Nangarhar University
International Journal of Biosciences

Received: 20 Sep 2022
Accepted after revision: 15 Nov 2022
Published Online: 25 Feb 2023

ORAGINAL ARTICLE Vol 2, Issue 1 (2023) e-ISSN: 2957-9988

Blood Metabolites and Hormonal Profiles in Dairy Cows during Estrus Cycle in Jalalabad Afghanistan

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ABSTRACT

Background: The study was aimed to investigate the productivity and fertility status of the crossbred and Watani (local) cows in Jalalabad and role of blood metabolites in reproductive cyclicity.

Materials and Methods: A total of 100 dairy cows were selected in five zones comprising city, north, west, south and east of Jalalabad. Fifty crossbred cows (French Friesian x Watani) and 50 Watani cows were selected. Blood samples were collected (10 ml) for blood metabolites and hormonal profiles on day 1, 4 and 17th of the estrus cycle and analysed through UV/Vis spectrophotometer and hormonal profiles through ELISA using Humareader.

Findings: The concentrate intake in crossbred cows was 2.00 ± 0.20 ranging from 1.63 to 2.42 kg day^{-1}, while in Watani cows the values were 2.20 ± 0.21, 1.80 and 3.30 kg day^{-1}, respectively. The milk production for crossbred cows was 7.64 ± 0.14 ranging from 7.28 to 8.00 kg day^{-1}, while in Watani cows the values were 3.75 ± 0.49, 2.00 and 4.88 kg day^{-1}, respectively. Mucus discharge in crossbred cows was 2.00 ± 0.22 (1.50 to 2.50 scales) against 2.45 ± 0.19 in Watani cows. Standing for mounting in crossbred cows was higher (0.43 ± 0.86) than Watani cows (0.26 ± 0.09; scale 0 to 4), Triglycerides varied from 100.94±2.79 to 117.52±5.77 mg/dl (P=0.024) with significant effect of estrus days but no effect of region. Serum glucose in crossbred cows was 67.18±1.50 mg/dl while in Watani cows, it was 65.20±1.33. Protein was 7.62±0.11 in crossbred cows and 6.99±0.13 g/dl in Watani ones. The respective values for triglycerides were 110.69±3.43 and 104.03±3.40 mg/dl. Progesterone concentrations were 0.581±0.076 ng/ml and 0.569±0.09 ng/ml while LH was 28.41±3.31 and 24.20±2.64 ng/ml in the two breeds. All the blood metabolites and hormonal concentrations were not different among regions, however triglycerides showed a constant increase during the succeeding phases while glucose manifested an opposite pattern during the estrus cycle. The level of glucose in the city animals was higher (69.21±2.37 mg/dl, (P=0.127). Progesterone concentration increased on day 4th and declined on 17th day while LH showed a consistent increase during the period.

Conclusion: This study concluded that breeding of the local Watani cows with French Frisian semen resulted in production of crossbred cows. The crossbred cows showed higher concentrations of blood glucose, triglycerides, proteins, progesterone and LH hormones, reflecting higher metabolic activities than the local Watani cows.

Keyword: Blood; Metabolite; Hormone; Dairy Cow; Estrus Cycle

INTRODUCTION

The breeding efficiency of dairy cows gets lowered with a number of reproductive disorders like endometritis, anoestrus and repeat breeding. It adversely affects the productive and reproductive performance of cows, and results in great economic losses to dairy farmers. The basic causes of the reproductive problems in a herd are multiple. They include management, nutritional and pathological factors. The dairy farming has been supporting national economy through production of milk and beef for meeting the protein demand of the rapidly expanding human population (Qureshi, 2007., Dutta et al., 1988). Reproductive and productive disorders are associated with crossbreeding in cattle. Out of the existing cattle population of Pakistan, more than 25% are crossbred animals while the remaining 75% are local cows. According to the breeding policies, the indigenous cows in plain-irrigated areas are crossed with exotic Holstein-Friesian breed while the cows in hilly and arid areas are crossed with Jersey breed. However under the existing artificial insemination practices more than 50% of exotic blood level is used which leads to a decreased immunity and resistance to stress conditions. Increase in genetic purity coupled with poor nutritional and management conditions affect the reproductive and productive performance badly. The stress comprises nutritional, health and thermal factors and is revealed by the inability of an animal to cope with its environment, a phenomenon that is often reflected in a failure to achieve genetic potential (Qureshi, 2007).

Blood glucose, total proteins, urea and cholesterol levels in cyclic, non-cyclic, and endometritic crossbred cows in Seventy-five crossbred cows kept at the Livestock Experimental Station, Qadirabad, and District Sahiwal. The animals were divided into three equal groups i.e. cyclic, non-cyclic and endometritic. The results revealed significantly higher values of glucose and cholesterol in endometritic cows as compared to cyclic and non-cyclic cows. Protein level was highest in endometritic, followed by non-cyclic and lowest in cyclic cows. However, serum level of urea did not differ in cyclic, non-cyclic and endometritic animals (Ahmad et al., 2004).

