

# Bulk Cations and Trace Elements in the Nili-Ravi Buffalo and Crossbred Cow Bull Semen

KANWAL, M.R., N.U. REHMAN, N. AHMAD, H.A. SAMAD, ZIA-UR-REHMAN, N. AKHTAR AND S. ALI  
*Department of Animal Reproduction, University of Agriculture, Faisalabad-38040, Pakistan*

## ABSTRACT

A total of 36 semen samples, obtained from two Nili-Ravi buffalo and two Sahiwal X Friesian crossbred cow bulls were analyzed for four major cations and three trace elements. Among major cations, the concentration of sodium, potassium, calcium and magnesium averaged  $319.2 \pm 51.5$ ,  $64.5 \pm 16.1$ ,  $30.0 \pm 5.2$  and  $5.00 \pm 0.9$  mg/dl, respectively in the Nili-Ravi buffalo bull semen. The corresponding values for the crossbred cow bull semen were  $460.3 \pm 48.7$ ,  $177.9 \pm 29.3$ ,  $25.3 \pm 2.5$  and  $5.5 \pm 0.9$  mg/dl. The cow bull semen had significantly higher ( $p < 0.01$ ) sodium and potassium but lower calcium contents than the buffalo bull semen, while the difference in magnesium content between the two species was non-significant. Average concentrations of both copper and iron were significantly higher ( $p < 0.01$ ) in the crossbred cow than in the Nili-Ravi buffalo bull semen while the zinc concentration was comparable between bulls of the two species. In the Nili-Ravi buffalo bulls, the ejaculatory volume showed a significantly positive correlation ( $p < 0.05$ ) with magnesium and iron contents and a significantly negative correlation with zinc contents of semen. Similarly, the percentage of dead sperm was positively correlated with magnesium and copper contents of the semen while negatively correlated with zinc contents. In the crossbred cow bulls, only the iron contents of semen showed a significantly positive correlation with the motile sperm percentage, while all other correlations were non significant. These results emphasize the need for separate extenders for the proper preservation of semen from bulls of the two species.

**Key Words:** Bulk cations; Trace elements; Buffalo; Crossbred bull; Semen

## INTRODUCTION

Biochemical parameters of semen are of vital importance from its fertility and preservation aspects. Information on the extent of variation of seminal characteristics between bulls of different breeds is an essential pre-requisite for effective preservation of their germ plasm to be used in artificial insemination program. A large number of reports on the biochemical composition of cattle semen have been published (Singh *et al.*, 1969; Igboeli & Rakha, 1971; Gupta *et al.*, 1983; Rattan, 1990). But there is scarcity of parallel information about the semen of the Nili-Ravi buffalo and Sahiwal X Friesian crossbred cow bulls maintained under local conditions of Pakistan. The available data in this respect indicate that biochemical properties of buffalo bull semen differ from those of cattle semen in several aspects (Ganguli, 1980). This may be one of the reasons of poor preservability of the buffalo bull semen, as most of the extenders designed for cow bull semen are being used as such for preservation of buffalo bull semen.

This study was carried out to determine the concentrations of bulk cations (sodium, potassium, calcium and magnesium) and trace elements (copper, zinc and iron) in whole semen of the Nili-Ravi buffalo and Sahiwal X Friesian crossbred cow bulls. Attempts were also made to study the correlation coefficients between various physical and biochemical parameters of

semen collected from bulls of the two species. It is hoped that the results obtained will be useful for formulating semen extenders for bulls of the two species.

## MATERIALS AND METHODS

Four breeding bulls, including two Nili-Ravi buffalo (B1 and B10) and two "Sahiwal x Friesian" crossbred cow bulls SF1 and SF2, aged 6-8 years, maintained under similar managerial conditions at the Semen Production Unit, Department of Animal Reproduction, University of Agriculture, Faisalabad were used in this study. Semen was collected twice weekly from each bull with an artificial vagina. In all 36 semen samples, nine samples from each bull, were available for further processing.

Immediately after collection, semen samples were transferred to a water bath maintained at  $37^{\circ}\text{C}$ . Each sample was examined for its physical characteristics (color, ejaculatory volume, motile sperm percentage, sperm concentration and dead sperm percentage). The concentrations of bulk cations (sodium, potassium, calcium and magnesium) and trace elements (copper, zinc and iron) in the whole semen collected from bulls of the two species were also determined.

The volume and color of the ejaculates were recorded directly from the graduated vials used for semen collection. Motile sperm percentage was determined under light microscope with warm stage

maintained at 37°C, while the sperm concentration was determined using counting chamber (Hafez, 1987). The dead spermatozoa percentage was assessed by eosin-nigrosin staining technique (Campbell *et al.*, 1956). Two slides were prepared for each sample and at less 100 spermatozoa were counted per slide.

