

MEASUREMENT OF BLOOD UREA NITROGEN (BUN) LEVEL AND ITS EFFECT ON CONCEPTION RATE IN DAIRY COWS AT DISTRICT ABBOTTABAD, PAKISTAN

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ABSTRACT: Milk production and reproductive efficiency are significant indicators for the grading of dairy cows. Native breeds of the Pakistan having 4- 8 liters milk production per day with average reproductive efficiency. Productive and reproductive performance is mainly dependent upon the balanced feeding. Mainly animals of the remount area are deficient in energy and protein diets. They also need a balanced ration to give better output. However, exotic breeds of high yielder dairy cows have been introduced both on small and large scale dairy farming. These breeds have very optimized feeding plan. Balanced animal nutrition is of vital importance for good metabolic and reproductive output in dairy cows. Best calving interval of one year is profitable for dairy farmer. Genetics and environmental factors are associated for good performance of dairy animals. One major metabolic product blood urea nitrogen (BUN) or plasma urea nitrogen (PUN) have been addressed to affect predominantly the reproductive efficiency of the dairy cows. Blood urea is a product of protein metabolism. Supplementary nitrogen is provided in the animal feed to fulfill protein need. As a result of nitrogen utilization, urea is produced that is absorbed in the blood stream. To investigate the effect of blood urea in dairy cows, 100 samples of blood from four groups of dairy cows i.e. C1, C2, C3 and C4 have been analyzed for blood urea analysis. Significant variations between the four groups of lactating cows have been noticed for BUN levels. With a mean BUN level of 17.78 mg/dL, group C1 has the highest level, group C2, with a mean BUN level of 16.47 mg/dL, the mean of group C3 is 16.99 mg/dL. While the mean of group C4 is 17.61 mg/dL, reveals a balanced protein status. Individual cow responses to nutritional intake vary widely, as seen by the standard deviations, which reveal that Group C2 has the most variability (3.78) and Group C4 has the lowest variability. Reproductive performance in terms of conception rate (CR) and successive pregnancy has been found highest in group C4 probably due to proper metabolic output in terms of BUN lower than detrimental level of 20 mg/dL. Optimized protein feeding is important for good conception rates. Moreover, over protein feeding will also increase the feeding cost.

Key words: Blood Urea Nitrogen, dairy cows, conception rate, reproductive efficiency.

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INTRODUCTION

Milk production and reproductive efficiency are important parameters for the assessment of the grading in dairy cows. Genetics and environment are significantly important for profitable dairy farming. Expression of genetic traits is dependent upon the environmental and managerial factors. Ideally one year (365 days) is best calving interval for a profitable dairy farming. Many factors influence the Fertility rate in cattle, including genetics, management practices, environmental conditions, and nutritional status (Michael *et al.*, 2019). Balanced animal nutrition is main managerial input for the good performance of the animals by putting their metabolic process in proper line. Proper nutrition around breeding time is essential for dairy animals because they have to maintain their health, produce milk, increasing their live weight. Ensuring they receive the right nutrients is crucial for them to stay healthy, come into heat sooner,

and exhibit stronger heat signs. Cow management in the first 30 days post-calving plays a vital role in fertility. Additionally, the nutrition of dry cows significantly impacts fertility, as the egg for the next pregnancy develops during this period. Providing the balanced feeding at these critical times can make a substantial difference in your herd's reproductive success.

Two main feeding ingredients are energy and protein. Both these affect productive and reproductive efficiency of the cattle. Reproductive efficiency of the cattle is a key factor of profitability in dairy farms (Santos *et al.*, 2013). Protein metabolism is one of the important elements that affects reproductive performance. (Sammad *et al.*, 2020). Nitrogen is provided in the diet of dairy cow generally in the form of protein. Extra dietary protein that the cow doesn't require is often broken down into ammonia in the rumen, ammonia is hazardous to mammalian tissues thus the liver quickly changes it into urea. This urea is then released into the bloodstream,

which can be measured as plasma urea nitrogen (PUN). The term "blood urea nitrogen" (BUN) is frequently used to refer to the urea that circulates in the blood circulation and is quantified as urea nitrogen in either plasma (PUN) or serum fractions (Lavery and Ferris, 2021).

