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Relationship of blood metabolites with reproductive cyclicity in dairy cows

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ABSTRACT

A total of 40 dairy cows in early lactation (60 to 90 days) of four different breeds were selected, comprising 10 animals each from Holstein Friesian (HF), Jersey (J), Achai (ACH) and F_1 (HF x Sahiwal). All cows were multiparous with body weight 250-400 kg and milk production ranged from 3 to 12 kg/day. Blood samples were collected from each animal at fortnight intervals for 90 days. In the present study, 65 % of cows reestablished estrus while 35 % cows remained anestrous, 80 % of Holsteins showed estrus followed by 70 % of Jersey and F_1 cows while in Achai breed showed only 40 % and 60 % cows remained anestrous. Blood glucose and daily milk yield significantly affected post partum estrus (P<0.01) while serum total protein and triglycerides concentration in blood did not affect post partum estrus. The serum glucose level was lower (39.93 ± 3.14 mg/dl) two months before and showed an increasing trend (49.63 ± 2.47 mg/dl) towards commencement of estrus as well as during estrus (48.20 ± 2.42 mg/dl) and then a declining trend was observed. Mean concentration of serum glucose was significantly higher in Jersey (52.50 ± 2.09 mg/dl) followed by F_1 (39.68 ± 1.45 mg/dl), HF (38.85 ± 1.77 mg/dl) and Achai (33.30 ± 2.17 mg/dl) respectively. Breed type significantly affected blood glucose (P < 0.05) in jersey cows whereas both blood glucose and triglycerides were significantly affected (P < 0.001 and P < 0.05, respectively) in F_1 cows.

Key words: Blood metabolites, Glucose, Post partum estrus, Triglycerides, Total proteins.

INTRODUCTION

In dairy industry, fertility is crucial for the genetic improvement in a herd and the replacement of culled cows. The ever increasing demand for milk and meat has compelled both farmers and researchers for selection of genetically superior cows. Genetic improvement of dairy cows has markedly increased milk yield over the last three decades but it has been associated with decreased reproductive efficiency (Lucy 2001). High milk yield requires high dietary intake and altered patterns of metabolism and these outcomes seem to be associated with sub fertility (Gutierrez *et al.*, 2006). The modern high-producing dairy cow partitions a greater proportion of available nutrients towards milk production at the expenses of body reserves and reproduction (Collard *et al.*, 2000). Selection for increased milk yield has produced cows that are better able to mobilize body tissue reserves to support milk production (Pryce *et al.*, 2001). Consequently, even when nutrient intake is increased to high levels, the outcome is simply an increase in milk production without necessarily an improvement in reproductive performance (Horan *et al.*, 2004). Increased genetic potential for milk production has been associated with a decline in fertility of lactating dairy cows.

Impaired fertility has a multifactorial background. One of the most important factors influencing the reproductive cyclicity of cattle is the presence of comprehensive metabolic changes around parturition and in early lactation. The metabolic demands of higher production may be related to the decline in reproductive performance of cows. During early lactation, increasing dietary intake fails to keep pace with rising milk production. The resultant negative energy balance (NEB) and rate of mobilization of body reserves appear directly related to the postpartum interval, to first ovulation and lower conception rate (Butler *et al.*, 1981). Negative energy balance probably acts similarly to under-nutrition and may manifest in delayed ovarian activity by impinging on pulsatile secretion of LH. Lower availability of glucose and insulin may also decrease LH pulsatility or limit ovarian responsiveness to gonadotropins.

Physiological pathways by which the hypothalamic-pituitary-ovarian axis is informed about the energetic status of the animal are very complex and involve several metabolites and hormones. Among the metabolites which are required for proper function of the reproductive processes in dairy cows, glucose is the most important (Short and Adams, 1988). Glucose acts as primary metabolic fuel for the central nervous system and inadequate availability of utilizable glucose reduces hypothalamic release of GnRH (Keisler and Lucy, 1996; Wetteman *et al.*, 2003). The hypothalamus detects low blood glucose in a threshold-

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dependent manner in such a way that GnRH secretion will be impaired in case of inadequate glucose availability (Randel, 1990; Dhuyvetter and Caton, 1996). Stimulation beyond the threshold to promote GnRH secretion is possible by increasing gluconeogenesis via dietary manipulation (Randel, 1990).

Although it is difficult to ascertain if specific nutrients limit reproduction through common or discrete mechanisms, appropriate quantities of the nutrients are required for optimal reproduction. The lack of any proper ration formulation practices lead to excessive or deficient intake of some nutrients which may affect the reproductive performance of cows. The present study is therefore designed with the following objectives:

1. To study the reproductive cyclicity of four breeds (Jersey, Holstein Friesian, F_1 and Achai) of dairy cows.
2. To study the relationship of blood glucose, total protein and triglycerides levels with reproductive cyclicity.

MATERIALS AND METHODS

The present study was designed to investigate the relationship of blood metabolites with reproductive cyclicity of various breeds of dairy cows. The study was performed at the Government Cattle Breeding and Dairy Farm (CB&DF) Harichand, District Charsadda.

Selection of animals : A total of 40 dairy cows in early lactation (60 to 90 days after parturition) of four different breeds were selected from a herd of 96 lactating cows at CB&DF Harichand. These included 10 Holstein Friesian (HF), 10 Jersey (J), 10 Achai (ACH) and 10 F_1 (HF x Sahiwal) cows. Reproductive cyclicity and blood metabolites were monitored. All cows were multiparous with body weight 250-400 kg and milk production ranged from 3 to 12 kg/day.

