EFFECT OF UN-DEGRADABLE PROTEIN SUPPLEMENTATION ON SEMEN QUALITY OF BUFFALO BULLS UNDER HEAT STRESS CONDITIONS

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ABSTRACT

A study was conducted to investigate the effect of feeding un-degradable protein (UDP) supplement on the semen quality of buffalo bulls during hot and humid part of summer season in Peshawar. Six Nili-Ravi buffalo bulls maintained at a semen production unit, Peshawar were randomly divided into two equal groups (control and treatment). Both groups were fed a basal diet of maize fodder (15 Kg/day/bull) and wheat straw (6 Kg/day/bull). Bulls in the control group received 5 kg /day/head of a commercial concentrate while the treatment group was fed 3 Kg commercial concentrate and 1 Kg UDP supplement. Both supplements were iso-nitrogenous and provided 150g nitrogen /head/day. The diets were fed in a switch over design over two periods. Each period lasted for 32 days. One-week adaptation period was allowed at the start of each experimental period. *In-sacco* protein degradability at 12 hours incubation for commercial concentrate and UDP supplement was 72.87 and 43.46%, respectively. Mean ambient temperature, humidity and temperature-humidity-index were 32.01°C, 66.66% and 84.97, respectively. Semen volume of bulls in the control and treatment groups did not vary and averaged 6.87 ± 0.41 and 7.41 ± 0.56 ml /collection day with a mean sperm concentration of 1004.5 ± 69.06 and 969.14 ± 77.88 $x10^6$/ml, respectively. Number of defective sperms (head abnormalities, mid-piece defects, proximal droplet and tail defects) in the control and treatment groups was not different. Feeding of UDP supplement did not influence the blood concentrations of calcium, phosphorus, urea and glucose. The absence of UDP effect on semen volume and quality could be attributed to low thermal stress, feeding small quantity of UDP and less number of replicate bulls used in the present study.

Key words: Buffalo bulls, heat stress, semen quality, un-degradable protein.

INTRODUCTION

Water buffalo has an ancient history of domestication, which in China and Indian sub-continent reaches at least 2500 BC (Sidhu and Guraya, 1985). In spite of great usefulness of buffaloes, very little work has been done to exploit both its productive and reproductive potentials. At present 164 artificial insemination (A.I.) centers are actively serving the farmer community throughout the NWFP. Both fresh and frozen semen is being used at these centers. Annually, these centers are providing services to about 21,000 buffaloes and 136,000 cows (Anonymous, 1996). Field observations have indicated that the success rate of AI in buffaloes is lower than in cows. In addition to many other factors, poor quality of semen is considered as one of the important reasons of low success rate of AI in buffaloes. The quality of a buffalo bull depends both on its genetic make-up and environmental influences such as temperature, humidity, nutrition and management, which seriously affect the breeding performance of the bull (Ahmad *et al*., 1964).

In Peshawar valley, summer generally extends from May to September. During this period, temperature may peak to 45°C with a maximum humidity of 95%. These environmental extremes may affect normal physiological functions and may lead to the production of low quality semen. There are reports that nutritional manipulations can reduce the adverse effects of environmental stress (Collier *et al*., 1982). The present study was therefore conducted to examine the quality and the quantity of semen produced by the breeding buffalo bulls exposed to summer heat stress and to investigate their response to partial replacement of conventional concentrate with a concentrate high in rumen un-degradable protein.

MATERIALS AND METHODS

Experimental design and animals

The experiment was conducted in a 2X2 switch over design, consisting two diets and two periods of 32 days each. Six Nili-Ravi buffalo bulls (age about 7 years) kept at the Semen Production Unit, Surezai were used. The bulls were randomly divided into two equal groups. All the bulls were kept in individual pens where clean drinking water was available all the time. Daily

temperature and humidity in the bull shed was recorded with the help of a hygrothermograph. Bulls were weighed fortnightly and rectal temperature was recorded twice a day at 6 AM and 6 PM, using a clinical thermometer.