The level of PUN or BUN indicates the balance between protein intake and nitrogen excretion, offering valuable insights into the animal's nutritional status (Marín-García *et al.*, 2022).

The performance of dairy cows with respect to reproductive efficiency and protein intake have been linked in several studies; nevertheless, feeding dairy cows too much protein may be harmful to their fertility (Butler *et al.*, 2000). Conception and start of pregnancy involve an organized series of interconnected processes involving all of the reproductive tracts like tissues follicular development leading to ovulation, fertilization of the oocyte, transportation and development of the embryo, maternal recognition, and implantation. Theoretically, interference at one or more of these processes by ammonia, urea, or another harmful byproduct of protein metabolism could reduce the effectiveness of cow reproduction (Izquierdo *et al.*, 2021). Over the past spans, decreasing cattle fertility rate has been interconnected with nutritional strategies (Łakoma *et al.*, 2023). Among the various physiological parameters that influence fertility, plasma urea nitrogen (PUN) has emerged as a significant marker of metabolic status with potential implications for reproductive outcomes (Olorunkoya, 2022). Cows fed excess dietary protein had increased blood urea levels that altered uterine fluid composition, decreased uterine pH, and reduced conception rates (Mekuriaw, 2023). Plasma progesterone concentrations were reportedly lower in cows fed with high dietary protein. In relating dietary protein degradability to fertility, reported that blood urea nitrogen concentrations exceeding 20mg/dL were associated with reduced conception rates in lactating cows (Chaveiro *et al.*, 2011). Consequently, monitoring of BUN levels is important for dairy farmers to correctly formulate protein feeding to enhance cattle health, productivity, and reproductive performance (VanDerWal *et al.*, 2020). BUN measurements have given researchers a helpful marker for examining the relationship between dietary protein metabolism and reproductive effectiveness (Lu *et al.*, 2020). Understanding the relationship between blood urea nitrogen levels and conception rates is paramount for effective herd management. This study aimed to assess the relationship between BUN level and cow fertility status.

Objectives of the research study

1. To evaluate blood urea nitrogen level in lactating cows.
2. To find out the effect of blood urea nitrogen levels on conception rate.

MATERIALS AND METHODS

Proposed study was carried out at district Abbottabad. Blood samples were collected from dairy cows and different localities of district Abbottabad

Sample Size: Randomly one hundred (100 Number) blood samples were collected from dairy cows before their breeding through artificial insemination. Feeding, breeding and management history was collected from the farmer.

Sample Collection: Using sterile disposable syringes aseptically, approximately 3 ml of blood was collected from the jugular vein of each selected animal. The syringe and collection tube were marked using a permanent marker for identification of the sample. Before inserting the sterile needle of the syringe, the area was disinfected properly using 70% alcohol. Blood was collected in a vacutainer tube and taken to Veterinary Research & Disease Investigation Center (VR&DIC) Abbottabad under proper shipment procedure. The blood samples were allowed to clot by placing the vacutainers overnight at room temperature. The next day serum was separated and analyzed. The samples were refrigerated at -20 °C until analyzed.

Sample analysis: Other than fresh blood samples, frozen samples were thawed before use. The blood chemistry analyzer model URIT-800 Vet was setup according to the manufacturer's instructions and BUN test was done by using Merck's Blood Urea Nitrogen kit and method was employed as per below manufacturer's instruction:

Absorbance Wavelength: 340nm

Working temperature of machine: 37 °C

Blank measurement: against distilled water

Reaction mixture preparation: Following reaction mixture was prepared using different chemical components of reagent 1 (4 volumes) with reagent 2 (1 volume) as shown in table 1 below.

Table 1: Reaction mixtures

Reagents	Sample	Standard
Reaction mixture	1000µl	1000µl
Standard	-	10µl
Sample	10µl	-

Readings were noted through a Semi-Automatic Uret Analyzer.

Statistical analysis of the data: Data was properly recorded on a computer in Microsoft Excel sheets during the analysis of the samples and analyzed through Chi square test.

