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COMPARATIVE STUDY OF HEMATOLOGICAL AND BIOCHEMICAL PROFILES OF REPEAT BREEDING ZEBU COWS

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ABSTRACT

The study was undertaken to know the hematological and biochemical profiles of repeat breeding zebu cows. Blood samples from cows with repeat breeding syndrome were collected for hematological, biochemical and humoral immune response analysis. The erythrocyte sedimentation rate (ESR) was higher and hemoglobin (Hb) percentage was lower in repeat breeding cows than the normal cyclic cows. The packed cell volume (PCV) was lower in repeat breeding cows than in normal cyclic cows. Total erythrocyte count (TEC) is not significantly different whereas total leukocyte counts (TLC) significantly higher in repeat breeding cows. The bilirubin concentration was not significantly higher ($P< 0.01$) in repeat breeding cows compared to the control groups of cows. The mean serum IgG was lower in repeat breeding cow than control groups of cows.

Keywords: hematological profile, biochemical profile, repeat breeding, zebu cows

INTRODUCTION

Repeat Breeding in cows is an important constraint to the dairy farmers (Serur *et al.*, 1982; Albino *et al.*, 1989; Jainuddin and Hafez, 1993). Defective management can increase the incidence of repeat breeding cases (Shamsuddin *et al.*, 1988). Repeat breeding is the most important reproductive disorders encounters in Bangladesh (Samad *et al.*, 1978; Vale, 1997) causing considerable economic loss (Jainuddin and Hafez, 1993). The recent evidence suggested that the early repeat breeders are heifers (Albin *et al.*, 1989). Several reports have implicated various blood chemical constituents in repeat breeding cows (Serur *et al.*, 1982; Albin *et al.*, 1989; Dhahiwal *et al.*, 1996; Hoeben *et al.*, 2002) but no such reports are available in Bangladesh.

The zebu cows in a tropical or subtropical environment do not increase their metabolism to the same extent when external temperature rises above 73^0F as the European cattle (Van Heerden, 1963). About 64%, 32.4% and 2.8% of repeat breeding cows diagnosed from delayed ovulation, metritis and ovobursal adhesion respectively (Al-sultan *et al.*, 1998). The highest seasonal incidences of repeat breeders are in Fall and Winter with the lowest incidence in the Spring and Summer (Hewett, 1968). Repeat breeding in local Iraqi cows occurred mainly due to fertilization failure (Al- Sultan *et al.*,1998). For cows, the incidence of persistence corpus luteum was greater in cows and it is about 19.86%. The present investigation was undertaken to evaluate the hematological and biochemical profiles of cows with repeat breeding syndrome in Bangladesh. It is found that the incidence of repeat breeding in heifers that had calved for first time is 5.2%, 13.3% dairy cows over 7 years of age and 15% in cows under 3 years of age (Slack *et al.*, 1964). The fertility decreased from 68% to 58% as animals grew older and herd size increased. There was decreased infertility and greatest fertility was on farms raising grade cattle (Van Dieten, 1968).

MATERIALS AND METHODS

Animal selected

A total of 10 repeat breeder zebu cows weighing 220-355 kg were selected for this study. These cows were suffering from repeat breeding syndrome for more than 180 days. All were out patients at the veterinary

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clinic of Bangladesh Agricultural University (BAU). Another 10 cyclic lactating multiparous zebu cows were selected from BAU dairy farm and from the zebu cows belong to small holders where only 1 to 15 cows were kept and treated as control group. They normally maintain their animals on rice straw cut and-carry grass and limited grazing on road side and community land as forage and milling by-product as concentrate (rice polish, wheat bran, mustard and till oil cakes), fed 1-2 kg per 1.5 -2.0 liters of milk. There is no system of estrous detection, but farmers relied on visible sign of estrus.

Blood sampling

Five (5) ml blood was collected from each cow by jugular vein puncture using ten (10) ml plastic syringe on the first day of examination and every week for three weeks. Half of the blood sample was kept with heparinized saline for routine hematological examination; rest of sample was taken in vials for collection of serum. Serum samples were kept -20^0 for further analysis.