Diets and feeding

In both control and treatment diets the roughage part provided 15 kg green maize fodder and 6 kg wheat straw/bull/ day. Concentrate mixture on iso-nitrogen basis (150 g N/head/day) was fed to bulls of both groups. A commercial concentrate mixture (18.78% CP in dry matter) was fed to the control group at the rate of 5 Kg/bull/day while bulls in the treatment group were given 3 Kg commercial concentrates and one Kg test concentrate. The test concentrate was prepared by mixing 30% blood meal, 50% corn gluten meal and 20% cottonseed meal and contained 43.40% crude protein of low rumen degradability. The nutrient composition of feed ingredients used in the study is given in Table 1.

Di-calcium phosphate 100 gm/head/day was fed to all the bulls. Concentrate and di-calcium phosphate were mixed with wheat straw and offered in the morning, while chopped maize fodder was offered in the evening. One-week adjustment period was allowed at the start of each experimental period. Representative samples of all the feed ingredients were collected fortnightly and analyzed for dry matter, ash, crude protein, ether extract and crude fiber according to the standard procedures of AOAC (1990).

Measurement of protein degradability

Rumen degradability of the commercial concentrate and the ingredients of the test concentrate were measured with the Dacron bag technique, as described by Orskov *et al.* (1980). Dacron bags each containing about 5 gm samples were incubated in three rumen fistulated buffalo steers for 2, 4, 8, 12, 24 and 48 hours. All the bags were washed, dried and weighed, as described by Qureshi *et al.* (1987). The bag residues were analyzed for CP (AOAC, 1990) and DM and CP degradability were calculated.

Semen collection

Semen was collected early in the morning on each collection day (twice a week) by using the artificial vagina method. Prior to semen collection, each bull was cleaned and sexually stimulated to mount on a dummy. After one false mount the semen was collected. Separate artificial vagina for one ejaculate per collection with an inside temperature 37°C to 42°C was used for each bull. A total of 53 ejaculates from the control group and 51 ejaculates from the treatment group were collected during the experimental period of 64 days.

Physical examination of semen

Immediately after collection, the semen ejaculates were incubated in a water bath at 37°C for 5 minutes. Semen volume was measured in a graduated test tube while color of the semen was evaluated on 0-4 scale, where 0 was used for watery and 4 for thick creamy semen (Ala-ud-Din, 1985). Seminal pH was recorded with the help of a digital pH meter. Mass motility was determined by examining of a thick drop of semen under a microscope (low power x10 and reduced light) for the presence of waves on a numerical scale (Ala-ud-Din, 1985).

For the estimation of sperm concentration, a well-mixed semen sample (0.2 ml) was transferred to a small clean vial and a few drops of methylene blue solution were added. Semen was slowly sucked in a RBC pipette up to the mark 1 and diluted 100 times by drawing 3% NaCl solution into the pipette (up to the mark 101). After mixing and discarding initial few drops, the diluted semen was placed on a counting chamber under a cover slip. The number of spermatozoa in 1 ml was calculated by multiplying the spermatozoa count in 80 small squares with 5,000,000 (Ala-ud-Din, 1985).

Morphological examination of semen

Morphological examination of semen was carried out only once a week. For each bull three slides per ejaculate were prepared and stained with Eosin-Nigrosin stain (Ala-ud-Din, 1985). At random 200 spermatozoa in each slide were counted under a microscope. The proportion of sperms having the following abnormalities were recorded.

i. Head abnormalities (large, small, elongated, detached).
ii. Mid-piece abnormalities (broken, swollen).
iii. Tail abnormalities (bent, coiled).
iv. Proximal droplets.

Measurement of blood metabolites

Jugular blood samples (10 ml) were collected from all the bulls two hours after feeding on day 1, 15, 30, 40, 55, and 70 of the experiment. The plasma was separated and stored at -20°C until analyzed. Plasma samples were assayed in duplicate for glucose, urea, calcium and phosphorus concentrations using commercial kits (Clonital, New Jersey, USA).

