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IMPACT OF DIETARY RESTRICTION ON FOLLICULAR WAVES IN CATTLE WITH PARTICULAR REFERENCE TO ANOESTRUS

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ABSTRACT

Reproductive failure (anoestrus) is a one of the major problem in cattle production of Bangladesh due to under feeding and unavailability of balanced ration. This study was conducted for a period from July 2009 to June 2010 to determine the nutritional impact on the follicular growth using blood hormone assays and the rate of anoestrus in cows following dietary restriction and to develop a feasible feeding technique concerning the available feed ingredients in local market. A uniform group of 2-3 years old 15 heifers were selected and were grouped randomly into three named T_1(60% feeding restriction), T_2(40% feeding restriction) and T_3 (standard feeding). Body condition score (BCS) and body weight were measured by standard method. Hormonal assays were analyzed to determine the impacts of restricted feeding on the ovarian follicular cycle using blood sera. The average BCS and body weight was decreased significantly ($p<0.01$) in the animals of T_1 and T_2 compared to control T_0. The average values of estrogen and progestrogen was decreased significantly ($p<0.01$) in restricted feeding heifers (T_1& T_2) than that of standard feeding heifers (T_3). About 50% heifers showed irregular estrus and 50% showed anoestrus signs in the animals of T_1 group. In addition, a total of 60% animals showed irregular estrus and 40% showed anoestrus condition in animals of T_2 group. On the other hand, the animals of control group showed regular estrous cycle with good hormonal assays. Standard feeding could improve the cattle production efficiency by overcoming the reproductive failure using available feed ingredients.

Keywords: Nutrition, follicular waves, anoestrous, heifers, restricted feeding.

INTRODUCTION

Nutrition is one of the most important factors which have many adverse effects on estrus and ovarian cycles in cows. Underfeeding or overfeeding heifers have significant consequences on their development. Under feeding may result in sub-normal conception rate, underdeveloped mammary glands, and greater incidence of calving problems (Petterson *et al.*, 1992). Overfeeding often results in weak expression of estrus, sub-normal conception rate, high embryonic mortality, decreases mammary gland development and reduced milk production (Murphy *et al.*, 1991). Correlation between body weight gain and age at puberty indicate that increased growth rate of heifers results in younger age at puberty (Oyedipe *et al.*, 1982). In Bangladesh major problems in dairy and beef cows are poor conception rate, early embryonic death, irregular cycles and failure of animals to return in heat (Shamsuddin, 1995). Throughout the bovine estrous cycle, follicular development progress in waves was determined by blood serum hormone assay. Follicular growth in cows occurs either in two (Ginther *et al.*, 1989a, b) or three waves (Savio *et al.*, 1988, Sirois and Fortune, 1988). In Bangladesh, dairy animals in most of the dairy farms represent low fertility, weak and insignificant heat, conception failure and repeat breeding which are the common cases (Hossain *et al.*, 2001). Inadequate nutrition and underfeeding might have contribution on reproductive parameters. Little works on the effects of under nutrition and/or restricted nutrition on cow's estrous cycles/waves have been conducted so far throughout the world and limited work has been conducted in Bangladesh. For efficient dairy production management system, the knowledge of nutritional effects on the estrous cycle or ovarian cyclicity is essential. To develop a scientific knowledge for better and efficient dairy management and feeding strategies, the research work was designed and undertaken.

MATERIALS AND METHODS

Study period and site

The study was conducted from July 2009 to June 2010 in Sylhet Govt. district dairy farm, Sylhet, Bangladesh.

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Experimental animals

A uniform group of 21 heifers was selected initially from which 15 animals were used in this experiment. They were selected randomly from the herd and were numbered numerically. All the animals were born and reared under same farming condition. The BCS scale was measured from 1 to 5. All the experimental heifers were fed allowing the standard Thumb rule of feeding system (Banergee, 1998) to raise their BCS up to 4.0. After reaching the BCS, 15 animals were selected and were grouped into three treatment groups- T_1 (60% restricted feeding), T_2 (40% restricted feeding) and T_3 (Standard feeding considerd as control group). Initially all the animals were treated with 4 fortnightly concurrent doses of Levamizole hydrochloride (7.7 mg per kg body weight), albendazole (7.5 mg per kg body weight), Fenbendazole (10 mg per kg body weight) and Ivermectin (0.2 μg per kg body weight).

