Low dietary intake altered the serum biochemical profile of cross breed heifers eliciting irregular estrous cycle

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Abstract: In Bangladesh, under feeding or over feeding is one of the major concerns to overcome the reproductive difficulties (anestrus) of cattle. The information on standard feeding using available local feed ingredients could be helpful to overcome the problem. The study was conducted to determine the influence of dietary intake on serum biochemical profile which prompts the estrous induction of cross breed heifers. A total of 12 of cross breed heifers were randomly selected having same age and body weight which were divided into three uniform groups namely T0 (Control or standard feeding), T1 (40% restricted or less feeding) and T2 (60% restricted feeding) were fed green grasses, concentrate and adlibitum water after frequent deworming. The blood samples were collected aseptically from jugular veins every 15 days intervals of post treatment. The blood sera were used to analyze the biochemical profile using available commercial kits with standard methods. The animals of T1 and T2 groups were found significantly (p<0.01 and p<0.05) low glucose, total protein, calcium and iron values. On the other hand, sera phosphorus, manganese and cobalt level were found significantly (p<0.05) decrease in the animals of T2. In addition, no significant changes were found in serum zinc and copper values. The heifers of T0 exhibited regular estrous whereas the heifers of T1 and T2 showed irregular estrus signs followed by anestrus. In this study, it is revealed that standard feeding is very important to maintain the biochemical profile and estrus induction in heifers.

Key words: Nutrition, blood, heifers, calcium, phosphorus and iron.

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I. Introduction

Livestock has a direct impact in our national economy about 6.5% of total gross domestic product (GDP) and about 13% of total foreign exchange earnings (GoB, 1991) by supplying egg, meat, milk as animal protein, provides bio-fertilizer and a source of draught power. In Bangladesh, dairy farm is generally very small often having only one lactating cow. But reproductive failure or anestrus of dairy cattle is one of the major concerns in all over the country, which causes a huge economical loss to the dairy farmers. There is a report that anestrus cows had the lowest P, Zn and Fe, the highest Mn levels and subnormal Cu level in whole blood (Prasad and Rao et al., 1997, Kumar and Sharma, 1991). Moreover, total protein, cholesterol, glucose, calcium, inorganic phosphorus, sodium, chloride, potassium, alkaline phosphate and SGOT were significantly lower in the anestrous cows than those in the normal cycling counterparts (Arosh et al., 1998). For development and functions of reproductive organs, feeding of balanced ration is of utmost importance, because the most field cases of reduced fertility or sterility are of nutritional origin (Singh and Vadhare, 1987). To breed regularly, the animal has to have active ovaries, regular oestrus signs, and ability of mating, high conception rate and sustain embryonic growth (Hidiograll, 1979; Duttaet al., 1988). In the past, researchers were found that the ovaries remain inactive basically due to a lack of follicle stimulating hormone from the anterior lobe of the pituitary gland. This may be predisposed by nutritional, hormonal, pathological, genetical, or environmental factors, singly or in combination each other (Palmer et al., 1935; Durrell, 1951 & 1955; Eckleset al., 1935). Improper feeding cause minerals deficiency, especially phosphorus may predispose cattle to anestrous indirectly by triggering inappetence or pica. It is imperative to establish how changes in nutrition, whether of short or long term duration, specifically affect the cattle’s serumbiochemical-profile during estrous cycle and anestrous condition. This study was conducted to determine the serum biochemical status of cattle in order to formulate a standard dairy feed which could able to maintain regular estrous cycle as well as the fertility rate.
II. Materials and Methods

Experimental Animals
About 2.0 to 3.0 years of age a uniform group of 12 heifers were selected from a herd. The animals were born and reared under farm condition. They were treated with 4 fortnightly concurrent doses of anthelmintic for nematodes and trematodes. Napier grass (*Pennisetum purpureum*) and Para grass (*Brachariamutica*) were given to all animals following the instructions given by Banerjee (1998). Afterwards when all experimental animals was achieved asimilar body condition score (BCS), the experimental animals were divided randomly into three groups named T<sub>0</sub> (Control feeding), T<sub>1</sub> (40% restricted feeding) and T<sub>2</sub> (60% restricted feeding). A standard concentrate mixture and green grasses were given daily to the animals of T<sub>0</sub> as per recommendation of the thumb rule of Banerjee (1998). Water was made available to each animal at all time in a separate container. Animals in T<sub>1</sub> and T<sub>2</sub> groups were fed with 40% and 60% restricted (less) feeding, respectively. About 8 kg green grass + 3 kg concentrate (as standard feeding). 4.8 kg green grass + 1.8 kg concentrate (as 40% restricted feeding) and 3.2 kg green grass + 1.2 kg concentrate (60% restricted feeding) of high quality feeds were fed to each animals of T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> per day, respectively.

