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Prediction of postpartum performances of transition Zebu (*Bos indicus*) cows using receiver operating characteristics analysis

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ABSTRACT

Receiver Operating Characteristics (ROC) analysis is a popular method to discriminate between the two conditions of tested animals. In this study, we estimated accuracy and threshold values of metabolic (Dry matter Intake; DMI and Body Condition Score: BCS, NEFA and BHBA) and immune indicators (Haptoglobin: Hp, Serum Amyloid A: SAA, IL-6, TNF-α, IL-1β, and IL-8) during transition period (–21, –14, –7, 0, +3, +7, +14 and +21 days) to predict the high yielding (HY) and pregnant Deoni cows. ROC analysis revealed that SAA (–21 d), IL-6 (–21 and –7 d), BCS (–7 d) and BHBA (–7 d) during pre-partum period, differentiated HY from low or medium yielder (LY/MY) cows with moderate to excellent accuracy (AUC >0.8). During postpartum period, IL-6 (+7 d), TNF-α (+21 d), DMI (+21 d), NEFA (+14 d and +21 d) and BHBA (+21 d) levels had moderate to excellent accuracy to differentiate HY from LY or MY cows. IL-6 (–14 d and –7 d), TNF-α (–14 d) and DMI (–21 d; above 2 kg/100 kg BW) during pre-partum period while, SAA (+3 d and +7 d), IL-6 (+3 and +21 d) and TNF-α (+7 and +21 d) during postpartum period were significantly predicted the pregnant cows with moderate to excellent accuracy. Altogether, it is concluded that SAA, IL-6 and TNF-α levels had higher accuracy in discrimination of HY and pregnant cows from LY or MY and non-pregnant cows, respectively. Therefore, their corresponding threshold values could be used for predicting HY and pregnant Zebu (Deoni) cows.

Keywords: Acute phase proteins, Deoni cows, Energy indicators, Inflammatory cytokines, Milk yield, Reproductive performance, Transition period

Transition period, the interval between 3 weeks pre-partum to 3 weeks post-partum period (Grummer 1995, Drackley 1999) is characterized by physiological, nutritional, behavioural, metabolic, and immunological changes (Ingvartsen and Andersen 2000, Sordillo *et al.* 2009, Sundrum 2015). It is believed that all these changes arise as physiological adaptation mechanism towards non-lactating, pregnant state to lactating, non-pregnant condition (Trevisi *et al.* 2016). Among the various challenges, metabolic and immunological changes play a key role in deciding post-partum performances, which starts several weeks before parturition and influences the health and production for weeks to months later (Leblanc 2010, Kehrli 2015, Wankhade *et al.* 2017). Therefore, these metabolic and immune indicators were explored as predictive markers to identify the risky animals and use as routine cow monitoring tools during early lactation (Brodzki *et al.* 2015, Shin *et al.* 2018, Wisnieski *et al.* 2019, Macmillan *et al.* 2020). There is a paucity of information on association of metabolic inflammation with postpartum performances in Deoni (*Bos indicus*) cows, an important dual-purpose indigenous cattle breed of southern and western India due to more disease resistant and heat tolerance capacity. Receiver Operating Characteristics (ROC) analysis is normally used to discriminate the two conditions of tested animals through selection of threshold values, particularly when a test (e.g. ELISA) that yields continuous results. Recently, we reported that the plasma levels of certain immune and metabolic indicators are related to postpartum productive and reproductive performances of transition Zebu (Deoni) cows (Wankhade *et al.* 2018, 2019). In continuation, the current study estimated the threshold values and likelihood ratio were established for prediction of milk yield and conception in these animals using ROC analysis.