Endocrines and milk yield of crossbred cows treated with recombinant bovine (rbST) somatotropin to investigate the effect of rBST on the blood metabolites, hormones and milk yield in lactating crossbred cows. Thirty lactating cows were divided into 2 groups as control (n=10) and treated (n=20). Treated animals were injected subcutaneously with 250 mg of rbST at 0, 14 and 28 days, whereas control animals were given place of 2 ml normal saline. Blood metabolites (glucose, blood urea nitrogen (BUN), triglycerides, total proteins, albumin, globulin, sodium and potassium) and hormones (thyroxine (T4) and insulin) were not altered by (rbST) injection. Serum growth hormone (GH) increased significantly ($P<0.001$) due to rbST injection but not milk GH. However, a significant ($P<0.001$) decrease in triiodothyronine (T3) level was observed in rbST-treated group compared to control group. The weekly average milk yield showed a significant increase of 33% in the rbST-treated group compared to the control group. It was concluded that 250 mg rbST could be used for short duration to increase the milk yield of crossbred cows (Qureshi et al., 2002).

This study was designed to monitor the changes of the blood metabolites and hormonal profiles in crossbred and Watani dairy cows during estrous cycle in Jalalabad, Afghanistan, with the following objectives:

1. To document the lactation and fertility status of crossbred dairy cattle in comparison to Watani cows under field conditions in Jalalabad, Afghanistan.

2. To investigate the relationship among the status of reproductive cyclicity, hormonal status and blood metabolites in crossbred and Watani (Local) dairy cows.

MATERIALS AND METHODS

Study Design

This study was conducted in Jalalabad and surrounding villages in three months in private farms; 5-10 cows from each farm. Fifty animals were selected from each; F1 cross of French Friesian with Watani cows and fifty pure Watani cows. Lactation status, fertility indicators and nutritional status were recorded for each animal. Lactation status comprised the level of daily milk yield (kg/day^{-1}) and lactation length. The fertility indicators comprised age at puberty, service period, calving interval, services per conception and number of calves born. Data on nutritional status was collected having concentrates which comprising the cotton seed cake and wheat bran 2-3 kg/day^{-1} and the quantity of 20-30 kg/day^{-1} of berseem were provided. T test was applied for determining difference between local and crossbred cows' concentrates intake, milk yield, mucus discharge and standing for mounting. Analysis of variance was applied for determining means for the five zones, in the result of the aforementioned parameters.

Selection of Animals

Total number of 30 cows including 15 each of F1 cross of French Friesian with Watani cows and pure Watani cows were selected for this study. The animals were divided into younger and older groups falling with an age range 4 to 8 years and 9 to 11 years respectively.

Data Collection

The data on milk yield and quantity of concentrates was collected on the pre –designed questionnaire. Each animal was studied for one estrus cycle. Animals were selected from five zones comprising city, north, west, south and east of Jalalabad.

Blood sampling and analysis

To determine the hormonal and metabolic profiles of the experimental cows (10 ml) samples of blood were collected on day 1, 4 and 17th of the estrus cycle. The samples for blood were collected during June, July and August where the temperature was in the range of 40^{0C} .The blood was collected from jugular vein of all selected animals with 16 gauge sterile needle at 8:00 AM to 9:00 AM after 2 hours of feeding time. Serum was separated from the blood after poured into centrifuge tubes, was kept for 40 minutes up to formation of clot and was finally centrifuged at 2000 rpm for about 10 minutes then it was stored in a freezer at -20^{0}C till further analysis. Blood metabolites including total blood protein, glucose and triglycerides were determined through the available kits.

Determination of Protein levels

The protein level was determined by Biuret reaction method using the commercial protein kit (AMEDA Labordiagnostik GmbH. Krenngasse 12, 8010 Graz, Austria). Twenty µL serum samples were dispersed into labeled test tubes and an amount of 1.0 ml of the available reagent was added. Then the prepared mixture was incubated for 10 minutes at 37^{0}C. Then absorbance was read for the standard (AbsStd) and the sample (AbsS) at wavelength of 540nm against the blank reagent using (UV/ Vis Spectrophotometer (model U2020 Geesthacht Germany). The result was expressed in (g/dl).

Determination of Glucose levels

The level of glucose was determined by enzymatic – colorimetric method using the commercial glucose kit (AMP Medizintechnik GmbH. Stattegger Strasse 31b, 8045 Graz, Austria). 10 µL serum samples were dispersed into labeled test tubes and an amount of 1.0 ml of the available reagent was added. The prepared mixture was incubated for 10 minutes at 37^0C. Then absorbance was read for the standard (Abs Std) and the sample (Abs S) at wavelength of 510 nm against the blank reagent using (UV/ Vis Spectrophotometer (model U2020 Geesthacht Germany). The result was shown in mg /dl.

Determination of Triglyceride levels

The level of serum triglycerides was determined by enzymatic – colorimetric method using the commercial triglyceride kit (AMEDA Labordiagnostik GmbH. Krenngasse 12, 8010 Graz, Austria). 10 µL serum samples were dispersed into labeled test tubes and an amount of 1.0 ml of the available reagent was added. Then prepared mixture was incubated for five minutes at 37^{0C}. Then absorbance was read for the standard (AbsStd) and absorbance for sample (AbsS) at wavelength of 500 nm against the blank reagent by (UV/ Vis Spectrophotometer model U2020 Geesthacht Germany). The result was shown in mg /dl.

