Haryana Vet. **49** (December, 2010), pp 56-58

Research Article

FOLACIN, COBALAMIN AND IRON-BINDING CAPACITIES IN RESPONSE TO PARENTRAL HAEMATINIC SUPPLEMENTATIONS IN BLOOD OF BUFFALOES

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ABSTRACT

To know the effects of parentral haematinic supplementations on serum folacin, cobalamin, iron, iron binding capacities and haematological parameters, six buffalo-bullocks were offered standard rations for one month. The animals were then half fed for one week to simulate the condition of simple anorexia followed by full rations later on. On day 1 of half-feeding, each animal was given an iron supplement (500 mg) and a mixed supplement of folacin (150 mg) and cobalamin (5 mg) intra-muscularly. The supplementation was repeated twice a week for four weeks. Blood samples were taken before starting half feeding and later on at weekly intervals for four weeks. Serum levels of folacin and cobalmin were determined using radio-immuno-assay kits, whose concentrations increased with the supplementation reaching maximum levels after the fourth injection and decreased later. Results showed that well-fed buffaloes had levels of serum iron, (121 µg/dl); latent iron-binding capacity (LIBC; 128 µg/dl), total iron-binding capacity (TIBC; 249 µg/dl); folacin (22.8 ng/ml) and cobalamin (344 pg/ml); the supplementation raised them to 199 µg/dl, 214 µg/dl, 369 µg/dl, 36.0 ng/ml and 518 pg/ml, respectively. Haemoglobin, RBC and PCV increased non-significantly by the supplementation. Four, bi-weekly injections of haematinics were adequate to maximize their blood levels, further dosing was futile.

Key words: Haematinic supplementation, serum iron and iron binding capacity, folacin, cobalamin, haematology

Malnutrition is often a cause, a concomitant or a consequence in numerous diseases. Supplementation of minerals and vitamins to neutralize the effects of malnutrition is likely to be beneficial in veterinary cases. The feed intake of animals during disease and recovery will vary considerably with the stage of the disease. Thus, it would be difficult to decide the exact extent of deficiency in the dietary intake of an animal. Nutritional deficiencies, which are known to occur in different types of diseases, are of proteins, vitamins and minerals such as iron, calcium, etc. Supportive therapies with haematinics like iron, folate or vitamin B_{12} are well known. Parentral administration may serve as a research tool to determine requirements by overcoming problems associated with dietary supplementation. It is to be studied whether haematinic supplementation is of any use to under-fed animals and if so, the dosage required. In view of the above, a study was conducted to determine the effects of parentral haematinic supplementation in half-fed buffaloes.

MATERIALS AND METHODS

Animals: Six buffalo-bullock of body weight 270-450 kg were offered standard rations as per the National Research Council (NRC, 1988) for one month and their actual weekly consumption of both concentrates and wheat straw was noted. The animals were then half fed the feed that they had earlier for one week. Later on, the animals were fed full standard rations (standard feeding, SF) for three more weeks.

Haematinic administration: On the first day of half-feeding, each animal was given 500 mg of elemental iron as 10 ml of an iron-dextran injection ('Imferon', Rallis, India) and 10 ml of Vitcofol (FDC, Bombay) consisting of cyanocobalamin (5000 µg), folic acid (150 mg) and niacin (2000 mg) by intra-muscular route twice a week for four weeks. Hence, a total of eight injections each of iron and the vitamins were given to each animals.

Blood Sampling: Samples of blood were taken from the jugular vein before the first feed of the day. Samples were collected once a week before starting half feed and later. An aliquot of the sample was used for the common haematological parameters like cell counts, haemoglobin, haematocrit etc. by standard manual methods (Schalm *et al.*, 1975). The serum was separated and stored at -20°C until assay.

Iron and iron-binding capacity: The serum iron, total iron-binding capacity (TIBC) and latent iron binding capacity (LIBC) were determined by the method of O' Malley *et al.* (1970).

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Vitamin B_{12} and folic acid assays: The serum levels of vitamin B_{12} and folic acid were determined using radioassay kits having ^{57}Co-vitamin B_{12} (Cobalamin) and ^{125}I-folic acid (Folacin) as tracer (Diagnostic Products Corporation, Los Angeles, USA) as per the method of Kubasik *et al*. (1980). Radioactivity was measured on a suitably calibrated computerised Rackbeta Scintillation Counter (LKB Model 1209, Finland).

Statistical analysis: The data were subjected to analysis based on complete Randomized Block design (Snedecor and Cochran, 1968) for statistical significance.

RESULTS AND DISCUSSION

Haemoglobin (Hb) and haematocrit (PCV) concentrations (Table 1) increased slightly with supplementations. The RBC and WBC increased with half-feeding (HF) plus heamatinics and a peak value was observed after two weeks, thereafter the counts decreased (Table 1). The mean corpuscular volume (MCV), means corpuscular heamoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) showed minor fluctuation (Table 1). The static levels of Hb even when more iron was supplemented indicated its adequacy for normal erythropoiesis. Similarly, Welchman *et al.* (1988) found that in calves up to 16-20 weeks of age, increase of the total dietary iron to approximately 12500 mg raised the Hb to a mean value of 12.4 g/dl, similar to the controls. There was no further increase in Hb even when more iron was fed. However, Reece *et al.* (1985) reported an immediate increase in the values of Hb and PCV after an injection of iron-dextran. The effects of 100 ppm of iron in a milk replacer for calves were effective in increasing the Hb only in the anaemic, but actually decreased it in those initially well (Miltenburg *et al.,* 1991a).

The commercial preparation of the vitamins, used in this experiment contained niacinamide; the effects of niacin on erythropoiesis are negligible and so it was considered to be just an additional material of no consequences to the haemotology of buffaloes.