Table 1. Standard concentrate feed used in the experiment

SL	Feed Ingredients	Quantity (in grams)	Composition %
1.	Corn	750	25
2.	Wheat bran	600	20
3.	Rice polish	450	15
4.	Khesari	600	20
5.	Soya bean meal	510	17
6	DCP	60	2
7.	Salt	30	1
	Total	3000 g (3 kg)	100

Feeding management before restricted feeding

Napier grass (*Pennisetum purpureum*) and para grass (*Bracharia mutica*) were fed to fulfill the dry matter requirement of the animals. The grass has been provided to all animals followed by the instructions given by Banerjee (1998). A concentrate mixture was given daily to all animals following the thumb rule methods of Banerjee (1998). The ingredients of mixed concentrate feeds were procured from the open market at Sylhet of Bangladesh. Water was made available to each animal for 24 hrs.

Feeding management during restricted feeding

Napier grass (*Pennisetum purpureum*) and para grass (*Brachariamutica*) were made available to satisfy the dry matter requirement of the animals. The grasses were provided to animals of T_3 group following the instructions given by Banerjee (1998). The animals in T_1 and T_2 were restricted by 60% and 40% green grass, respectively. Similarly, three concentrate mixtures were given daily to animals in age as 60% in T_1, 40% in T_2 and 0% in T_3 groups, respectively. The ingredients of mixed concentrate feeds were procured from the local market of Sylhet in Bangladesh. *Ad libitum* water was supplied available to each animal at all times.

Ultrasound imaging

The ovarian follicles were monitored regularly by using ultrasonography machine (Easy-scan™ curve bovine ultrasound machine, BCF technology, UK).

Collection of blood sample and analysis

Blood was collected every 3 days interval by jugular venepuncture. Samples were kept at room temperature for 1 h, and at 4 ^{0}C for 24 h. Serum was collected before being centrifuged at 3000 rpm for 10 minutes and stored at -20°C until assayed for estrogen and progesterone concentrations using ELISA kit.

Determination of hormone concentration

Progesterone concentrations in blood serum were determined through solid phase competitive ELISA by using commercially available kit (Bio Check, Inc. USA, Lot. RN-28387) (Ali *et al.,* 2009**)**. The sensitivity of the progesterone ELISA assay was 0.3 ug/ml, intra-assay precision was less than 7.1 % and inter-assay precision was less than 12.6%. Estradiol 17-β concentrations in blood serum were determined through ELISA by using

commercially available kit (Bio Check, Inc. USA, Lot. RN-27637).The sensitivity of the estradiol ELISA assay was 10 pg/ml, intra-assay precision was less than 24.1% and interassay precision was less than 26.7%.

Statistical analysis

The collected data were analyzed statistically through analysis of variance, using completely randomized design. Significant means were compared using Duncan's Multiple Range (DMR) test (Steel and Torrie, 1990). The obtained data in two years of experiment were statistically analyzed in the laboratory using standard methods.

RESULTS

The experiment was started by selecting 15 heifers bearing same age and the body condition score (BCS) having an average of 3.5. The animals of T_1 group were fed subsequent restriction up to 60%, animals in T_2 were fed with 40% restricted feeding and animals in T_3 group were fed by standard feeding named control group i.e. no restriction to feed (standard feeding). Afterwards the restriction feeding, the BCS of T_1, T_2 and T_3 heifers were shown 2.43 ± 0.09, 3.58 ± 0.09 and 3.98 ± 0.06 after completion of first year and were shown 2.54 ± 0.08, 3.65 ± 0.09 and 4.15 ± 0.05 after completion of second year of experiment, respectively (Table 2 and 3, respectively). The ultrasonography was performed to all experimental animals in every three days intervals. The ultrasonography results of restricted feeding animals were found unclear due to irregular estrus or anoestrus condition but follicular growth of control group were found good.

Table 2. Feeding and BCS after completion of first year of the experiment

ITEM		[1]Treatment-1 (T_1)	[2]Treatment-2 (T_2)	[3]Treatment-3 (Control, T_3)
Feed/Animal/Day	Green Grass	3.2 kg	4.8 kg	8 kg
	Concentrate	1.2 kg	1.8 kg	3 kg
Percent of restriction of feeding		60% less	40% less	0
Body Condition Score (1-5 Scale) (Mean SD/SE)		2.43±0.09**	3.58±0.09**	3.98±0.06
Body weight (kg) (Mean SE/SD)		136.28±2.81**	161.13±2.81**	186.10±1.99

[1, 2, 3] Average of five replications, ** Significant (P<0.01) at 95% CI, Initial body weight of all animals in each group was around 170.65±3.11 and BCS was 3.5.