Determination of luteinizing hormone (LH)

Luteinizing hormone (LH) was determined by using commercial ELISA kit (AmGEnix International, INC. 3444 Pinotin Ct. San Jose, CA 95148, USA). The principle for the Enzyme Linked Immune Sorbent Assay for LH is based up on the direct binding of LH to sheep polyclonal antibodies. The assay system consists of sheep polyclonal anti LH antibodies coated in the micro titration plate. The mouse monoclonal antibodies conjugate with enzyme that is horseradish peroxidase. The LH molecule is sandwiched between these antibodies and by adding substrate they read enzyme and produce color. The LH absorption is directly proportional to the color intensity of the last sample. In procedure of LH 50 µl, standards, specimens, and controls were pipette in micro titer plate. After that 100µl of enzyme conjugate reagent was added in every well and mixed for 39 seconds. Incubation was made in room temperature (18-25^0C) for 45 minutes. Then extra mixture was removed from the exist plate and wells were washed five times by distilled water. If residues were found, they were removed with paper towels. 100µl of TMB reagent was added in each well and they were gently mixed for five seconds. The micro titer plate was incubated in room temperature in the dark place for twenty minutes. The action was stopped adding up 100µl of stop solution to every well and slowly mixed for 5 more seconds. The color changed from blue to yellow. Absorbance was read at 450 nm with ELIZA plate reader (Humareader Plus.Cat-No.18500 P. Human GmbH Diagnostica Worldwide D65205 Wiesbaden Germany) within 15 minutes.

Progesterone Assay for Serum

The Progesterone level was determined by using the commercial progesterone kit (AmGEnix International .INC. 3444 Pinotin Ct. San Jose, CA 95148, USA).The progesterone ELISA is based on the principle of competitive binding between progesterone in the last sample and progesterone – HRP for a constant amount of rabbit, anti-progesterone antibodies. During incubation fixed amount of progesterone- HRP conjugate, compete with the progesterone of the standard and last sample for a fixed number of binding sites of the specific progesterone antibodies. Thus the amount of progesterone- HRP conjugate immunologically binds to the wells. It will be decreased as the concentration of progesterone in the standard and test sample. Thus the intensity of the color formed is directly proportional to the progesterone-HRP conjugate and as inversely proportional to the progesterone (unlabeled progesterone) of the standard in test sample.

In procedure of progesterone 25µl standards, specimens and controls were pipette into appropriate wells. Then 100µl of Working Progesterone – HRP and Conjugated Reagent were added in each well. Then 50µl of Rabbit Anti –Progesterone Reagent was added to each well and mixed for 30 seconds. Incubation was made at room temperature (18-25^{0}C) for 90 minutes. The micro wells were washed for 5 times with distilled or deionized water. 100 µl of TMB reagent was dispensed into each well. They were gently mixed for 10 seconds. The microtiter plate was again incubated at room temperature (18- 25^{0}C) for 20 minutes. The reaction was stopped by adding 100 µl stop solution to each well and mixed for 30 seconds. The reaction was completed when blue color changed to yellow. Absorbance read at 450 nm through ELIZA plate reader (Humareader Plus.Cat-No.18500 P. Human GmbH Diagnostica Worldwide .D65205 Wiesbaden Germany) within 15 minutes.

Rectal Palpation and Insemination

The experimental cows were clinically examined. Data was recorded and rectal examination was conducted for estrus detection and status of the reproductive tract. Reproductive parameters were recorded comprising mucus (scale 0-4) and standing for mounting (scale 0-1). The animals were artificially inseminated at appropriate time in consultation with the owner and it was palpated for pregnancy diagnosis per rectum on 60th day post-breeding.

Data analysis

The data was collected and maintained in computer based MS Excel files. SPSS-19 software was used for data analysis using guidelines provided by Steel and Torrie (1980). Means were compared through analysis of variance for zones. The dependent parameters were blood glucose, triglycerides, total protein, progesterone and luteinizing hormones.

RESULTS

Effect of feeding status and region on fertility and milk yield

Measure for the daily concentrates intake in two breeds (Watani and crossbred) cows and their association with fertility and milk yield are reported in **Table 1**. Statistical analysis of the data indicated that the average concentrates intake was higher in Watani than crossbred dairy cows. It was 2.20 kg versus 2.00 kg day^{-1}. The average milk yield of Watani cows was lower than the crossbred cows and was 3.75±0.49 versus 7.64±0.14 kg day^{-1}. The table shows that the local Watani cows consumed more concentrates while produced less milk than the crossbred cows.

Fig. 1 & 2 show the daily intake of concentrates and milk yield in different regions. Highest concentrates intake was found in west region in Watani dairy cows the two parameters showed opposite pattern, the west was followed by north, south, city and east reaching to the final values of 1.8 concentrates and 4.5 kg day^{-1} milk. In the crossbred cows the concentrates feeding and daily milk yield was almost parallel across the four regions. The north zone showed the best efficiency producing 8 kg day^{-1} milk with consuming 2.3 kg concentrates as compared to the east zone showing milk yield the same level 7.2 kg day^{-1} against concentrate intake. Higher concentrates intake (2.7 kg day^{-1}) was found in city region.

