INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 6, p. 115-125, 2019

RESEARCH PAPER OPEN ACCESS

# Impact of vitamin C and electrolytes supplementation on semen quality of exotic and indigenous poultry under thermal stress

**Muhammad Inam[1], Sarzamin Khan[2], Naila Imtiaz[2], Asad Sultan[2], Nazir Ahmad Khan[2], Rafiullah[2], Naseer Ahmad[2], Emad Mohammed Elken[3], Hai-peng Zhang[4], Yi-ming Wang[4], Kokou Ayefounin Odah[4], Ling-Cong Kong[4*]**

[1]*Shaheed Benazir Bhutto University Sheringal Dir Upper, Pakistan*

[2]*Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Pakistan*

[3]*Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Nasr City, Cair, Egypt*

[4]*College of Animal Science and Technology, Jilin Agricultural University, Xincheng Street No.2888, Changchun 130118, China*

**Key words:** Semen, Breed, Antioxidants, Thermal, Stress.

http://dx.doi.org/10.12692/ijb/15.6.115-125 Article published on December 18, 2019

## Abstract

Warm and stressful environment adversely affect poultry but to search out the reasons for reduced fertility during extremes of summer was scanty. The Antioxidants may counter balance stress and improve semen quality. Therefore, the current study was designed to study the effects of vitamin C/electrolytes supplementation, breed and semen collection frequency on semen quality using 243 roosters in a 3 x 3 x 3 factorial design (n = 9/group). Semen volume, sperm concentration, motility, viability, and morphology were evaluated. There were effects ($P < 0.05$) on sperm concentration, motility, and viability in the roosters receiving 1 mL (4.6± 0.12 x $10^9$ sperm/mL, 53.7 ± 1.03 %, and 53.6 ± 1.01%, respectively) or 2 mL of supplementation (4.6 ± 0.13 x $10^9$ sperm/mL, 54.9 ± 1.04%, and 55.2 ± 1.02%, respectively) when compared to control (4.3 ± 0.11 x $10^9$ sperm/mL, 48.6 ± 1.01%, and 46.8± 1.04%, respectively). Semen volume was less in indigenous roosters (0.146 ± 0.01 mL) than in Cobb-500 and Starbro roosters (0.306 ± 0.01 and 0.284 ± 0.01 mL, respectively). However, sperm concentration, motility and viability were greater in indigenous roosters (5.98 ± 0.13 x $10^9$ sperm/mL, 76.8 ± 1.0%, and 74.4 ± 1.2%, respectively) than in Cobb-500 (4.11 ± 0.11 x $10^9$ sperm/mL, 40.1 ± 0.9%, and 40.7 ± 1.1%, respectively) and Starbro (3.33 ± 0.12 x $10^9$ sperm/mL, 40.4 ± 1.0%, and 40.8± 1.0%, respectively). No significant effect was observed on sperm morphology. In conclusion, the supplementation revealed in better results in indigenous than exotic poultry.

* **Corresponding Author:** Ling-Cong Kong ✉ konglingcong@jlau.edu.cn

## Introduction

Semen evaluation is not only important for selection of good breeding males but also to monitor reproductive performance (Cheng *et al.,* 2002). Evaluation of motility, live-dead sperm and morphological defects are indicators for fertilizing ability in chicken semen (Tabatabaei*et al.,* 2009). For proper evaluation of semen the ejaculate should not be mixed in order to assess the visual observation for colour, volume and pH as well as for the evaluation of reproductive potentials (Holsberg*et al.,* 1998). The main cause of decline in fertility in natural mating is the selection of heavy broiler breeder (McDaniel, 1978). In future, if the broiler breeders become so large as like turkey then natural mating will be less successful and artificial insemination will be applied. Keeping in view the future planning artificial insemination is of greater importance (Reddy, 1995).Semen quality should be examined prior to artificial insemination in order to enhance the fertility by evaluating different parameters (Dumpala *et al.,* 2006). Different methods are used for determination of semen quality by checking different parameters like motility, viability, colour, volume, concentration and sperm morphology (Donoghue and Wishart, 2000).

In the areas having hot climate the poultry faces problems of high environmental temperature which results in adverse effect on poultry production (McDaniel*et al.,* 1995, Karaca *et al.,* 2002 a,b). Semen quality, fertility, semen quantity and hatchability decreases with high temperature which have detrimental effects on fertilization (Karaca*et al.*,2002 a,b).The detrimental effects of high environmental temperature can be eradicated or reduced by different methods which include short term fasting, manipulation of diet, antioxidant nutrients supplementation in the form of Melatonin and ascorbic acid (Altan *et al.*,2000 a,b, Sahin *et al.*,2004) but the literature regarding effect of summer stress on broiler breeder and indigenous cocks in Pakistan is scanty.The mating frequency is reduced with high environmental temperature which results in reduction of fertility as well as semen quality and production (Galah, 2008). Reproduction of poultry can be improved by supplementation of ascorbic acid (McDaniel,*et al.*,1995). Likewise, the fertility decreases by the use of artificial insemination when the broiler breeder males are heat stressed. Infertility of broiler breeder in males is due to changes in semen characteristics during summer stress (Karaca, 2002) and need for ascorbic acid in chicken may be increased due to heat stress The ascorbic acid produced during heat stress cannot fulfill the physiological needs of the body in chicken (Pardue and Thaxton, 1986).

