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Short Communication

Impact of Blood Metabolite Profile and Milk Yield on Fertility of Dairy Cows

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ABSTRACT

This study was conducted to evaluate the influence of blood metabolites (glucose and urea) and milk yield on fertility of local non–descript dairy cows. For this purpose, blood samples were collected from one hundred cows on day 1 (pre–insemination) and day 21 (post insemination). Data pertaining to age, body condition score, milk yield and fertility attributes of selected animals were recoded using a structured questionnaire. Contrary to non–pregnant cows, the pregnant animals demonstrated relatively lower blood glucose levels. Glucose concentration in the blood was directly proportional to body condition score. Blood urea concentration varied significantly between conceived and non–conceived groups. Non–pregnant group exhibited comparatively lower blood urea levels as compared to pregnant group. Different age groups displayed a non–significant disparity in terms of blood metabolites concentration. Difference in milk yield between the pregnant and non pregnant cows was statistically non–significant. This study validated that blood glucose, blood urea and milk yield could not be regarded as specific determinants of fertility in non–descript dairy cows.

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Livestock sector plays important role in the improvement of rural economy and its production is the best suitable fashion to enhance the revenue of the small farmers (Iqbal and Ahmad, 2002; Khan et al. 2013). The economic value of livestock production is predominantly attributed to the reproductive performance of animals. Nevertheless the indigenous cows of Pakistan are inferiorly ranked in terms of their fertility status when compared with internationally documented values. Reproductive disorders like anestrus, endometritis and repeat breeding have been implicated for negatively affecting the productivity as well as reproductive efficiency of cows, thereby instigating huge economic losses to dairy industry (Dutta et al., 1988). Moreover, the fertility of dairy cows can be influenced by several factors like nutrition, health, metabolic status, productivity and management (Clark et al., 2000; Mwaanga and Janowski, 2000; Roxstrom et al., 2001). Maintenance of optimal blood levels of different biochemical ingredients is vital for physiological adequacy of various body systems including the reproductive system (Azab et al., 1993). Modification in various biochemical constituents can provoke reproductive failure (Ahmad et al., 2004). Consequently, appraisal of blood biochemical profile could reflect metabolic perturbation, inappropriate nutrition and subclinical disease. Blood glucose and urea are considered as pivotal nutrients regulating the cyclicity in farm animals. Milk production is the primary emphasis as far as the dairy animals are concerned. Although increased milk yield is the most widely projected parameter, it can instigate mastitis, milk fever and negative energy balance. This project was designed to ascertain the impact of blood glucose, blood urea and milk yield on post–partum conception rate of indigenous dairy cows.

This study was conducted on one hundred indigenous non–descript cows with different age groups summarized in (Table 2), randomly selected from district Peshawar, Pakistan. Data pertaining to age, body condition score, milk yield and fertility attributes of animals were recoded by means of a structured questionnaire.

Body condition scores for all animals were documented following the method recommended by Peter and Ball (1987). Following this method, lumber and tail head area was measured and a score ranging from zero (very weak) to 5 (very fat) was assigned to each animal.

A total of 10 ml blood sample was obtained from the jugular vein of each animal on day 1 (pre–insemination) and day 21 (post–insemination). The blood samples were subsequently placed in sterilized test tubes and allowed to clot at room temperature. Later on the tubes were kept in an icebox and transported to the laboratory. Next, the samples were centrifuged at 3000 rpm for 10 minutes. Following separation and labeling, the sera samples were stored at –20 ^{o}C for further analysis.

Blood glucose concentration was estimated through a commercial kit being operated on enzymatic colorimetric mechanism (Trinder, 1969). Blood glucose level was determined after enzymatic oxidation elicited by the catalytic action of glucose oxidase. The subsequent reaction of formed H_2O_2 with phenol and 4–amino antipyrine was catalyzed by peroxidase enzyme and it yielded guinoneimine. About 10 µL of sample or standard was properly mixed with 1000 µL of working reagent and then incubated at 37 °C for 10 minutes. Direct exposure to sunlight was precluded and the absorbance was quantified at 550 nm. The experiment was executed at 25–37 °C and colour stability was sustained for a period of 30 minutes.

