



Metabolism and immune status during transition period influences the lactation performance in Zebu (*Bos indicus*) cows

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ABSTRACT

We evaluated the changes in the concentration of innate immune molecules (haptoglobin: Hp, serum amyloid A: SAA, IL-6, TNF- α , IL-1 β , and IL-8), energy indicators [NEFA, dry matter intake (DMI) and body condition scoring (BCS)] during the transition period in dual-purpose Zebu (Deoni breed) cows in relation to milk yield. Blood collection was done at weekly intervals (-21 ± 2 , -14 ± 1 , -7 ± 1 , d pre-partum, day 0 (date of calving) and 3 ± 1 , 7 ± 1 , 14 ± 1 , 21 ± 2 d postpartum period) for estimation of above plasma variables using commercially available bovine specific ELISA kits. We also recorded DMI and BCS during the corresponding period. Transition cows were classified based on their milk yield during the study period as high (6), medium (6) and low (6) yielding cows and data were analyzed by using Mixed-model repeated measure analysis. High yielding (HY) cows had significantly higher concentrations of SAA, TNF- α , and IL-6 during pre-partum and early postpartum period than low yielding (LY) cows. DMI was significantly higher in HY cows than MY (3rd and 7th d) or LY cows (21st d) while, BCS was significantly higher in HY than LY cows during pre-partum period (-7^{th} d). LY cows had significantly higher concentration of NEFA during the postpartum period (14th and 21st d). It is concluded that the active functioning of the immune system and more dry matter intake in transition Deoni cows enabled to synthesise more milk during the postpartum period.

Key words: Acute phase proteins, Deoni cows, Energy indicators, Inflammatory cytokines, Milk yield, Transition period

The transition phase from non-lactating, pregnant stage to non-pregnant, lactating stage (3 weeks before to 3 weeks after calving), is the most challenging and critical period to ensure health, productive and reproductive performance of dairy animals. The importance of transition period is mostly due to the occurrence of physiological, nutritional, behavioural, metabolic, and immunological changes during this period (Sundrum 2015). Among the various challenges, metabolic and immunological changes play a key role in lactation performance of animals and these changes start several weeks before calving and have effects on health and production for weeks to months later (Leblanc 2010, Kerhli 2015). On the other hand, cows must adapt these

metabolic and inflammatory changes during the transition period to ensure its potential milk production in the ensuing lactation. In fact, occurrences of clinical and subclinical metabolic disorders were mostly due to cow's inability to cope with metabolic demand or disturbances in the metabolic process. Occurrences of metabolic or infectious disease in 30–50% of dairy cows around calving period substantiate the importance of understanding about metabolic inflammation (LeBlanc 2013).

Immune suppression during transition period has been linked with negative energy balance (NEB) in high yielding cows which in turn cause increased transition cow disorders (Wathes *et al.* 2009, Sordillo and Raphael 2013). Aitken *et al.* (2011) reported that the delicate balance between a sufficient inflammatory response for optimal pathogen clearance and the prompt return to immune homeostasis is required during transition period. Farney *et al.* (2013) suggested that inflammation, in some cases, is an adaptive rather than pathological phenomenon and some degree of inflammation might even be required for successful metabolic adaptation. On the other hand, reduced milk productivity is a consequence of reduced feed intake and metabolic adaptations (Majkic *et al.* 2017). Banos *et al.*

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(2013) reported that cellular immune traits associated with improved health or reproductive performance were not associated with reduced milk productivity. The existing correlation of metabolic inflammation during transition period with the efficiency of milk production in high yielding cows is not known in moderate yielding Zebu cows. Sundrum (2015) reported that the understanding of metabolic process during transition period in apparently healthy cows is more challenging. Majority of the studies associated the metabolic and immune indicator levels using various threshold levels with disease outcome and studies in apparently healthy cows are scarce. On the other hand, altered physiological functions in healthy animals are believed as responsiveness against various challenges which are often unnoticed in dairy animals (Sundrum 2015). Combined investigation of immune system traits, energy balance, and production is recently recognized approach to predict future health and productivity of dairy animals (McLean 2017). Accordingly, we hypothesized that metabolic inflammation is altered during the transition period in relation to milk yield in apparently healthy Zebu (Deoni) cows. We studied the levels of innate immune molecules, energy metabolites and dry matter intake in relation to the different ability of milk yield in apparently healthy Deoni cows.