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MATERIALS AND METHODS

Experiment animals and management: The experiment was conducted at LRC, SRS, ICAR-NDRI, Bengaluru, Karnataka (India), which is located in southern Plateau and Hill agro-climatic zone on 12°97′ N latitude and 77°56′ E longitude. The experimental procedures were duly approved by Institute Animal Ethical Committee. All animals were apparently healthy and free from any diseases while enrollment. The experimental cows were maintained under individual tie barn housing system and provided space as per Bureau of Indian Standards (BIS). The shed used for this experiment were open from all side, concrete paved with tiled roof. All the cows were fed according to NRC (2001) recommendation using institute grown seasonal green fodders like maize, jowar, hybrid napier, paragrass, guinea grass and cowpea along with dry fodder (ragi straw) and commercially available concentrates (Nandini gold-cow feed containing 16–18% crude protein, 70–72% TDN, 2.5–3.5% fat, 5.5–6% crude fibre, 1–1.5% acid insoluble ash and 10–11% moisture, M/s Karnataka Milk Federation, Bengaluru). The cows were fed 15–20 kg green fodder, 2–3 kg ragi straw and 1.5–2.5 kg concentrates during pre-partum transition period. After calving the animals were offered 18–22 kg green fodder, 2–3 kg ragi straw and 2.5–3 kg concentrate divided in equal proportion fed at the time of milking during morning and evening hours based on milk production of individual cow. The animals were provided with clean drinking water four times in a day.

Grouping of animals: A total 29 transition Deoni cows were enrolled a month before the expected date of calving and followed up to 45 days after calving. Of 29 pregnant cows, few cows were eliminated due to post-partum complications and missing sampling. Thus, 18 multiparous cows were considered for grouping based on milk yield as high (n=6), medium (n=6) and low (n=6) yielding cows. The overall average lactation milk yield of Deoni cows was reported as 779 kg with a lactation length of 187 days (Das *et al*. 2011). In the present study, we classified the group into high (>600 kg), medium (300–600 kg) and low yielding (<300 kg) cows and details of parity, body condition score (BCS), and milk yield of study animals are presented in Table 1. The lactation length of high, medium and low yielding cows was 16–36, 15–33 and 11–21 weeks, respectively.

After a voluntary waiting period of 45 to 60 days, all the animals were routinely observed by trained personnel for estrus, two times a day. Animals identified to be in estrus were confirmed for their proper stage to receive semen by Veterinarian. Animals detected in estrus in the early morning were artificially inseminated (AI) in morning of the same day with frozen semen and those cows detected in estrus in the late evening and night were inseminated in the morning of the next day. Two inseminations were done for each cow at 12 hrs interval. Pregnancy diagnosis was done 45 to 60 after AI through rectal palpation and transition cows were grouped as pregnant (n=7) and non-pregnant (n=11) even after breeding period of 180 days in milk.

Blood sampling and biochemical analysis: Blood samples collected from 18 cows during transition period (–21±2, –14±1, –7±1, 0, 3±1, 7±1, 14±1 and 21±2 days relative to calving) were used for estimation of plasma concentrations of inflammatory [Haptoglobin (Hp), Serum Amyloid A (SAA), Interleukin 6 (IL-6), Interleukin 1β (IL-1β), Tumor Necrosis Factor α (TNF-α), Interleukin 8 (IL-8)] and metabolic [NEFA, BHBA] indicators using bovine specific ELISA kits. The Dry Matter Intake (DMI) and Body Condition Score (BCS) were also estimated during same time period and reported (Wankhade *et al.* 2018; 2019).

Statistical analysis: The data was analyzed by using mixed-model repeated measure analysis with statistical software package SPSS version 22 (SPSS for windows, V22.0; M/s SPPS Inc., Chicago, IL, USA). Further, the interaction between two groups at each time point of intervals (i.e. from –21 to +21 days) and interaction within a group was analyzed by using univariate method. The difference of means was considered as significant if probability was $\leq$0.5 and the results are presented as Mean±SEM. The detailed results of immune and metabolic indicators during transition period (i.e. days at –21, –14, –7, 0, +3, +7, +14, +21) in relation to milk yield (high, medium and low) and fertility (pregnant and non-pregnant) performance of this study animals are reported in Wankhade *et al.* (2018) and Wankhade *et al.* (2019). The summary statistics of metabolic and immune indicators during transition period in relation to milk yield and fertility are presented in Table 2 and Table 3.