Blood urea level was assessed using commercial kit governed by the principle that reagent containing salicylate and hypochlorite reacted with ammonium ions to yield a green coloured complex (called 2.2 dicarboxylindophenol). The procedure involved mixing of 10 µl of sample with 1ml of the working reagent followed by incubation at 37 °C for the 3 minutes. The result was computed by quantifying the standard and sample against reagent blank within 2 hours.

The data were subjected to statistical analysis using statistical package for social sciences (SPSS, version–20)Simple Independent t–test considering maximum level of significance is 0.05 was employed for the assessment of conception groups (pregnant and non–pregnant) and body condition score (moderate and high) . The effect of age on blood metabolites (glucose and urea) and milk yield was evaluated as per the method of Steel and Terrie, (1980) as mentioned above. Briefly, the independent t–test is employed where we want to compare the population mean with sample means. It is also assumed that population is normally distributed with mean µ and variance σ^2

The influence of blood glucose level on conception rate has been illustrated in Table 1. It is evident that the correlation coefficient between blood glucose level and conception rate was statistically non–significant (p>0.01) on day 1 and day 21. Pregnant cows exhibited relatively lower blood glucose concentration in contrast to non–pregnant cows on day–1 (41.22±0.79 mg/dl and 40.88±1.07 mg/dl respectively). Similarly the blood glucose content measured on day 21 (post–insemination) was higher in non–pregnant group (41.86±0.97 mg/dl) as compared to pregnant group (40.15±1.24 mg/dl).

Blood urea concentration varied significantly (p≤0.01) between conceived and non–conceived groups (Table 1). Blood urea level was considerably lower in non–pregnant group as compared to pregnant group on day 1 (34.19±1.41 mg/dl vs. 40.34±2.00 mg/dl). Mean values of blood urea concentration for non–pregnant and pregnant groups on day 21 were 36.18±1.42 mg/dl and 42.22±2.19 mg/dl respectively.

Table 1: Descriptive statistics and t–test for variation in blood metabolites and milk yield for two groups in local (non–descript) dairy cows

Parameters	Groups	Mean±S.E	P– value
Urea (day 1)	Negative	34.19±1.41 mg/dl	0.012
	Positive	40.34±2.00 mg/dl	
Urea (day 21)	Negative	36.18±1.42 mg/dl	0.019
	Positive	42.22±2.19 mg/dl	
Glucose (day 1)	Negative	41.22±0.795 mg/dl	0.796
	Positive	40.88±1.07 mg/dl	
Glucose (day 21)	Negative	41.86±0.97 mg/dl	0.284
	Positive	40.15±1.24 mg/dl	
Milk yield	Negative	5.79±0.17 Lit./day	0.320
	Positive	5.48±0.27 Lit./day	

Negative = Non–Pregnant, Positive = Pregnant group

Table 2: Effect of various parameters on the blood metabolites in local (non– descript) dairy cows

Age (year)	No. of animals	Average Values of blood metabolites Urea (day 1) mg/dl	Glucose (day 1)mg/dl	Glucose (day 21)mg/dl	Urea (day 21) mg/dl
Younger (≤6 year)	19	31.45	42.52^b	42.91	32.04
Moderate (7 year)	23	41.27	41.46^a	42.00	40.89
Mature (8year)	24	38.22	37.82^b	42.06	37.28
Adult (9 year)	34	34.77	41.79^a	40.81	38.63
P–value		0.142	0.043	0.850	0.376
Body condition score (1–5)					
Moderate (2–3)	41	36.92	40.13	40.36	38.44
High (>3)	43	36.61	41.43	46.85	28.42
P–value		0.398	0.414	0.433	0.069

The estimated milk yield of pregnant and non–pregnant cows was 5.79 and 5.48 liters/day respectively. Difference in milk yield between the pregnant and non pregnant cows was statistically non–significant (P=0.320), as shown in Table 1.

The blood glucose levels of younger (42.52 mg/dl), moderate, (41.46 mg/dl) mature (37.82 mg/dl) and adult (41.79 mg/dl) cows significantly varied when measured on day 1. Mature animals (8 years of age) possessed the lowest level (37.82 mg/dl) of blood glucose as compared to other age groups on day 1 as shown in Table 2. However the blood glucose concentrations measured on day 21 (post–insemination) indicated the lack of a distinct variation among various age groups.