Blood collection
Approximately 5 ml of blood samples were collected aseptically from the jugular vein of experimental heifers without anticoagulantsevery 15 days interval (at day 0, day 15, day 30, day 45, day 60, day 75, day 90 and day 105). The serum samples were separated and stored at -20°C for minerals estimation.

Biochemical profile analysis
The stored serum sample were used for analysis of glucose and total protein according to the description of Teitz (1986), and other trace minerals (Zn, Cu, Mn and Co) using atomic absorption spectrophotometer [Perkin Elmer Analyst 100, USA] as per recommendation of commercial kits of Human Gesellschaft fur Biochemica and Diagnostica GmbH, Wiesbaden, Germany. The values of serum calcium, phosphorus and iron was determined by semi-automatic analyzer (3000 evolution analyzer, UK, code RM 4030) that completely managed by micro-processor and equipped with a big graphic display using commercially available kits (Vitro Scient, Medical device safety Services, MDSS GmbH, Hannover, Germany). All tests were performed in the department of Pharmacology Laboratory, Sylhet Agricultural University, Bangladesh and calculation was analyzed automatically with printed out results directly bearing measuring units.

Heat detection/ Determination of estrus cycle
Estrus cycles of the experimental heifers were determined by observing the signs of estrus followed by established estrus signs of cattle during the study period.

Statistical Analysis
The data were subjected to statistical analysis by logistic regression with the help of statistical software program STATA 8.0 and unpaired one tailed student t-test using Microsoft Excel-2010.

III. Results

Among the biochemical profile, all parameters were measured and the experimental animals were showed approximately equal values before starting the restricted feeding. The values of biochemical profile were varied after treatment with time. A detail report on biochemical profiles are shown in table 1.

**Effects of dietary feeds on serum glucose and total protein level of cross-bred heifers**

The heifers of restricted feeding (T<sub>1</sub> and T<sub>2</sub>) were found significantly (p<0.01) decrease glucose and total protein compared to control (T<sub>0</sub>) feeding animals. The values of glucose (mg/dl) were 55.62, 30.23 and 25.12 in the animals of T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> respectively. The total protein (g/dl) was found at a level of 6.33, 4.26 and 4.01 in the animals of T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> group respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T&lt;sub&gt;0&lt;/sub&gt; (Mean±SE)</th>
<th>T&lt;sub&gt;1&lt;/sub&gt; (Mean±SE)</th>
<th>T&lt;sub&gt;2&lt;/sub&gt; (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>55.62±1.80</td>
<td>30.23±2.11</td>
<td>25.12±1.34</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.33±0.18</td>
<td>4.26±1.07</td>
<td>4.01±1.02</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>13.07±0.38</td>
<td>8.73±0.85</td>
<td>7.03±0.73</td>
</tr>
<tr>
<td>Inorganic Phosphorus (mg/dl)</td>
<td>6.95±0.77</td>
<td>5.24±0.43</td>
<td>4.82±0.79</td>
</tr>
<tr>
<td>Iron (µmol/L)</td>
<td>30.33±1.13</td>
<td>18.02±2.21</td>
<td>14.31±1.96**</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>1.04±0.05</td>
<td>1.0±0.03</td>
<td>0.9±0.01</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>0.9±0.05</td>
<td>0.8±0.01</td>
<td>0.8±0.08</td>
</tr>
<tr>
<td>Manganese (ppm)</td>
<td>1.14±0.05</td>
<td>0.9±0.04</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td>Cobalt (ppm)</td>
<td>1.13±0.41</td>
<td>1.03±0.31</td>
<td>0.7±0.22</td>
</tr>
</tbody>
</table>

** indicate 99% significant level (p<0.01)
* indicate 95% significant level (p<0.05)
Nutritional impacts on serum trace minerals of cross-bred heifers

Initially the serum calcium in animals of all treatment groups (T₀, T₁ and T₂) was approximately similar. At the end of the experiment (day 105), the average values of serum calcium (mg/dl) in animals of T₀, T₁ and T₂ groups were found at an amount of 13.07, 8.73 and 7.03, respectively (Figure 1). The serum calcium decreased significantly (p<0.01) in animals of T₁ and T₂ compared to the animals of T₀ (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Average values of serum calcium (mg/dl) in different treatment groups. The calcium level declined with days of post treatment in T₁ and T₂ groups. On the other hand, the calcium values of standard feeding heifers remained stable with time.

