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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.Thestudy was conductedto determine the influence of dietaryintake on serum biochemical profile which prompts the estrous induction of cross breed heifers. A total of 12 of cross bred heifers were randomly selected bearing same age and body weight which weredivided into three uniform groups namelyT_0 (Control or standard feeding), T_1 (40% restricted or less feeding) and T_2 (60% restricted feeding)were fed green grasses, concentrate and adlibitum water after frequentdeworming. 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 T_1 and T_2groupswerefoundsignificantly ($p<0.01$and/or $p<0.05$)lowglucose, 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 T_2. In addition, no significant changes were found in serum zinc and copper values. Theheifersof T_0exhibited regular estrous whereasthe heifers of T_1 and T_2 showedirregular estrus signs followed by anestrus. In thisstudy, it is revealed that standard feeding is very important tomaintaintheblood 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 Vadnere, 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 (Hidiroglou, 1979; Dutta*et 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;Eckles*et al.*, 1935). Improper feeding cause minerals deficiency, especially phosphorus may predispose cattle to anestrous indirectly by triggeringinappetence or pica. It is imperative to establish how changes in nutrition, whether of short or long term duration, specifically affect the cattle's serumbio-chemical 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 (*Pennisetumperpureum*) 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_0 (Control feeding), T_1 (40% restricted feeding) and T_2 (60% restricted feeding).A standard concentrate mixture and green grasseswere given daily to the animals of T_0 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_1 and T_2 groups were fed with 40% and 60% restricted (less) feeding, respectively. About8 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_0, T_1 and T_2 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 -20^0 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 Aanalyst 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 bya 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_1 and T_2) were found significantly ($p<0.01$) decrease glucose and total protein compared to control (T_0) feeding animals. The values of glucose (mg/dl) were 55.62, 30.23 and 25.12 in the animals of T_0, T_1 and T_2 respectively. The total protein (g/dl) was found at a level of 6.33, 4.26 and 4.01 in the animals of T_0, T_1 and T_2 group respectively.

Table 1 Serum biochemical profile of heifers following standard and restricted feeding at day 105 of post treatment (Mean±SE) in different treatment group

Parameters	T_0 (Mean±SE)	T_1 (Mean±SE)	T_2 (Mean±SE)
Glucose (mg/dl)	55.62±1.80	30.23±2.11**	25.12±1.34**
Total protein (g/dl)	6.33±0.18	4.26±1.07*	4.01±1.02**
Calcium (mg/dl)	13.07±0.38	8.73±0.85*	7.03±0.73**
Inorganic Phosphorus (mg/dl)	6.95±0.77	5.24±0.43	4.82±0.29*
Iron (µmol/L)	30.331±1.13	18.025±2.21*	14.312±1.96**
Zinc (ppm)	1.04±0.05	1.0±0.03	0.90±0.01
Copper (ppm)	0.90±0.05	0.81±0.01	0.80±0.08
Manganese (ppm)	1.14±0.05	0.93±0.04	0.89±0.01*
Cobalt (ppm)	1.13±0.41	1.03±0.31	0.70±0.22*

** 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 (T_0, T_1 and T_2) was approximately similar. At the end of the experiment (day 105), the average values of serum calcium (mg/dl) in animals of T_0, T_1 and T_2groups 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 T_1 and T_2 compared to the animals of T_0(Figure 1).

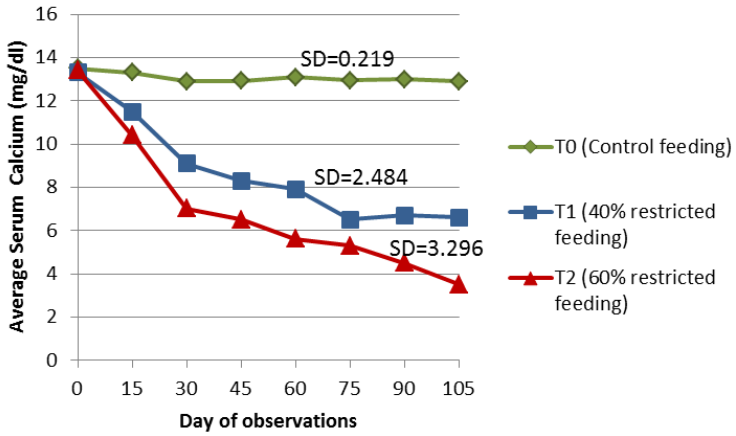

Figure1. Average values of serum calcium (mg/dl) in different treatment groups. The calcium level declined with days of post treatment in T1 and T2 groups. On the other hand, the calcium values of standard feeding heifers remained stable with time.

In this study, the observed serum phosphorus levels (mg/dl) of cross-breed heifers were 6.95, 5.24 and 4.82 in animals of T_0, T_1 and T_2 group heifers, respectively on day 105(Figure 2). The serum phosphorus decreased significantly ($p<0.01$) in animals of 40% restricted feeding (T_1) and 60% restricted feeding (T_2) compared to the animals in control T_0.