In Pakistan broiler breeder or grandparent stocks are mostly from European countries. These require time for adaptation to summer stress of Pakistan. Faisal (1998) reported lower hatchability in breeder flocks at Abbot Abad and Mansehra. This decline in hatchability may be associated with low fertility of males in summer season. Present study was therefore, planned to compare the semen from Starbro, Cobb-500 broiler breeders and indigenous cocks (Non-Descriptive) under summer stress of Peshawar and to study the effect of collection frequency and vitamin C and electrolytes supplementation on semen.

## Materials and methods

The present study was conducted at the Department of Poultry Science, Faculty of Animal Husbandry and Veterinary Sciences; The University of Agricultural Peshawar- Pakistan. Total of 81 cocks of approximately same age were selected. All the cocks were vaccinated and dewormed before subjecting to the experiment. The cocks were reared on the litter floor. Three breeds of cocks were used for the present experiment comprising 27 each of Starbro, Cobb-500 broiler breeders and Indigenous cocks (Non-Descriptive). The exotic broiler breeder were obtained from Geo Chicks Poultry Breeding Farm, Shinkyari; Mansehra Khyber Pakhtunkhwa. The cocks of the same age (30 weeks) were selected for the accurate semen evaluation because the age may affect different parameters of the semen.The study was conducted in summer season and no cooling supply was given to the house except the room electric fans because heat

factor was involved in the study and 16 hours light was provided to the cocks during experimental period. The ambienttemperature was between 38-44°C during experimental period. The semen collection and evaluation was done in the favorable environment in the Semen Production Unit having the temperature of 15-20°C. Each group of the birds was brought to SPU in order to avoid the collected semen from environmental stress and lethal effects of the heat and light. The collection was made by handling the bird gently between the legs due to heavy weight and massaging and squeezing the area around the cloaca before milking the semen in the test tube (Lake, 1995).The flock was divided into three groups designated as Group St, Cb and Ic, representing starbrow, Cobb-500 and Indigenous cocks breeds respectively. These groups were divided into three subgroups comprising nine cocks each. One sub group was kept control having no vitamin C and electrolytes supplementation. In other two subgroups one was provided with one ml vitamin C and electrolytes solution per liter of drinking water while the other was provided with two ml vitamin C and electrolytes solution per liter of drinking water.

Vitamin C and electrolytes supplement solution was composed of 60 grams ascorbic acid, 3.9 grams sodium citrate, 9.5 grams sodium chloride and 1.5 grams potassium chloride per liter. Each sub group was maintained in separate partition of the same environment. All cocks were provided with routinely used male breeder ration at the rate of 120 grams per day and water was provided *ad libitum*.

After the adaptation period of 2 weeks semen collection was started with the following frequencies. Total 243 samples were collected in the whole experimental period.In daily collection the samples were obtained in the morning for consecutive three days from all cocks of all groups. A day rest was given to the birds for alternate day collection. Alternate day collection was made at every other day up to three sampling days and then again rest of three days was given to the birds and three collections were obtained each with three days interval.

Volume of the semen was measured by collecting the semen in graduated test tube of 1.5 ml.For the determination of motility fresh semen were diluted at 1:200 (Tabatabaei, 2009) with Modified Ringer's Solution (Martin, 1957). One drop of diluted semen was placed on slide and cover slip was kept over the semen drop for equal distribution.

The observation was performed with 400 microscopic magnifications and at least 10 microscopic fields were observed.Sperm concentration was performed with heamocytometer. The whole semen was diluted with spermicidal solution (Qureshi, 2011, Chaudry *et al.*, 2000). A drop of diluted semen was placed on both end of the heamocytometer. The sperm count in five square of the heamocytometer was made and the total count was calculated. These observations were done on the 400 microscopic magnifications. The sperm count was calculated according to the following formula.

$C = 50{,}000 \times N \times D$

Where: C = Concentration of Sperm per Volume (ml)

N = Number of Spermatozoa

D = Dilution rate

The live-dead sperm count was obtained on the basis of 300 spermatozoa by smear staining with eosin-negrosin (Tabatabaei*et al.*, 2009. Qureshi, 2011). The live-dead sperm count was done by microscopic observation (400 magnifications).

The live sperm were seen as white and the dead as red in colour (Qureshi, 2011).The stained slide was observed for morphological defects on the basis of 300 cells for each preparation. Various defects of acrosome, head, mid-piece and tail were observed for each preparation (Tabatabaei*et al.*, 2009).

The experiment was laid out in Randomized Complete Block Design with split plot arrangements. Three factors i.e. vitamin C and electrolytes supplementations, breed of cocks and collection frequency were observed for all parameters.