Statistical analysis

The data were analyzed with the analysis of variance using general linear model of SAS (2000). Means were compared with the Least Significant Difference procedure (Steel and Torrie, 1981), where necessary.

RESULTS AND DISCUSSION

Ambient temperature and humidity

Ambient temperature and humidity were recorded daily throughout the experiment and are summarized in Table 2. For breeding buffalo bulls the environmental temperature from 10°C to 35°C is considered as comfortable without having any adverse effect on semen production or its characteristics (Abbhi, 1968). The data in Table 2 suggest that ambient temperature during the study remained in the upper limit of this comfortable zone. During the experiment, ambient temperature peaked to 37.5°C for a few days. However, no adverse effects were noted on the quality of the semen produced during these days. Generally, high temperature combined with high humidity is more stressful to farm animals. Temperature humidity index (THI) from 78 to 89 may cause intermediate stress in dairy cattle (Armstrong, 1994). However, this may be true for temperate zone cattle. In tropical and sub tropical regions, native Zebu cattle and buffaloes are adapted to a relatively high ambient temperature. Therefore, the level of THI recorded in the present study apparently did not cause stress and therefore, quality and quantity of the semen produced was not adversely affected.

Chemical composition and protein degradability of feed ingredients

Nutrient composition of the feed ingredients used in the experiment is given in Table 1. All the three test concentrate ingredients (blood meal; cottonseed meal and corn gluten meal) were higher in protein contents than the commercial concentrate ($P<0.01$). Results on protein degradability are summarized in Table 3. At all incubation times, protein degradability of blood meal was lowest ($P<0.05$) followed by corn gluten meal, cottonseed meal and commercial concentrate mixture. Except blood meal, the degradability of other ingredients of the test concentrate was not different from the commercial concentrate at 48 hours of incubation. As the proportion of blood meal in the test concentrate was low (30%), the test concentrate may not have appreciably improved the overall supply of un-degradable protein (UDP) to the small intestine of the buffalo bulls. Cottonseed meal has been reported as a good source of by-pass protein (Preston and Leng, 1987). However, in the present study, the UDP fraction of cottonseed meal was not high, presumably due to different processing methods used for oil extraction in the local industries.

Physical characteristics of semen

Semen volume, sperm concentration and mass motility

A total of 53 ejaculates from the control group and 51 ejaculates from the treatment group were collected during the experimental period of 64 days. The average volume of semen produced by the bulls in control and treatment groups on each collection day was 6.87 ± 0.41 and 7.41 ± 0.56 ml, respectively (Table 4). The semen volume tended to be higher in the buffalo bulls of the treatment group than in the control group. However, the difference was statistically not significant, presumably due to high variation caused by the bulls. The semen volume varied from 1 to 10 ml and resulted in a co-efficient of variation of 40.93%. Similarly, the average sperm concentrations in the semen of control and treatment bulls were 1005.45 ± 69.06 and 969.14 ± 77.88 x 10^6/ml respectively, which were not different.

The semen volume produced by the buffalo bulls in the present study was higher than the values (3.4 ml and 3.2 ml) reported by Ahmad *et al.*, (1964) and Malik *et al.*, (1974) respectively. Bajwa *et al.* (1982) attributed low semen volume produced in their study to the scarcity of green fodder and increased heat stress. Present study was also conducted during summer; however, bulls were fed both green fodder and concentrate in adequate quantities that provided nutrients according to those recommended by NRC (1988).

According to Rekwot *et al.* (1988), both ejaculate volume and sperm concentration can respond to variations in protein intake. In the present study, daily protein consumption in all the bulls remained the same. The average sperm mass motility ranked on a scale 0 to 4 was recorded as 2.50 and 2.33 for control and treatment groups, respectively (Table 4) and was not different.