Plasma metabolic and immune indicators that showed a significant (P<0.05) difference between groups at various time of transition period (Wankhade *et al.* 2018, 2019) were

Table 1. Overview of study animals in different experimental groups

Parameter	HY	MY	LY	Pregnant	Non-pregnant
Parity	4.33 (2–8)	3.4 (3–4)	3.4 (3–4)	4 (2–8)	3.55 (3–5)
Average daily milk yield	5.98 (4.84–6.67)	3.25 (2.10–3.88)	1.77 (1.0–2.16)	4.02 (3.22–4.59)	4.18 (3.40–4.71)
Total lactational yield	955.93±92.24 (616.22–1168.91)	461.60±30.37 (406.01–578.98)	165.15±31.37 (107.77–215.83)	647.74±164.77 (107.77–1168.91)	572.12±113.50 (171.86–1098.78)
BCS at the start of experiment	3.42±0.11 (3–3.75)	3.35±0.10 (3–3.5)	3.30±0.15 (3–3.75)	3.36±0.11 (3–3.75)	3.36±0.08 (3–3.75)

HY, High yielder; MY, Medium yielder; LY, Low yielder.

Table 2. Summary statistics of metabolic and immune indicators during transition period in relation to milk yield

Parameter	HY		MY		LY	
	Mean±SE	Minimum-Maximum	Mean±SE	Minimum-Maximum	Mean±SE	Minimum-Maximum
SAA (ng/mL)	1.56±0.06	1.35–1.85	1.18±0.04	1.00–1.34	1.29±0.06	1.04–1.51
Hp (µg/mL)	4.43±1.50	2.43–14.85	7.91±4.85	2.35–41.79	5.10±1.93	2.65–18.54
IL-6 (pg/mL)	113.16±12.70	68.64–166.20	119.25±10.99	88.91–163.20	78.06±8.54	53.69–120.41
TNF-α (pg/mL)	66.78±1.48	60.46–74.27	50.43±1.03	46.96–56.09	51.66±2.20	44.55–60.86
IL-1β (pg/mL)	30.15±1.41	25.78–36.57	28.73±1.17	26.06–35.34	30.32±1.59	25.32–35.32
IL-8 (pg/mL)	94.32±5.19	69.87–112.18	102.12±6.92	67.06–132.10	98.41±8.70	61.65–130.30
NEFA (µmol/L)	1.01±0.08	0.77–1.29	0.91±0.05	0.67–1.05	1.53±0.10	1.16–1.97
BHBA (µmol/L)	452.39±27.33	370.83–596.11	304.75±10.73	257.38–343.32	117.64±4.24	94.08–130.17
DMI (per 100 kg BW)	2.02±0.09	1.70–2.42	1.80±0.09	1.51–2.23	1.95±0.10	1.59–2.34
BCS (1–5 scale)	3.21±0.10	2.88–3.67	3.16±0.09	2.95–3.55	3.05±0.07	2.85–3.30
Milk yield (kg)	5.98±0.16	4.84–6.67	3.25±0.16	2.10–3.88	1.77±0.11	1.00–2.16
Fat (%)	3.91±0.13	3.43–4.22	3.59±0.14	3.24–4.14	3.68±0.22	2.83–4.33

HY, High yielder; MY, Medium yielder; LY, Low yielder.