MATERIALS AND METHODS

Experimental animals and management: The experiment was conducted at Livestock Research Centre (LRC), Southern Regional Station (SRS), ICAR-National Dairy Research Institute, 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 (IAEC). Transition Deoni cows (29) were enrolled 1 month before the expected date of calving and followed up to 45 days after calving. All animals were apparently healthy and free from any diseases while enrollment. The experimental cows were maintained under tie barn housing system. The sheds used for this experiment were open from all side, concrete paved with tiled roof. The housing space for cows was as per BIS standard. 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. The animals were provided with clean drinking water four times in a day.

Estimation of milk yield and fat per cent: During the initial 5 days of postpartum colostrum period, the cows were allowed for suckling twice a day. Thereafter, lactating cows were allowed for partial suckling for 1 min by their calves for the letdown of milk, before actual milking, and milk yield (kg) was recorded with electronic weighing balance. The milk samples were collected in 50 ml sterilized centrifuge tubes from individual animals at 7±1, 14±1, 21±1, 28±1, 35±1 and 42±1 days postpartum period for

milk fat estimation using Gerber's method (IDF 1991).

Estimation of dry matter intake (DMI) and body condition score (BCS) of individual cows: The DMI of the individual cows was estimated on weekly basis before calving, on the day of calving, afterward on third day and then on weekly basis up to third week after calving. It was calculated by the amount of feed offered and amount of residue obtained on next day (i.e. 24 h) and then DMI was estimated per 100 kg body weight. An individual cow BCS (on a 5-point scale) was also assessed during the transition period (Kellogg 2017).

Blood sampling and biochemical assays: Blood samples were collected from cows using vacutainer tubes with heparin as anti-coagulant on -21±2, -14±1, -7±1 days before the expected date of calving, on the day of calving (day 0), and 3±1, 7±1, 14±1 and 21±2 days after calving. The blood samples were centrifuged within 30 min after collection, at 3,000 rpm for 10 min and plasma was stored at -20°C until further analysis. Blood plasma variables were analyzed using commercially available bovine specific ELISA kits and microplate reader (Multiskan GO, M/s Thermo Scientific, USA). The ELISA kits used for haptoglobin (Hp) was based on a solid phase ELISA and obtained from M/s Life Diagnostics, Inc., West Chester, PA (USA). The sandwich ELISA based kits for estimation of serum amyloid A (SAA), interleukin 6 (IL-6), interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), interleukin 8 (IL-8) were obtained from M/s Cloud-Clone Corporation, Houston, TX (USA) and competitive ELISA based kit for NEFA estimation was obtained from M/s MyBiosource, Southern California, San Diego (USA).

During the study period, all the experimental cows were observed daily for any abnormalities. The samples of all primiparous cows and animals suffered from any postpartum complication like abortion, dystocia, stillbirth, retained placenta, uterine infections (metritis, endometritis) and clinical mastitis were not considered for analysis. Thus, 18 multiparous cows were considered for grouping based on milk yield as high (6), medium (6) and low (6) yielding cows and biochemical assay. 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 into above average or high (>600 kg), average or medium (300–600 kg) and below average or low yielding (<300 kg) cows and found that 956 (range 616–1168), 462 (range 427–578) and 165 (range 108–216) kg as average milk yield in these groups, respectively during study period (8.5 months). The high yielding cows gave milk from 16 to 36 weeks, while medium yielders gave 15–33 weeks and low yielders from 11–21 weeks. Therefore, we considered the minimum lactation length of 11 weeks for statistical comparison of milk yield in different groups.