For the determinations of bulk cations and trace elements, the whole semen samples (one ml each) were digested separately with concentrated nitric acid and perchloric acid (Richard, 1954). Sodium and potassium determinations were made with a flame photometer according to the procedure described by Igboeli and Rakha (1971). Concentrations of calcium, magnesium, copper, zinc and iron were measured by using atomic absorption spectrophotometer using the respective hollow cathode lamps for each element (David, 1960; Willis, 1960a,b).

For statistical analysis, mean and standard deviation were calculated for each parameter. Student-t test was applied to see the magnitude of variation in various physical and biochemical parameters of semen quality between the Nili-Ravi buffalo and crossbred cow bulls. Correlation co-efficients between different physical and biochemical parameters for bulls of each species and their statistical significance were also computed (Snedecor & Cochran, 1967).

## RESULTS AND DISCUSSION

The mean ejaculatory volume of the Nili-Ravi buffalo bulls ( $4.7 \pm 1.51$  ml) was significantly lower ( $P < 0.05$ ) as compared to  $5.92 \pm 1.77$  ml for crossbred cow bulls (Table I). Suryaprakasam and Rao (1993) also reported similar findings for the Murrah buffalo and crossbred cow bulls. The percentage of dead spermatozoa was significantly higher ( $P < 0.01$ ) in the semen of crossbred cow as compared to that of the Nili-Ravi buffalo bulls. Other physical characteristics of

semen quality i.e. motile sperm percentage and sperm concentration did not differ between bulls of the two species (Table I). Among bulk cations, the concentration of sodium was the highest, followed by potassium and calcium, while the magnesium content was the lowest in the semen collected from bulls of both species (Table II). Concentrations of sodium and potassium were significantly lower ( $P < 0.01$ ) in the Nili-Ravi buffalo bull semen as compared to that of crossbred cow bulls (Table II). These results are in line with the findings of Kanakraj and Krishnamurthy (1984), Ezekwe and Orji (1988) and Dhami *et al.* (1991).

Contrary to sodium and potassium contents, mean concentration of calcium was higher ( $P < 0.01$ ) in the Nili-Ravi buffalo bull semen than that in crossbred cow bull semen (Table II). Similar results were obtained in previous investigations on the Murrah buffalo vs Hariara cow bulls (Kapoor, 1978) and the Nili-Ravi buffalo vs Sahiwal cow bulls (Humayun, 1984).

Mean concentration of magnesium in the semen of the Nili-Ravi buffalo and Sahiwal X Friesian crossbred cow bulls differed non-significantly (Table II). Similar observations have been reported for Surti buffalo and crossbred cow bulls (Gupta *et al.*, 1983; Misra *et al.*, 1989).

Among trace minerals, mean concentration of iron was the highest, followed by zinc and then copper in the semen of Sahiwal X Friesian crossbred cow bulls semen. In the Nili-Ravi buffalo bull semen, average concentrations of iron and zinc were almost similar and about seven times higher than the copper concentration (Table II). A previous investigation (Misra *et al.*, 1989) on semen of crossbred cow bulls revealed copper concentration to be lower than that recorded for the crossbred cow bulls used in this study. Cragle *et al.* (1958) and Misra *et al.* (1989), who reported  $< 0.1$  mg/dl,  $0.61$  mg/dl and  $0.28 \pm 0.12$  to  $0.4 \pm 0.037$  mg/dl, respectively.

**Table I. Mean values ( $\pm$  SE) of physical characteristics of Nili-Ravi buffalo and crossbred cow bull semen.**

Bull Number	Ejaculatory volume (ml)	Sperm motility (%)	Sperm concentration (billion/ml)	Dead sperm (%)
<b>Buffalo bulls</b>				
B1	$3.73 \pm 1.13$	$68.3 \pm 3.54$	$1.036 \pm 0.117$	$6.69 \pm 2.78$
B10	$5.67 \pm 1.19$	$68.33 \pm 3.54$	$1.236 \pm 0.145$	$10.15 \pm 1.12$
Mean	$4.70 \pm 1.51^*$	$68.33 \pm 3.43$	$1.136 \pm 0.164$	$8.42 \pm 2.72^{**}$
<b>Cow bulls</b>				
SF1	$6.38 \pm 1.95$	$69.40 \pm 6.35$	$1.090 \pm 0.138$	$14.32 \pm 1.02$
SF2	$5.44 \pm 1.53$	$72.22 \pm 3.63$	$1.153 \pm 0.142$	$13.49 \pm 1.58$
Mean	$5.92 \pm 1.77^*$	$70.83 \pm 5.22$	$1.121 \pm 0.140$	$13.91 \pm 1.36^{**}$

\*Mean values in the same column differ significantly ( $p < 0.05$ ); \*\*Mean values in the same column differ significantly ( $p < 0.01$ ).