All the cows were kept on the same ration during 90 days experimental period. The experimental animals were offered green fodder (berseem, barley and oats) ad libitum and farm mixed concentrate mixture containing 18% crude protein and 72% TDN at a scale of 1kg per 3kg milk produced as per prevailing practice at the farm. The concentrate mixture consisted of wheat bran (25%), cotton seed cakes (23%), maize gluetin (30%), rice polish (10%), molasses (10%), DCP (1%) and sodium chloride (1%).

Blood sample collection: Blood samples from each cow of all four breeds were collected through jugular vein puncture at fortnight intervals to estimate blood glucose, protein and triglycerides levels. Blood sample of about 8-10 ml was taken into a test tube without anti-coagulant and allowed to clot at room temperature and subsequently kept in refrigerator at 4^{o}C for half an hour. Serum was separated through centrifugation at 1500 rpm for 15 minutes and was stored at -20^{o}C until analyzed. During the experimental period, a total of 240 serum samples were collected for analysis.

Serum analysis: The blood serum was analyzed for glucose, total protein and triglycerides using commercial kits. Manufactures instructions were followed for sample preparation and analysis, names of manufacturer companies and country are given under respective headings below. Absorbance of sample was recorded through spectophotometry and the results were interpreted using guidelines given by the manufactures.

Determination of blood glucose : Blood glucose levels were determined through commercial Human kit made Centronic, Germany which works on the Enzymatic Colorimetric principle (Trinder 1969). Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. Hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-amino-antipyrine to form a red violet dye quinoneimine. The intensity of the color is proportional to the glucose concentration in the sample.

About 10 μl of serum sample or standard was pipetted into 1000 μl of working reagent (supplied with the kit) and after mixing, incubated for 10 minutes at 37^{o}C. Exposure to direct sunlight was avoided. The absorbance was read at 550 nm using spectrophotometer. The test was performed at 25-37^{o}C and the colour stability was upto 30 minutes. Concentration of glucose in the serum was determined as follow:

$$\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 100 = \text{Concentration (mg/dl)}$$

Determination of total blood protein: Serum Total proteins were determined using commercial kit of DiaSys international France which works on the principle of Biuret method as described by Slater (1986). Under alkaline conditions substances containing two or more peptide bonds form a purple complex with copper salts in the reagent.

Serum sample or standard of 20 μl was mixed with 1000 μl of reagent 1 (sodium hydroxide 0.2 M and sodium potassium tartrate) and incubated for 5 minutes at 37^{o}C. 250 μl of reagent 2 (sodium hydroxide 0.2 M, sodium potassium tartrate, potassium iodide and copper sulfate) was added followed by incubation for 5 minutes. The absorbance was recorded through spectrophotometer at 540 nm. Although the color was stable, all readings were taken within 10 minutes of sample preparation.

Determination of blood triglycerides : Serum triglycerides were determined using commercial kits of Centronic, Germany (Koditschek 1969).

Serum triglycerides were hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. In the presence of Adenosine-tri-phosphate and glycerol kinase, the glycerol was converted to glycerol-3-phosphate, which then was oxidized by glycerol phosphate oxidase to yield hydrogen peroxide. The oxidative condensation of ADPs (N-Ethyl-N-3-sulfopropyl-3-methoxyaniline) and 4-aminophenazone in the presence of peroxidase and hydrogen peroxide produced a rose colored dye 4-p-benzoquinone-monoimino-phenazone (Young, 2000).

10 µl serum sample was mixed with 1000 ìl of working reagent (supplied with the kit) and was incubated for 5 minute at 37^0C. The absorbance was read at 550 nm using spectrophotometer and the results were calculated as under.

$$\text{Conc. Triglycerides (mg/dl)} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{Cal.}STD}} \times 200$$

Clinical examination of cows: The selected cows were examined clinically three times on different days in a week while rectal palpation of the cows was performed when needed. For detection of cows in estrus, three daily visual observations for 30 minutes at 0700, 1500, and 1900 h were made. Estrus detection was based upon observation of "standing heat" of the cows. Estrus symptoms included vaginal mucus, bellowing, mounting and standing to be mounted. Estrus was also confirmed through rectal palpation of the reproductive organs and mucus discharge on squeezing.

Cows in standing estrus were inseminated artificially. Repeat breeders after 4 inseminations were sired naturally. The cows with the history of prolonged absence of estrus were examined twice at 11days interval to assess the ovarian status and those with smooth ovaries on both examinations were confirmed as cases of anestrous.

Data analysis: The collected data were maintained in Microsoft Excel files. Means of blood metabolites were compared for various groups through analysis of variance for breed and post partum estrus interval (PPEI). Computer software SPSS-10 (1999) was used for data analysis. Means were compared and ranked through Duncan's multiple range tests as dscribed by Steel and Torrie (1982).

RESULTS AND DISCUSSION

This research work was conducted to study the relationship of blood metabolites with reproductive cyclicity in dairy cows. The results are reported under various sub-sections dealing with effect of age and breeds, postpartum day and relationship of occurrence of estrus with blood metabolites levels in dairy cows.