Statistical analysis: The data were analyzed by using mixed-model repeated measure analysis with statistical software package SPSS version 16 (SPSS for windows, v16.0; M/s SPPS Inc., Chicago, IL, USA). Further, the

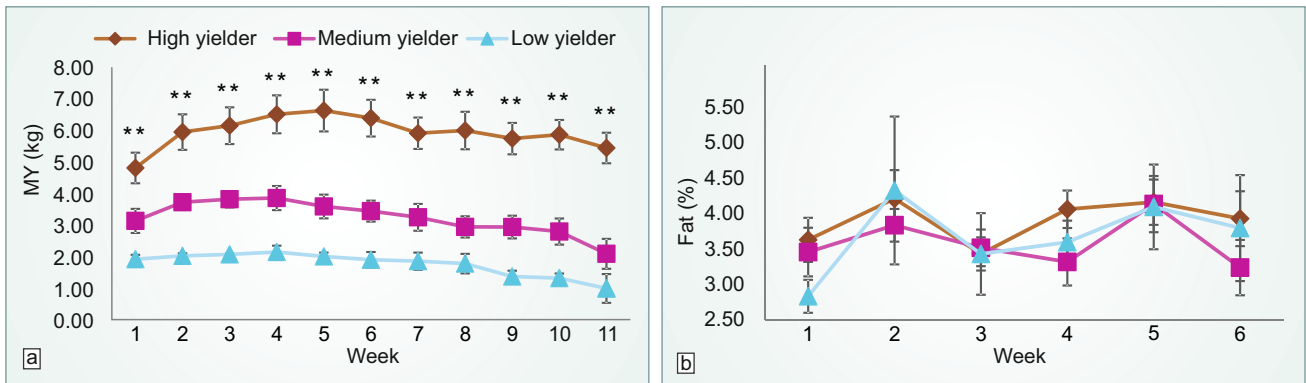


Fig. 1. Weekly average milk yield (1a) and milk fat % (1b) in high, medium and low yielding cows. First week observation was considered after colostrum period of 5 days. Lines bearing marks differ significantly (** $P < 0.01$) between the group at each intervals.

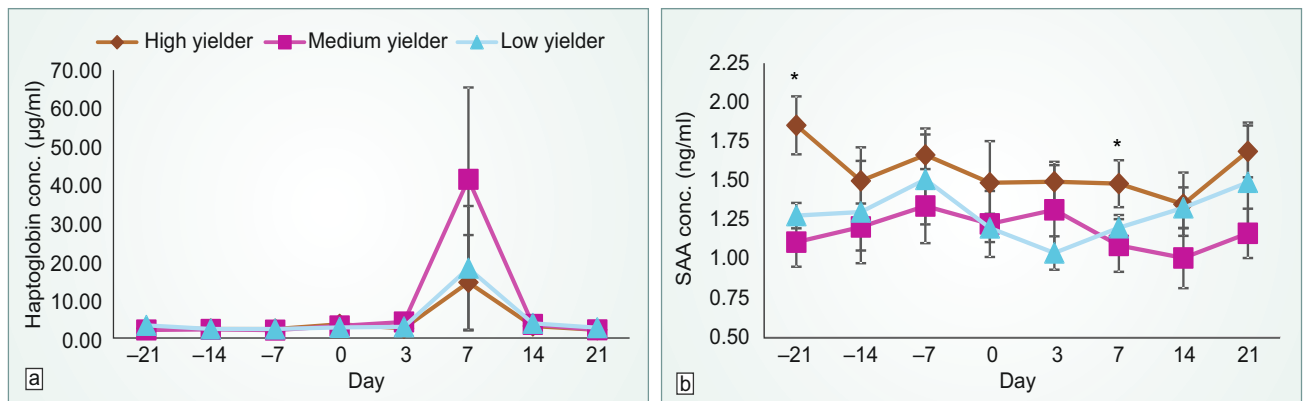


Fig. 2. Plasma concentrations of haptoglobin (2a) and serum amyloid A (SAA; 2b) in relation to milk yield. Lines bearing marks differ significantly (* $P < 0.05$) between the group at each intervals.

interaction between 2 groups at each time point of intervals (i.e. from -21 to +21 days) and interaction within a group was analyzed by using the univariate method. The data were expressed as mean \pm SE and the differences between groups were considered as significant when $P < 0.05$.

RESULTS AND DISCUSSION

We found significantly ($P < 0.01$) higher weekly average milk yield in high yielding (HY) than low yielding (LY) cows (Fig. 1a). We did not find any significant changes in milk fat percentages between different yielding groups during early postpartum period (Fig. 1b).

