Semen lysozyme levels and semen quality in Turkeys (Meleagris gallopavo) fed with various dietary protein levels

L. SOTIROV, S. DIMITROV and E. JELIAZKOV

SUMMARY

Twelve male turkeys from the White Imperial Breed, divided into 2 groups of 6 birds each were studied. The first group received a diet containing 14 % protein whereas the second — diet with 17 % protein. The simultaneous influence of dietary protein content in blood and semen lysozyme concentration upon ejaculate volume, sperm cell concentration, motility, live, dead or abnormal spermatozoa was followed out. It was concluded that the higher dietary protein content improved both the quantitative and the qualitative parameters of semen and the different lysozyme concentrations in blood and semen influenced significantly the spermatozoa motility, live and dead spermatozoa.

KEY-WORDS: turkey - proteins - lysozyme - semen.

1. Introduction

Protein level is a limiting factor in the diet of birds. Its optimal content is a prerequisite not only for a rapid growth, but also for the normal condition of breeders by influencing the quantitative and qualitative parameters of semen.

The studies of MEYER et al. [17] about the volume of ejaculate and the concentration of spermatozoa showed that the feeding of male turkeys with diets containing 12 % and 17 % protein respectively resulted in nonsignificantly better results when the higher dietary protein level was not used.

According to others [7, 3, 5, 6, 8, 18], the low dietary protein content (12.8 %) decreases the quality of semen. The use of 17 % dietary protein levels up to the age of 28 weeks and 8 % protein thereafter results in a satisfactory sperm production up to end of the breeding period (age of 47-52 weeks).

The quality of semen is further influenced by other factors related directly or not directly to the level and quality of feeding. KUZMIN et al. [13] reported that the decreased spermatozoa motility in humans is closely related to the decreased semen lysozyme levels and proposed the use of this parameter for a development of test for determination of the fertility of spermatozoa.

A number of other studies have evidenced that the enzyme preparation Roxazym G (Hoffman La Roche, Austria) and the probiotic Lacto-Sacc (Alltech Inc., USA) increased serum lysozyme and complement activities in broiler chickens and turkeys [19, 20, 21].

ARTICLE ORIGINAL

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Thus, the aim of the present study was to determine lysozyme concentrations in seminal plasma and blood serum as well its relationship with some semen characteristics in turkeys fed with different dietary protein levels.

2. Material and methods

A) BIRDS AND EXPERIMENTAL DESIGN

The experiment was performed on 12 male turkeys from the White Imperial breed, divided into 2 groups with 6 birds in each. Up to the age of 31 weeks, both groups were given standard diets. From the age of 32 weeks onward, they were fed diets containing 14 % and 17 % protein respectively (Table I).

The birds were floor housed under a light regimen of 14 h daylight/10 h darkness. The water supply was done by automated waterers. The feeding was ad libitum.

B) SEMEN COLLECTION AND EVALUATION

The semen was obtained by massage according to the method of BURROWS and QUIN (1937) obtaining 3 ejaculates per week by an aspirator (IMV Technologies, L'AIGLE, France).

Obtained ejaculates were individually assessed using the following parameters: volume - by a graduated micropipette with a precision of 0.01 ml; number of sperm cells - with the Thoma counting chamber; percent of motile spermatozoa - subjective evaluation under microscope on a scale ranging from 0 to 100 %. Nigrosin/eosin stains were used for determination of live/dead and abnormal sperm under a light microscope. This semen quality tests were described by KUBRATOV et al. [12], BAKST and CECIL [1].

C) ARTIFICIAL INSEMINATION

The fertilizing ability of semen was assessed by intravaginal artificial insemination of 30 hens per each protein treatment. The females were inseminated with fresh semen with 250 millions spermatozoa per dose every 10 days.

The fertility (fertilized/incubated eggs x 100) was determined by candling the eggs after 7th day of incubation. Early dead embryos were considered as fertile for calculation of percentage of true fertility.

D) DETERMINATION OF LYSOZYME CONCENTRATION

Sperm and blood serum lysozyme concentrations were determined according to the method of LIE [15], after centrifugation at 2000 rpm/min for 10 min. Briefly 20 ml of 2 % agarose (ICN, UK, Lot 2050) dissolved in sodium phosphate buffer (pH = 6.2) was mixed with 20 ml suspension of 24 hours culture of Micrococcus lysodeicticus at 67°C. This mixture was poured out in Petri’s dish (14 cm diameter). After solidifying at room temperature 32 wells were made (5 mm diameter). Fifty microliters of undiluted sera and sperm supernatants were poured out in each well. Eight standard dilutions (from 0.025 to 3.125 µg/ml) of lysozyme (Veterinary Research Institute, Veliko Tarnovo) were used in the same quantity as well. The samples were incubated for 20 hours at 37°C and lytic diameters were measured.

<table>
<thead>
<tr>
<th>Components</th>
<th>14 % protein</th>
<th>17 % protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>65.50</td>
<td>54.50</td>
</tr>
<tr>
<td>Corn</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Sunflower oil meal</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Creda</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Salt</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>1 kg contain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>13.40</td>
<td>16.76</td>
</tr>
<tr>
<td>ME, MJ/kg</td>
<td>12.15</td>
<td>11.60</td>
</tr>
<tr>
<td>Methionine + cystine, %</td>
<td>0.52</td>
<td>0.61</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>0.48</td>
<td>0.67</td>
</tr>
<tr>
<td>Arginine, %</td>
<td>0.76</td>
<td>1.00</td>
</tr>
<tr>
<td>Isoleucine, %</td>
<td>0.54</td>
<td>0.68</td>
</tr>
<tr>
<td>Triptophan, %</td>
<td>0.15</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table I. — Composition of the diets of breeder toms.
E) STATISTICAL ANALYSIS

A one way MANOVA AND MANCOVA with fixed effects statistical analysis were used. Like cofactors we considered the semen and blood lysozyme concentrations. The post hoc comparisons were done by SCHEFFE test.