In this study, the observed serum phosphorus levels (mg/dl) of cross-bred heifers were 6.95, 5.24 and 4.82 in animals of T₀, T₁ and T₂ group heifers, respectively on day 105 (Figure 2). The serum phosphorus decreased significantly (p<0.01) in animals of 40% restricted feeding (T₁) and 60% restricted feeding (T₂) compared to the animals in control T₀.

![Figure 2](image2.png)

**Figure 2.** Average values of serum phosphorus (mg/dl) in different treatment groups. The phosphorus level declined with days of post treatment in T₁ and T₂ groups. The values of phosphorus in standard feeding heifers remain stable with time.

The average amounts of serum iron (µmol/L) were 30.331, 18.025 and 14.312 in the heifers of T₀, T₁ and T₂, respectively (Figure 3). Most of the iron in the body bound with hemoglobin molecules in the red blood cells. Usually about 3-4% of iron found in myoglobin molecules and 30% of iron found to store as ferritin or hemosiderin in the spleen, bone-marrow and the liver in the body. Transferrin produced in the liver bound with one or two irons and used to move these stores of iron to areas where it might be required. The serum iron level decreased significantly (p<0.01) in animals of T₁ and T₂ with restricted feeding compared to animals of T₀ (Figure 3).
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Effects of nutrition on estrus cycle and anestrous condition of heifers

The experimental animals were observed for three months to determine the effects of nutrition on estrus and anestrous condition of heifers. The feeding of animals is very important to perform their physiological functions of the body. The highest percentage (89%) of heifers showed regular estrus sign in the animals of T₀ with standard feeding and the least percentage (32%) of estrus showed by the animals of T₂ group (Figure 4).

IV. Discussions

Randomly selected 12 cross bred heifers were divided into three uniform groups namely T₀, T₁, and T₂. The heifers of T₀ were fed with standard feeding i.e. no feeding control, the animals of T₁ were fed with 40% restricted or less feeding and T₂ were fed up to 60% restricted feeding. The blood samples were collected aseptically from jugular veins every 15 days intervals of post treatment. The biochemical profile was found marked decrease in the heifers of T₁ and T₂ which showed irregular estrus cycle. Standard feeding is very important for heifer to maintain their growth and reproduction. In heifers, nutritional deficiency causes various reproductive abnormalities. Estrus cycle is usually influenced by several factors like as nutrition, breed, environment etc. This study was determined the effects of standard daily feeding on serum minerals levels as well as estrus induction of heifers.