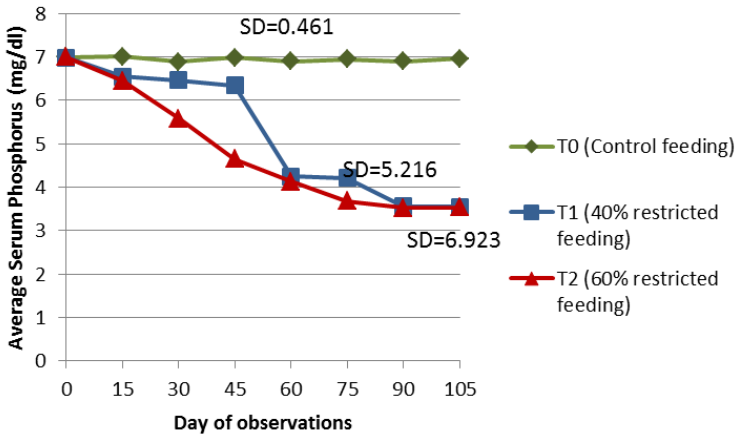

Figure 2. Average values of serum phosphorus (mg/dl) in different treatment groups. The phosphorus level declined with days of post treatment in T1 and T2 groups. The values of phosphorus in standard feeding heifers remain stable with time.

The average amounts of serum iron (µmol/L) were 30.331, 18.025 and 14.312 in the heifers of T_0, T_1 and T_2, 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 T_1 and T_2 with restricted feeding compared to animals of T_0 (Figure3).

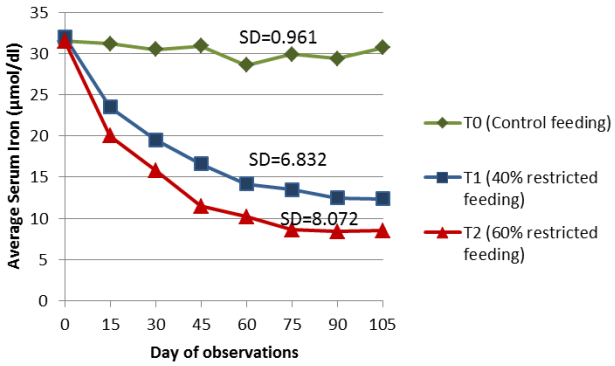

Figure 3. Average values of serum iron (µmol/L) in different treatment groups. The iron level declined with days of post treatment in T1 and T2 groups. On the other hand iron levels of standard feeding heifers remain stable with time.

Among trace minerals manganese and cobalt were decreased in the sera of the restricted feeding heifers of T_2 compared to standard feeding (T_0). No significant change was found among the values of zinc and copper in different treatment groups (Table 1).

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 anoestrus 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 inthe animals of $T_0$with standard feeding and the least percentage (32%) of estrus showed by the animals of T2 group (Figure 4).

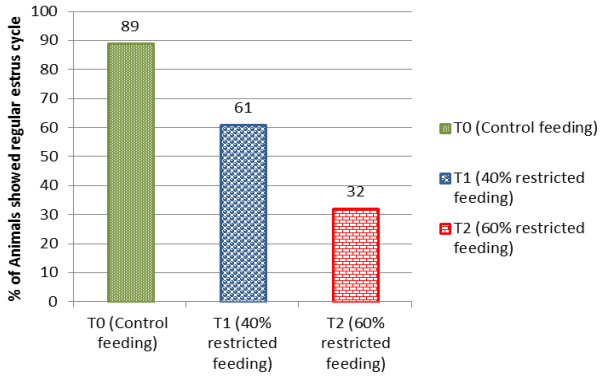

Figure4. Heifers showed regular estrus cycle (%) in different treatment groups. Least percentage of heifers of T_2 group showed regular estrus and the highest percentage of standard or control feeding heifers showed regular estrus.

IV. Discussions

Randomly selected 12 cross bred heifers were divided into three uniform groups namely T_0, T_1 and T_2. The heifers of T_0 were fed with standard feeding i.e. no feeding control, the animals of T_1 were fed with 40% restricted or less feeding and T_2were 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 T_1 and T_2which 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 standarddaily feeding on serum mineralslevels as well as estrus induction of heifers.

In the present study, significantly ($P<0.05$) high serum glucose level was observed in T_0 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

anoestrus animals (Kumar and Sharma 1991). In the present investigation, the low blood glucose level observed in anoestrus 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 anoestrus cows. The values of serum total protein were decreased significantly in the 40% and 60% feeding restricted animals which showed anestrus 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 anoestrus cows and heifers have been reported by previous researchers (Arosh*et al.* 1998; Singh *et al.* 2004; Muthukumar*et 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/dland the range of serum-Ca is 8.65-11.65 mg per 100 ml, or± 15 % around the mean (Allcroft and Green, 1934). Kumar and Sharma (1991) reported that there is no significant difference between serum Ca level of cyclic cows and anoestrus cows and heifers which corroborated to this finding. In contrast to this, higher serum Ca concentration in cyclic cows than that of the anoestrus 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 founddecline with time in the animals of group T_1 and T_2 due to low dietary intake. Duringthe 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 ($p<0.01$) declineserum phosphorus valuewas observed in animals of 40% restricted feeding (T_1) and 60% restricted feeding (T_2) which showed irregular estrous signs. The higher serum P concentration in cyclic cows than that of anoestrus 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 anoestrum and reduced ovarian activity (Pugh et al. 1985). Anoestrus, suboestrus, irregular and delayed sexual maturity have been occureddue 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.* 2002 c).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 (T_1 and $T_{2)}$ compared to animals of T_0 (Figure3). 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 Wenzlf 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 animalsthoughirregular estrous cycle as well as anestrous condition was found in the experimental animals of restricted feeding. The anestrus 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 anestrus 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 anestrous 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 writtenby S. Begum. Review and final correction of the manuscript were done by M.M.R. Howlader.

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