Semen color and pH

The average score of semen color on scale 0-4 was 2.11 and 2.16 (light creamy) for control and treatment groups, respectively and was not affected by the diets used in the study (Table 4). Lack of difference in semen color of control and treatment bulls observed in the present study may be attributed to the absence of difference in semen volume and sperm concentration (Saeed, 1988).

Mean pH values of the semen collected from control and treatment bulls were 6.68 ± 0.10 and 6.64 ± 0.12, respectively (Table 4). The pH of the semen was not influenced by the difference in source of dietary protein.

Sperm abnormalities

Average sperm abnormalities observed in the

Table 1. Nutrient composition of feed ingredients used in the experiment

Feed Ingredients	Dry matter %	Percent in dry matter			
		Ash	Crude protein	Crude fiber	Ether extract
Blood meal	88.01	4.12	60.12	0.80	0.58
Corn gluten meal	87.69	1.19	39.21	8.35	4.96
Cottonseed meal	90.11	10.02	29.13	10.33	2.71
Commercial concentrate	86.75	14.06	18.78	23.57	3.21
Green maize fodder	89.72	19.56	9.72	20.60	1.8
Wheat straw	92.00	12.41	4.46	42.73	0.17

Table 2. Average values of ambient temperature, humidity and temperature humidity index during the experiment

Parameters	Period I			Period II		
	Min.	Max.	Av.	Min.	Max.	Av.
Ambient temperature (°C)	32.1	37.5	33.75^{a}	23.5	32.7	30.27^{b}
Humidity (%)	58.4	84.7	67.95^{a}	53.2	77.7	65.30^{b}
Temperature-humidity index	81.5	95.5	88.5^{a}	69.17	85.5	77.34^{b}

Means in the same row with different superscripts are significantly different (P<0.05)

Table 3. Protein degradability of feed ingredients at different incubation times (% of total CP present)

Incubation hours	Feed ingredients			
	Blood meal	Cotton seed meal	Corn gluten meal	Commercial concentrate
2	4.92^{b}	53.09^{a}	56.61^{a}	53.09^{a}
4	5.75^{c}	55.98^{a}	58.36^{a}	62.10^{a}
6	6.93^{c}	64.75^{b}	59.10^{b}	75.66^{a}
8	8.30^{c}	72.73^{a}	64.06^{b}	73.02^{a}
12	10.63^{c}	77.36^{a}	63.69^{b}	72.87^{a}
24	18.12^{c}	87.75^{a}	74.72^{b}	83.22^{a}
48	42.66^{b}	92.22^{a}	91.73^{a}	86.47^{a}

Means in the same row with different superscripts are significantly different (P<0.05)

Table 4. Seminal characteristics of experimental buffalo bulls (mean ± SE)

Seminal parameters	Control group	Treatment group*
Volume (ml)	6.87 ± 0.41	7.41 ± 0.56
Sperm concentration (10^6/ml)	1005.45 ± 69.06	969.14 ± 77.88
PH	6.68 ± 0.1	6.64 ± 0.12
Color (0-4)	2.11	2.16
Mass motility (0-4)	2.5	2.33
Total sperm abnormalities (%)	7.76	6.69

*Differences between the two groups for all the parameters are non-significant.

Table 5. Sperm abnormalities (%)in experimental buffalo bulls (mean ± SE)

Sperm abnormalities	Control group*	Treatment group*
Head abnormalities	4.05 ± 1.09	2.73 ± 0.85
Mid-piece abnormalities	0.27 ± 0.19	0.24 ± 0.11
Proximal droplets	0.21 ± 0.08	0.55 ± 0.18
Tail abnormalities	3.23 ± 0.46	3.17 ± 0.49
Total	7.76 ± 1.82	6.69 ± 1.63

*Differences between the two groups for all the parameters are non-significant.