The whole calculations were made by statistical package Statistica of StatSoft Int.

3. Results and discussion

The results for the influence of dietary protein levels upon some semen parameters in turkeys are presented in Table II. Significant differences were observed by respect to spermatozoa motility (p = 0.008) and live spermatozoa (p = 0.003) with more favorable results in the group fed 17 % protein. The other parameters showed a stable tendency towards improvement in the same group. This was valid for the lysozyme level in seminal plasma and blood serum of experimental turkeys. The values of semen parameters and fertility trend to increase in the group fed with higher protein content. The average blood serum lysozyme concentrations in the group with 17 % protein were by 27.05 % higher than those with 14 % protein.

The MANCOVA analysis was presented in Table III. The blood and semen lysozyme values, combined with the higher dietary protein content, influenced statistically significantly the motility of spermatozoa (p = 0.017) and live and dead spermatozoa (p = 0.007 : p = 0.0192). From 14 % till 54 % of the variability of semen parameters are due to the different levels in blood and semen lysozyme values. The other parameters were not significantly different, but turkeys fed with the 17% protein diet and possessing higher blood lysozyme levels showed better parameters compared to the group with lower dietary protein level and lower blood lysozyme values.

Our data gave the reason to suggest that the high blood lysozyme concentrations influenced favourably the quantitative and qualitative semen parameters in turkeys. Similar results were reported by KUZMIN et al. [13]. The addition of lysozyme to human ejaculates was reported to increase the functional activity of spermatozoa. On the basis of this fact, methods of treatment of sterile couples have been proposed.

Table II shows that semen lysozyme concentrations in both groups were very high (7.382 μg/ml in group I and 6.304 μg/ml in group II). Thus, the question arises about the origin of lysozyme : whether there is a diffusion from blood into semen or it is secreted in testes ? In order to answer those questions, we analyzed lysozyme levels in the blood serum of birds. The difference between serum and semen lysozyme concentrations was huge - 29 times higher in the to answer those questions, wa analyzed lysozyme levels in the blood serum of birds. The difference between serum and semen lysozyme concentrations was hyge - 29 times higher in the semen group and 23 times in group II. Therefore, the only possible answer is that semen lysozyme is secreted in testes. Furthermore, we hypothesized that specific genes, expressed in the testicular tissue, are responsible for that event. Our hypothesis is further supported by the studies of several investigators. IRWIN et al. [11] reported for genes controlling lysozyme secretion in the stomach of ruminants. YEH et al. [22] found out that in mice, two different genes were responsible for lysozyme synthesis in intestines (P) and macrophages (M). In rats, according to the same authors, a single gene does this. In dogs, the synthesis of lysozyme in milk and spleen is controlled by two different genes [10]. A gene, specific for the gastric lysozyme in swine was reported by YU and IRWINE [23].

The favourable effect of lysozyme on semen quality was possibly due to its extremely important protective characteristics. According to BUHARIN and VASSILEV [2] lyso-

<table>
<thead>
<tr>
<th>semen parameters</th>
<th>groups in respect to protein levels</th>
<th>p-levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 %</td>
<td>17 %</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>n</td>
</tr>
<tr>
<td>volume of ejaculate (cm³)</td>
<td>0.178</td>
<td>6</td>
</tr>
<tr>
<td>concentration of spermatozoa (x10⁹/cm³)</td>
<td>3.452</td>
<td>6</td>
</tr>
<tr>
<td>motility of spermatozoa (%)</td>
<td>63.513</td>
<td>6</td>
</tr>
<tr>
<td>abnormal spermatozoa (%)</td>
<td>10.025</td>
<td>6</td>
</tr>
<tr>
<td>live spermatozoa (%)</td>
<td>79.023</td>
<td>6</td>
</tr>
<tr>
<td>dead spermatozoa (%)</td>
<td>21.043</td>
<td>6</td>
</tr>
<tr>
<td>lysozyme in sperm (µg/ml)</td>
<td>7.382</td>
<td>6</td>
</tr>
<tr>
<td>lysozyme in sera (µg/ml)</td>
<td>0.207</td>
<td>6</td>
</tr>
</tbody>
</table>

* - statistically significant

Table II. — Analysis of variance of some semen parameters of turkeys fed with different protein levels.
zyme is very active against Gram-positive bacteria. Interacting with the complement system, it was reported to be active against the gram-negative organism *E. coli* [9].

Others reported that lysozyme was active against some viruses too. Lysozyme performed a lytic activity against the rabbies virus in experimental infection [16].

4. Conclusions

The higher dietary protein level improved the quantitative and qualitative parameters of semen.

The different lysozyme concentrations in blood and semen influenced significantly the spermatozoa motility, live and dead spermatozoa.

**References**


2. — BUKHARIN O.V. andVASILEV N.V. : Effect of lysozyme on experimental infectious, in Lysozyme and its role in Biology and Medicine, Tomsk, Russia, University of Tomsk, 1974, 145-153 (Ru).


