SEMINAL PROTEINS OF HOLSTEIN-FRIESIAN BULLS ASSOCIATED WITH VITAMIN C SUPPLEMENTATION

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ABSTRACT

Semenal total protein (STP) as well as percentage of albumin, α-, β- and γ-globulins were examined in Holstein – Friesian bulls as affected by vitamin C supplementation during hot season (August – September) in Iraq. A total of nine bulls (3 – 3.5 years old) were randomly assigned to 0 (Control, C), 5g (T1) and 10g (T2) vitamin C dissolved in drinking water for 5 weeks. STP tended to be higher (P < 0.16) in T2 – bulls during week 3 onward. During week 2, 3 and 4, percent seminal albumin was greater (P < 0.01) in T2-group, whereas a slight decline (P = 0.10) was noticed in T1-bulls during week 5. No overwhelming change was observed in α-globulin percentage during first 4 weeks, while it decreased (P < 0.01) in group 1 during week 5. Vitamin C supplementation relatively increased (P < 0.06) seminal β-globulin percentage of group T2 during week 5. Excluding data of week 1, percent seminal γ-globulin was not changed among groups during the whole experimental period. It was concluded that variable responses of Holstein bulls to different levels of vitamin C were noted. However, weekly treatment with 5g vitamin C had a positive effect on seminal β-globulin and reduce seminal albumin. This could improve bull fertility in Iraq and further investigations are warranted.

INTRODUCTION

To maintain health, productivity, reproductive performance as well as to increase the capacity of the immune system in stress conditions (e.g. heat), vitamin C supplementation is advisable (9,12, 18, 20). If plasma level of vitamin C are substantially reduced, less is taken up into cells, this in turn interferes with various biosynthetic processes (10).

Deficiency for three weeks led to reduction in weight of the testes, the various parts of the epididymis, the seminal vesicles and the prostate of guinea pigs (2). Moreover, sperm count fell substantially and sperm motility was reduced in Holstein bulls (9).

In Iraq, semen collection at AI centers was impaired during summer (July – September) because of heat-stressed bulls. Since stressors increases vitamin C consumption and reduce its plasma level(11), the accessory sex glands and their secretions could be affected by the lack of vitamin C in the diet. Also, no previous studies concerning the influence of vitamin C on seminal proteins in bulls.

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were reported. These observations prompted us to schedule this work to inspect the influence of vitamin C supplementation on seminal total protein (STP) and percent of seminal albumin, α-, β- and γ-globulins of Holstein – Friesian bulls during hot season in Iraq.  

MATERIALS AND METHODS  

Animals and Treatment  

Nine mature Holstein – Friesian bulls of proven fertility (3 – 3.5 years old) were used during the period from beginning of August to mid of September, 2000. These bulls were housed at Al center in Abu-Ghraib, Baghdad. Animals were kept in a semi closed single pens with simulated natural photoperiod and maintain under routine management conditions. Bulls were randomly assigned to grouping receiving 0 (Control, Group C), 5g vitamin C (Group T1) or 10 g vitamin C (Group T2) dissolved in drinking water tanks (capacity = 50 L). Treatment schedule was done as twice weekly. During the 5 weeks treatment period, semen was collected weekly using an artificial vagina and teaser cow.  

Obtaining of seminal plasma and proteins assay:  

Seminal plasma was separated by spinning the semen sample (4000 rpm / 30 min.). The supernatant was recentrifugate and used for the determination of seminal total protein (STP) and percent of seminal α-, β- and γ-globulins. Biuret method (19) was used for STP evaluation. Electrophoresis was performed on cellulose acetate membranes in 0.05 M-sodium barbitol buffer to determine the percent of protein fractions (17), scanning was undertaken by using elphograph 3 scanner. An average of STP and protein fractions were calculated within each bull.  

Statistical analysis  

Statistical computations were performed using General Linear Model (GLM) procedure of SAS (15). The general model for analysis of variance was:

\[ Y_{ij} = \mu + T_i + W_j + e_{ij} \]

Where \( Y_{ij} \) = dependent variable (STP and α-, β- and γ-globulins); \( \mu \) = overall mean; \( T_i \) = effect of ith treatment (I = 0 (C), 5 (T1) and 10 g vitamin C (T2)); \( W_j \) = effect of jth week (W = 1, 2, 3, 4, 5); \( e_{ij} \) = error term.  

Differences between means were compared by Duncan's multiple range test (4).  

RESULTS AND DISCUSSION  

A great deal is heard today about stress. Anti-stress properties are exhibited in particular by vitamin C. Where the intake of vitamin C is inadequate, more severe stress can produce adverse effects, especially for reproduction (9, 12).  