Table 3. Summary statistics of metabolic and immune indicators during transition period in relation pregnancy status

Parameter	Pregnant		Non-pregnant	
	Mean±SE	Minimum-Maximum	Mean±SE	Minimum-Maximum
SAA (ng/mL)	1.49±0.04	1.32–1.69	1.25±0.06	1.05–1.57
Hp (µg/mL)	8.70±6.24	2.09–52.36	3.41±0.27	2.69–4.93
IL-6 (pg/mL)	121.61±14.64	83.67–186.24	86.87±7.55	60.78–118.85
TNF-α (pg/mL)	63.07±1.65	57.32–72.32	49.51±1.62	45.06–60.01
IL-1β (pg/mL)	29.96±1.95	24.41–40.15	29.61±0.81	26.23–32.41
IL-8 (pg/mL)	94.58±10.89	42.50–140.44	114.95±6.77	85.06–153.47
NEFA (µmol/L)	1.32±0.07	1.10–1.62	0.83±0.06	0.61–1.13
BHBA (µmol/L)	917.85±20.53	828.98–1008.00	199.20±19.58	92.94–299.86
DMI (per 100 kg BW)	2.02±0.02	1.93–2.13	1.79±0.13	1.37–2.27
BCS (1–5 scale)	3.15±0.08	2.93–3.50	3.10±0.08	2.93–3.45
Milk yield (kg)	4.02±0.15	3.22–4.60	4.18±0.11	3.40–4.71
Fat (%)	3.58±0.17	3.16–4.23	3.91±0.12	3.64–4.43

analyzed by receiver operating characteristics (ROC) method using Sigmaplot 11 software package (Systat software, Inc, California, USA). The accuracy of all the parameters was interpreted based on Area under the ROC Curve (AUC) as per Gardner and Greiner (2006) and other researchers (Peterson *et al.* 2008, Patbandha *et al.* 2012, Patbandha *et al.* 2018).

RESULTS AND DISCUSSION

The immune indicators such as acute phase proteins and their regulatory cytokines along with metabolic indicators like NEFA, BHBA, DMI and BCS plays very significant role in homeostasis during the transition period (Manimaran *et al.* 2016). Earlier data from same animals revealed that more active functioning of immune system, stable DMI, and limited fat mobilization during transition period were associated with more milk yield and early conception in zebu (Deoni) cows during post-partum period (Wankhade *et al.* 2018; 2019). In this study, threshold values and likelihood ratio were established for prediction of high milk yield (Table 5) and pregnancy (Table 6) in these animals using ROC analysis. The confusion matrix for binary outcomes is presented in Table 4.

Table 4. Confusion matrix for binary outcomes

Outcome based on diagnostic indicator test	Outcome based on gold standard test	
	Positive	Negative
Positive	TP	FP
Negative	FN	TN

TP, True Positive [number of high yielder (HY) and pregnant cows having immune and metabolic indicators (except NEFA) above the threshold value]; FP, False Positive [number of low/ medium yielder (LY/MY) or non-pregnant cows having these indicators (except NEFA) above the threshold value]; TN, True Negative [number of HY and pregnant cows having these indicators below the threshold value]; FN, False Negative [number of LY/MY or non-pregnant cows having these indicators below the threshold value.

Acute phase proteins as a predictor of high milk yield and pregnancy in Deoni cows: In the present study, the AUC of SAA for prediction of HY cows was observed to be 0.87 (P=0.04) on day –21 with moderate accuracy (87%), while its accuracy for prediction of pregnancy is less (77–

79%). The plasma SAA threshold value of 1.70 ng/mL on day –21 and 1.18 ng/mL on day +7 was considered as optimum level for prediction of HY cows with 100% Sp. It indicated that all LY cows could be correctly identified (TN cases) by using plasma SAA level. Similar to our earlier findings (Wankhade *et al.* 2018, 2019) of non-significant role of Hp, ROC analysis also revealed lesser accuracy of Hp than SAA for differentiating Deoni cows based on lactation and reproductive performance. Zhang *et al.* (2018) reported that combined circulating concentrations of SAA, TNF-α, and BHBA at 4 weeks pre-partum had the best predictive ability for postpartum performance of dairy cows. Therefore, combined accuracy of SAA, IL-6, BCS and BHBA levels during prepartum for better prediction of high yielding cows should be studied further in these animals.