Table 3. Feeding and BCS after completion of second year of the experiment

ITEM		[1]Treatment-1 (T_1)	[2]Treatment-2 (T_2)	[3]Treatment-3 (Control) (T_3)
Feed/Animal/Day	Green Grass	3.2 kg	4.8 kg	8 kg
	Concentrate	1.2 kg	1.8 kg	3 kg
Percent of restriction of feeding		60%	40%	0
Body Condition Score (1-5 Scale)		2.54 ± 0.08 **	3.65 ± 0.09 **	4.15 ± 0.05 **
Body weight (kg)		132.16±2.16 **	155.35±2.55 **	178.15±1.86 **

[1, 2, 3] Average of five replications, ** Significant (P<0.01) at 95% CI

Heifers in group of T_1, T_2 showed significantly (P<0.01) decrease values of estrogen (Table 4) such as 178.65±6.13 and 184.83±7.18pg/ml, respectively, compared to control group (197.65±5.91). Where as the average progesterone values of T_1, T_2 were decreased significantly (P<0.01) which was 0.40±0.12, 0.34±0.11 respectively in contrast to control group T3 (0.28±0.11). From the hormonal analysis it was concluded that the animals of treatment groups T_3 group perform better and showed more regular estrous or heat than T1 and T2 groups (Table 4) i.e. the standard feeding would be helpful for normal follicular growth and for showing regular estrus cycle.

Table 4. Estrus cycle and average hormonal status of heifers

Parameters	[1]Treatment-1 (T_1)	[2]Treatment-2 (T_2)	[3]Treatment-3 (T_3)(control)
Estrogen(pg/ml)	178.65±6.13**	184.83±7.18**	197.65±5.91
Progesterone(ng/ml)	0.40±0.12**	0.34±0.11**	0.28±0.11
Estrus cycle	Irregular (50% Heifers), Anoestrus (50%)	Irregular (60% Heifers), Anoestrus (40%)	Regular (100% Heifers)

[1, 2, 3] Average of five replications, ** Significant (P<0.01) at 95% CI

DISCUSSION

Daily nutritional requirement of cross-breed heifers are very important to develop and keeping functioning of all the vital organs. In our present study we have developed a standard feeding practice using locally available feed ingredients followed by the instruction of Banergee (1998) to maintain the reproductive efficiency of heifers. Feeding restriction of heifers was caused a reduced BCS and developed a gradual of anoestrus in experimental heifers. Nutritional deficiency causes a reduce muscular activity with gonadotrophin depression. In this study, the feeding restriction was reduced the body weight as well as body condition score (BCS) in the heifers. Earlier researcher was reported that supplementation of different feed ingredients increase the live weight of animals. Lindt and Blum (1994) who reported that the carcass yield of calves increased by supplementation of iron with dietary feeds. Similarly, Vatn and Framstad (2000), Vellema *et al.* (1997) reported that cobalt increases live weight in sheep. So, the standard feeding to heifers could be able to improve the body physiology as well as body weight.

In our study we found that the heifers with restricted feeding were developed an irregular estrus followed by anestrus condition. On the other hand, the heifers of standard feeding showed regular estrus cycle with remarkable gonadotrophin secretions. This finding is partially similar to that finding of Wiltbank *et al.* (1962). They reported that a greater proportion of cows was failed to show estrus due to low energy level after calving. Underfeeding delays puberty in heifers (Joubert, 1954) and stops estrus and ovarian cyclicity in heifers that are already cyclic (Terqui *et al.*, 1982). Nutritional restriction has got immediate negative effects on follicular growth and cyclicity (Mackey *et al.*, 1997; 1998; 1999). The dietary energy restriction might influence the LH release directly at the pituitary level as well as indirectly through effects on ovarian steroid production (Beal *et al.*, 1978).The energy restriction decreased the incidence of heifers having estrous cycles and the anestrous heifers did not respond to a progestin-estrogen treatment for induction of estrus (Imakawa *et al.*, 1986). Cessation of ovulation observed in nutritionally induced beef heifers by Bossis *et al.* (1999). LH secretion is inhibited by E2 in the nutritionally anestrous heifer and is accepted following supplementation of high energy diet (Imakawa *et al.*, 1984). Energy intake has influenced on a wide variety of endocrine, neural and metabolic mechanisms (Rhodes *et al.*, 1996) *viz.* changes of gonadotropic hormone secretion from the pituitary, production of progesterone during both the estrous cycle and pregnancy, differential sensitivity of the pituitary-hypothalamus to steroids and releasing hormones and changes in ovarian activity measured by hormone secretion, follicular development and ovulation (Short and Adams, 1988). Less feeding reduces all the functional activities of the body like as growth and reproduction.

CONCLUSION

The study demonstrates that the restricted dietary feeding poses to reduce the average body weight and the estrogen values along with low progestogen. Therefore, animals provided with standard feeds using locally available feed ingredients could perform equally in respect to reproductive hormone production like estrogen and progesterone with regular estrus cycle.

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