The average mucus discharge of Watani cows was higher (2.45±0.19) on estrus day, ranging from 2.00 to 2.90 than the crossbreed cows (2.00±0.22) ranging from 1.50 to 2.50. Standing for mounting in Watani cows was found as 0.26±0.09 on estrus days, ranging from 0.00 to 0.54 as compared to 0.43±0.86, ranging from 0.28 to 0.67 in crossbred cows.

Mucus discharge and standing for mounting in the city were the highest values of those parameters while the west zone showed the lowest values in Watani cows. For crossbred cows showed the most vigorous stress symptoms

in southern zones, mucus discharge was lowest in the north and standing for mounting was lowest in the city and east.

Table 1. Concentrates feed offered, Daily milk yield, Mucus discharge and Standing for mounting in Watani (local) and crossbred dairy cows.

	Breed	Min	Max	Mean±SE
Concentrates offered (kg day^{-1})	Watani	1.80	3.30	2.20±0.21
	crossbred	1.63	2.42	2.00±0.20
Milk yield (kg day^{-1})	Watani	2.00	4.88	3.75±0.49
	crossbred	7.28	8.00	7.64±0.14
Mucus discharge scale (0-4)	Watani	2.00	2.90	2.45±0.19
	crossbred	1.50	2.50	2.00±0.22
Standing for mounting scale (0-1)	Watani	0.00	0.54	0.26±0.09
	crossbred	0.28	0.67	0.43±0.86

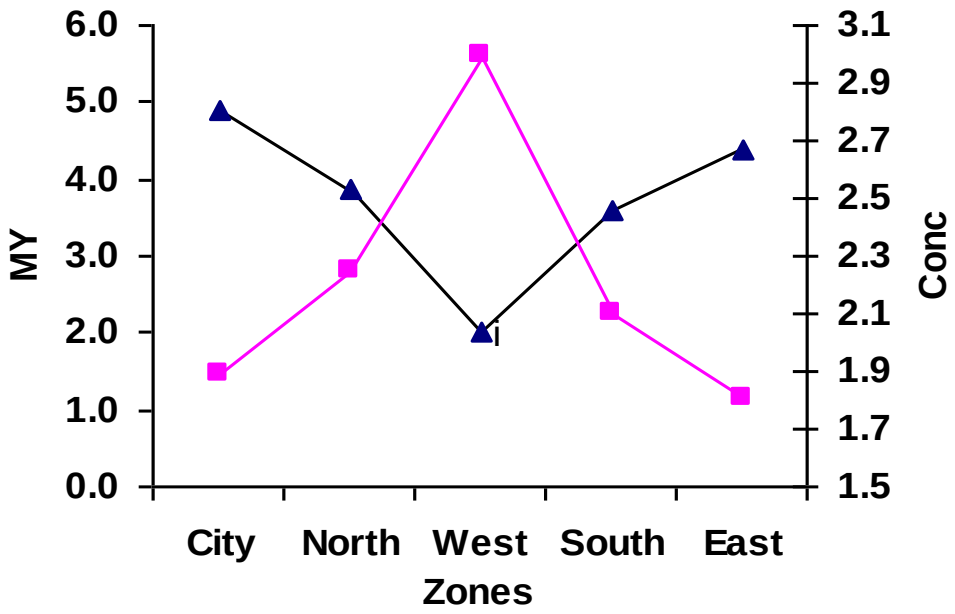

Fig.1. Milk Yield (kg day^{-1}, (▲) and concentrate intake (Conc. kg day^{-1}, (■) by Watani Dairy Cows in Jalalabad.

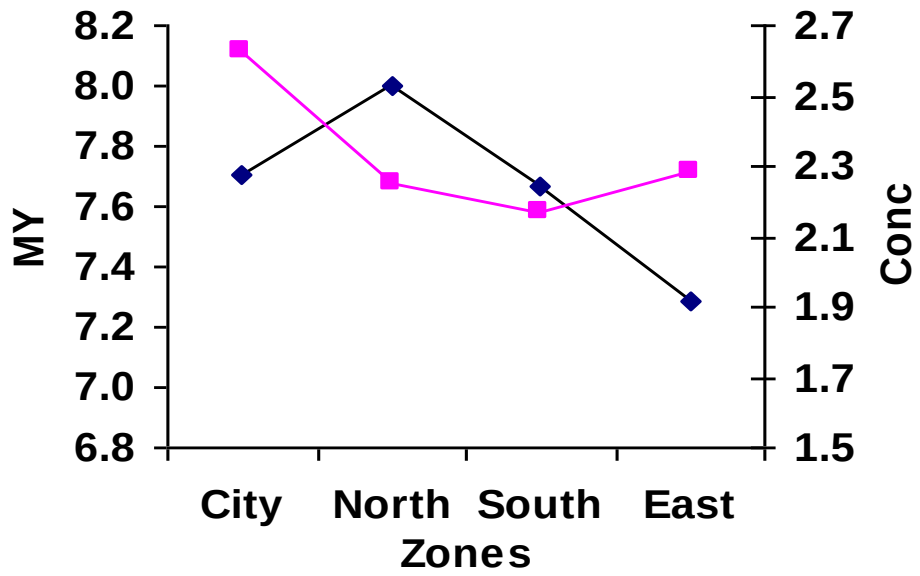

Fig. 2. Milk Yield (kg day^{-1}, (■) and concentrate intake (Conc, kg day^{-1}, (■) by Crossbred Dairy Cows in Jalalabad.