Relationship of blood metabolites with occurrence of estrus

Serum glucose: Blood glucose significantly affected post partum estrus (P<0.01) as illustrated in Table 1. The serum glucose level was lower (39.93 ± 3.14 mg/dl) two months before and showed an increasing trend (49.63 ± 2.47 mg/dl) towards commencement of estrus i.e. one month before estrus. At the time of estrus serum glucose level was 48.20± 2.42 mg/dl. Afterwards a declining trend in the glucose level was observed (Table 1 and figure 1). These findings clearly indicate the significant effect of serum glucose in supporting the occurrence of estrus. The lowest serum glucose was found in anestrous cows 35.74 ± 1.57 mg/dl.

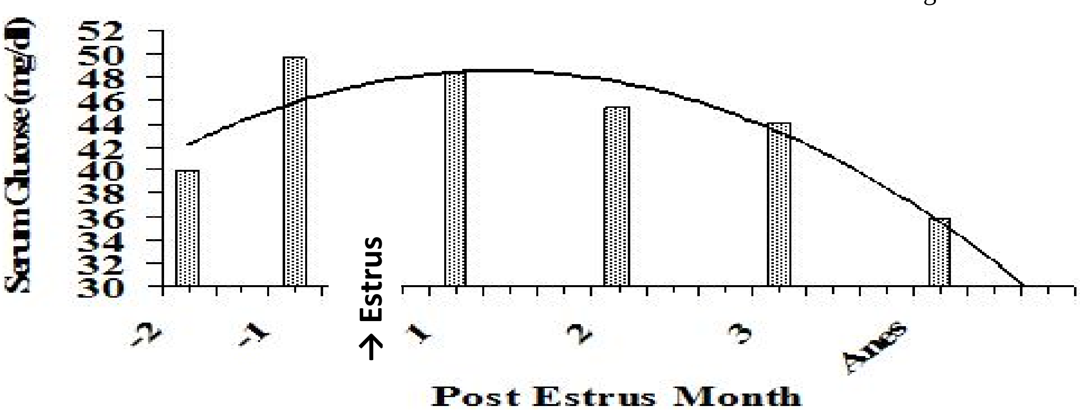

Fig 1: Changes in serum glucose in relation to occurrence of estrus in dairy cows.

Table 1: Changes in blood metabolites levels in relation to occurrence of estrus in dairy cows (Means ± SE).

Post estrus interval (in months)	Glucose (mg/dl)	Total Protein (g/dl)	Triglycerides (mg/dl)	Daily Milk Yield (kg/day)
-2	39.93^c ± 3.14	14.90 ± 0.69	18.68 ± 1.32	11.30^a ± 0.60
-1	49.63^a± 2.47	17.34 ± 0.78	18.00 ± 1.58	10.52^{ab} ± 0.80
1	48.20^a ± 2.42	16.05 ± 0.52	16.40 ± 0.85	9.91^{bc} ± 1.04
2	45.29^{ab} ±4.10	15.92 ± 0.65	17.43± 2.14	9.00^c ± 0.99
3	44.06^{ab} ± 3.95	16.07 ± 0.87	20.11 ± 2.17	5.83^d ± 1.12
Anestrous	35.74^c±1.57	16.14 ± 0.38	19.17 ± 0.84	6.95^d ± 0.43
Probability level	0.01	0.09	0.72	0.01

[a,b,c,d] Means within the same column having different superscript are significantly different.

Serum total protein: Non significant effect of serum total protein on post partum estrus was observed (Table 1). The serum total protein recorded two months before estrus was 14.91 ± 0.69 g/dl. One month before occurrence of estrus, serum total protein increased to 17.34 ± 0.78 g/dl. After occurrence of estrus, serum total protein decreased to 16.05 ± 0.52 g/dl one month after estrus and 15.92 ± 0.65g/dl two months after estrus. Serum total protein recorded in anestrus cows was 16.14 ± 0.38 g/dl. Although the effect of serum total protein on occurrence of estrus was statistically non significant, the concentration increased to 17.34 ± 0.78 g/dl one month before estrus and then showed a declining trend afterwards (figure 3).

Serum triglycerides: The effect of triglycerides on post partum estrus was found non significant. The serum triglycerides level was higher two months before estrus (18.68 ± 1.32 mg/dl) followed by decline near the occurrence of estrus (18.00 ± 1.58 mg/dl) and one month after estrus (16.40 ± 0.85 mg/dl) while further increasing trend was observed later on (Table 1 and figure 2). Serum triglycerides recorded in anestrous cows were 19.17 ± 0.84 mg/dl. The overall effect of serum triglycerides on the occurrence of estrus was non significant statistically.

Daily milk yield: The effect of post partum estrus on daily milk yield was statistically significant (Table 1). Average daily milk yield was higher (11.30 ± 0.60 kg/day) two months before estrus which gradually declined one month before estrus to 10.52 ± 0.80 kg/day. This decline in daily milk yield persisted to 9.91 ± 1.04, 9.00 ± 0.99, and 5.83 ± 1.12 kg/day one month, two months and three months after occurrence of estrus respectively (figure 2). The average milk yield recorded in anestrous cows was 6.95 ± 0.43.

The Breed effect: Results of blood metabolites (mean ± SE) in various breeds of cows are given in Table 2.

On overall basis, breed type has significantly affected the glucose concentration while protein and triglycerides did not respond to difference in breed. Mean concentration of serum glucose was significantly higher (P < 0.001) in Jersey (52.50 ± 2.09 mg/dl) followed by F_1, HF and Achai (39.68 ± 1.45 mg/dl, 38.85 ± 1.77 mg/dl and 33.30 ± 2.17 mg/dl respectively (figure 4).