Pro-inflammatory cytokines as a predictor of high milk yield and pregnancy in Deoni cows: The IL-6 had significant (P<0.05) accuracy (86–90%) for prediction of HY Deoni cows during –21st day, –7th day pre-partum and +7th day postpartum period. The corresponding optimum threshold values were 104 and 98 pg/mL, respectively for differentiating HY cows from their LY counterparts. The AUC of IL-6 for prediction of pregnant was also significantly (P=0.01) high (93%) on day 3 of postpartum period with threshold value of 69 pg/mL (Table 6). The AUC of TNF-α for predicting HY and pregnant cows were 1.00 (P=0.02) and 0.88 (P=0.02) respectively, on +21st day postpartum period (Table 6). The plasma IL-6 concentrations are positively associated with inflammatory response in early-lactating cows (Trevisi *et al.* 2012) and it could be a predictive marker for diagnosis of animals that may fail to have a successful transition period (Trevisi *et al.* 2015) and identifying risky animals for developing diseases during postpartum period (Amadori *et al.* 2015). Dervishi *et al.* (2016) also reported similar range of IL-6 concentration in healthy cows as observed in our study and

Table 5. Receiver operating characteristics (ROC) analysis of plasma metabolic and immune indicators as predictor of high milk yielding Deoni cows

Day*	AUC	P value	Optimum threshold value	Se (95% CI)	Sp (95% CI)	LR+
SAA (ng/mL)						
Day –21 (Ref, MY)	0.87±0.12	0.04	>1.68	66.67 (22.28–95.67)	100 (47.82–100)	Infinite
Day –21 (Ref, LY)	0.87±0.12	0.04	>1.70	66.67 (22.28–95.67)	100 (47.82–100)	Infinite
Day +7 (Ref, MY)	0.63±0.19	0.47	>1.2	66.67 (22.28–95.67)	60.00 (14.66–94.73)	1.67
Day +7 (Ref, LY)	0.63±0.19	0.47	>1.18	66.67 (22.28–95.67)	60.00 (14.66–94.73)	1.67
IL-6 (pg/mL)						
Day –21 (Ref, LY)	0.90±0.11	0.03	> 104.2	83.33 (35.88–99.58)	100 (47.82–100)	Infinite
Day –7 (Ref, LY)	0.87±0.14	0.04	> 126.3	83.33 (35.88–99.58)	100 (47.82–100)	Infinite
Day +7 (Ref, LY)	0.87±0.12	0.04	>97.81	66.67 (22.28–95.67)	100 (47.82–100)	Infinite
TNF-α (pg/mL)						
Day +3 (Ref, LY)	0.81±0.17	0.14	>53.36	75.00 (19.41–99.37)	75.00 (19.41–99.37)	3.00
Day +14 (Ref, LY)	0.56±0.27	0.77	>69.20	50 (6.75–93.26)	100 (39.76–100)	Infinite
Day +21 (Ref, LY)	1.00	0.02	>52.55	100 (39.76–100)	100 (39.76–100)	Infinite
Day +3 (Ref, MY)	0.86±0.14	0.08	>53.09	75.00 (19.41–99.37)	75.00 (19.41–99.37)	3.00
Day +14 (Ref, MY)	0.69±0.25	0.39	>51.48	75.00 (19.41–99.37)	75.00 (19.41–99.37)	3.00
Day +21 (Ref, MY)	0.94±0.09	0.04	>56.19	100 (39.76–100)	75.00 (19.41–99.37)	4.00
DMI (per 100 kg BW)						
Day +3 (Ref, MY)	0.77±0.16	0.14	>1.89	66.67 (22.28–95.67)	80.00 (28.36–99.49)	3.33
Day +7 (Ref, MY)	0.83±0.13	0.07	>2.02	83.33 (35.88–99.58)	80.00 (28.36–99.49)	4.17
Day +21 (Ref, LY)	0.93±0.08	0.02	>2.01	100 (54.07–100)	80.00 (28.36–99.49)	5.00
BCS (based on 1–5 scale)						
Day –7 (Ref, LY)	0.80±0.14	0.1	>3.63	66.67 (22.28–95.67)	80.00 (28.36–99.49)	3.33
NEFA (µmol/L)						
Day +14 (Ref, LY)	0.90±0.12	0.03	<1.26	100 (54.07–100)	80.00 (28.36–99.49)	5.00
Day +21 (Ref, LY)	0.90±0.1	0.03	<1.02	83.33 (35.88–99.58)	80.00 (28.36–99.49)	4.17
BHBA (µmol/L)						
Day –7 (Ref, LY)	1.00	0.01	>196.70	100 (47.82–100)	100 (39.76–100)	Infinite
Day +21 (Ref, LY)	1.00	0.01	>123.70	100 (47.82–100)	100 (39.76–100)	Infinite