Effect of estrus days on blood metabolites and hormonal profiles

Table 2 shows the effect of estrus days on blood metabolites and hormonal profiles determined in dairy cows. Statistical analysis of data showed that the glucose remained higher on day 1 and 4 and decreased on day 17 (P= 0.650). Triglycerides showed an upward trend throughout the study period and is varied from 100.94±2.79 to 117.52±5.77 mg/dl (P=0.024) and the result was significant. Protein remained higher on day 1 and 4 and decreased on day 17 (P=0.385) which varied from 7.39 to 7.42 g/dl and the result was non-significantly different. Progesterone was lower on day 1 and increased gradually on day 4 with the developing of luteal tissue constituting the luteal phase up to day 16. Later on progesterone level went on decreasing on day 17 (P= 0.231). LH hormone was lower from day 1 to day 4 of estrus cycle increased from day 4 till it reached the maximum level on day 17. (P= 0.655).

LH hormone was lower from day 1 to day 4 of estrus cycle, but increased from day 4 till it reached the maximum level on day 17. The ovulation is expected on day 21 which needs the highest level of LH in the form of LH surge. Progesterone was lower on day 1 increased gradually on day 4 with the developing of luteal tissue constituting the luteal phase up to day 16. Later on progesterone level went on decreasing due to regressing of corpus leutum reaching its minimum on the day of ovulation.

Table 2. Blood Metabolites and Hormonal concentration as influenced by the days of estrus cycle in dairy cows (Mean ± SE, n=81)

Day	Glucose mg/dl	Triglyceride mg/dl	Protein g/dl	Progesterone ng/ml	LH ng/ml
1	67.18±1.67	100.94±2.79	7.39±0.15	0.47±0. 01	25.48±3.16
4	67.12±1.74	106.23±3.83	7.42± 0.15	0.71±0.16	25.70±3.68
17	64.23±1.85	117.52±5.77	7.11±0.17	0.54±0.03	28.48±4.56
P. value	0.650	0.024	0.385	0.231	0.655

Fig.3 shows that both glucose and protein were higher and parallel, on the day of estrus and remained so up to day 4. This may probably be supportive for estrus activities exhibited by the animals through enhanced metabolism, mucus secretion, locomotion and other signs of estrus. This was reflective of higher metabolic rates and the metabolites may have been derived from the nutrient pools in the body reserves.

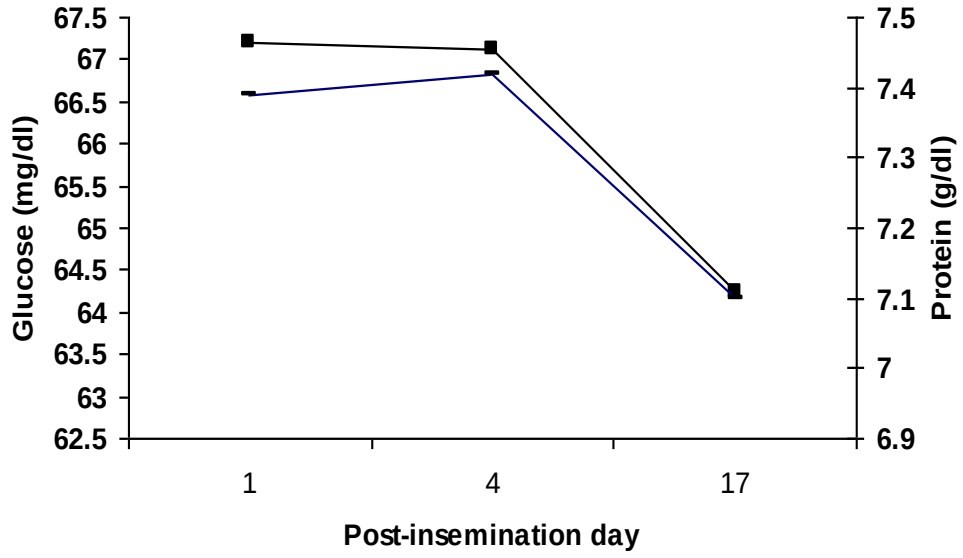

Fig. 3. Changes in blood glucose (solid squares) and protein (lines) levels in dairy cows with the post-insemination day

Effect of Regions on Blood metabolites and hormonal profiles

Data regarding effect of regions on blood metabolites and hormonal profiles in dairy cows in Jalalabad is reported in **Table 3** .Statistical analysis indicates that the average serum glucose concentration was similar in all regions, the differences being non-significant. Triglyceride and Protein concentration in serum were almost similar in all regions, triglycerides (P=0.624) and protein 7.33±0.09 (P=0.104) the result were non-significant. LH and Progesterone hormones concentration in serum was also non-significant difference among regions, the average of LH was 26.39±2.13 (P=0.450) and Progesterone was 0.57±0.059 (P=0.340).