The serum iron (mean±S.E.) levels increased significantly ($P<0.05$) from 121±3.47 ug/dl to 180±7.8 ug/dl after two weeks of supplementation. Half-feeding with supplementation increased the levels further. Thereafter, it again decreased and after four weeks, the levels increased significantly ($P<0.05$) to 199±0.04 g/dl (Table 2). The TIBC levels increased significantly from 249±4.0 µg/dl to 369±6.29 µg/dl due to supplementation. Then it started decreasing and returned to the level of SF after three weeks. Later, it again increased (Table 2). The levels of LIBC decreased significantly ($P< 0.05$) from 214±6.29 µg/dl to 87±5.7 µg/dl. Percent saturation increased significantly and a peak (69.9±2.24%) was obtained after two weeks and later on it decreased. Variations in serum iron may be because of iron turnover as also reported by Miltenburg *et al.* (1991b) on ferrokinetic studies in calves. Changes in serum iron-binding capacities may be due to the turnover of the transferrin and are similar to the findings of Furugouri (1984).

The folacin and cobalamin increased slightly (Table 2) with supplementation during half-feeding. A peak level of both the vitamins was observed after the fourth injection i.e. 36.0±4.0 ng/ml (folacin) and 518±48.2 pg/ml (cobalamin). Thereafter, the concentrations started

Table 1
Average concentrations of blood parameters with or without haematinic supplementation in buffalo bullocks

Parameter	Treatments				
	SF	W_1	W_2	W_3	W_4
Haemoglobin (g/dl)	10.9^a±0.06	11.3^a±0.92	11.3^a±0.09	11.3^a±0.10	11.3^a±0.15
Packed cell volume (%)	31.0^a±0.23	32.7^a±0.40	32.0^a±0.29	32.7^a±0.40	30.8^a±0.55
Red blood cells (10^6/cu mm)	5.8^a±0.04	6.3^{ab}±0.04	6.4^b±0.09	6.1^{ab}±0.08	5.9^a±0.13
White blood cells (10^3/cu mm)	7.4^a±0.12	7.9^a±0.14	10.1^b±0.22	8.4^a±0.33	8.9^{ab}±0.33
Mean corpuscular volume (µM^3)	53.3^a±0.60	52.2^a±0.64	50.8^b±1.01	54.3^a±0.98	53 .0^a±0.86
Mean corpuscular haemoglobin (ng)	18.7^{ab}±0.17	18.0^a±0.20	17.9^a±0.31	18.7^{ab}±0.28	19.5^b±0.32
Mean corpuscular haemoglobin concentration (%)	35.1^{ab}±0.22	34.5^{ac}±0.19	35.3^{abc}±0.14	34.5^{ac}±0.16	36.8^b±0.23

Values with common superscripts (a,b,c) for a parameter do not differ significantly at $P <0.05$
SF- after standard feeding; W_1 to W_4 after weeks of supplementation

Table 2
Blood parameters (Mean±S.E.) with or without haematinic supplementation in buffalo bullocks

Parameter	Treatments				
	SF	W_1	W_2	W_3	W_4
Serum iron (µg/dl)	$121^{a}\pm3.47$	$155^{ab}\pm5.59$	$180^{bc}\pm7.75$	$127^{a}\pm6.00$	$199^{c}\pm0.04$
LIBC (µg/dl)	$128^{a}\pm4.04$	$214^{b}\pm5.67$	$87^{c}\pm5.67$	$130^{a}\pm7.43$	$172^{d}\pm6.65$
TIBC (µg/dl)	$249^{a}\pm4.04$	$369^{b}\pm6.29$	$257^{a}\pm6.25$	$241^{a}\pm3.31$	$355^{b}\pm5.51$
Iron saturation (%)	$48.7^{ac}\pm1.33$	$41.9^{a}\pm1.35$	$69.9^{b}\pm2.24$	$53.6^{c}\pm2.89$	$56.0^{c}\pm2.12$
Vitamin B_{12} (pg/ml)	$344^{a}\pm26.12$	$475^{b}\pm59.20$	$518^{b}\pm48.17$	$476^{b}\pm40.82$	$370^{a}\pm17.02$
Folic acid (ng/ml)	$22.8^{a}\pm0.86$	$25.4^{a}\pm2.20$	$36.0^{b}\pm4.0$	$31.6^{ab}\pm2.65$	$27.0^{ab}\pm2.65$

Values with common superscripts (a.b.c) for a parameter do not differ significantly at $P < 0.05$
SF - after standard feeding; W_1 to W_4 after weeks of supplementation
LIBC - Latent Iron Binding Capacity; TIBC - Total Iron Binding Capacity

decreasing inspite of further supplementation. After the eighth injection, the levels of cobalamin decreased significantly while the folacin levels decreased slightly as compared to controls. The lack of response to continued intra-muscular injections of folic acid might be due to its rapid clearance; this being a water-soluble vitamin. The high concentration of serum folates after fourth injection might have accelerated renal excretion of folates which could also be partly explained by the absence of folate-binding proteins; these proteins were detected in cow sera (Ford *et al.*, 1972; Markkanen *et al.*, 1974) which can protect folates against renal excretion (Fernandes Costa and Metz, 1979). Girard and Matte (1997) reported that the percentage of the dose of folic acid injected intra-muscularly recovered in urine was higher in milk-fed than in weaned calves and it decreased with age. The reason for decreased concentration of cobalamin, inspite of supplementation, could be their increased excretion in urine and faeces as reported by Alexander and Davies (1969) in horses.

This study revealed that four bi-weekly injections of haematinics were adequate to maximize their blood levels; further dosing was futile. It is suggested that such a programme of parentral haematinic dosage would be useful to determine haematological norms for species, breeds and other physiological conditions such as growth, pregnancy or lactation.

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