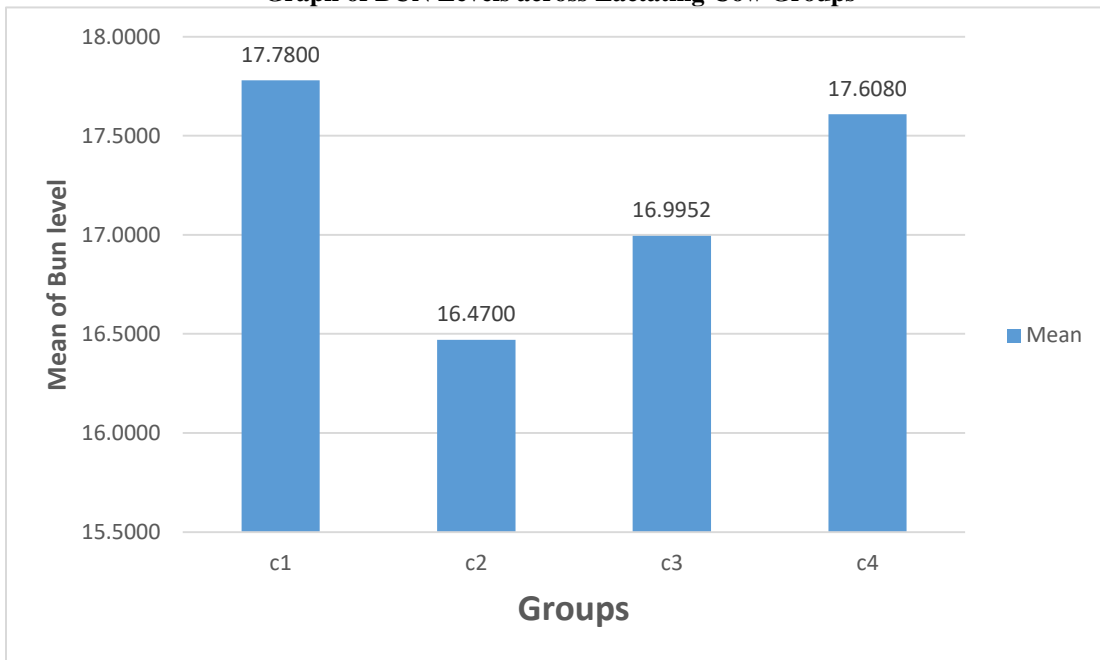
RESULTS AND DISCUSSION

Table 2: Evaluating Blood Urea Nitrogen (BUN) levels in lactating cows

Group	Mean BUN Level (mg/dL)	Sample Size (N)	Std. Deviation
C1	17.78	25	3.46
C2	16.47	25	3.78
C3	16.99	25	3.36
C4	17.61	25	3.08
Total	17.21	100	3.42

Significant variations between the four groups of lactating cows have been noticed for BUN levels. With a mean BUN level of 17.78 mg/dL, group C1 has the highest level, group C2, with a mean BUN level of 16.47 mg/dL, the mean of group C3 is 16.99 mg/dL. While the mean of group C4 is 17.61 mg/dL, reveals a balanced protein status. Individual cow responses to nutritional intake vary widely, as seen by the standard deviations, Group C2 has highest variability (3.78) and Group C4 has the lowest variability (3.08).

Graph of BUN Levels across Lactating Cow Groups



The graph illustrating mean BUN levels across the four groups presents a clear comparative analysis of nitrogen metabolism among lactating cows. Group C1 has the highest mean BUN level of 17.7800 mg/dL. Group C2 shows the lowest mean at 16.4700 mg/dL, suggesting optimal nitrogen utilization, which may be useful both for health and fertility outcomes. Group C3, with a mean of 16.9952 mg/dL, comes between group C2 and C1, indicating a moderately efficient nitrogen status, while Group C4, with a mean of 17.6080 mg/dL, approaches group C1's levels, suggesting that multiparous cows may experience similar metabolic challenges

although with good conception rate with better optimal protein utilization. Two groups of animals were made based upon BUN levels. One group with BUN level 11.18.5 mg/dL and was comprising 47 cows. In this group pregnancy was recorded for 45 cows with an impressive pregnancy rate of 96% while there were two non-pregnant cows. Similarly, other group was comprised of 53 cows with BUN level 19-24.5 mg/dL. There was no pregnant animal showing 100 percent non conception rate. There is need to investigate further details of the ration compositions offered to dairy animals with different protein levels.