Table 3. Blood Metabolites and Hormonal concentration as influenced by Region wise in dairy cows in Jalalabad. (Mean ± SE, n=81)

Region	Glucose mg/dl	Triglyceride mg/dl	Protein g/dl	Progesterone ng/ml	LH ng/ml
City	69.21±2.37	106.72±4.64	7.48±0.18	0.48±0.01	28.08±4.77
North	68.04±1.17	110.45±5.30	7.42±0.1	0.65±0.17	23.73±3.42
West	64.57±1.84	105.94±6.48	7.12±.26	0.69±0.23	29.14±5.94
South	63.49±2.68	105.91±5.79	6.96±.19	0.50±.02	23.02±4.44
East	63.51±2.77	108.3±5.80	7.54±0.31	0.59±0.06	27.85±5.22
P. value	0.127	0.624	0.104	0.340	0.450

Fig. 4 the level of glucose present in the city area is higher than those of north, west, south and eastern zones, 69, 68, 64, 63 and 63 mg/ dl. The protein also follows the same pattern as the glucose with the exception of the east, in which the protein level is highest (7.5 g/ dl) and lowest is in the south (6.9 g /dl). The region has a non-significant effect on serum glucose (P=0.127) and protein (P=0.104). **Fig. 5** shows that protein varied from 6.6 to 7.6g/dl shows the highest level in east followed by city, north, west and south; Triglycerides follows the same pattern except for the city.

Fig. 4. Effect of Region on Blood metabolites Glucose (Square) and Protein (Triangle)

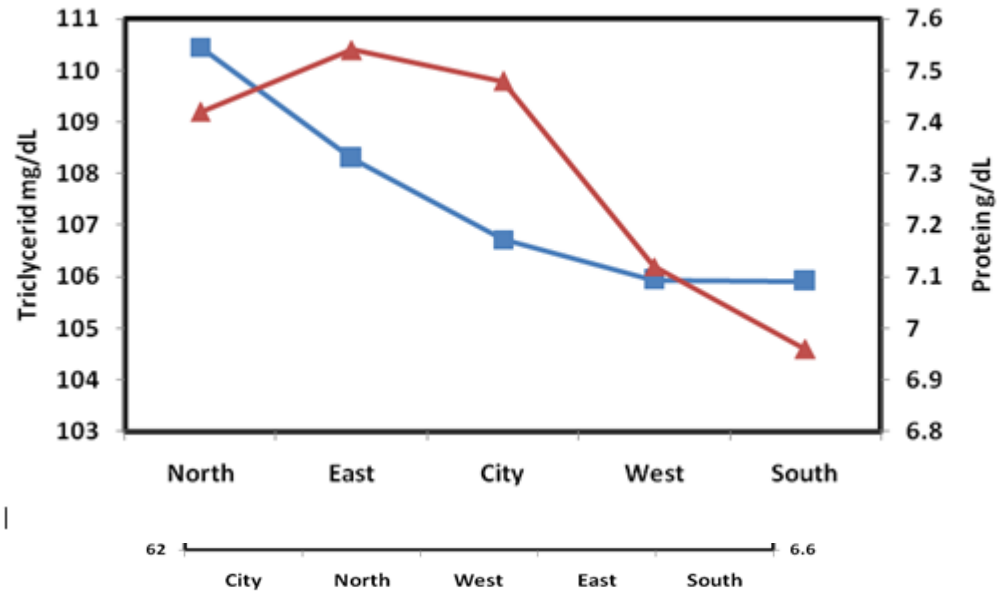

Fig. 5. Effect of Region on Blood metabolites (Triglycerides (Triangle) and Protein (Square)

Effect of breeds on Blood metabolites and hormonal profiles

Data regarding blood metabolites and hormonal concentration of crossbred and Watani dairy cows in Jalalabad is reported in **Table 4**. Statistical analysis indicated that the average serum glucose concentration in crossbred cows was higher 67.37±1.50 than Watani cows 65.20±1.33 mg/dl, the result was not significant.

Triglyceride concentration were also higher in crossbred than Watani cows 110.69±3.43 and in Watani was 104.03± 3.40 Protein concentration in crossbred was 7.62±0.12, and in Watani it was 6.99±0.13 mg/dl. Triglyceride in breeds was not significant while protein was significant in them (P=0.001).

LH and Progesterone hormones concentration in serum were also higher in crossbred and lower in Watani cows. The average of LH in crossbred was 28.41±3.31 and 24.20±2.64 ng/ml while Progesterone was 0.581±0.076 and 0.569±0.09 ng/ml in the two breeds respectively.