Hematological studies

Erythrocyte sedimentation rate (ESR), packed cell volume (PCV), hemoglobin percentage (Hb %), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were carried out as described by Coffin (1953).

Total Serum Protein Assay

Total blood protein was measured using Folin-phenol method (Lowry *et al.*, 1951). Briefly a standard curve was made with equilibrated (200 µl distilled water) bovine serum albumin solution (1mg/ml) ranging from 0 to 60 µl. Unknown serum samples (10µl) were taken in separate tubes after similar equilibration. After addition of prepared solution (one ml), each tube was incubated in a dark place for 30 minutes at room temperature. The absorbance of standard proteins at 660 nmol using spectrophotometer was plotted against concentration of BSA and concentration of proteins was extrapolated by putting the value of optical density (OD) in the standard curve. Serum bilirubin was measured using the standard technique of Harisson and Barlow (1989). Briefly, five (5) ml methanol, one (1) ml diazo reagent and four ml diazo blank were taken as blank sample. For unknown serum samples, five (5) ml methanol and one ml diazo reagent and four (4) ml atl: 10 diluted serum were mixed thoroughly and incubated for 30 minutes at room temperature. The blank was set at 100% at 540 nm and the percentage of transmission (T) was read in the unknown sample. The result of percentage transmission (T) was then converted to mg/dl.

Serum immunoglobulin–G (IgG)

Immunoglobulin was estimated by the single radial immune-diffusion techniques (Mancini *et al.*, 1965). The polyclonal antibody IgG was raised by immunizing New Zealand White Rabbit with purified 0.5 mg/ ml Bovine IgG (Sigma, USA) Blood (Serum) was collected from the ear vein every week after first booster dose. Sera were used to check the antibody response and quantify the antigen by double immune-diffusion test and single radial immune diffusion techniques respectively, following the procedures described by Bari (1997). The anti-bovine IgG was striated using ELISA technique (Bari, 1989) in 5 fold dilutions starting from 1 in 50. All the data were analyzed by comparison between groups using student's t- test (Bailey, 1981).

RESULTS AND DISCUSSION

Hematological Examination

The results hematological parameters of repeat breeding cows are shown in (table 1). The mean values of erythrocyte sedimentation rate (ESR) in mm/24 hour were 11.1 ± 5.6; and 7.5±1.2 in repeat breeding and control groups of cows, respectively. The mean values of ESR were significantly higher in repeat breeding cows than in control group of cows. The mean hemoglobin (Hb) % in repeat breeding and control group of cows were 9.6 ± 1.1, and 13.1±2.6, respectively. The hemoglobin (Hb) concentration was significantly lower in repeat breeding ($A<0.05$) than control group of cows ($P<0.01$). The mean packed cell volume (PCV) % in repeat breeding group and control group of cows were 28.7 ± 7.0 and 43.2±7.9, respectively. Packed cell volume (PCV) in repeat breeding group of cows was significantly lower than control group of

cows. The total erythrocyte count (TEC) of repeat breeding and control group cows were 5.1±1.0 and 4.5±1.5 million/µl, respectively. The differences between repeat breeding and control group of cows were not significant. The mean total leukocyte count (TLC) in repeat breeding and control group of cows were 10.0 ± 5.7 and 6.5±1.5 respectively. The Total leukocyte Count (TLC) in repeat breeding group of cows is significantly higher than the control group of cows.

Table1. Hematological values of repeat breeding cows

Parameters	Repeat breeding group (A) n=10	Control group (B) n=13
	Mean ±SD	Mean ±SD
ESR mm/24hr	11.1±5.6***	7.5 ±1.2
Hb gm.%	9.6 ± 1.1**	13.1 ±2.6
PCV %	28.7 ±7.0***	43.2 ±7.9
TEC million/µl	5.10 ±1.0	4.5 ± 1.5
TLC thousand/µl	10.0 ±5.7**	6.5 ± 1.5
Serum protein (mg/dl)	3.7 ± 1.1***	5.2 ±0.8
Serum bilirubin mg/dl)	0.3 ±0.2*	0.3± 0.3
IgG(µg/dl)	869.6 ±109.4*	1222.2 ±178.9

In the above table, * p<0.05, ** p<.01, *** p<.001.