**Results**

*Volume*

The present study was conducted for the comparative evaluation of semen from chicken and the effects of three factors on chicken semen were observed. Five parameters of semen were studied in response to vitamin C-Electrolytes supplementation and collection frequency. The mean volume obtained from Starbro, Cobb-500 broiler breeders and Indigenous cocks were 0.284, 0.306 and 0.146 ml respectively as given in Table 1.

**Table 1.** Mean volume (ml) of semen as affected by breed, vitamin C-Electrolytes supplementation and collection frequency.

| Breed | Vitamin C-Electrolytes supplementation | Collection Frequency | | | Mean |
|---|---|---|---|---|---|
| | | Daily | Alternate Day | Twice Weekly | |
| | | BR × VE × CF | | | BR × VE |
| St | Control | 0.223 | 0.267 | 0.317 | 0.269 |
| St | 1ml/L | 0.263 | 0.313 | 0.310 | 0.296 |
| St | 2ml/L | 0.280 | 0.303 | 0.283 | 0.289 |
| Cb | Control | 0.277 | 0.323 | 0.317 | 0.306 |
| Cb | 1ml/L | 0.283 | 0.327 | 0.327 | 0.312 |
| Cb | 2ml/L | 0.280 | 0.333 | 0.290 | 0.301 |
| Ic | Control | 0.127 | 0.163 | 0.157 | 0.149 |
| Ic | 1ml/L | 0.133 | 0.137 | 0.157 | 0.142 |
| Ic | 2ml/L | 0.130 | 0.143 | 0.170 | 0.148 |
| - | - | | BR ×CF | | Mean |
| - | - | - | - | - | BR |
| St | - | 0.256 | 0.294 | 0.303 | 0.284[a] |
| Cb | - | 0.280 | 0.328 | 0.311 | 0.306[a] |
| Ic | - | 0.130 | 0.148 | 0.161 | 0.146[b] |
| - | - | | VE ×CF | | Mean |
| - | - | - | - | - | VE |
| - | Control | 0.209 | 0.251 | 0.263 | 0.241 |
| - | 1ml/L | 0.227 | 0.259 | 0.264 | 0.250 |
| - | 2ml/L | 0.230 | 0.260 | 0.248 | 0.246 |
| - | Mean | 0.222[b] | 0.257[a] | 0.259[a] | - |

*Motility*

The mean sperm motility in semen from Starbro, Cobb-500 broiler breeder and Indigenous cocks were 40.386, 40.077 and 76.843% respectively as shown in Table 2. A significantly higher motility ($p<0.05$) was observed in Indigenous cocks followed by Starbro and Cobb-500 broiler breeders. Similarly vitamin C-Electrolytes supplementations also resulted into significantly ($p<0.05$) higher sperm motility. However, the effect of collection frequency on sperm motility was non-significant ($p>0.05$).

*Concentration*

The sperm concentration per ml of chicken semen in experimental birds is given in the Table 3. The mean sperm concentration per ml of semen from Starbro, Cobb-500 broiler breeders and Indigenous cocks were 4.335 $\times 10^9$, 4.110 $\times 10^9$ and 5.979 $\times 10^9$ respectively. Significantly higher sperm concentration ($p<0.05$) per ml of semen was observed in the Indigenous cocks than starbro and cobb-500 broiler breeders. Sperm concentration per ml of semen with vitamin C-Electrolytes supplementation at 1ml and 2ml/L was

significantly higher ($p<0.05$) than control groups.There was no significant difference among sperm concentration per ml of semen in all the breeds with collection frequencies. The combine interaction of breed, vitamin C-Electrolytes supplementation and collection frequency for sperm concentration was non-significant ($p>0.05$) statistically.

**Table 2.** Mean motility (%) of semen as affected by breed, vitamin C-Electrolytes supplementation and collection frequency.

| Breed | Vitamin C-Electrolytes supplementation | Collection frequency | | | Mean |
|---|---|---|---|---|---|
| | | Daily | Alternate Day | Twice Weekly | |
| | | BR × VE × CF | | | BR ×VE |
| St | Control | 37.780 | 36.340 | 35.560 | 36.560$^{cd}$ |
| St | 1ml/L | 39.113 | 43.337 | 41.113 | 41.188$^{bc}$ |
| St | 2ml/L | 43.560 | 42.113 | 44.557 | 43.410$^{b}$ |
| Cb | Control | 35.780 | 32.670 | 37.450 | 35.300$^{d}$ |
| Cb | 1ml/L | 44.670 | 38.003 | 39.227 | 40.633$^{bc}$ |
| Cb | 2ml/L | 48.003 | 42.447 | 42.447 | 44.299$^{b}$ |
| Ic | Control | 72.003 | 74.447 | 76.117 | 74.189$^{a}$ |
| Ic | 1ml/L | 76.560 | 78.340 | 82.780 | 79.227$^{a}$ |
| Ic | 2ml/L | 77.337 | 78.003 | 76.003 | 77.114$^{a}$ |
| - | - | | BR × CF | | Mean |
| - | - | - | - | - | BR |
| St | - | 40.151$^{bc}$ | 40.597$^{bc}$ | 40.410$^{bc}$ | 40.386$^{b}$ |
| Cb | - | 42.818$^{b}$ | 37.710$^{c}$ | 39.708$^{bc}$ | 40.077$^{b}$ |
| Ic | - | 75.300$^{a}$ | 76.930$^{a}$ | 78.300$^{a}$ | 76.843$^{a}$ |
| - | - | | VE × CF | | Mean |
| - | - | - | - | - | VE |
| - | Control | 48.521 | 47.819 | 49.709 | 48.683$^{b}$ |
| - | 1ml/L | 53.448 | 53.227 | 54.373 | 53.683$^{ab}$ |
| - | 2ml/L | 56.300 | 54.188 | 54.336 | 54.941$^{a}$ |
| - | Mean | 52.756 | 51.744 | 52.806 | - |