We did not find any significant difference in plasma Hp concentrations between HY, MY and LY group (Fig. 2a) during the transition period. Plasma SAA concentration was significantly higher in HY cows than MY ($P < 0.01$) and LY ($P < 0.05$) cows during pre-partum (-21st d) and the postpartum period (7th d; $P < 0.05$) (Fig. 2b). Plasma IL-6 concentration was significantly higher in HY and MY than LY cows during pre-partum (-21st; $P < 0.01$ and -7th d; $P < 0.05$) and early postpartum period (3rd and 7th d) (Fig. 3a). Similarly, the TNF- α concentration was significantly ($P < 0.05$) higher in HY than LY cows during early postpartum period (3rd, 14th and 21st d) (Fig. 3b). We did not find any significant difference in IL-1 β (Fig. 3c) and IL-8 (Fig. 3d) levels between different milk yielding groups at any time of the transition period.

During postpartum period, HY cows consumed significantly ($P < 0.05$) higher quantity of feed on dry matter basis (Fig. 4a) than MY (on 3rd and 7th d) and LY cows (on 21st d), while BCS (Fig. 4b) was significantly ($P < 0.05$) higher in HY than LY cows during pre-partum period (-7th d). Over the transition phase, BCS was significantly ($P < 0.05$) reduced from pre-partum period to on the day of calving (average of 3.36 to 2.93), which was maintained similarly during subsequent postpartum period (average of 2.95–3.03) in all these groups. But, the magnitude of loss in HY cows was greater than remaining groups. The NEFA concentration (Fig. 5) was significantly higher in LY than HY group during early postpartum period (14th and 21st d).

We studied inflammatory response through positive bovine major acute phase proteins (APPs) and cytokines level during the transition period in relation to milk yield in apparently healthy Zebu cows and found that HY cows had a higher concentration of SAA and cytokines (IL-6 and TNF- α) than LY cows. Higher levels of APP and cytokine clearly indicating a more immune response in these cows. The possible source of cytokines under this normal physiological circumstance would be adipose tissues, placenta or central nervous system (Loor *et al.* 2005). They reported that adipose tissue and central nervous system are sensitive to inflammatory conditions which occur during normal parturition in dairy cows. Cytokines such as IL-6, TNF- α , and IL-1 β are key inflammatory mediators to