Although the breed effect on concentration of serum total protein was non-significant, the data trend showed higher concentration in Jersey followed by Holstein Friesian, Achai and F_1.

The effect of breed on serum triglyceride levels was also non significant but the data trend revealed higher level found in Achai was 21.93 ± 1.42 mg/dl followed by HF (18.08 ± 0.95 mg/dl), F_1 (17.42 ± 0.87 mg/dl) and Jersey (16.52 ± 0.90 mg/dl).

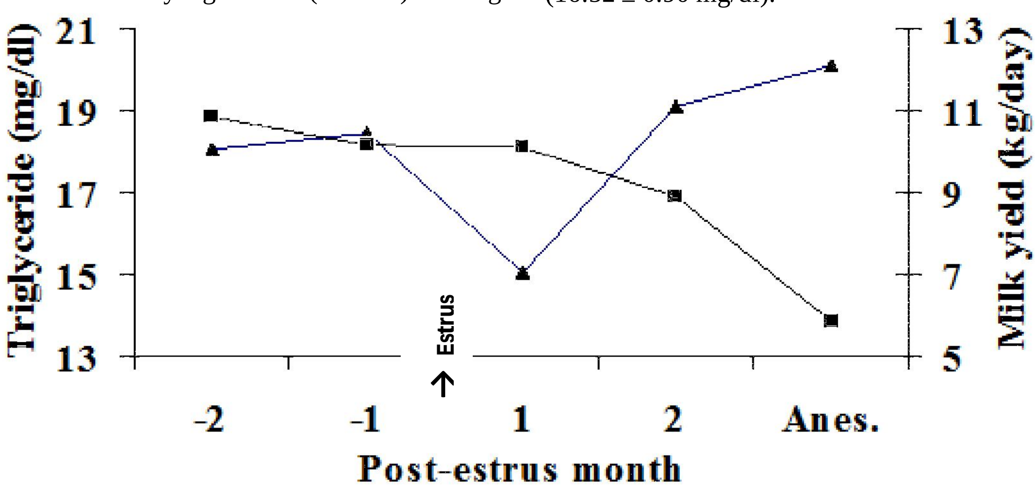

Fig 2: Changes in blood triglycerides (solid squares) concentration and milk yield (solid triangles) in relation to occurrence of estrus in dairy cows.

Table 2: Effect of breed on blood metabolites in dairy cows (Means ± SE).

Cow Breed	Blood metabolites		
	Glucose (mg/dl)	Total Protein (g/dl)	Triglycerides (mg/dl)
Holstein Friesian	38.85[b] ± 1.77	16.25 ± 0.47	18.08 ± 0.95
Jersey	52.50[a] ± 2.09	16.43 ± 0.49	16.52 ± 0.90
F_1 (HF x Sahiwal)	39.68[b] ± 1.45	15.63 ± 0.53	17.42 ± 0.87
Achai	33.30[c] ± 2.17	16.05 ± 0.45	21.93 ± 1.42
Probability level	P<0.001	0.44	0.64

[a,b,c] Means within the same column having different superscript are significantly different.

Metabolites effects within breed

Holstein Friesian breed: Blood metabolites had non significant effect upon the occurrence of estrus in Holstein Friesian cows (Table 3). The blood glucose and total protein elevated before the occurrence of estrus while triglycerides level showed a decline towards estrus. In anestrus cows, higher concentration of blood triglycerides and lower blood glucose and total protein levels were observed.

Jersey breed: Post-partum estrus was significantly affected by blood glucose ($P < 0.05$) in Jersey breed while total protein and triglycerides showed non significant effect (Table 4). Blood glucose level remained lowest in anestrus animals (44.16 ± 4.28 mg/dl), increased two months before estrus up to 51.00 ± 3.30 mg/dl and increased further to 57.12 ± 5.14 mg/dl one month before the occurrence of estrus; remaining higher later on (figure 5).

Crossbred cows: Occurrence of estrus in F_1 cows was significantly affected by blood glucose and triglyceride levels

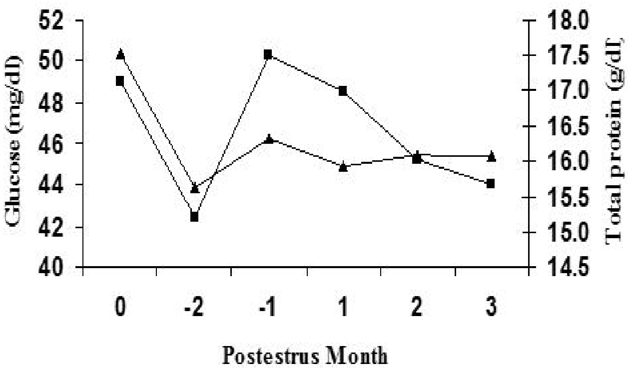

Fig 3: Changes in serum glucose (solid squares) and total protein

($P < 0.001$ and $P < 0.05$, respectively) whereas total protein had non significant effect upon the occurrence of estrus in this breed. Blood glucose level increased form 44.74 ± 4.93 mg/dl to the higher level of 48.56 ± 3.00 mg/dl one month before estrus occurrence (Table 5). Lowest glucose level of 33.63 ± 2.20 mg/dl were recorded in anestrus cows. Triglyceride level showed totally opposite trend in F1 cows as compared to other breeds studied in this experiment. Triglyceride level two months before the occurrence of estrus was 16.50 ± 3.68 mg/dl which elevated to 18.44 ± 3.06 mg/dl prior to estrus commencement. Higher triglyceride level (22.75 ± 2.73 mg/dl) was recorded two months after occurrence of estrus and lower level (16.00 ± 0.91 mg/dl) was observed in anestrus cows.