Total serum Protein (mg/dl)

The mean total serum protein in repeat breeding and control group cows were 3.7 ± 1.1 and 5.2±0.8 mg/dl respectively. It was significantly (p<0.001) lower in repeat breeding group than control group cows (Table 2). The mean concentration of serum bilirubin in repeat breeding and control group of cows were 0.3±0.2 and 0.3±0.3, respectively. The mean bilirubin concentration in repeat breeding group of cows was not significantly variable than control group of cows.

The mean concentration of IgG in repeat breeding and control group of cows were 869.6 ±109.4 and 1222.2 ±178.9 respectively. The concentration in repeat breeding group of cows is significantly lower than control group. The results show that the 3rd bleeding before 3rd booster had highest concentration of antibody.

Table 2. Titration of anti-bovine IgG by ELISA in repeat breeding cows

Serum dilution	A (Mean ±SD)	B (Mean ±SD)	C (Mean ±SD)	D (Mean ±SD)
Blank	0.16 ± 0.00	0.19 0.00	0.21 ± 0.00	0.25 ± 0.00
0.00000128	0.24 ± 0.00	0.43 0.03	0.48 ± 0.01	0.55 ± 0.01
0.0000064	0.38 ± 0.00	0.50 0.03	0.65 ± 0.03	0.89 ± 0.03
0.000032	0.45 ± 0.00	0.73 0.02	0.89 ± 0.01	1.23 ± 0.03
0.00016	0.57 ± 0.00	1.39 0.01	1.51 ± 0.06	2.10 ± 0.01
0.008	0.78 ± 0.01	2.10 0,02	2.74 ± 0.01	3.02 ± 0.02
0.004	0.92 ±.00	2.83 0.04	3.09 ± 0.05	3.60 ± 0.03
0.02	1.06 ± 0.1	3.313 0.01	3.28 ± 0.03	4.10 ± 0.01

SD = Standard deviation

A= control, B= first boostered bleeding, C= second boostered bleeding, D= third boostered bleeding.

The erythrocyte sedimentation rate (ESR) is generally high in chronic infection and malnutrition (Samad *et al.*, 1978; Dutta *et al.*, 1991; Islam *et al.*, 1999). Low hemoglobin (Hb) percentage indicates anemia and its values are significantly low in repeat breeding groups of cows than control group of cows. The hemoglobin concentration is lower in repeat breeding group of cows than control group of cows (Baki and Rahman, 1981). The animal in the present study were outpatients at the veterinary clinic and had been suffering from

gastrointestinal parasites causing anemia and hypoprotenemia (Murthy *et al.*, 1975). The packed cell volume (PCV) % was significantly decreased only in the repeat breeding group of cow compared to the control group of cows. Packed cell volume (PCV) % is another index of anaemia in hematological parameters (Samad *et al.*, 1978; Baki and Rahman, 1981; Islam *et al.*, 1999). Total leukocyte count (TLC) was significantly higher in repeat breeding group of cows than in control group of cows. Indeed chronic microbial infection may cause endometritis that may be responsible for repeat breeding in cows (Jahan and Myenuddin, 1996). Total serum protein was low in all repeat breeding cows than control group of cows.

In the present investigation, total serum bilirubin was higher in repeat breeding group of cows than control group of cows. The serum IgG in repeat breeding cows was significantly lower than in control group of cows. This could indicate that the lower humoral immunity is a factor of repeat breeding in zebu cows. Indeed, humoral immunity is low in cows with a repeat breeding syndrome (Dhaliwal *et al.,* 1996; Sanin *et al.,* 1999). The result showed that the 3rd bleeding before 3rd booster dose had highest concentration of antibody against bovine IgG. On the other hand, the mean value of anti-bovine IgG were highly significant ($P<0.01$). The total anti-bovine IgG was higher probably due to immune-potentiating action against bovine IgG treated rabbit.

It is concluded that there is no specific hematological, biochemical and immunological marker for repeat breeding anestrous in zebu cows. Non-specifically ESR values were high in repeat breeding zebu cows; however the Hb% and PCV%values were low. Humoral immune responses are non- specifically lower in repeat breeding cows than in control group cows.

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