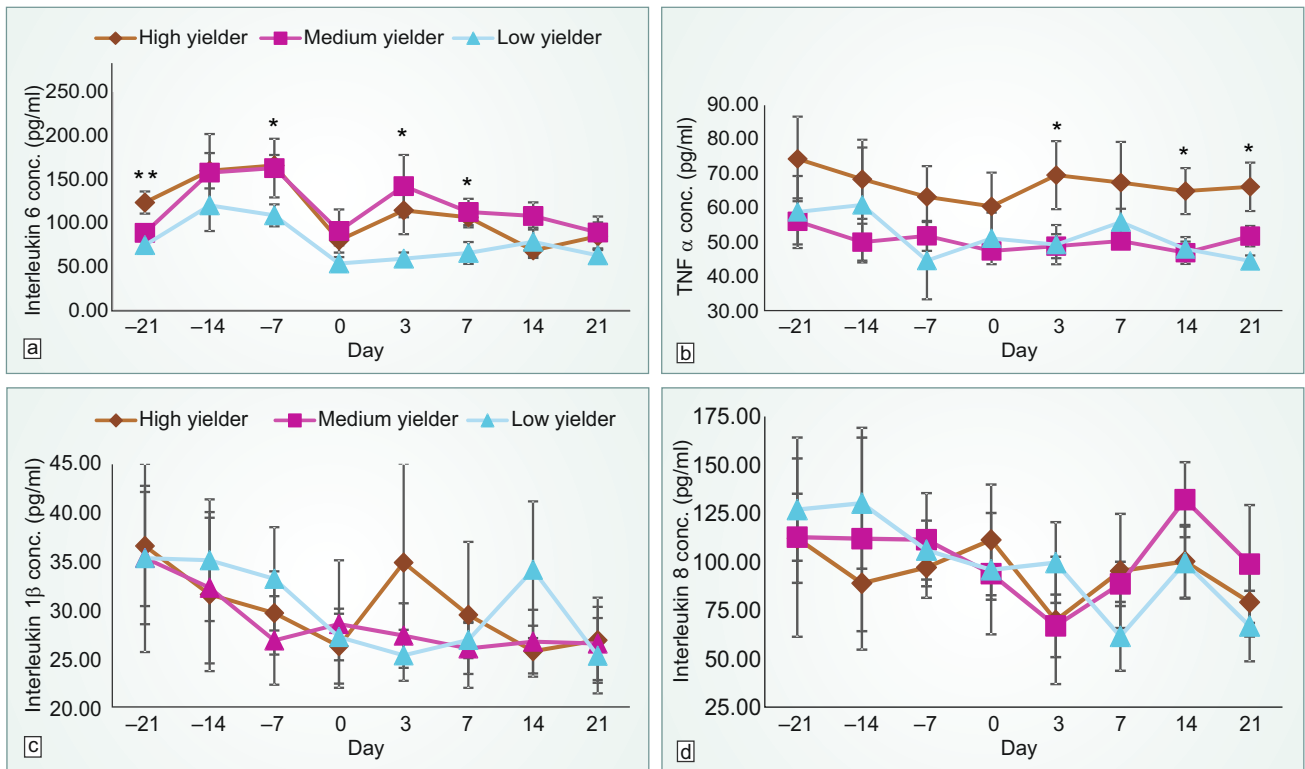


Fig. 3. Plasma concentrations of IL-6 (3a), TNF-α (3b), IL-1β (3c) and IL-8 (3d) in relation to milk yield. Lines bearing marks differ significantly (*P<0.05; **P<0.01) between the group at each intervals.

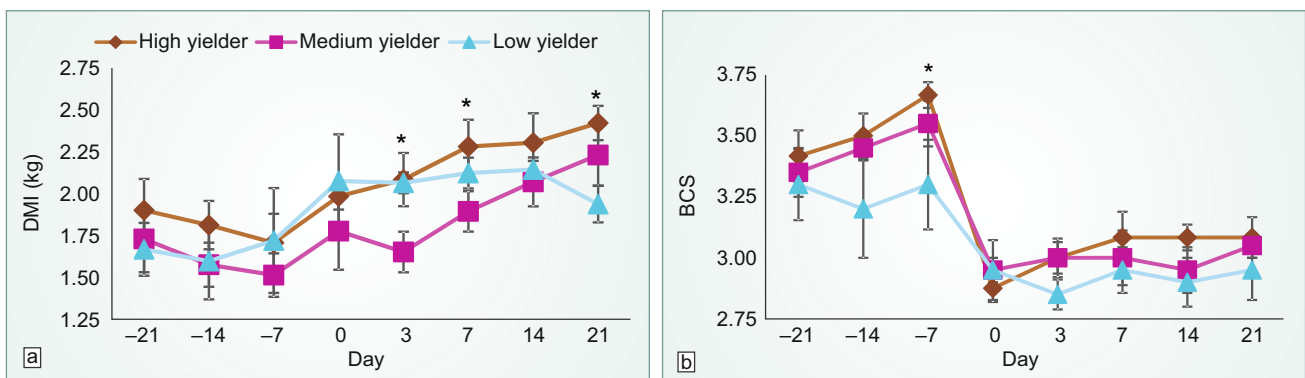


Fig. 4. Dry matter intake (DMI/100 kg BW) (4a) and body condition score (BCS) (4b) during transition period in relation to milk yield. Lines bearing marks differ significantly (*P<0.05) at each intervals.

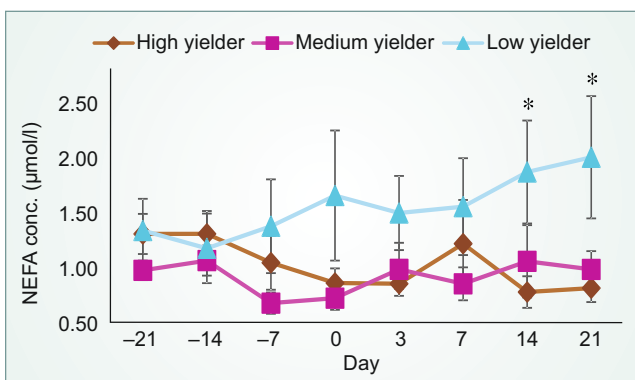


Fig. 5. Plasma concentration of NEFA in relation to milk yield. Lines bearing marks differ significantly (*P<0.05) between the group at each intervals.