Achai breed: In the present study, the post partum estrus in Achai cows was not affected by blood metabolites (Table

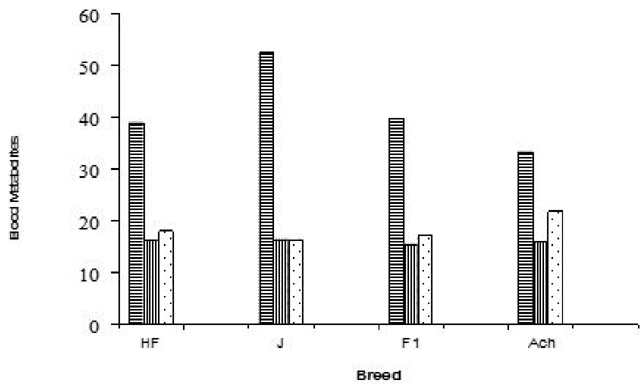

Fig 4: Effect of breed on blood metabolites concentrations (blood glucose, mg/dl, horizontal lines; total protein, g/dl, vertical lines; and triglycerides mg/dl, dots) in dairy Cows: (HF= Holstien Fresien. J= Jersey. F_1=Sahiwal x HF. Ach= Achai). Concentration of serum glucose was significantly higher in Jersey ($P < 0.05$).

Table 3: Changes in blood metabolites with pre and post estrus intervals in Holstein Friesian breed (Means ± SE).

Post estrus interval (in months)	Glucose (mg/dl)	Total Protein (g/dl)	Triglycerides (mg/dl)
-2	34.00 ± 2.86	15.4 ± 0.83	19.69 ± 1.79
-1	44.88 ± 4.28	19.01 ±1.36	17.00 ± 3.14
1	47.00 ± 4.93	14.87 ± 1.08	15.38 ± 2.35
2	38.00 ± 1.72	16.03 ± 1.24	13.50 ± 0.64
3	36.00 ± 5.13	15.04 ± 3.08	13.33 ± 5.90
Anestrous	37.35± 2.96	16.44 ± 0.83	20.00 ± 1.48
Probability value	0.22	0.19	0.28

Table 4: Changes in blood metabolites with pre and post estrus intervals in Jersey breed (Means ± SE).

Post estrus interval (in months)	Glucose (mg/dl)	Total Protein (g/dl)	Triglycerides (mg/dl)
-2	51 ± 3.30 [b]	15.32 ± 1.23	18.86 ± 1.62
-1	57.12 ± 5.14 [ab]	16.8 ± 1.12	19.75 ± 2.47
1	57 ± 1.99 [ab]	16.87 ± 0.43	15.4 ± 0.91
2	61.5 ± 3.57 [ab]	15.39 ± 1.44	12.37 ± 2.88
3	71.33 ± 5.18 [a]	15.12 ± 2.69	19.33 ± 1.20
Anestrous	44.16 ± 4.28 [b]	16.96 ± 0.66	16.25 ± 1.54
Probability level	0.03	0.77	0.34

[a,b] Means within the same column having different superscripts are significantly different.

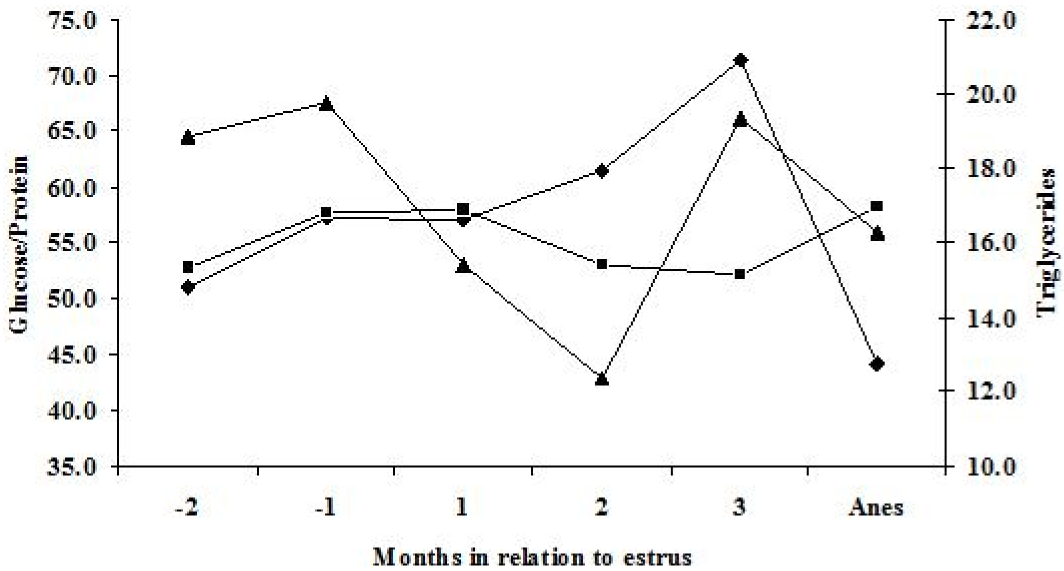

Fig 5: Changes in blood metabolites (blood glucose, mg/dl, ♦; total protein, g/dl, ■; and triglycerides, mg/dl, ▲) with pre and post estrus intervals and anoestrus (Anes) in Jersey breed. Blood glucose was significantly different ($P < 0.05$) during various months.