*Viability*

The mean viability percentage of sperm in the semen during summer stress is given in the Table 4. The mean viability of starbro, cobb-500 broiler breeders and Indigenous cocks was 40.756, 40.657 and 74.361% respectively. Significantly higher ($p<0.05$) sperm viability was observed for the Indigenous cocks then starbro and cobb-500 broiler breeders.

Sperm viability in the groups of all breeds treated with both one and two ml vitamin C-Electrolytes supplementation was highly significant ($p<0.05$) than control group.

*Morphological defects*

The morphological defects of starbro, cob-500 broiler breeders and local cocks were 9.867, 10.930 and 11.670% respectively as shown in Table 5. A non-significant affect ($p>0.05$) of all the three factors (breed, vitamin C-Electrolytes supplementation and collection frequency) over morphological defects for all breeds was observed.

**Discussion**

*Volume*

The volume obtained from starbro, cobb-500 broiler breeders and Indigenous cocks in our experiment was

0.284, 0.306 and 0.146 ml respectively which was significantly affected ($p<0.05$) by the breeds. Similar results were given by Pater *et al.,* (2008) who worked on semen quality traits of seven strain of chicken raised in humid tropics and reported that semen volume was significantly affected by the strain of chicken.

**Table 3.** Mean Sperm concentration/ml of semen as affected by breed, vitamin C-Electrolytes supplementation and collection frequency.

| Breed | Vitamin C-Electrolytes supplementation | Collection Frequency | | | Mean |
|---|---|---|---|---|---|
| | | Daily | Alternate Day | Twice Weekly | |
| | | | BR × VE × CF | | BR × VE |
| St | Control | 3.127x10$^9$ | 3.237 x10$^9$ | 3.290 x10$^9$ | 3.218 x10$^9$ |
| St | 1ml/L | 3.427 x10$^9$ | 3.517 x10$^9$ | 3.517 x10$^9$ | 3.487 x10$^9$ |
| St | 2ml/L | 3.323 x10$^9$ | 3.213 x10$^9$ | 3.367 x10$^9$ | 3.301 x10$^9$ |
| Cb | Control | 4.003 x10$^9$ | 3.637 x10$^9$ | 4.123 x10$^9$ | 3.921 x10$^9$ |
| Cb | 1ml/L | 4.470 x10$^9$ | 3.727 x10$^9$ | 4.440 x10$^9$ | 4.212 x10$^9$ |
| Cb | 2ml/L | 3.837 x10$^9$ | 4.437 x10$^9$ | 4.313 x10$^9$ | 4.196 x10$^9$ |
| Ic | Control | 6.273 x10$^9$ | 5.880 x10$^9$ | 5.293 x10$^9$ | 5.803 x10$^9$ |
| Ic | 1ml/L | 6.357 x10$^9$ | 6.023 x10$^9$ | 6.413 x10$^9$ | 6.264 x10$^9$ |
| Ic | 2ml/L | 6.103 x10$^9$ | 5.737 x10$^9$ | 5.770 x10$^9$ | 5.870 x10$^9$ |
| - | - | | BR × CF | | Mean |
| - | - | - | - | - | BR |
| St | - | 3.292 x10$^9$ | 3.322 x10$^9$ | 3.391 x10$^9$ | 3.335 x10$^{9b}$ |
| Cb | - | 4.103 x10$^9$ | 3.933 x10$^9$ | 4.292 x10$^9$ | 4.110 x10$^{9b}$ |
| Ic | - | 6.232 x10$^9$ | 5.880 x10$^9$ | 5.826 x10$^9$ | 5.979 x10$^{9a}$ |
| - | - | | VE × CF | | Mean |
| - | - | - | - | - | VE |
| - | Control | 4.456 x10$^9$ | 4.251 x10$^9$ | 4.236 x10$^9$ | 4.314 x10$^{9b}$ |
| - | 1ml/L | 4.751 x10$^9$ | 4.422 x10$^9$ | 4.790 x10$^9$ | 4.654 x10$^{9a}$ |
| - | 2ml/L | 4.421 x10$^9$ | 4.462 x10$^9$ | 4.483 x10$^9$ | 4.456 x10$^{9ab}$ |
| - | Mean | 4.543 x10$^9$ | 4.379 x10$^9$ | 4.503 x10$^9$ | - |

Likewise Zaharaddeen *et al.,* (2005) worked on local and exotic turkeys raised in tropical environment and significantly greater volume was obtained from exotic than local turkeys. Nwachukwu *et al.,* (2006) worked on the effect of genotype on semen characteristics of local cocks and reported significant differences of genotype on semen volume.According to our results greater volume was obtained in twice collection per week than daily collection.

