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Effect of strategic mineral supplementation on the occurrence of hemoglobinuria in pregnant buffaloes

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Abstract
The supplementation study was carried out in 20 advanced pregnant buffaloes selected from the area of study. Availability of major elements (Ca, P and Mg), trace elements (Fe, Cu, Mn, Zn,) for animals were calculated on the basis of mineral composition of feedstuffs and feed intake. The mineral availability of individual animal was compared with the nutrient requirements calculated with the help of feeding standards. Results indicated that there was shortage of phosphorus 40.34%, zinc 86.34% and copper 46.26%. But calcium, magnesium, iron, and manganese was shown to be supplied in excess 394.87%, 177.22%, 309.09% and 16.22% respectively on daily basis.The amount of strategic mineral supplement containing deficient minerals was added in the normal routine diet of advanced pregnant buffaloes. Supplementation of phosphorus, zinc and copper during gestation period of buffalo was found beneficial and has successfully prevented the occurrence of hemoglobinuria in advanced pregnant buffaloes.

Keywords: Advanced pregnant, buffalo, minerals, hemoglobinuria

1. Introduction

India has huge and tremendous population of livestock. According to 20th livestock senses of our country the total livestock population is 535.78 million that showing an increase of 4.6% over Livestock Census 2012. Out of this the total buffaloes in the country are 109.85 million showing an increase of about 1.0% over previous census. In Madhya Pradesh the total buffalo population is 10.3 million which shows an increase of more than 25% over previous censes (Livestock censes, 2019) [1].

Achieving higher production from dairy animals enhances the chances of metabolic disorders in high producing buffaloes. Digraskar *et al.* (1991) [6] reported that in advanced gestation, more phosphorus and calcium are required for the developing fetus.

Pre-parturient hemoglobinuria is one of the dangerous metabolic disease reported in pregnant buffaloes of Malwa region. It causes a great economic loss to animal owners. It is a non-infectious hemolytic syndrome of buffaloes and cattle which is characterized by intravascular haemolysis, anaemia and hemoglobinuria (Akhtar *et al.,* 2007) [2]. Similar type of hemoglobinuria has also been reported in late pregnancy in Egyptian buffaloes (Radostits *et al.,* 2010) [13]. Development of the disease in pregnant buffaloes during advanced pregnancy is presumed to be due to increased demand of the developing fetus (coupled with dietary deficiency Dhonde *et al.*, 2007) [5]

Adult buffaloes are usually affected with higher frequency during advanced pregnancy (8-9 months) and the immediate post-partum period (1-60 days post-partum) (Bhikane *et al.,* 2004; Dalir *et al.,* 2006; Gahlawat *et al.,* 2007 and Durrani *et al.,* 2010) [3, 4, 8, 7].

The exact cause of disease is still under study. Few reporting has been done on pre-parturient hemoglobinuria. Clinicians suggest that deficiency of phosphorus is responsible for the problem. Phosphorus deficiency plays a key role in causing hemoglobinuria which is manifested by acute intravascular hemolysis, hemoglobinuria, anemia, and hypophosphatemia (Resum *et al.,* 2017) [14]. Research on feeding aspect suggests that feeding of leguminous straws is the main cause in Malwa region (Jain *et al.,* 2012) [9]. Significantly decreased copper levels in blood of hemoglobinuric dairy animals could be attributed to a 3-way interaction between copper, molybdenum, and sulphur as this interaction can occur with concentrations of molybdenum and sulphur naturally present in feed stuffs, and involved in formation of thiomolybdates in the rumen. It binds with copper and form a highly insoluble complex, that does not release copper even under acidic conditions and renders it unavailable to the animals for utilization resulting in hypocupraemia Suttle (1991) [17].

Alternatively most farmers are of view that "No milk - No Concentrate", only some offer little amount of concentrate/greens without any mineral mixture during advanced pregnancy. This could greatly imbalance the micro nutrient availability to animals and also their utilization (Thakur *et al.*, 2016) [18]. A decrease in the incidence of the disease was reported after copper supplementation of cattle in a copper-deficient area (Smith *et al.*, 1975) [16].
Looking to the paramount importance of the problem present study was planned to study the effect of strategic mineral supplementation on occurrence of hemoglobinuria in pregnant buffaloes.