6). The blood glucose level in Achai cows showed an increasing trend towards commencement of estrus and after estrus occurrence, the trend became inconsistent. Total protein remained almost persistant throughout all the pre and post partum estrus months.

The present study was conducted to evaluate the effect of blood metabolites (Glucose, total serum protein and triglycerides) and their relation with reproductive cyclicity of different breeds of cows i.e. Holstein Friesian, Jersey, Crossbred (F_1) and Achai. For this purpose a total of 240 blood samples were collected and daily milk yield of selected cows were recorded for 90 days.

Relationship of blood metabolites with occurrence of estrus

In the present study, 65 % of cows reestablished estrus while 35 % cows remained anestrous during the experimental period. 80 % of Holsteins showed estrus followed by 70 % of Jersey and F_1 cows. The reestablishment of postpartum estrus was very low in Achai and only 40 % of Achai cows were cyclic and 60 % remained anestrous.

The effect of blood glucose and daily milk yield on post partum estrus was found significant. Several reports indicated a close association between glucose availability, serum LH, and reproductive function (McClure *et al.*, 1978;

Table 5: Changes in blood metabolites with pre and post estrus intervals in F_1 cows (Means ± SE).

Post estrus interval (in months)	Glucose (mg/dl)	Total Protein (g/dl)	Triglycerides (mg/dl)
-2	44.74 ± 4.93^{ab}	12.41 ± 0.79	16.50±3.68^{ab}
-1	48.56 ± 3.00^{a}	14.62± 1.45	18.44±3.06^{ab}
1	44.36 ± 2.73^{ab}	15.87 ± 0.88	14.27±1.14^{b}
2	37.63 ± 3.44^{ab}	16.02 ± 1.08	22.75±2.73^{a}
3	43.75 ± 5.69^{ab}	15.02 ± 1.60	22.00±5.40^{a}
Anestrous	33.63 ± 2.20^{b}	16.04 ± 1.05	16.00±0.91^{ab}
Probability level	0.001	0.66	0.05

[a,b] Means within the same column having different superscript are significantly different.

Table 6: Changes in blood metabolites with pre and post estrus intervals in Achai cows (Means ± SE).

Post estrus interval (in months)	Glucose (mg/dl)	Total Protein (g/dl)	Triglycerides (mg/dl)
-2	39.00 ± 3.69	16.63 ± 1.35	12.50 ± 1.32
-1	47.25 ± 2.46	16.50 ± 0.59	18.00 ± 4.60
1	42.17 ± 6.74	16.58 ± 1.57	23.33 ± 4.64
2	24.50 ± 1.01	16.31 ± 1.45	28.00 ± 5.48
3	40.80 ± 4.06	18.47 ± 1.59	19.00 ± 4.02
Anestrous	32.92 ± 2.70	15.50 ± 0.63	22.89 ± 1.86
Probability level	0.16	0.61	0.37

Richards *et al.*, 1989; Funston *et al.*, 1995). Glucose is recognized as a major source of energy for the ovary (Rabiee *et al.*, 1999). This study demonstrates that with approaching estrus, the blood glucose levels inclined to produce the threshold for estrus.

Patil and Deshpande (1979) reported a distinct rise in blood glucose concentration in cows during estrus due to excitement and increased metabolic rate. Zaman *et al.*, (1985) observed that glucose level around estrus especially in the fertile phase was greater in normal cycling dairy animals than non-cycling or sub estrus cows which is in line to the findings of the present study. The findings of the present study is in strong agreement to Wagner and Oxenreider, (1971); Folman *et al.*, (1973) and Patil and Deshpande, (1979) who reported detrimental effects of under nutrition and low blood glucose concentrations on postpartum cyclicity of dairy cows. Poor body condition in Achai cows exacerbated the effects of suckling and markedly extended the postpartum anovulatory interval partially due to the negative influence of energy deprivation on hypothalamic GnRH release (Rasby *et al.*, 1992) which is in strong agreement to Short *et al.*, (1990); Randel, (1990) and Jolly *et al.*, (1995).

When cows are weaned, even if temporarily, glucose availability increases along with serum IGF-1 and a return to reproductive cyclicity (Randel, 1990; Jolly *et al.*, 1996). Lowest serum glucose level (35.74 ± 1.57 mg/dl) and increased triglyceride level (19.17 ± 0.84 mg/dl) were observed in anestrous cows which is in strong agreement to Sathish and Sharma (1991) who noticed a lower blood glucose in non fertile animals which is an indication of subnormal energy status of cows. 35 % animals remained anestrous during present study.