The results are similar with observation of Zaharaddeen *et al.,* (2005) that turkey semen volume can be increase by once collection per week as compared to twice and trice collection per week and decreased collection frequency was resulted in greater semen volume. Noirault and Brillard (1999) also worked on the effects of collection frequency on semen in turkeys and reported that high collection frequency reduces semen volume and significant effect of collection frequency was shown on the semen volume. Significantly decreased volume of semen in Domyati Ducks was reported by Ghonim *et al.,* (2009) at high collection frequency. Anon-significant effect ($p<0.05$) of vitamin C and electrolytes supplementations was observed for semen volume according to our results but a slightly greater volume was observed in 1 ml vitamin C and electrolytes supplementation. Similar results can be derived from the experiment of McDaniel *et al.,* (2004) who attempted for the reduction of heat stress infertility through dietary ascorbic acid. He stated that feed consumption reduce during severe heat stress and

recovery period as compared to control period, so volume could be slightly reduced during due to low feed intake as observed in our control groups.

*Motility*

The results of broiler breeders are opposed by Tabatabaei *et al.,* (2009) by comparing the semen quality of indigenous and Ross broiler breeders. The fertility reported by him was 82.25 and 78.25% under favorable environmental condition.

Likewise, Peters *et al.,* (2008) reported that highest motility was 82.54 and least 62.55 % from different local strains in humid tropics which are similar to the motility of our local cocks.

**Table 4.** Mean (Viability %) of semen as affected by breed, vitamin C-Electrolytes supplementation and collection frequency.

| Breed | Vitamin C-Electrolytes supplementation | Collection Frequency | | | Mean |
|---|---|---|---|---|---|
| | | Daily | Alternate Day | Twice Weekly | |
| | | | BR × VE × CF | | BR × VE |
| St | Control | 36.447$^{cdef}$ | 35.780$^{cdef}$ | 30.337$^{f}$ | 34.188$^{c}$ |
| St | 1ml/L | 40.670$^{bcde}$ | 44.223$^{bcd}$ | 46.893$^{b}$ | 43.929$^{b}$ |
| St | 2ml/L | 45.333$^{bc}$ | 39.557$^{bcdef}$ | 47.560$^{b}$ | 44.150$^{b}$ |
| Cb | Control | 36.337$^{cdef}$ | 34.113$^{def}$ | 33.447$^{ef}$ | 34.632$^{c}$ |
| Cb | 1ml/L | 47.337$^{b}$ | 37.890$^{bcdef}$ | 40.557$^{bcde}$ | 41.928$^{b}$ |
| Cb | 2ml/L | 47.227$^{b}$ | 45.560$^{bc}$ | 43.450$^{bcde}$ | 45.412$^{b}$ |
| Ic | Control | 70.893$^{a}$ | 69.560$^{a}$ | 74.893$^{a}$ | 71.782$^{a}$ |
| Ic | 1ml/L | 74.670$^{a}$ | 71.780$^{a}$ | 79.223$^{a}$ | 75.224$^{a}$ |
| Ic | 2ml/L | 76.003$^{a}$ | 77.780$^{a}$ | 74.447$^{a}$ | 76.007$^{a}$ |
| - | - | | BR × CF | | Mean |
| - | - | - | - | - | BR |
| St | - | 40.817 | 39.853 | 41.597 | 40.756$^{b}$ |
| Cb | - | 43.633 | 39.188 | 39.151 | 40.657$^{b}$ |
| Ic | - | 73.856 | 73.040 | 76.188 | 74.361$^{a}$ |
| - | - | | VE × CF | | Mean |
| - | - | - | - | - | VE |
| - | Control | 47.892 | 46.484 | 46.226 | 46.867$^{b}$ |
| - | 1ml/L | 54.226 | 51.298 | 55.558 | 53.694$^{a}$ |
| - | 2ml/L | 56.188 | 54.299 | 55.152 | 55.213$^{a}$ |
| - | Mean | 52.769 | 50.694 | 52.312 | - |