Different age groups demonstrated a non–significant variation in terms of blood urea levels as measured on day 1

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and day 21. The blood urea concentration manifested an increasing trend in animals less than 7 years of age. While blood urea concentration progressively declined for cows older than 7 years of age. Animals belonging to moderate age group (7 years) possessed the highest (41.27 mg/dl) blood urea concentration as compared to young (≤6 years) animals (31.45 mg/dl).

Blood glucose concentration was directly proportional to BCS on day 1 and day 21. Cows with moderate BCS were having relatively lower blood glucose levels (40.13 mg/dl VS 40.36 mg/dl) as compared to cows with high BCS (41.43 mg/dl VS 48.65 mg/dl). Contrary to cows with high BCS, moderate BCS–possessing cows exhibited a consistent trend in blood glucose levels on day 21. But the difference was statistically non significant.

The average blood urea level on day 1 for cows with moderate BCS was 36.92 mg/dl as compared to high BCS–possessing cows (36.61 mg/dl). However mean blood urea concentration of cows with moderate BCS, was comparatively greater (38.44 mg/dl) than cows with high BCS (28.42 mg/dl) as measured on day 21 post–insemination.

According to Table 3, A strong and positive correlation (P<0.000) existed between the blood glucose concentrations measured on days 1 and 21 (r = 0.426).. Blood urea levels calculated on days 1 and 21 also correlated positively (r = 0.505) which was statistically significant (P<0.000). Blood urea level on day 21 negatively correlated with blood glucose concentration (r = – 0.512) on day 21. Strong negative correlation was also noted between age and BCS (r = – 0.237). Blood urea level on day 1 and blood glucose concentration on day 21 demonstrated a weak negative association (r = – 0.216). Blood urea concentration on day 21 and blood glucose concentration on day 1 displayed a weak negative correlation (r = – 0.232). Finally the blood urea level estimated on day 21 also exhibited a weak negative correlation with milk yield (r = – 0.190).

Table 3: Correlation among various parameters of local non–descript dairy cows

Parameters	Glucose (day 1)	Urea (day 21)	Glucose (day 21)	Milk yield	BCS	Age
Urea (day 1)	– 0.159 (0 .115)	0 .505 (0.000)	– 0.216 (0.031)	– 0.113 (0.261)	– 0.136 (0.177)	– 0.066 (0 .950)
Glucose (day 1)	––	– 0.232 (0.020)	0 .426 (0 .000)	0.073 (0.468)	0 .074 (0.467)	– 0.072 (0 .474)
Urea (day 21)	––	––	– 0.512 (0 .000)	– 0.190 (0.059)	0 .133 (0 .186)	0 .155 (0 .123)
Glucose (day 21)	––	––	––	0.178 (0.076)	0 .164 (0 .103)	– 0.103 (0 .307)
Milk yield	––	––	––	––	0 .145 (0 .150)	– 0.067 (0 .509)
BCS	––	––	––	––	––	– 0.237 (0.018)

The conception rate of dairy cattle is considered to be directly or indirectly affected by blood metabolite profile, lactation and feeding status of animals. Nevertheless the association between nutrition and reproduction is complicated and not precisely understood (Boland and Lonergan, 2003).

Different cows vary in terms of their partitioning of energy for various body functions (Veerkamp, 1998). This partitioning of energy depends upon the age, breed and physiological status of cows. The lowest blood glucose concentration of mature animals could be caused by the shifting of more glucose towards the reproductive tract to ascertain reproductive performance. While young and older cows tend to be relatively less active in terms of their reproductive performance. Young animals having relatively underdeveloped endocrine function exhibit inadequate partitioning of nutrients and energy (Wathes *et al.*, 2007). These results signify the deployment of more glucose for reproductive activity with increasing age, sparing little for general circulation. Nevertheless with further increase in age, this utilization is diminished shifting more glucose towards general circulation. Although carbohydrate containing feed constitutes the primary source for the derivation of glucose, animals receiving carbohydrate deficient feed synthesize glucose through the mechanism of gluconeogenesis. Therefore cows with high BCS revealed comparatively high blood glucose levels owing to ample adipose tissue content. While blood glucose concentration has been deemed as a definite marker for the impact of nutrient intake on reproductive performance, literature data indicate conflicting evidences. High pregnancy rates have been documented in cows exhibiting high plasma glucose levels (Westwood et al., 2002). However, results of the current study illustrated that mean glucose concentrations of pregnant and non–pregnant cows did not significantly (p>0.05) fluctuate on day 1 and day 21 post–insemination. These results are in compliance with the findings of a previous study demonstrating a non–significant difference in blood glucose levels of conceived and non–concieved cows (Dehkordi et al., 2012). Ahmad et al., (2004) found the lack of considerable variation between cyclic and non–cyclic crossbred cows in terms of blood glucose concentration. Comparable results were also observed by Russel and Wright (1983), who reported that assessment of plasma glucose is not valuable in estimating the energy status of cows at various stages of the reproductive cycles. Moreover, intravenous infusion of glucose did not improve postpartum rebreeding in beef cows (McCaughey, 1988). Kenny et al. (2002) found that blood glucose concentration did not vary with the stage of estrous cycle. Similar results have also been reported by Jordan et al. (1983). Hayhurst et al. (2007) and Kadivar et al. (2012) found no relationship between blood glucose concentration and reproductive performance of cows. Threshold value of blood glucose concentration is critical to regulate reproduction. Even though sub–threshold level of blood glucose may instigate serious consequences (such as anoestrus) but exceeding the threshold level does not impart any extra benefit. The intrinsic tendency of animals to uphold relatively steady glucose concentrations in the blood may elucidate the lack of a dependable correlation between circulating glucose concentrations and reproductive performance.