The present study showed that there was an increase in serum total protein towards estrus which is in line to the findings of Burle *et al.*, (1995) who reported significantly higher value of total serum protein in cyclic cows. The results of the present study are different from the findings of Hewett (1974) who reported that higher level of total serum protein was associated with low fertility. However, the mechanisms by which high level of protein adversely affect reproduction in dairy cows is unknown (Randel, 1990). The expression of estrus signs were more prominent in Jersey than F_1 cows which is in agreement to the findings of Beareten and Fuquary (1992) who suggested that deficiency in protein induced weak expression of estrum. It may be due to the deficiency of certain amino acids required for synthesis of gonadotropins due to low level of serum protein (Vohra *et al.*, 1995). The present study shows that values of serum total protein remained steady after parturition in cows which did not show estrus within 90 days but increased significantly in those which did. This finding is in line with the results of Park *et al.*, (1980) and Thomas and Chiboka (1984) who concluded that the composition of serum total protein was not altered by source or amount of protein in the ration.

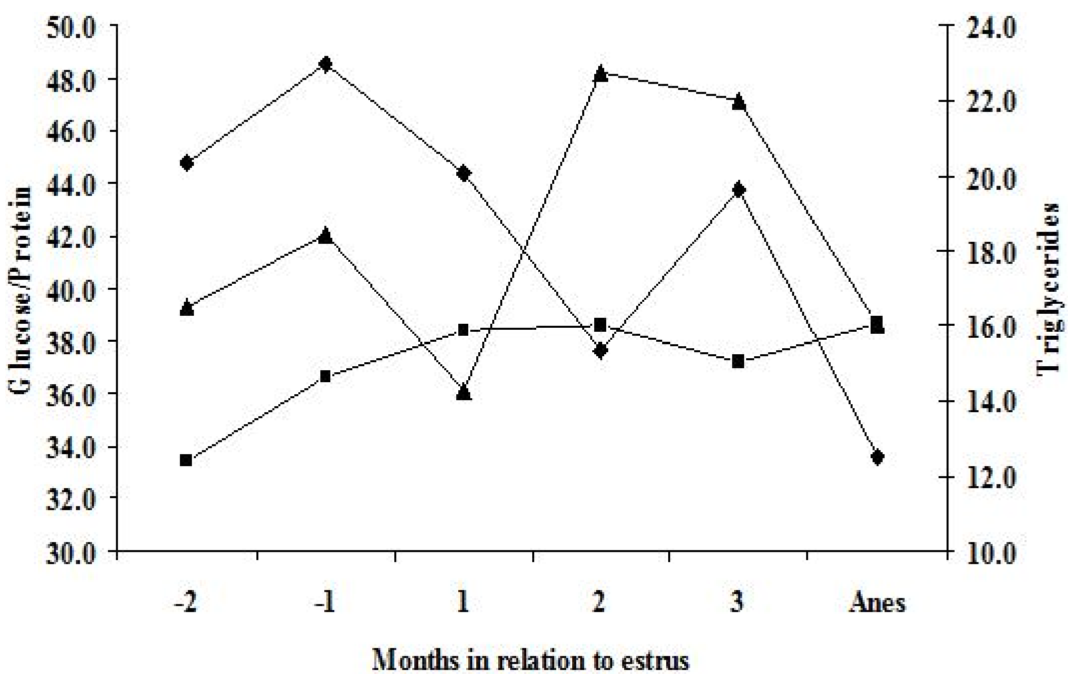

Fig 6: Changes in blood metabolites (blood glucose mg/dl ♦, total protein g/dl ■ and triglycerides mg/dl ▲) with pre and post estrus intervals and anestrus (Anes) in crossbred cows. Blood glucose and triglycerides concentration were significantly different ($P < 0.001$ and $P < 0.05$, respectively) during various months.

In the present study, the relationship of serum triglyceride with postpartum estrus was non significant. Triglyceride showed fluctuating trend. Firstly it showed a decrease towards estrus and up to one month after occurrence of estrus and then increased. Highest triglyceride level was found three months after estrus which may be attributed to the decrease in daily milk yield. Cow's milk their heaviest during the first 2 to 3 months of lactation, this heavy milking with related increase in thyroid activity could account for the low triglyceride during these months. During mid-lactation, cows are generally in a positive nutrient balance i.e. nutrient intake exceeds nutrient outgo. As a result, losses of body weight during heavy milking are starting to be regained at this time. Increasing weight gain and decelerated thyroid activity may explain the increase in triglyceride during mid-lactation. Triglyceride decreased slightly during later months of lactation; this decrease of triglyceride may reflect increasing nutrient demands of the developing fetus as well as increasing estrogen and progesterone in blood.

The result of the present study is in line to the findings of Bronson and Manning (1991) who argued that body fat does not have a direct causal role in regulating ovulation. But it is clear that in situations of negative energy balance, the quantity of fat stored in the body will influence the composition of body tissue mobilized (Wright and Russel 1984) and the size and nature of the metabolic pool (Oldham, 1984). The mechanisms by which triglyceride may influence the fertility of dairy cattle is unclear. Improved fertility of cows with higher concentrations of serum triglyceride in present study may reflect other aspects of a more positive energy balance, rather than a causal relationship between higher triglyceride and fertility.

The present study shows a significant relationship between daily milk yield and occurrence of estrus ($P<0.01$) which is probably due to nutrient sparing effect of decreased milk yield chanellizing some of the metabolites towards reproductive cyclicity. It is in line with the findings of Lucy, (2001); Washburn *et al.,* (2002); de Vries and Risco, (2005) who reported that prior to the occurrence of estrus, decrease in milk yield allowed dairy cows to partition some nutrients towards cyclicity. They reported negative association between fertility and milk yield. However this relationship cannot be considered as a cause-and-effect relationship, but rather partly explained by the antagonistic genetic correlation between milk yield and fertility (Dematawewa and Berger, 1998 and Hansen, 2000).