The sperm motility reported by Nwachukwu *et al.,* (2006) ranged from 60 to 77 % in local cocks of Nigeria which support our results. According to literature we got no report of sperm motility of broiler breeder that has been presented under heat stress having the temperature alike our experimental period. The above observation indicates that summer stress of Peshawar has severely affected sperm motility of starbro, cobb-500 broiler breeders while our indigenous cocks were resistant to summer stress of Peshawar and not a prominent change was observed for sperm motility. It could be hope that greater motility percentage can be obtain in favorable condition from our indigenous cocks as compare to motility of the cocks in summer season.Zaharaddeen *et al.,* (2005) reported anon-significanteffect of collection frequency over motility percentage of sperm in the semen of two breeds of turkey raised in tropical environment. Likewise, three different collection frequencies were applied by Ghonim *et al.,* (2009) to Domyati Ducks for semen collection and no significant difference for sperm motility was observed among all collection frequencies. According to Bonato *et al.,* (2011) sperm motility percentage in semen of

ostrich was not affected by collection frequency. A contrast with the results of Fan *et al.,* (2004) was found who stated that slightly better sperm motility could be obtain from Taiwan country chicken in high collection frequencies. The percentage in sperm motility was slightly increased in the groups supplemented with 1 and 2 ml vitamin C and electrolytes supplementation which indicate the detrimental effect of summer stress on motility of sperm. Semen characteristics were severely affected through heat stress according to McDaniel *et al.,* (2004) while working on alleviation of heat stress infertility. It was also found by Joshi *et al.,* (1980) that elevated environmental temperature decrease motility percentage. Likewise increased sperm motility percentage of chicken semen was reported by Tabatabaei (2011) through 3% ascorbic acid supplementation as compare to control group during liquid storage.

**Table 5.** Mean Morphological Defects (%) of semen affected by breed vitamin C-Electrolytes supplementation and collection frequency.

| Breed | Vitamin C- Electrolytes supplementation | Collection Frequency | | | Mean |
|---|---|---|---|---|---|
| | | Daily | Alternate Day | Twice Weekly | |
| | | | BR × VE×CF | | BR × VE |
| St | Control | 11.893 | 8.893 | 9.670 | 10.152$^{abc}$ |
| St | 1ml/L | 9.890 | 9.447 | 9.893 | 9.743$^{bc}$ |
| St | 2ml/L | 9.737 | 10.780 | 9.003 | 9.707$^{bc}$ |
| Cb | Control | 7.227 | 10.227 | 10.227 | 9.227$^{c}$ |
| Cb | 1ml/L | 11.337 | 11.560 | 13.447 | 12.114$^{ab}$ |
| Cb | 2ml/L | 10.337 | 11.447 | 12.560 | 11.448$^{abc}$ |
| Ic | Control | 12.783 | 12.227 | 12.890 | 12.633$^{a}$ |
| Ic | 1ml/L | 10.337 | 10.783 | 11.337 | 10.819$^{abc}$ |
| Ic | 2ml/L | 10.333 | 12.337 | 12.003 | 11.558$^{abc}$ |
| - | - | | BR × CF | | Mean |
| - | - | - | - | - | BR |
| St | - | 10.373 | 9.707 | 9.522 | 9.867 |
| Cb | - | 9.633 | 11.078 | 12.078 | 10.930 |
| Ic | - | 11.151 | 11.782 | 12.077 | 11.670 |
| - | - | | VE × CF | | Mean |
| - | - | - | - | - | VE |
| - | Control | 10.634 | 10.449 | 10.929 | 10.671 |
| - | 1ml/L | 10.521 | 10.597 | 11.559 | 10.892 |
| - | 2ml/L | 10.002 | 11.521 | 11.189 | 10.904 |
| - | Mean | 10.386 | 10.856 | 11.226 | - |

*Concentration*

Tabatabaei*et al.,* (2009) also founded significant effect of breed over sperm concentration of semen in indigenous and Ross broiler breeders. Similar to our results significant effect of strain on sperm concentration of semen was found by Peters *et al.,* (2008) in chicken raised in the humid tropics. Significant effect of genotype on sperm concentration of semen was also reported by Nwachukwu *et al.,* (2006) for the local cocks of Nigeria. The above statements support our results. According to Malecki *et al.,* (1997) no difference in sperm concentration in per ejaculate was found at different collection frequencies which strongly support our results. According to our results only slightly greater sperm concentration was observed in daily collection than alternate day and twice collection per week. Results were opposed by Ghonim *et al.,* (2009) who stated that sperm concentration was decrease in Domyati Ducks by high collection frequencies. Likewise, Noirault and Brillard (1999) opposed our results working on semen characteristics of turkey. Sperm concentration through vitamin C and electrolytes supplementation was significantly increased ($p<0.05$)

in all breeds according to our results. Similar results were given by Monsi and Onitchi (1991) who stated that sperm concentration could be increase by ascorbic acid supplementations. Likewise, Dobrescu (1987) founded increase sperm concentration through dietary ascorbic acid supplementation. As sperm concentration is reduced by elevated ambient temperature according to Joshi *et al.,* (1980).