**Table II. Mean concentrations (mg/dl,  $\pm$  SE) of various minerals in the whole semen of the Nili-Ravi buffalo and**

**crossbred cow bulls**

Bull Number	Na	K	Ca	Mg	Cu	Zn	Fe
Buffalo bulls							
B1	316.7±52.0	70.8±17.9	26.9±5.5	4.3±0.1	0.11±0.01	1.12±0.09	0.71±0.28
B10	321.4±54.1	58.2±11.7	33.1±2.1	5.7±0.6	0.16±0.02	0.80±0.46	1.18±0.33
Mean	319.2±51.5*	64.5±16.1*	30.0±5.2*	5.0±0.9	0.14±0.03*	0.96±0.36	0.95±0.38*
Cow bulls							
SF1	422.8±20.9	201.3±14.3	25.7±2.4	5.8±0.6	0.25±0.02	0.89±0.09	1.16±0.33
SF2	497.8±38.0	154.4±19.5	25.0±2.7	5.2±0.3	0.21±0.06	0.94±0.13	1.56±0.28
Mean	460.3±48.7*	177.9±29.3*	25.3±2.5*	5.5±0.9	0.23±0.05*	0.92±0.11	1.36±0.35*

Cragle *et al.* (1958) reported less than 0.2 mg/dl of zinc in cow bull semen. Differences in the dietary mineral level may be one of the factors responsible for these variations (Underwood & Somers, 1969).

Among the trace minerals, the concentrations of copper and iron were significantly higher ( $P<0.01$ ) in the semen of crossbred cow bulls as compared to the Nili-Ravi buffalo bulls. However, the concentrations of zinc in the semen did not vary among bulls of the two species (Table II).

In the Nili-Ravi buffalo bulls (Table III), the ejaculatory volume showed a significantly positive correlation ( $p<0.05$ ) with magnesium ( $r = 0.621$ ), and iron ( $r = 0.47$ ) contents and a significantly negative correlation with zinc contents of semen ( $r = -0.559$ ). Similarly, the percentage of dead sperm was positively correlated with magnesium ( $r = 0.623$ ) and copper ( $r = 0.492$ ) contents of the semen while negatively with zinc contents of the semen ( $r = -0.571$ ). Correlations between other parameters were non significant (Table III).

**Table III. Correlation coefficients between various parameters of semen quality in the Nili-Ravi buffalo bulls**

Semen parameters/minerals	EV	Motile sperm (%)	Sperm conc.	Dead sperm (%)
Motile sperm	-0.37			
Sperm conc.	.345	0.495*		
Dead sperm	.352	0.128	0.332	
Sodium	.069	0.026	0.064	0.061
Potassium	-0.124	0.165	-0.241	-0.229
Calcium	0.366	-0.003	0.248	0.402
Magnesium	0.621*	-0.216	0.343	0.623*
Copper	0.430	0.09	0.445	0.492*
Iron	0.470*	0.17	0.370	0.335
Zinc	-0.559*	0.20	-0.206	-0.571*

EV= Ejaculatory volume;

\* = Significant correlation at  $p<0.05$ .

However, in the crossbred cow bulls, only the iron contents of semen showed a significantly positive correlation ( $r = 0.529$ ,  $p<0.05$ ) with motile sperm percentage, while all other correlations were non significant (Table IV). This further emphasizes the need

for separate extenders for the proper preservation of semen from bulls of the two species. Further studies are imperative to formulate suitable semen extenders for bulls of the two species keeping in view differences in the bulk cations and trace elements contents of semen of these species.

**Table IV. Correlation coefficients between various parameters of semen quality in the crossbred cow bulls**

Para-meters	EV	MS (%)	SC	DS (%)
MS	-0.598*			
SC	-0.097	-0.181		
DS	0.015	0.063	0.028	
Na	-0.383	0.263	0.306	-0.454
K	0.811	-0.149	-0.106	0.433
Ca	-0.227	0.090	0.174	-0.133
Mg	0.148	0.001	-0.245	0.247
Cu	0.109	-0.395	-0.282	0.148
Fe	-0.287	0.529*	-0.072	-0.257
Zn	-0.268	0.044	0.187	0.058

EV= Ejaculatory volume; MS= Motile sperm; SC= Sperm concentration; DS= Dead sperm; \* = Significant correlation at  $p<0.05$ .