*Day in which plasma immune and metabolic indicators were showed a significant (P<0.05) difference between groups (Wankhade *et al.* 2018). Se, proportion of HY cows with immune and metabolic indicators above the threshold value [(TP)/(TP+FN)]; Sp, proportion of LY cows with these indicators below the threshold value [(TN)/(TN+FP)]. LR+ (positive likelihood ratio: number of times a cow with these indicators level above threshold value is more likely to produce more milk [LR+=Se/(1–Sp)] compared to below the threshold value. Parameters with reference group of MY at different time point of transition period are non-significant with LY.

several researchers suggested that IL-6, TNF-α, and SAA as very early indicators of inflammatory processes in transition dairy cows (Ishikawa *et al.* 2004, Kuhla 2020).

DMI, BCS and energy metabolites as a predictor of high milk yield and pregnancy in Deoni cows: During transition period, the primary challenge faced by dairy cows is a sudden increase of nutrient requirements for milk production at a time when DMI lags behind. Thus, the cows enter a physiologically unavoidable negative energy balance status (Grummer *et al.* 2004, Ingvartsen 2006). The DMI of >2 kg/100 kg BW had significant (P=0.02) accuracy (93%) for differentiating the HY cows from LY cows on +21st day postpartum period. Similarly, the AUC for pregnant cows to differentiate them from non-pregnant counterparts was 0.89 (P=0.01) on –21st day pre-partum period with optimum threshold value of 2.10 kg/100 kg BW (Table 6). Perez-Baez *et al.* (2019) also reported pre-partum DMI as a better predictor of metritis.

Body condition score (BCS) is an assessment of the fat reserves, which signifies the overall energy balance of the cow (Roche *et al.* 2009). In this study, BCS of 3.6 and above was considered optimum to differentiate HY and LY cows on –7th day pre-partum period (Table 5). Several researchers reported that pre-partum BCS is more important determinant of postpartum performances and pre-partum BCS of 3.75 associated with better body fat mobilization to support milk

Table 6. Receiver operating characteristics (ROC) analysis of plasma immune and metabolic indicators as predictor of pregnancy in Deoni cows