In the present study, significantly (P<0.05) high serum glucose level was observed in T₀ heifers which showed regular cycle than that of restricted feeding heifers. The blood glucose level has been acclaimed to be a reasonably accurate test to measure the energy status of the animal (Madan 1979). Presence of high serum glucose level in cyclic cows has been observed by the past researchers (Arosh et al. 1998; Singh and Singh 2005), while some researchers did not find any significant difference in serum glucose level between cyclic and
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anoestrous animals (Kumar and Sharma 1991). In the present investigation, the low blood glucose level observed in anoestrous heifers which received restricted dietary intake than cyclic heifers of standard feeding. This finding indicates that the poor energy status have co-relation with consequent infertile condition of the anoestrous cows. The values of serum total protein were decreased significantly in the 40% and 60% feeding restricted animals which showed anoestrous signs (Table 1 and Figure 4). The lower levels of energy and/or protein associate with the ovarian inactivity and anoestrus (Wiltbank et al., 1965) as the negative energy balance depresses the ovarian activity by inhibiting pulsatile LH release (Butler and Smith, 1989). The higher values of serum protein in cyclic cows than the anoestrous cows and heifers have been reported by previous researchers (Arosh et al. 1998; Singh et al. 2004; Muthukumaret al. 2004; Kumar et al. 2005; Singh and Singh 2005). Rowlands et al. (1980) reported that there was a direct relationship between concentration of globulin and number of services required for conception. The declined globulin concentration is suggested to be influencing the biosynthesis of gonadotropins and gonadal hormones in cattle (Singh et al. 2004). In general, the average serum-Ca of the normal bovine is around 10 mg/dl and the range of serum-Ca is 8.65-11.65 mg per 100 ml, or± 15 % around the mean (Alcroft and Green, 1934). Kumar and Sharma (1991) reported that there is no significant difference between serum Ca level of cyclic cows and anoestrous cows and heifers which corroborated to this finding. In contrast to this, higher serum Ca concentration in cyclic cows than that of the anoestrous cows and heifers has been observed by many researchers (Das et al. 2002c; Singh and Singh 2005). In the study, the value of calcium was found to decline with time in the animals of group T1 and T2 due to low dietary intake During the growing stage of animals, the blood calcium level may be completely depended on dietary source due to high metabolic activity. It has been revealed that serum calcium level decreased with the restricted feeding i.e. serum calcium level decreased with less intake of dietary feed. In the present study, significantly (p<0.01) declines serum phosphorus value was observed in animals of 40% restricted feeding (T1) and 60% restricted feeding (T2) which showed irregular estrous signs. The higher serum P concentration in cyclic cows than that of anoestrous cows was corroborated to the findings of many researchers (Arosh et al. 1998; Das et al. 2002c and Singh and Singh 2005). Hypophosphatemia adversely affects the cell functions, as P is an integral component of nucleic acid, nucleotides, phospholipids and some protein. Phosphorus is essential for transfer and utilization of energy, phospholipid metabolism and huge co-enzyme activation (Hurley and Doane1989). Inorganic P plays a major role in accelerating the process of ovulation and fertilization in cows (Stephan 1971). Hence, phosphorus is frequently associated with reproduction in cattle and its deficiency induces anoestrus and reduced ovarian activity (Pugh et al. 1985). Anoestrus, suboestrus, irregular and delayed sexual maturity have been observed due to P deficiency (Blood et al. 1994). Even a marginal deficiency of P was found to be sufficient enough to cause disturbances in pituitary-ovarian axis without manifestation of deficiency symptoms and might be a cause for inducing infertility (Das et al. 2002c). The calcium and phosphorus ratio may be maintained by body physiology from dietary source in growing animals. On the other hand, the serum iron level decreased significantly (p<0.01) in animals of restricted feeding (T1 and T2) compared to animals of T0 (Figure 3). The results of this study are consistent with the findings of Sumati and Kapoor (1986), who obtained significantly increased value for dietary iron treated Wister rats. Similar results were also reported by Wenzlif and Erhardt(1991), who observed the high values of iron dextran treated lambs. This finding is slightly similar to that of Begum et al., (2010). They found that restricted dietary feeding decrease the hemoglobin content of whole blood in cross breed heifers. Iron is one of the components of hemoglobin. So, the dietary source might influence to maintain the iron level in the blood stream. In addition, no significant differences were found among the values of zinc and cobalt in the restricted feeding animals throughout irregular estrous cycle as well as anoestrous condition was found in the experimental animals of restricted feeding. The anoestrous condition of heifers may develop due to nutritional deficiency. The findings of present study are similar to the findings of Anderson(1933), Phillips (1942), and Williams (1939). They found that deficiencies of energy (carbohydrate), protein, and water induce poor body condition and anoestrus in heifers. Cessations of estrus cycle in nutritionally induced heifers were reported by Stagg et al.(1995). It is revealed that the amount of feeds greatly influences to carry out the regular estrus and anoestrous condition of cattle but mechanism not well understood.

V. Conclusion

The result reveals that the farmers should give attention to the standard feeding which could be an effective way of increasing cattle production by conserving optimum serum biochemical profile for cattle in Bangladesh. It also concludes that dietary deficiency reflects on the biochemical profile of animals and a strategic supplementation is needed to the animals for exploitation of their genetic potential for optimum production and reproduction.

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Authors Contribution
S. Begum and M.M.R. Howlader are directly involved with the research work. Data analysis and the first draft of the manuscript have been written by S. Begum. Review and final correction of the manuscript were done by M.M.R. Howlader.

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