activate local and systemic host defense system including APPs synthesis in the liver of dairy animals. SAA is IL-6 and TNF-α dependent APP (Uhlar and Whitehead 1999) and reacts faster than Hp against acute phase response. The observed active immune response in HY cows might have helped for avoiding infections during transition period and thereby improved health and production during the subsequent postpartum period. Bionaz *et al.* (2007) reported that stimulated production of positive APPs indicates a normal functioning of liver, which is necessary to obtain high performance in dairy cows during early lactation period. Inflammatory cytokines are the central integrator of metabolism and immune function (Sordillo *et al.* 1995) and direct stimulation of adipose tissue mobilization during transition period is one of the most important metabolic

effects of cytokines in dairy cattle (Kushibiki *et al.* 2001). Therefore, the combined observation of immune stimulation and fat mobilization in HY animals are part of adaptation mechanism.

BCS provides an indication of the energy status of lactating dairy cattle. In this study, BCS was significantly ($P < 0.05$) higher in HY than LY cows during pre-partum period (-7th d) and over the transition phase, BCS was significantly ($P < 0.05$) reduced from pre-partum period to on the day of calving in all cows. But, the magnitude of loss was more in HY cows. The higher tendency of fat mobilization to cover milk production demands in high genetic merit dairy cattle had been reported by several researchers (Pryce *et al.* 2002, Jilek *et al.* 2008). We found that HY cows had significantly higher DMI than MY and LY cows during postpartum period. Conversely, LY cows had a significantly higher concentration of NEFA than HY cows during postpartum period. But, NEFA level is within normal range of cows in both the groups (Adewuyi *et al.* 2005). It indicated that lower DMI might have stimulated more fat mobilization or LY cows could not have effectively utilized the mobilized fat (through esterification and β -oxidation of fatty acids) (Ospina *et al.* 2010). Decreased DMI leads to a low-energy state, which causes adipose tissue triglycerides to be broken down by hormone-sensitive lipase and increases NEFA in the blood (Gruffat *et al.* 1996, Herdt 2000). Whereas lesser concentration of NEFA in HY cows might be due to effective utilization of mobilized NEFA for milk synthesis as revealed in these cows (the average lactation milk yield of HY and LY yielding cows was 956 and 165 kg, respectively). In fact, the NEFA are utilized for about 40% of milk fat synthesis and milk produced from body tissue reserves with an efficiency of 82 to 84% during early lactation (Moe *et al.* 1971, Adewuyi *et al.* 2005). Milk yield is the outcome of efficient feed utilization and metabolic adaptation of dairy animals during transition period. Mobilization of NEFA from adipose tissue and consequent re-esterification in the mammary gland is one of the most important homeostatic adaptation mechanisms in early-lactation cows to manage a high volume of milk synthesis (Mathews *et al.* 2016). Looor *et al.* (2005) also reported that metabolic adaptation is mainly regulated by cytokines, APPs and serum NEFA as observed in this study.

The average weekly milk yield (11 weeks) of HY, MY and LY cows was 5.98, 3.25 and 1.77 kg, respectively. We calculated average gross feed efficiency (the ratio of milk produced to feed consumed) in HY, MY and LY cows during early postpartum period as 0.68, 0.45 and 0.24, respectively. It indicated that the metabolic adaptation in HY or MY cows is different from LY cow. Variation in metabolic adaptation and negative effect poor adaptation on milk yield was reported by Duffield and Leblanc (2009). However, further studies are required with suitable sample size to understand the role of various factors in metabolic adaptation among healthy animals, before establishing the relationship of these metabolites with disease outcome in Zebu cows.

Identification of such factors may assist to establish the precise cutoff values of these metabolites against various health conditions and to limit the negative effects of a poor adaptive response on productive and reproductive performance in these cows. We found a positive correlation between cytokines (IL-6 and IL-8) with NEFA during the transition period in HY cows. We also found positive correlation between SAA and BCS during early postpartum period (data not presented). Several researchers reported a positive correlation between APPs, cytokines and energy indicators (Kovac *et al.* 2009, Gabler *et al.* 2010, Tothova *et al.* 2014, El-Deeb and El-Bahr 2017) as observed in our study. Taken together, it is concluded that an inflammatory stimulation, more dry matter intake and efficient metabolic adaptation during transition period enable more synthesis of milk in zebu (Deoni) cows.

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