Seminal total protein did not differ significantly among groups, however, it tended to be higher in Group T2 at weeks 3 (+11, +9.3%), 4 (+18, +18.3%) and 5 (+7, +13%) compared with groups C and T1 respectively (Table 1). Regardless vitamin C treatment, STP in the present work exceeded those previously obtained by Juma (10) in Holstein – Friesian bulls examined in different seasons in Iraq (4.23 – 7.70 g / 100 ml). In the presence of androgens, seminal proteins are secreting from epididymis, prostate and vesicular glands (6, 7, 13). As vitamin C is required for the synthesis of testosterone in the interstitial cells (3), it is worthy to mention that more relatively testosterone synthesized in the testes of T2 – bulls may stimulate seminal proteins production by the epididymis and accessory sex glands than other groups. On the other hand, deficiency of vitamin C for three weeks led to a reduction in the weight of the testis, the various parts of the epididymis, the vesicular glands and the prostate of guinea pigs (2). This may alters STP production of group C.  

Seminal albumin was greater (P < 0.01) in Group T2 at weeks 2 (30.00 ± 0.50%), 3 (60.00 ± 13.41%) and 4 (41.70 ± 7.15%) than the other experimental groups. Interestingly, T1 – bulls exhibited a slight decline in seminal albumin at week 5 (12.50 ± 2.50%) compared to others (Table 2).
Table 1. Seminal total protein (g/100 ml) of Holstein – Friesian bulls treated with O (C), 5 (T1) or 10 (T2) g vitamin C/week during the experimental period (Mean ± S.E.)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>9.37±1.07a</td>
<td>8.30±0.65a</td>
<td>8.90±0.55a</td>
<td>8.57±0.90a</td>
<td>8.70±0.85a</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>9.15±1.35a</td>
<td>8.47±0.67a</td>
<td>9.07±1.16a</td>
<td>8.67±0.72a</td>
<td>8.13±0.28a</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>9.05±1.52a</td>
<td>9.00±1.52a</td>
<td>10.00±0.46a</td>
<td>10.50±0.45a</td>
<td>9.35±0.25a</td>
</tr>
</tbody>
</table>

Means with similar superscripts within each column did not differ significantly.

Table 2. Percent seminal albumin of Holstein – Friesian bulls treated with O (C), 5 (T1) or 10 (T2) g vitamin C/week during the experimental period (Mean ± S.E.)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>11.67±4.67a</td>
<td>20.67±1.45b</td>
<td>20.33±12.03b</td>
<td>21.16±2.14b</td>
<td>22.33±1.33a</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>9.00±1.06a</td>
<td>13.50±0.50b</td>
<td>16.67±3.53b</td>
<td>16.00±3.45b</td>
<td>12.50±2.50a</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>20.50±0.50a</td>
<td>30.00±0.50a</td>
<td>60.00±13.41a</td>
<td>41.70±7.15a</td>
<td>25.50±7.50a</td>
</tr>
</tbody>
</table>

Means with different superscripts within each column differ significantly (P < 0.05)

Bulls of high fertility, seminal albumin is usually the smallest protein fraction whereas in animals with defective spermiogenesis and high incidence of abnormal spermatozoa, that fraction seems to be usually high perhaps because of an accelerated rate of albumin passage from the blood plasma into the inflamed or otherwise pathologically altered the testis and epididymis (15). It is tempting to speculate that higher seminal albumin percentage of Group T2 may have related with the reverse effect of high vitamin C level (10 g) on the whole reproductive tract. In contrary, the slight decline of seminal albumin of group T1 at week 5 (12.50% - 2.50%) indicated that low vitamin C supplementation (5g) is enough to fulfill the physiological needs of bulls and to improve semen quality during heat stress period in Iraq (9). The current results were inconsistent with the previous reports (8, 10) whom observed a considerable reduce in seminal albumin percentage of Iraqi bucks and Friesian bulls examined in different seasons (5.083 – 19.95%). This inconsistency may due to the experimental conditions and vitamin treatment.

Excluding data of weeks 5, vitamin C did not influence seminal α-globulin percentage during overall experimental period. An obvious decrease (P < 0.01) was noticed in percent seminal α-globulin in Group T2 at week 5 (15.00 ± 0.04%) in relation with the groups (Table 3). The present results of seminal α-globulin in further confirmed by contemporaneous findings of Juma (10) in Iraq Friesian bulls (28.946 – 50.08%). Of particular interest, α-globulin had an important role in binding of serum lipids and steroids including testosterone (5). Therefore, increasing in seminal α-globulin percentage could be a function of enhanced testicular activity of T1-bulls and depressed of T2-bulls (week 5). This result strengthen the evidence that high level of vitamin C might have an adverse influences on the reproductive tract and immune system related (12).