Table 6. Serum concentration of different metabolites in experimental buffalo bulls (mean ± SE)

Blood metabolites	Control group*	Treatment group*
Calcium (mg/lit)	95.83 ± 2.73	98.91 ± 2.51
Phosphorus (mmol/lit)	1.80 ± 0.07	1.91 ± 0.08
Urea (mg/dl)	39.20 ± 0.96	38.10 ± 1.38
Glucose (mg/dl)	88.40 ± 8.18	76.50 ± 6.49

*Differences between the two groups for all the parameters are non-significant.

present study were 7.76 and 6.69% in control and treatment groups respectively (Table 4), which were not different. Sperm abnormalities in the range of 10-11% are usually acceptable (Saeed, 1988). However, large variation in sperm abnormalities (4 to18.5%) for buffalo bulls has been reported in the literature (Gill *et al.*, 1974; Malik *et al.*, 1974). Environment, staining technique, human error and reaction time are the factors generally causing sperm abnormalities (El-Wishy, 1978).

Head abnormalities of sperm were 4.05 ± 1.09 and 2.73 ± 0.85% in control and treatment groups, respectively (Table 5). Apparently, head abnormalities of spermatozoa in control bulls were higher than the treatment group but the difference was not significant. In the present study, the number of sperms with elongated, detached, small and large heads was also observed. Total head abnormalities observed in the present study were not high and were below the accepted limits (11.72%) of a fertile buffalo bull, as reported by Malik *et al.* (1974).

The average values of mid piece abnormalities non significantly varied from 0.27 ± 0.19% in control to 0.24 ± 0.11% in the treatment group (Table 5). Thus diets used in the present study did not produce any effect on mid piece abnormalities in the sperms of the buffalo bulls. Similar values for mid piece abnormalities (0.2%) were also reported by Malik *et al.* (1974) and Saeed (1988) in Nili Ravi buffalo bulls. Mid piece abnormalities observed in the present study included broken mid piece and swollen mid piece.

Mean values of sperm proximal droplets observed in the present study were 0.21 ± 0.08 and 0.55 ± 0.18% in control and treatment groups, respectively (Table 5) the difference was not different. Malik *et al.* (1974) and Saeed (1988) also reported similar values for proximal droplet in the sperms of buffalo bulls. However, large variation among the bulls irrespective of the diets was observed in both the groups.

The present study did not reveal any difference in tail abnormalities of spermatozoa produced by the bulls given the two different diets (Table 5). The average tail abnormalities were 3.23 ± 0.46 and 3.17 ± 0.49% in control and treatment groups, respectively.

Blood metabolites

Calcium and phosphorus

No difference in blood concentrations of calcium and phosphorus was observed between the two treatment groups of buffalo bulls (Table 6). Serum calcium levels can be depressed when diets are low in calcium or high in phosphorus. A positive correlation exists between phosphorus intake and blood phosphorus levels, however, it does not always reflect phosphorus intake (Topps and Thompson, 1984). In the present study, all buffalo bulls were fed same quantity of di-calcium phosphate as a source of dietary calcium and phosphorus which may explain the lack of difference in calcium and phosphorus between the two groups.

Urea and glucose

Blood urea levels in the experimental buffalo bulls of both the groups were also not different (Table 6). As both the groups were fed iso-nitrogenous diets and the degradability of protein in the two concentrates did not vary markedly, the blood urea also remained the same. In the present study, plasma glucose levels in all the bulls were in the higher range and were not different due to diet (Table 6).

Body weight and rectal temperature

Source of supplementary protein did not influence body weights of the bulls and changes in body weight remained the same on both the diets. The rectal temperature of bulls varied from 37.8 to 38.4°C. The ambient temperature during the experiment did not affect rectal temperature of the buffalo bulls.

The present study revealed that partial replacement of conventional concentrate with UDP protein source did not cause marked differences in the levels of calcium, phosphorus, glucose and urea in the circulatory blood, which may explain the lack of difference in semen volume, pH, color, sperm concentration, mass motility and morphological abnormalities of sperms under the conditions of the present study. It is assumed that relatively large proportion of UDP in the concentrate may be required to illicit marked improvement in the semen quality of breeding bulls exposed to thermal stress.

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