2. Material and Methods

The supplementation study was carried out in 20 advanced pregnant buffaloes selected from the area of study. These pregnant buffaloes were divided in two groups having 10 buffaloes in each as group (1) Control/non-supplemented and group (2) supplemented. As shown in table no. 01.

Table 1: Experimental design for supplementation trial

Group 1 n= 10	Group 2 n= 10
Normal diet without supplement	Normal diet + mineral supplement

2.1 Feed intake

Average daily feed intake of each pregnant buffalo was calculated by measuring feed offered (morning and evening) and residue left by individual animal for 3 consecutive days.

2.2 Body weight

Body wt. (Kg) of buffaloes of supplement receiving group (for calculating the deficiency) was calculated by using the Shaeffer's formula (Sastry *et al*., 1982) (15) as given below.
Shaeffer's formula

$$\text{Body Weight (Kg)} = \frac{\text{Length X Girth}^2}{660}$$

(Length and girth was taken in inches)

2.3 Mineral availability

Availability of major elements (Ca, P and Mg), trace elements (Fe, Cu, Mn, Zn,) for animals were calculated on the basis of mineral composition of feedstuffs and feed intake. Finally, the mineral availability of individual animal was compared with the nutrient requirements calculated with the help of feeding standards suggested by Kearl (1982) [10]; NRC (1989) [11] and Paul and Lal (2010) [12] to work out the mineral deficiencies/excess.

2.4 Preparation of strategic mineral supplement

For preparation of strategic mineral supplement sodium di-hydrogen orthophosphate dehydrate was used as source of phosphorus. Zinc oxide and copper sulphate were used as source of Zn and Cu respectively (table 02). Then deficiency of minerals was calculated and required quantity of salts was weighed. After weighing phosphorus supplement was packed separately in zip lock polythene bags. While zinc and copper supplements were packed in gelatin capsules. The supplementation was given to the pregnant buffaloes till the period of parturition along with feed. Pregnant buffaloes of both groups were observed for occurrence of hemoglobinuria during supplementation study.

Table 2: Mineral salts used for preparation of strategic mineral supplement

Mineral	Ingredients used	Percentage availability of element
Phosphorus	Sodium Dihydrogen Ortho phosphate dihydrate ($Na\ H_2PO_4.2H_20$) Ortho Phosphate Dihydrate ($NaH_2PO_4.2H_2O$)	20%
Zinc	Zinc oxide (ZnO)	80%
Copper	Cupper sulphate ($CuSO_4.5H_2O$)(Cu,25%)	25%

3. Results and Discussion

On studying the feeding of pregnant buffalos it was found that farmers were only offering the leguminous straw to their pregnant buffaloes. The details of supplementation study have been shown in Table nos.3-5. The average body wt of pregnant buffaloes were found 538.55 ± 1.6 kg. The average DMI intake was 10.23±0.10 kg. The daily major mineral availability of Ca, P and Mg was 153.41±1.46, 14.32±0.14 and 28.64±0.27 gm/day respectively. While trace mineral availability of Fe, Cu, Mn and Zn was 2250.01±21.40, 59.11±0.56, 511.37±4.86 and 59.22±0.56 mg/day respectively.
Daily requirements and availability of minerals in advanced pregnant buffaloes are presented in Table 04. Results indicated that there was shortage of phosphorus 40.34%, zinc 86.34% and copper 46.26%. When the availability of other minerals was compared with standard requirements, calcium, magnesium, iron, and manganese was shown to be supplied in excess 394.87%, 177.22%, 309.09% and 16.22% respectively. This may be due to supply of higher amounts of leguminous straws in their ration. Leguminous straw over supplies calcium, magnesium iron and manganese.
Feeding a balanced ration to buffaloes in the last trimester of pregnancy through the breeding season is critical. Nutritional demands increase greatly in late gestation and even more in early lactation. Vitamins and minerals play a vital role in metabolism, normal growth, production and reproduction (Thakur *et al.*, 2016) [18]. As per the deficiency status of the nutrients, a strategic mineral supplement was prepared using Sodium Dihydrogen Ortho phosphate dihydrate, Zinc oxide and copper sulphate.
The amounts of strategic mineral supplement was added in the normal routine diet of advanced pregnant buffaloes as presented in Table 05. Measured amounts of trace minerals were supplemented by placing them in gelatin capsules. while phosphorus supplement was packed in zip lock polythene bag.
After completion of supplementation study it has been observed that buffaloes of group 1 were calved successfully however 3 buffaloes of group 1 were suffered from hemoglobinuria during their period of study and were treated accordingly. Similar findings were reported by Bhikane *et al.* (2004); Dalir *et al.* (2006); Gahlawat *et al.* (2007) and Durrani et *al.* (2010) [3, 4, 8, 7]. They stated that adult buffaloes are usually affected with higher frequency during advanced pregnancy (8-9 months) and the immediate post-partum period (1-60 days post-partum)
In group 2 all buffaloes calved successfully without occurrence of hemoglobinuria. The present findings were in accordance to Smith *et al.* (1975) [16] who observed decreased incidence of hemoglobinuria after copper supplementation of cattle in a copper-deficient area.