Table 4. Descriptive statistics for blood metabolites and hormones in crossbred and Watani dairy cows. (Mean ± SE, Minimum, Maximum)

	Glucose mg/dl	Triglyceride mg/dl	Protein g/dl	Progesterone ng/ml	LH ng/ml
Crossbred	67.371±1.50	110.69±3.43	7.62±0.11	0.581±0.076	28.41±3.31
	48.8	81.84	5.85	0.375	13.6
	87.2	167.60	9.68	3.630	109.6
	65.20±1.33	104.03±3.40	6.99±.13	0.569±.09	24.20±2.64
Local Watani	47.9	79.35	5.32	.375	13.6
	85.1	185.83	9.24	4.005	96.0
P. Value	0.288	0.173	0.001	0.919	0.328

DISCUSSION

The daily concentrates intake in Watani and crossbred cows and its association with fertility and milk yield was studied. The average concentrates intake was higher in Watani than crossbred dairy cows and (2.20 kg versus 2.00 kg day^{-1}) while the average milk yield of the former was lower (3.75±0.49 versus 7.64±0.14 kg day^{-1}). It shows an adverse effect of over feeding on milk yield in the low yielding breed. Watani cows have no genetic potential to utilize the extra amount of concentrates for milk synthesis. This phenomenon was extensively explored by our group (Qureshi, 2007). A same scale feeding was reported exposing the low yielding animals to adverse effects of over feeding. Intake of crude protein (CPI) varied between seasons and was positively correlated with serum urea levels (r=0.22, p<0, 01; Qureshi et al., 2002), In LMY buffaloes there seemed to be the stress of over feeding of degradable protein. In previous studies (Rahman et al., 1987; Husain and Mostafa, 1985; Khan and Khatun, 1998) it was observed that the daily milk yield was 8.10, 9.74 liters and 7.35 liters for SL x Pabna, Fx Pabna and Pabna x Pabna genetic groups respectively.

The present study shows that different regions have significant (P<0.05) effect on daily intake of concentrates and milk yield. In the crossbred cows the concentrates feeding and daily milk yield was almost parallel across the four regions. The north zone showed the best efficiency producing milk 8 kg day^{-1} while consuming 2.3kg concentrates as compared to the east zone showing milk yield is 7.2 kg day^{-1} consuming the same amount of concentrate intake. Higher concentrates intake (2.7 kg day^{-1}) was found in city region, while

higher milk yield (8 kg day^{-1}) was recorded in north zone. The best efficiency of the north zone may be attributed to the well-developed irrigation system supporting production of plenty of fodder which constitutes the best combination of practices for crossbred animals.

In the present study the Watani dairy cows show high mucus discharge than the crossbred dairy cows. Watani dairy cows are more adjusted to the local conditions as compared to the crossbred dairy cows, therefore they exhibit estrus symptoms with greater intensity as compare to the crossbred cows. The crossbred cows are not fully adapted to the local environment so fail to express full estrus signs.

The zones had a significant effect on mucus discharge and standing for mounting in Watani cows. The city showed the highest values of those parameters while the west zones showed the lowest values. The highest value is due to the special care, treatment facilities, feeding and attention of people of city, while the lowest value is due to low water availability, low land fertility, low treatment facility, feed scarcity and low attention of people of west zone.

In the current study, the mean concentration of glucose level in blood serum of local Watani and crossbred dairy cows during estrus cycle was (65.20±1.33 and 67.37±1.59 mg/dL) respectively. While Sarker et al. (2011) stated that blood glucose was 35.16±2.402 mg/ dl in a Red Chittagong (RC) cow and 35.37±2.403 mg/ dl in a local cow. Our result supported it. Anderson et al. (1930) stated that blood glucose level may alter because of the changing in its usage throughout the animal's life cycle. Ahmad et al. (2004) recorded blood glucose in cyclic cows as (50.72±1.12), and in non-cyclic as (50.56±1.13) mg/dl.

Glucose concentration varied with changing phases of estrus cycle on day 1 and 4 the concentration was higher while on day 17th it decreased by 3 mg/dl. The decreased concentration may be the result of glucose utilization for supporting estrus activities comprising restlessness, increase locomotion, mucus discharge and bellowing. Concentrations of glucose in plasma were affected by body condition score (Adams et al., 1987) and may influence reproductive performance in cattle.

Metabolism of glucose by bovine corpora luteal in vitro was influenced by the day of estrus cycle (Chase et al., 1992). The lack of estrus and failure of formation of functional corpora luteal in cattle was associated with inhibition of glycolysis (McClure et al., 1978). Glucose concentration increased and the GnRH- stimulated LH secretion with the infusion of propionate into heifers (Rutter et al., 1983). They reported cows that had luteal activity during the breeding season had 5.7 mg/dL more glucose than cows without luteal activity. Despite the significant difference, categorical data analysis indicated that individual glucose values were not determinants of luteal activity.

In our study total serum protein was 6.99±0.13 in Watani and 7.62±0.11 g/dl in crossbred cows. It observed from the present study that blood protein level increased from day 1^{s} to day 4th of estrus cycle and then 0. 28 g/dl blood protein level is reduced on day 17th of estrus activity, the decreased protein concentration maybe due to its utilization for supporting reproductive activities.