*Viability*

According to our results viability was significantly affected ($p<0.05$) by breed. Similar results were shown by Tabatabaei *et al.,* (2009) for the significant effects of breed over viability percentage of sperm in broiler breeder roosters. The contrast in the values percentage was due to the provision of favorable environment to the cocks by him. As a whole the effect of breed was shown the same for viability. It indicates that that heat stress of Peshawar has severely affected the viability of sperm in the semen of starbro and cobb-500 broiler breeders. According to Nawachukwu *et al.,* (2006) no significant effect of genotype was observed for viability percentage of sperm in the semen of indigenous chicken cocks of Nigeria which opposed our results. Peters *et al.,* (2008) founded significant effect of strain over the active and sluggish sperm in the semen. Viability percentage of sperm was significantly not affected ($p>0.05$) by collection frequency in our experiment. However the percentage was slightly increased in daily collection than alternate day and twice collection per week. Bonato *et al.,* (2011) stated that variation in viability percentage of sperm is found irrespective of the collection frequency. However, slightly increase in viability could be obtained in high collection frequencies. Noirault and Brillard (1999) opposed our results by observing significant increase in viability percentage in turkey semen at different collection frequencies. May be our result did not show significant difference in viability percentage due to severe heat stress at different collection frequencies. A significant affect ($p<0.05$) of vitamin C and electrolytes was observed for sperm viability percentage which was increased in the group supplemented with 1 and 2 ml vitamin C and electrolytes supplementations. McDaniel *et al.,* (2004) reported on significant effect of dietary ascorbic acid which opposed our results. The change could be due to the exposure of the cocks to different heat stress condition depending on severity. As it is mentioned before in our experiment that vitamin C and electrolytes supplementation has good effects on overall performance in summer stress of Peshawar therefore, the increase viability might be of this reason. Tabatabaei (2011) also reported better viability of sperm in semen with 1% ascorbic acid supplementation than control and other treatment groups during in vitro storage. He supported our results that vitamin C and electrolytes have positive effects on sperm viability.

*Morphological defects*

No significant effect of ($p>0.05$) of breed over morphological defects was found in our experiment. In broiler breeders cocks of our experiment the motility percentage was also very low than indigenous cocks, so this may be the cause for their low morphological defects that their defective sperm could not survive under summer stress of Peshawar. Similar results were provided by Nwachukwu *et al.,* (2006) for the non-significant effect of genotype over the morphological defects. An indirect contrast with the observation of Peters *et al.,* (2008) founded because he had mentioned significant effect of strain on percentage of active and sluggish sperm. The effect of collection frequency was not found significant statistically under summer stress of Peshawar however low morphological defects were observed at high collection frequencies. Similarly Ghonim *et al.,* (2009) reported low morphological defects on twice collection per week as compare to weekly collection of semen in the Domyati Ducks. Likewise Nwachukwu *et al.,* (2006) observed low morphological defects in twice collection per week than once collection weekly which support u results. The morphological defects were not affected by vitamin C and electrolytes supplementations according to our results and might be concern with other factors. Tabatabaei (2011) observed lower morphological defects with 1% ascorbic acid supplementation during liquid storage.

The dose we used for vitamin C and electrolytes supplementation might be non-effective for the morphological defects or the heat stress of our experimental period might be not coverable with vitamin C and electrolytes supplementation for morphological defects. Unfortunately no written reports of semen evaluation were provided for the Indigenous cocks of Pakistan.

Therefore, the results could not be compared with any indigenous cocks. Comparatively best results were obtain from Indigenous cocks in our study which show that Indigenous cocks have greater potential for survival under summer stress of Peshawar (Pakistan) and could be improve through selective breeding.

**References**

**Altan O, Altan A, Ozkan S, Acikgoz Z, Ozkan A.** 2000 a. Effect of dietary sodiu bicarbonate on egg production and egg quality of laying hen during high summer temperature. Archiv fur. Geflugelkundi. **64(6),** 260-272.

**Altan O, Ozkan S, Altan A, Akbas Y, Ayhen V, Ozkan K.** 2000 b. Effect of short term fasting and midnight lighting on egg production traits of laying hen during summer season. Archiv fur. Geflugelkundi **64(2),** 85-89.

**Bonato M, Rybnik PK, Malecki IA, Cornwallis CK, Cloet SWP.** 2011. Twice daily collection yields greater semen output and does not affect male libido in ostrich. Animal Reproduction Science **123(3-4),** 258-264.

**Cheng FP, Guo TJ, Wu JT, Lin TE, Ursem PJF, Colenbrander B, Fung HP.** 2002. Annual variation in semen characteristics of pigeons (*Columbia Livia)*. The Journal of Poultry Science **81,** 1050-1050.

**Donoghue AM, Wishart GJ.** 2000. Storage of poultry semen. Animal Reproduction Science **29,** 847-51.

**Dumpala PR, Parker HM, McDaniel CD.** 2006. The sperm quality index from fresh semen predicts chicken semen quality after storage. J. International Journal of Poultry Science **5(9),** 850-855.

**Faisal S.** 1998. Status of broiler breeder stock in Abbot Abad and Mansehra Pakistan. Thesis. M.Sc (Hons). KPK Agri, University Peshawar.