Table 3: Analyzing the Effect of BUN Levels on Conception Rate

BUN Category	Pregnant (1)	Not Pregnant (0)	Total	Chi-Square test
11-18.5 mg/dL	45	2	47	0.01
19-24.5mg/dL	0	53	53	
Total	45	55	100	

The analysis of BUN levels categorized into two groups (11-18.5 mg/dL indicating pregnancy in 47 cows, while, there were 53 non pregnant cows with blood urea level 19-24 mg/dL. Specifically, 45 out of 47 cows in the lower BUN category were pregnant, demonstrating an impressive pregnancy rate of 96%, while none of the 53 cows in the higher BUN category achieved pregnancy. Similar findings were reported by (Chaveiro *et al.*, 2011). This reflects that elevated BUN levels may negatively affect reproductive performance due to factors such as excessive protein intake, metabolic stress, or inadequate nitrogen utilization. It is also available information from different research studies that dairy cows may have a 43% reduced chance to conceive as a result of artificial having blood urea level above 7.0 mM or 19.3 mg/dL as reported by (Amundson *et al.*, 2015). These findings reflect the importance of maintaining optimal BUN levels in the lower range to enhance fertility outcomes, indicating a need for targeted nutritional management to support reproductive health in lactating cows. Overall, this analysis highlights the critical role of BUN levels in influencing conception rates, providing valuable insights for improving dairy herd fertility through better dietary strategies. (Sinclair *et al.*, 2000). The chi-square test results shows a significant association between BUN levels and conception rates at the time of artificial insemination to these lactating cows and a Pearson Chi-Square p-value of 0.013 indicates that cows with lower BUN levels (11-18.5 mg/dL) are more likely to be pregnant compared to those with higher BUN levels (19-24 mg/dL). This significant difference suggests that elevated BUN levels are linked to lower conception rates, indicating critical role of the nitrogen metabolism in reproductive performance of the dairy cows. These same findings have been reported by (Amundson *et al* 2015). Overall, these findings emphasize the need for careful nutritional management to maintain optimal BUN levels in dairy cows for improvement of fertility outcomes in lactating cows. Both high and low circulating urea concentrations, a product of protein metabolism, are associated with decreased fertility in dairy cows through poorly defined mechanisms. However, many researchers like (Amundson *et al* 2015), are suggestive that high level of feeding protein affects negatively to the uterine environment by altering the ionic composition of uterine fluid during the luteal phase ultimately decreasing fertility. Since the early bovine embryo cannot adapt to changes in the uterine environment, changes in the concentrations of ions (pH) in the uterus can be unfavorable to embryo development and survival therefore having negative effects on fertility. The present research study has been focused upon systemic circulatory concentrations of plasma urea nitrogen (PUN) it is presumed that high level of protein in a ration will elevate BUN to a level of concentration that has previously been reported to be negatively affecting

conception rate in dairy cows. The rate of involution and the endometrial ability to mount an adequate innate immune response after calving are both critical for subsequent fertility. Some researchers like (Mekuriaw, 2023). Overall, these findings emphasize the significance of managing dietary protein and nitrogen intake to enhance reproductive success in lactating cows indicating a potential excess of nitrogen or inadequate protein utilization, which could impair metabolic efficiency and reproductive performance reveals a strong correlation between lower BUN levels and higher conception rates in lactating cows. Furthermore, it is possible that circulating urea concentration is correlated to change expression levels of many genes in the endometrium shortly after calving. These gene expressions may be associated with associated with lipid metabolism, repair of tissue and innate immunity in the cows as mentioned by (Zhangrui *et al.*, 2015). Correlating the findings of the present research with findings of the related researchers, group C1, has highest BUN level and it may have problems with nitrogen metabolism or excess protein intake Group C2 implies effective nitrogen utilization but may also indicate insufficient protein under poor milk production.

Conclusion and Recommendations: Keeping in view the findings of the present study, it is concluded that high protein feeding in animal rations may affect negatively to the conception rate and hence reproductive performance of the dairy animals. Excess protein feeding is not only uneconomical feeding but it results poor conception rate which is much more economic loss to dairy farmer. The goal of a dairy farmer is to maintain good calving interval of not more than 400 days is mainly dependent upon good nutrition plan are important. It is recommended that balanced feeding especially protein and energy for dairy animals is utmost important. To prevent high blood urea levels, optimized protein level in the feed is important. This will also prevent wastage and economic loss of the dairy sector.

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