Day	AUC	P value	Optimum threshold value	Se (95% CI)	Sp (95% CI)	LR+
Haptoglobin (μg/mL)						
Day +7	0.69±0.15	0.170	>47.02	57.14 (18.41–90.10)	100 (75.92–100.00)	Infinite
SAA (ng/mL)						
Day +3	0.77±0.12	0.050	>1.15	85.71 (42.13–99.64)	69.23 (38.57–90.91)	2.79
Day +7	0.79±0.10	0.036	> 1.12	100 (59.04–100)	61.54 (31.58–86.14)	2.60
IL-6 (pg/mL)						
Day –14	0.89±0.08	0.01	>133.00	100 (59.04–100)	83.33 (51.59–97.91)	6.00
Day –7	0.87±0.08	0.01	>121.00	100 (59.04–100)	66.67 (34.89–90.08)	3.00
Day +3	0.93±0.06	0.01	>68.73	100 (59.04–100)	83.33 (51.59–97.91)	6.00
Day +21	0.75±0.12	0.06	>75.63	71.43 (29.04–96.33)	91.67 (61.52–99.79)	8.58
TNF-α (pg/mL)						
Day –14	0.85±0.11	0.03	>55.08	66.67 (22.28–95.67)	87.50 (47.35–99.68)	5.33
Day 0	0.90±0.09	0.01	>49.45	66.67 (22.28–95.67)	100 (63.06–100)	Infinite
Day +7	0.83±0.11	0.04	>51.48	83.33 (35.88–99.58)	75.00 (34.91–96.81)	3.33
Day +21	0.88±0.11	0.02	>47.09	100 (54.07–100)	75.00 (34.91–96.81)	4.00
IL-8 (pg/mL)						
Day +3	0.88±0.10	0.03	<81.25	100 (47.82–100)	75.00 (34.91–96.81)	4.00
DMI (per 100 kg BW)						
Day –21	0.89±0.08	0.01	>2.10	71.43(29.04–96.33)	92.86 (66.13–99.82)	10.00
NEFA (μmol/L)						
Day 0	0.87±0.08	0.01	>0.78	85.71 (42.13–99.64)	84.62 (54.55–98.08)	5.57
Day +3	0.87±0.08	0.01	>0.84	100 (59.04–100)	76.92 (46.19–94.96)	4.33
Day +7	0.85±0.09	0.01	>0.68	100 (59.04–100)	69.23 (38.57–90.91)	3.25
Day +14	0.82±0.10	0.02	>1.17	57.14 (18.41–90.10)	92.31 (63.97–99.81)	7.43
BHBA (μmol/L)						
Day –21	1.00	0.01	>306.00	100 (47.82–100)	100 (63.06–100)	Infinite
Day –14	1.00	0.01	>428.80	100 (47.82–100)	100 (63.06–100)	Infinite
Day –7	0.95±0.06	0.01	>261.60	100 (47.82–100)	87.50 (47.35–99.68)	8.00
Day 0	1.00	0.01	>447.60	100 (47.82–100)	100 (63.06–100)	Infinite
Day +3	1.00	0.01	>433.70	100 (47.82–100)	100 (63.06–100)	Infinite
Day +7	0.98±0.04	0.01	>218.60	100 (47.82–100)	87.50 (47.35–99.68)	8.00
Day +14	0.98±0.04	0.01	>222.90	100 (47.82–100)	87.50 (47.35–99.68)	8.00
Day +21	0.98±0.04	0.01	>213.60	100 (47.82–100)	87.50 (47.35–99.68)	8.00

*Day in which plasma immune and metabolic indicators were showed a significant (P<0.05) difference between groups (Wankhade *et al.* 2019). Se, proportion of pregnant cows with immune and metabolic indicators above the threshold value [(TP)/(TP+FN)]; Sp, proportion of non-pregnant cows with these indicators below the threshold value [(TN)/(TN+FP)]. LR+ (positive likelihood ratio: number of times a cow with these indicators level above threshold value is more likely to become pregnant [LR+=Se/(1–Sp)] compared to below the threshold value.