**Fan YK, Ju JC, Lee SL, Chen CF, Peb HC, Hsu JC, Lee YP.** 2004. High ejaculation frequencu enhances semen production in Thaiwan Country Chicken. Journal of Animal Science **17(7).** 924-929.

**Galah AI.** 2008. Effect of summer stress on poultry breeding stock. Journal Poultry Science. 1-6.

**Ghonim AIA, Awad AL, El-saway MA, Fatouh MH, Ibraheim Zenat A.** 2009. Effect of frequency of semen collection, dilution rate and insemination dose on semen characteristics and fertility of Domyati Ducks. Journal Egyptian Poultry Science **29(IV),** 1023-1045.

**Holsberg DR, Donghue AM, Froman DP, Ottinger MA.** 1998. Assest of ejaculate quality and sperm characteristics in turkey: Sperm mobility phenotype is independent of time. Journalof Poultry Science **77,** 1711-1717.

**Joshi PC, Panda B. Joshi BC.** 1980. Effect of ambient temperature on semen characteristics of white leghorn male chicken. Indian Veterinary Journal **57,** 52-56.

**Karaca AG, Parker HM, Yeatman JB, McDaniel CD.** 2002 a. The effect of heat and sperm quality classification on broiler breeder male fertility and semen ion concentration. Journal British Poultry Science **44,** 621-628.

**Karaca AG, Parker HM, Yeatman JB, McDaniel CD.** 2002 b. Role of seminal plasma in heat stress infertility of Broiler Breeeder Males. Journal of Poultry Science **81,** 1904-1909.

**Lake PE.** 1995. Historical perspective of artificial insemination technology. In:Bakst, M.R. and G.J. Wishart. Editors, 1995. Proc. *1st. int. sym. on artificial insemination of poultry,* Poultry Science Association. Savoy, IL.1-20.

**Malecki IA, Martin GB, Lindsay DR.** 1997. Semen production by the Emu (*Dromaius novaehollandiae*).2. Effect of collection frequency on the production of semen and spermatozoa. Journal of Poultry Science **76,** 622-626.

**Martin RD.** 1957. The male reproductive tract of fowl. Journal of Anatomy **91,** 116-129.

**McDaniel CD, Hood JE, Parkr HM.** 2004. An Attempt at Alleviating Heat Stress infertility in male broiler breeder Chicken with Dietary Ascorbic Acid. Journal of Poultry Science **3(9),** 593-602.

**McDaniel CD, Bramwell RK, Wilson JL, Howarth JRB.** 1995. Fertility of male and female broiler breeder following exposure to an elevated environmental temperature. Journal of Poultry Science **74,** 1029-1038.

**McDaniel G.** 1978. Low fertility in broiler hatching eggs. Journal of Poultry. Dig. p 446-448.

**Monsi A, Onitichi DO.** 1991. Effect of ascorbic acid (vitamin C) supplementation on ejaculated semen characteristics of broiler breeder chickens under hot and humid tropical conditions. Animal Feed Science and Technolgy **34,**141.

**Noirault J, Brillard JP.** 1999. Effect of frequency of semen collection on quantitative and qualitative characteristics of semen in turkey breeder males. Journal Poultry Science **78,** 1034-1039.

**Nwachukwu EN, Ibe SN, Amadi CU.** 2006. Effect of genotype and frequency of semen collection on semen characteristics of local chicken cocks. Journal Animal and Veterinary Advances **5(7),** 562-565.

**Peters SO, Shoyebo OD, llori BM, Ozejo MO, Ikeobi CON, Adebambo OM.** 2008. Semen Quality Traits of Seven Strains of Chicken Raised in the Humid Tropics. Journal of Poultry Science **7(10),** 949-953.

**Qureshi MS.** 2011.Reproductive Physiology of Domestic Animals. Edi.1st. p 198-200.

**Reddy RP.** 1995. Artificial insemination of broiler: Economic and Management implication. In:Balest, M.R., Wishart, G.J.(Eds).,Proc.1st int. Symp. On the Artificial insemination of poultry. Poultry Science Association. Savoy, IL, 73-89.

**Sahin N, Onderci M, Sahin K,Gursu M.** 2004. Ascorbic acid and melatonin reduce heat-induced performance inhibition and oxidative stress in Japanese Quails. Journal of British Poultry Science. **45,** 116-122.

**Tabatabaei S.** 2011. Effect of ascorbic acid on chicken semen quality during liquid storage. Comparitive Clinical Pathology **5,** 621-626.

**Tabatabaei S, Batavani RA, Talebi AR.**2009. Comparison of semen quality in Indigenous and Ross broiler breeder roosters. Journal of Animal and Veterinary Advances **8(1),** 90-93.

**Zaharaddeen D, Butswat ISR, Kalla DJU, Sir SM, Bukar MT.** 2005. Effect of frequency of ejaculation on semen characteristics of two breeds of turkeys (*Maleagris gallapavo*) raised in a tropical environment. Journal of Poultry Science **4,** 217-221.