This study shows that fertility and milk yield are related inversely; that is, when milk yield increases, fertility decreases over time. However, it does not mean that higher producing cows within a herd, are always less fertile than lower producing cows because high milk yield does not always exacerbate negative energy balance (Lucy, 2001; Tenhagen *et al.,* 2003). These authors reported that both Holstein and Jersey cows, being the highest milk producers, have better resumption of estrus. High milk yield is not always consistent with negative energy balance (Lucy, 2001) and they are not at risk for infertility when compared with low producing cows (Lucy *et al.,* 1992).

In the present study, despite very low daily milk yield of Achai, the reestablishment of estrus cyclicity was also very low. Only 4 out of 10 Achai cows resumed estrus cyclicity within the experimental period which guards the findings of Staples *et al.,* (1990) which states that milk production can be a poor indicator of reproductive status of dairy cattle.

The breed effect: In present study highly significant effect of breed on blood glucose concentration was observed ($P< 0.001$) showing the highest levels in Jersey followed by crossbred, HF and Achai. Post partum estrus was significantly affected by blood glucose ($P < 0.05$) in Jersey and crossbred cows ($P < 0.001$). The results of this study clearly indicate that Jersey has the highest serum glucose and total protein and lowest triglyceride level. Breed differences also would reflect any differences in body size and weight for the breed. For instance, large size does give greater consumptive capacity and, consequently, greater milk production. However the daily milk yield of Jersey cows was found very low as compared to their genetic potential which may be a potential cause of highest serum glucose level in these cows. This discrepancy may be related to the degree of acclimatization and/or the differences in milk production level of the studied animals.

The high serum blood glucose observed in the high-producing cows Holstein and crossbred may be ascribed to their increased feed intake necessary for sustained milk production and respiratory functions. Despite of low milk production, lowest blood glucose were recorded in Achai. The possible reason for this may be the slow increase in dry matter intake postpartum which resulted in poor milk production and reproduction. Contrary to Coppock *et al.,* (1974) who reported that dry matter intake progressively increased for the first 16 wk of lactation before starting to decline, Achai cows took a long time to reach peak feed intakes. Reid *et al.,* (1966) concluded that appetite was the greatest limitation to large milk yields.

The lowest concentration of serum glucose in Achai may also be attributed to suckling of their calves which is in a strong agreement to the study made by Marongiu *et al.,* (2002) in which glucose concentrations was affected by the suckling treatment ($P<0.01$) and increased (62 vs. 67 mg/dl) by suckling restriction. Negative energy balance during early lactation may be a contributory factor to lowest serum glucose level in achai. Highly significant effect of breed on blood glucose in this study contradict the findings of Shaffer *et al.,* (1981) who reported non significant effect of breed on blood glucose.

Occurrence of estrus in F_1 cows was significantly affected by blood triglycerides level ($P < 0.05$). However in other breeds, non significant effect of breed on serum total protein and triglyceride was noticed. Highest total protein was found in Jersey cows followed by Holsteins and Achai while crossbreds showed lowest level of total protein. The breed differences for total protein were small and non significant which is in agreement to Kirk and Davis (1970). Jersey cows are generally believed to calve in low body condition, lose less body weight during early lactation (Abe *et al.*, 1994; Mackle *et al.*, 1996). These observations suggest that the depth and duration of nutrient deficit is less severe in Jersey. Blood concentrations of non estrified fatty acids and 3-OH are lower and appetite is less depressed during early lactation than in cows and thus less body tissues are mobilized.

The findings of present study are in disagreement to Shaffer *et al.*, (1981) who showed significant effect of breed on total protein and triglyceride. Highest triglyceride level was found in Achai while lowest was recorded in Jersey cows. The possible justification for increased level of triglyceride may be the inhibition of fat mobilization from the adipose tissue, resulting in a sudden flux in the turnover rate of fatty acids and leading to a higher synthesis and release of triglycerides from the liver. It is hard to explain how such a large amount of triglycerides may be utilized by the mammary gland. The possibility that a fairly large portion of these triglycerides may leave the gland via the lymphatic system or be deposited in the glandular tissues cannot be ruled out. It has been shown that blood fi-lipoprotein triglycerides are more acceptable to the udder than the a-lipoprotein triglycerides (Welch *et al.*, 1963). In Jersey cows, enhanced mobilization of body reserves and partitioning of nutrients toward milk production may be the possible reason for low level of triglyceride. Within breed differences for triglyceride level were also evidenced.

CONCLUSIONS

(a) Based upon this study, we suggest that the rising levels of blood glucose one month prior to occurrence of estrus indicated the role of this metabolite in supporting fertility in dairy cows. Cows failing in exhibiting estrus were also deficient in blood glucose concentrations.

(b) Higher blood glucose and total protein concentration supported higher reproductive cyclicity in Jersey while higher triglycerides in Achai cows were associated with lower fertility.

(c) In crossbred cows, significant variations were observed in blood glucose and triglycerides level showing rising levels during pre estrus period indicating greater adaptability to the local environment.

Recommendations

(a) The feeding management should ensure sparing of glucose availability to support fertility in dairy cows after meeting other requirements of the animal.

(b) Further studies are recommended to document blood metabolic profiles of local breeds and their association with fertility.

(c) Crossbred cows need appropriate feeding strategies for expressing their fertility and lactation potentials.

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