yield as observed in our study (Alharthi *et al.* 2018, Zhao *et al.* 2019). In contrast, Gobikrushanth *et al.* (2019) reported optimum pre-calving BCS as 3.25 for primiparous and 3.0 for multiparous *Bos taurus* cows to avoid postpartum complications. However, such report in *Bos indicus* is scarce. Colakoglu *et al.* (2019) also suggested for monitoring BCS during two weeks before calving to identify risky cows. The data revealed excellent accuracy (90%) of NEFA (P=0.03) with threshold value of <1.0 and 1.3 µmol/L to differentiate HY from LY cows during early postpartum period (day +14 and +21 respectively) (Table 5). Similar range of threshold value of >0.7 µmol/L of NEFA was observed to differentiate pregnant from the non-pregnant cows at postpartum period (Table 6). The optimum threshold of BHBA for differentiating HY cows from LY cows was 197 µmol/L (P=0.01) during pre-partum period (–7 day) and 124 µmol/L (P=0.01) on +21 day postpartum (Table 5). The optimum threshold value of BHBA with excellent accuracy to differentiate pregnant cows from non-pregnant cows was >429 µmol/L during pre-partum period, >434 µmol/L during the postpartum period (Table 6). We reported earlier that different levels of NEFA and BHBA during transition period in relation to postpartum disease risk in dairy animals (Wankhade *et al.* 2017). But, no ROC studies are available among healthy animals to differentiate metabolic and immunological states, in relation to postpartum performances, though this physiological condition exists. Therefore, several factors should be considered for the interpretation of observed threshold value. For instances, though BHBA concentration >434 µmol/L during postpartum period can differentiate pregnant from non-pregnant cows, >670 µmol/L of BHBA and >0.3 mmol/L of NEFA during 3–14 days postpartum period were associated with retained placenta and metritis (Wankhade *et al.* 2017). Other researchers also reported higher risk of cows for metabolic disorders, delayed or decreased pregnancy, lesser milk production and more culling when animals had more postpartum NEFA concentrations (Ospina *et al.* 2010a, 2010b, Chapinal *et al.* 2012, Roberts *et al.* 2012). Patbandha *et al.* (2018) also reported that plasma NEFA on day 1st and 7th postpartum with excellent accuracy and BHBA on day 14th and 21st with excellent accuracy for prediction of puerperal metritic cows during postpartum period. Garverick *et al.* (2013) reported that cows with lesser NEFA concentration had more chance of becoming pregnant at first insemination compared to cows with higher NEFA concentration. Since the energy metabolites levels are influenced by breed (Guretzky *et al.* 2006, Brown *et al.* 2012) and minimum of 12 animals per herd should be sampled for establishing threshold values, it will be difficult to ensure the randomness of sampling of this study to relate the metabolic and inflammatory variables during transition period with pregnancy or lactation outcome. Although no information is available in dual-purpose *Bos indicus* cows, the observed plasma level of cytokines and energy metabolites were different from available reports from *Bos taurus* cows due to method of estimations or type of ELISA kits. Kuhla (2020) reported that level of serum pro-inflammatory cytokine concentrations during periparturient period varied enormously among studies due to variation in quality of commercial ELISA kits between manufactures and many of them are poor quality kits with little or no validation. For instances, the range serum IL-6 concentration was observed between from 120 to 350 pg/ml (Trevisi *et al.* 2012) and 1.5 and 3.5 ng/ml (Ishikawa *et al.* 2004). Pre-partum serum TNF-α and IL-1β concentrations also varied substantially between cows and studies, ranging from 30 to 900 to 60 to 320 pg/ml, respectively (Trevisi *et al.* 2012, 2015). Spectrum (number of case and controls), biasness and confounding factors in study plan are major concerns of any diagnostic tests. For examples, a greater number of samples in each group is required for evaluation of accuracy of specificity. Non-blinded studies with more number of diagnostic techniques may also leads to under or over estimation of AUC (Hajian-Tilaki 2013). Therefore, broad spectrum of samples with suitable control for biasness and confounding factors through appropriate study design and analytical methods are important in Zebu cows for meaningful interpretation of ROC output. Altogether, based on the finding of the present study it is concluded that SAA, IL-6 and TNF-α levels had higher accuracy in identification of HY and pregnant cows and thus their corresponding threshold values in combinations could be used for predicting postpartum performance of Zebu (Deoni) cows.

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