Table 3: Availability of minerals in advance pregnant buffaloes of group 2 before treatment

An. No.	Body Wt. (Kg)	Ca (g)	P (g)	Mg (g)	Fe (mg)	Cu (mg)	Mn(mg)	Zn(mg)
1.	535.52	152.26	14.21	28.42	2233.15	58.67	507.53	58.77
2.	533.30	149.04	13.91	27.82	2185.92	57.43	496.80	57.53
3.	538.41	156.4	14.60	29.19	2293.87	60.27	521.33	60.37
4.	541.73	158.24	14.77	29.54	2320.85	60.98	527.47	61.08
5.	544.58	159.62	14.90	29.80	2341.09	61.51	532.07	61.61
6.	541.73	149.04	13.91	27.82	2185.92	57.43	496.80	57.53
7.	544.58	159.62	14.90	29.80	2341.09	61.51	532.07	61.61
8.	541.73	151.8	14.17	28.34	2226.40	58.49	506.00	58.59
9.	533.30	149.96	14.00	27.99	2199.41	57.78	499.87	57.88
10.	530.62	148.12	13.82	27.65	2172.43	57.08	493.73	57.17
Mean± SE	538.55 ± 1.6	153.41±1.46	14.32±0.14	28.64±0.27	2250.01±21.40	59.11±0.56	511.37±4.86	59.22±0.56

Table 4: Average daily requirement and availability of nutrients in pregnant buffaloes

	Body Wt. (Kg)	*Ca (g)	*P (g)	***Mg (g)	**Fe (mg)	**Cu (mg)	**Mn(mg)	**Zn(mg)
Requirement	550.0	31.0	24.0	10.33	550.0	110.0	440.0	440.0
Supply	550.0	153.41	14.32	28.64	2250.01	59.11	511.37	59.22
Deficit(-)/ Excess(+)		+ 122.41	-9.68	+18.31	+ 1700.01	-50.89	+71.37	-380.78
Deficiency/Excess %		+ 394.87	-40.34	+177.22	+309.09	-46.26	+16.22	-86.54

* Kearl, 1982; **Paul and Lal, 2010, and ***NRC 1989

Table 5: Composition of strategic mineral supplement (per head/ day) for pregnant buffalos

Ingredients	Availability of mineral	Salt Quantity required	P (g) (Supply)	Zn (mg) (Supply)	Cu (mg) (Supply)
Sodium Dihydrogen Ortho phosphate dihydrate ($Na\ H_2PO_4.2H_20$)(20%,P)	Phosphorus 20%	48.4 gm	9.68	00	00
Zinc oxide (ZnO) (Zn, 80%)	Zinc 80%	476.0 mg	00	380.80	00
Cupper sulphate ($CuSO_4.5H_2O$)(Cu,25%)	Copper 25%	203.50 mg	00	00	50.89
Total supply			9.68	380.80	50.89
In terms of percentage			95.75%	3.76%	0.58%

4. Conclusion

It can be concluded that strategic mineral supplement fulfilled the mineral requirement during the advanced gestation period and successfully prevented the occurrence of hemoglobinuria. So, it can be suggested that supplementation of phosphorus, zinc and copper during gestation period of buffalo is beneficial and can able to prevent the occurrence of hemoglobinuria in advanced pregnant buffaloes.

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