Generally protein metabolism yields ammonia that is converted into microbial proteins by the ruminal microbes. However the provision of energy deficient feed saturates microbial protein synthesis thereby elevating the circulating ammonia concentration that is subsequently converted into urea by the liver (Carlsson and Pehrson, 1994). Variation in the level of blood urea could be attributed to altered feed intake and metabolic rate of young, mature and older animals. Moderate animals exhibit greater feed intake for the sake of maintenance and physiological attributes. Therefore these animals generate more urea as a result of protein catabolism. Young animals require comparatively less feed intake and thereby generate less amount of urea. Pregnant and non–pregnant cows exhibited significant difference in terms of blood urea concentration on mentioned days. These results are consistent with an earlier study demonstrating slightly elevated blood urea levels in crossbred dairy cows during early gestation (Alameen and Abdelatif, 2012). Moreover, slightly elevated blood urea levels did not adversely affect the plasma progesterone concentration, fertilization rate and embryo survival (Garcia–Bojalil et al., 1994; Kenny et al., 2002; Gath et al., 2012).

Milk yield being a multifactorial trait, is dependent upon genetics, breed, age, parity, stage of lactation, season, health status and nutritional as well as managemental factors. High milk yield has been linked with reduced fertility (Berger et al., 1981) and prolonged calving interval in cows (Oni et al., 2001). Conception failure in cows with high milk yield could be attributed to the antagonistic action of relatively enhanced level of prolactin on GnRH. The frequency and amplitude of GnRH secretion undergo diminution as a result of high level of prolactin (Senger, 2003). During lactation, glucose is primarily converted to lactose which predisposes the animal to negative energy balance thus diminishing the likelihood of conception (Bisinotto et al., 2012). But present study involved low milk–producing animals, therefore no significant correlation was found between milk yield and conception rate. Previous studies also failed to clearly establish any definite relationship between milk yield and conception rate (Fonseca et al., 1983; Eicker et al., 1996: Pryce et al., 2004).

The negative correlation observed between blood glucose and blood urea designates the extent of gluconeogenesis and ureagenesis. Proteins if subjected to gluconeogenesis increased blood glucose concentration but diminished blood urea level. While lack of appreciable gluconeogenesis in cows with optimal blood glucose levels resulted in the pre–eminence of ureagenesis. Gradual improvement occurred in BCS with increasing age due to progressive enhancement of fat deposition.

CONCLUSIONS

The partitioning of nutrients and energy being variable in different cows is governed by their age, endocrine function and physiological status. The blood glucose concentration of dairy cows remains under tight control in spite of undergoing slight fluctuation as result of nutritional and physiological modifications. Variation in blood urea levels of different cows could be attributed to altered feed intake and metabolic rate. No significant correlation exists between milk yield and conception rate in low–producing dairy cows. This study substantiated that blood glucose, blood urea and milk yield could not be considered as absolute determinants of fertility in non–descript dairy cows. Furthermore, the effect of other variables like environmental factors, parity, feed composition and hormonal profile should also be taken into account through the execution of additional studies.

CONFLICT OF INTEREST

There is no conflict of interest.

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