Sarker et al. (2011) studied blood metabolites of Red Chittagong (RC) and Local cattle at Chittagong, the total serum protein was recorded 8.98± 4.70 (g/dl) and 8.997±0.588 (g/dl) in RC cows and local cows, respectively. Kapale et al. (2008) reported that cows had low total protein than the adult Gaolao cows. Total protein and glucose of serum are the major blood components responsible for the maintaining homeostasis and metabolism. Protein deficiency resulted in delayed onset of puberty, increased days open, decreased dry matter

intake and lead to energy deficit (Gaikwad et al., 2007). They reported that adequate protein intake is necessary for normal fetal growth and development.

Ahmad et al. (2004) also reported that total protein level differed significantly ($P<0.05$) among cows of all the three groups, being highest in endometritic (19.16 ± 1.00), followed by non-cyclic (15.23 ± 0.89) and lowest in cyclic (9.19 ± 0.45) cows.

Triglycerides during estrus cycle show significant variation in the present study. We have observed that the blood serum triglycerides level consistently increased from day 1st to day 17th of estrus cycle. In Watani cows the mean was 104.03±3.40 and in crossbred cows it was 110.69±3.43 mg/dl. The increased concentrations were probably required for the milk fat synthesis, metabolic activity in lactating animal.

In previous study variation in blood cholesterol contents were reported during estrus and pregnancy, as precursor of the steroid hormones (Iriadam, 2007). Lipid profiles have been used to predict peripartum diseases; circulating blood triglycerides contribute significantly to milk fat synthesis (Nazifi et al., 2002). Relative to protein metabolism, a decrease in blood protein concentration during the late stages of gestation was observed in sheep, witnessing the utilization of amino acids for protein synthesis in the fetal muscles (Antunovic et al., 2002). It was also reported that plasma urea levels increased during week 10 of pregnancy, reaching a peak at parturition (El-Sherif and Assad, 2001), which in domestic ruminants was ascribed to the cortisol-stimulated catabolism of proteins in the body (Silanikove, 2000).

Nazifi et al. (2002) confirmed that a decrease occurring during lactation compared to dioestrus could be ascribed to the increased cholesterol uptake by tissue involved in milk synthesis because of the normal insulin responsiveness compared to late pregnancy. It was also observed that circulating blood triglycerides contribute significantly to milk fat synthesis. Karapehlivan et al. (2007) has also been reported that blood biochemical parameters including triglycerides, free fatty acids, total protein and urea are important indicators of the metabolic activity of lactating animals.

Schlumbom et al. (1997) reported that blood serum lipids profile is characterized by increase concentration of triglycerides, total cholesterol and lipoprotein due to the diminished responsiveness of target tissues toward insulin that together with an increased mobilization of fatty acids from adipose tissue make available new sources for fetal growth. While Leroy et al. (2004) found the triglycerides concentration in follicular liquid was improved with 43% as increased in the amount of follicle from (<4mm) to large (>10mm).

The present study showed that LH hormone in serum of Watani cow was 24.20±2.64 and in crossbred 28.41±3.31 ng/ml. Progesterone in Watani cows was 0.569±0.09 and in crossbred 0.581±0.076 ng/ml. LH was lower from day 1 to day 4 of estrus cycle but raised from day 4th till it reached the highest level on day 17th. The ovulation is expected on day 21 which needs the highest level of LH in the form of LH surge. Progesterone was lower on day 1 increased gradually on day 4 with the developing of luteal tissue constituting the luteal phase up to day 16. Later on progesterone level went on decreasing due to regressing of corpus leutum reaching its minimum on the day of ovulation.

Sprague et al. (1971) reported that in beef cows, LH peaked sharply from zero to between 2.5 and 61 ng/ml with occurrence of estrus cycle, while in second estrus the peak was less recognizable, after the detection of estrus cycle progesterone levels were lowest for 20hr while it peaked between days 2 to 3 and declined on day 4 to a level which was similar to that observed 20hr after the detection of estrus. Between days 6 and 12, the concentration was gradually rose which remained above 2.5 ng/ml with peaks on day 9 and 12 and declined

thereafter to the next estrus cycle. Haruna et al. (2009) studied five pairs of cows, on day 8 after insemination LH was higher in non-pregnant than in pregnant cows but they reverse on day 16.

CONCULSION

The average concentrates intake was higher in Watani than crossbred dairy cows while the milk yield was lower. It shows an adverse effect of over feeding on milk yield in the low yielding Watani cows having poor genetic potential to utilize the extra amount of concentrates for milk synthesis. The north zone showed the best efficiency producing 8 kg day^{-1} milk while consuming 2.3kg concentrates which may be attributed to the well-developed irrigation system supporting production of plenty of fodder. Watani dairy cows showed higher mucus discharge than the crossbred dairy cows which may be due to its adaptability to the local conditions. Blood glucose in local Watani and crossbred dairy cows was 65.20±1.33 and 67.37±1.50 mg/dl respectively and it decreased on 17^{th} day of estrus cycle probably due to its utilization for supporting estrus activities. Blood triglycerides consistently increased from day 1^{st} to day 17^{th} of estrus cycle. Serum LH was 24.20±2.64 ng/ml in Watani and 28.41±3.31 ng/ml in crossbred cows. The values for progesterone were 0.569±0.09 and 0.581±0.076 ng/ml respectively. LH was lower from day 1 to day 4 of estrus cycle but reached the highest level on day 17^{th} probably for supporting ovulation.

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