## ACKNOWLEDGEMENTS

The authors are thankful to Dr. Shafqat Nawaz and Mr. A. Sattar Khan, Department of Soil Science, University of Agriculture, Faisalabad for providing necessary facilities to carry out analytical work for this project.

## REFERENCES

- Campbell, R.C., H.M. Dott and T.D. Gloner, 1956. Nigrosin-eosin as a stain for differentiating live and dead spermatozoa. *J. Agri. Sci. Camb.*, 48: 1-8.
- Cragle, R.G. and J.H. Muntz, 1958. Mineral levels of bovine semen and seminal plasma. *J. Dairy Sci.*, 39: 922.
- Cragle, R.G., G.W. Salisbury and N.L. VanDemark, 1958. Sodium, potassium, calcium and chloride distribution in bovine semen. *J. Dairy Sci.*, 41: 1287.
- David, D.J., 1960. The determination of exchangeable sodium, potassium, calcium and magnesium in soil by atomic absorption

- spectrophotometry. *Analyst*, 85: 495.
- Dhami, A.J., M.T. Panchal and S.B. Kodagali, 1991. Comparative biochemical and enzymatic study of Jersey, crossbred and Surti buffalo semen with reference to freezability and fertility. *Indian J. Anim. Res.*, 24: 1–8.
- Ezekwe, A.G. and B.I. Oji, 1988. A comparative study of the seminal biochemistry in 'N' Dame (*Bos taurus*) and Muturu (*Bos brachyceros*) bulls. *Bull. Anim. Hlth. Prod. Africa*, 36: 180–84.
- Ganguli, N.C., 1980. Biochemistry of semen processing in buffalo reproduction and artificial insemination. Proceedings of the seminar sponsored by FAO/SIDA/Govt. of India. December 1978, National Dairy Research Institute, Karnal, India.
- Gupta, A.K., A.P. Asopa and A.K. Ghosal, 1983. Some observations on bulk cations and trace mineral levels in Surti buffalo bull semen. *Indian J. Anim. Sci.*, 53: 1337–8.
- Hafez, E.S.E., 1987. *Reproduction in Farm Animals*, 5th ed., Lea and Febiger, Philadelphia, USA.
- Humayun, F., 1984. Study on sodium, potassium, calcium, magnesium and chloride profiles in cattle and buffalo semen. *M.Sc. Thesis*, Univ. Agri., Faisalabad.
- Igboeli, G. and A.M. Rakha, 1971. Major cations in semen of Angoni (short horn zebu) bulls. *J. Reprod. Fert.*, 24: 377–81.
- Kanakraj, P. and U.S. Krishnamurthy, 1984. Influence of potassium and sodium on the quality of buffalo semen. *Cheiron*, 13: 48–50.
- Kapoor, R.D., 1978. Note on some chemical and biochemical characteristics of semen of Murrah and Haryana bulls in various months. *Indian J. Anim. Res.*, 12: 53–4.
- Misra, T.P., V.B. Saxena and S.S. Tripathi, 1989. Trace elements in seminal plasma of crossbred bulls. *Indian J. Anim. Sci.*, 59: 1245–8.
- Rattan, P.J.S., 1990. Physico-chemical constituents of buffalo bull semen. *Proc. 2nd wrld. Buff. Cong.* Indian Society of buffalo development and Indian Council of Agricultural Research, pp. 26–30.
- Richard, L.A., 1954. *United States Lab. Staff Agri. Handbook No.60*, United State Department of Agriculture, 129.
- Singh, B., B.B. Mahapatro and D.P. Sadhu, 1969. Chemical composition of cattle and buffalo spermatozoa and seminal plasma under different climatic conditions. *J. Reprod. Fert.*, 20: 175–8.
- Snedecor, G.W. and W.G. Cochran, 1967. *Statistical Methods*, Iowa State University Press, Ames, USA.
- Suryaprakasam, T.B. and A.V. Rao, 1993. Studies on seminal characteristics of multiple breed A.I. bulls. *Indian Vet. J.*, 70: 629–32.
- Underwood, E.J. and M. Somers, 1969. Studies of zinc nutrition in sheep. I. The relation of zinc to growth and testicular development. *Australian J. Agri. Res.*, 20: 889.
- Willis, J.B., 1960a. The determination of metals in blood serum by atomic absorption spectroscopy. I. Calcium. *Spectrochim. Acta*, 16: 259.
- Willis, J.B. 1960b. The determination of metals in blood serum by atomic absorption spectroscopy. II. Magnesium. *Spectrochim. Acta*, 16: 273.

(Received 18 August 2000; Accepted 20 September 2000)