There was a tendency (P = 0.17) toward increasing seminal β-globulin percentage in Group T1 at weeks 2 (+30%, 53.7%) and 4 (+3.7%, +23.7%) compared to that of group C and T1 respectively. Slight increases (P < 0.06) in seminal β-globulin was also observed in 5g vitamin C – treated group at week 5 namely 37.00 ± 7.00% (Table 4).

Seminal β-globulin has a crucial role as ions – binding proteins associated with spermatogonial division (16). Thus higher β-globulin percentage of 5g vitamin C – treated bulls at weeks 2, 4, and 5 may related to the effect of vitamin C on increasing sperm concentration of this group accordingly. This speculation is further supported by the findings of Al-Fahdawi (1) on Damascus bucks and Ishak et al. (9) on Holstein – Friesian bulls. A considerable increase in vitamin C consumption occurs during stress and in association with persistently high air temperature (12). Regardless of week 1, no remarkable changes was noted in seminal γ-globulin percentage during the whole experimental period. However, it tended (P = 0.11) to be lower in group T1 at week 5 (15.00 ± 3.00%) but still within the normal value in bulls (Table 5). The current data of seminal γ-globulin exceeded those previously noted by Juma.
(10) in Iraqi Holstein – Friesian bulls (5.99 – 8.317%). It is well known that in case of chronic prostatitis, \( \gamma \) component of seminal plasma was considerably increased (14). So, we expect that a slightly decline in \( \gamma \)-globulin value of Group T1 at week 5 may return to the positive effects on secretions of prostate (main resource of seminal \( \gamma \)-globulin) (20).

Table 3. Percent seminal \( \alpha \)-globulin of Holstein – Friesian bulls treated with O (C), T1 or 10 (T2) g vitamin C / week during the experimental period (Mean ± S.E.)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>37.00</td>
<td>19.66</td>
<td>20.33</td>
<td>30.13</td>
<td>32.67</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>42.50</td>
<td>18.25</td>
<td>32.00</td>
<td>31.75</td>
<td>32.50</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>20.50</td>
<td>26.00</td>
<td>20.00</td>
<td>24.44</td>
<td>15.00</td>
</tr>
</tbody>
</table>

Means with different superscripts for each week differ significantly (P < 0.05).

Table 4. Percent seminal \( \beta \)-globulin of Holstein – Friesian bulls treated with O (C), T1 or 10 (T2) g vitamin C / week during the experimental period (Mean ± S.E.)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>16.50</td>
<td>25.33</td>
<td>37.67</td>
<td>34.17</td>
<td>19.67</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>22.00</td>
<td>36.75</td>
<td>19.50</td>
<td>35.40</td>
<td>21.00</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>27.25</td>
<td>17.00</td>
<td>8.00</td>
<td>27.00</td>
<td>19.50</td>
</tr>
</tbody>
</table>

Means with different superscripts for each week differ significantly (P < 0.05).

Table 5. Percent seminal \( \gamma \)-globulin of Holstein – Friesian bulls treated with O (C), T1 or 10 (T2) g vitamin C / week during the experimental period (Mean ± S.E.)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>12.33</td>
<td>28.50</td>
<td>21.33</td>
<td>24.22</td>
<td>21.67</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>18.00</td>
<td>32.00</td>
<td>21.00</td>
<td>29.00</td>
<td>15.00</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>22.50</td>
<td>19.00</td>
<td>12.00</td>
<td>19.55</td>
<td>25.00</td>
</tr>
</tbody>
</table>

Means with different superscripts for each week differ significantly (P < 0.05).

It appears that the experimental period used presently (5 weeks) was not enough to distinguish the effect of vitamin C on seminal proteins profile in bulls. It is well established that vitamin C accumulates in the testes, the epididymis and the accessory sex glands in appropriate quantity before exert their effects on concerned target cells (2).

Overall, the influence of vitamin C to enhance seminal proteins secretions of Holstein – Friesian bulls during hot season in Iraq was not overwhelming except for \( \beta \)-globulins. Using vitamin C in drinking water as an anti-heat stress factor in bulls may consider, to the best of our knowledge, a new method to increase the heat tolerance of bulls and consequently the efficiency of artificial insemination during summer in Iraq. Further studies are needed to investigate the real effects of vitamin C on seminal proteins of Holstein – Friesian bulls by determining different routes and periods of supplementation.

REFERENCES