily used in mammary gland.

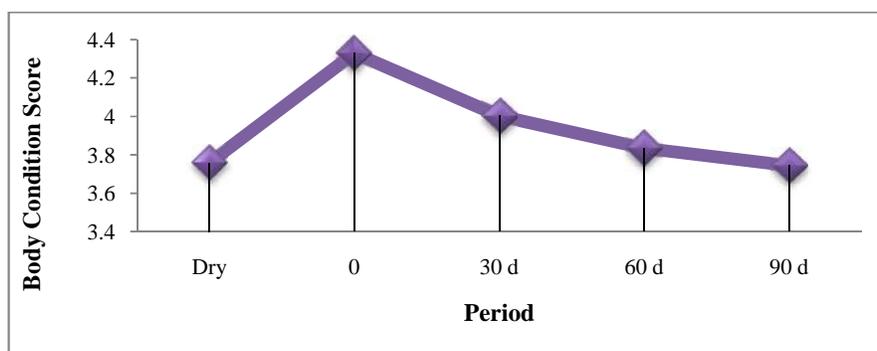


FIGURE 2. Pattern of Body Condition Score (BCS) during the experimental period

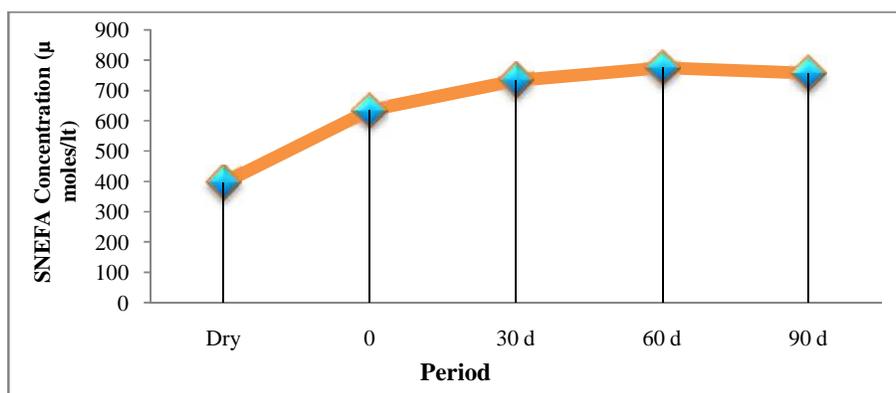


FIGURE 3. Pattern of Serum Non esterified Fatty acids (NEFA) concentration during the experimental period

The linear and multiple prediction equations developed for assessing the peak milk yield are presented in Table 4, the equations are ranked by R and R^2 values. The equations could be selected with respect to the available variables of the negative energy balance indicators. In general, the standardized deviation residuals were normally distributed. The wide variation in the independent variables included in the predictions ensure that the equations also are relevant and applicable to dairy buffaloes at various geographic and climatic locations, and managed on various feeding regimens. A significant ($P < 0.01$) relationship was found between peak milk yield (6% FCMY at 60 d postpartum), and the negative energy balance indicators during the entire experimental period. The current study also showed a closely inverse relationship of the amount of milk produced with BW, BCS, and SG concentration. Further, a strong correlation between milk production and SNEFA concentrations was noticed.

The Pearson correlation between the predicted peak milk yield and actual peak yield values i.e., ' R ' value was highest in the equation obtained by plotting the NEBI at 90 d postpartum indicating that 90 d serum metabolites are better predictors of milk yield. Among the predictors, physical indicators gave better equations with higher ' R ' value at precalving periods, and serum metabolites gave

more precise equations in postcalving periods. The preciseness of the equations was increased by using all the four parameters as predictors. However, dry matter intake is not calculated in the present experiment, and its incorporation as an independent variable might have added more accuracy to the equations developed (Khanh vu *et al.*, 2009).

The obtained tolerance values and variance inflation factors of the indicators at 30, 60, and 90 d postpartum were not optimum (data not shown). As per the SPSS manual, to avoid the higher multicollinearity and decrease the inflation of Standard errors of regression coefficients, the obtained tolerance percentage should be away from the Zero; and the variance inflation factor (VIF) should be less than 2. The lower tolerance value and higher VIF value in the present study indicates a higher percentage of variance in the individual variables that cannot be explained by other independent variables. The four predictors may be more influenced by the altered dry matter intake, as the buffaloes in this experiment were fed according to the 'scale feeding' method, i.e., the amount of feed was related to the buffaloes' milk production. Further, the increased role of DMI as a predictor might be due to the improved feed intake at 60 or 90 d postpartum, compared to the same at either calving or precalving periods (Reddy *et al.*, 2016).

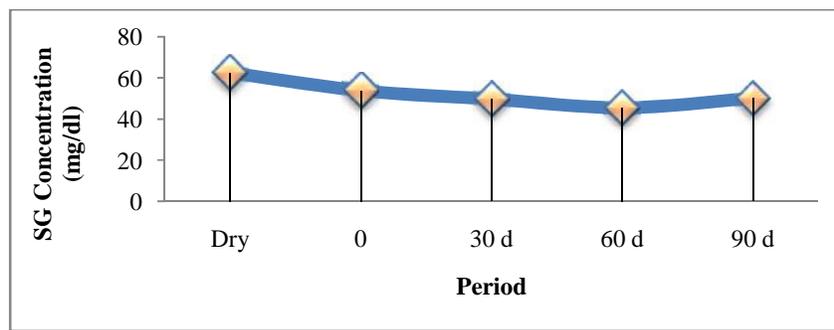


FIGURE 4. Pattern of Serum Glucose (SG) concentration during the experimental period

CONCLUSION

The current study showed that, with relatively few input variables those can quite easily be obtained and/or measured, it is possible to assess the milk production in high yielding Murrah buffaloes. In general, the equations became more precise when physical indicators are added as independent variables. The provided equations could be, not only used to establish tools for predicting milk production, but also for prevention of various metabolic disorders.

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