**Introduction**

Thus far, the theory of the various emotional sensitivity to stress in animals is based on the general adaptation syndrome and the activation of the hypothalamo-adreno-cortical system in conditions of higher environmental demands [20]. The external manifestations of emotion in animals are clearly mediated via motor, vegetative and other activities. The emotions evolved and improved through the course of evolution in order to achieve a better adaptation to the environment [22]. Behavioural differences are observed not only among species, but among the individuals of the same species as well. Even when animals are housed under the same conditions, significant behavioural variations could be noticed [1]. According to GRAY [14] two principal factors should be considered for studying emotional reactions in animals: the motivation and the temperament (individual emotional characteristics). The fearfulness (anxiety) is tightly related to lactation too. The lactation reflex in sheep is dependent on a number of factors that suppress or assists its normal development. Over 25% of sheep showed anxiety during the machine milking and exhibited problems, the most important being milk retention [3, 4, 6, 8]. On the other hand, stress influenced also the immune status in animals [11, 13, 23].

Because of all those facts, it would be of interest to study the influence of stress sensitivity (respectively resistance to stress) upon lysozyme and complement concentrations in dairy sheep, in order to detect an eventual immune deficiency in anxious animals.

**Material and methods**

**ANIMALS**

The experiment was performed in the sheep farm of the Research Institute of Sheep and Cattle Breeding in Stara Zagora, Bulgaria. One hundred and eighteen 2-3 year old sheep [dairy crossings Stara Zagora x East-Friesian and (Stara Zagora x East-Friesian) x Blackhead Pleven breeds] were used. Blood for analysis was sampled in 10 ml tubes from v. jugularis. The blood was allowed to clot for one hour at room temperature (25°C) and the samples were centrifuged at 2000 g for 10 min at room temperature.

**Methods**

**DETERMINATION OF THE SENSITIVITY (RESISTANCE) TO STRESS**

The temperament and welfare of dairy ewes milked in a milking parlour “Alfa Laval Ltd.” 2x24 were evaluated by a method reflecting factors influencing the individual behaviour. The detailed description of the method is already
published [5-7]. During a pilot study, 6 behavioural traits were retained for describing the sheep temperament in a milking parlour: 1) Taking position into the milking parlour; 2) Feed reaction; 3) Activity towards neighbours; 4) Feed reaction towards forage offered by hand (unknown person); 5) Reaction towards positioning teacups; 6) Persistency of taking place into the milking parlour.

Four degrees of responses for each behavioural trait were recorded. For example, superactivity, or activity with looking round, or changeable passiveness or passiveness could be observed towards neighbours. When forage was offered by hand, feeding with relish, or cautiously feeding, or rarely feeding, or no feeding were noticed. When teacups were positioned, sheep would be placid, or anxious only towards positioning teacups, or anxious during whole milking and would kick without getting down teacups, or would violently kick and get down teacups. Into the milking parlour, the attitude would be voluntary and persistent, or voluntary and no persistent, or no voluntary and no persistent, or, finally, no voluntary and persistent. Scores from 1 to 4 were given according to the animal reaction: score 4 represented the most favourable reaction and score 1 the most unfavourable. The scores obtained for each behavioural trait were added to form an individual complex score (CS). Six consecutive observations during morning machine milking were carried out. One month later, the same procedure, using another observer, was repeated. According to average complex scores, 3 significantly distinguishable (p < 0.001) temperaments were established: calm (resistant to fear stimuli, reacting adequately towards learning tests), nervous (sensitive to fear stimuli, reacting non adequately towards learning tests) and intermediate type ewes.

The tests used for evaluation of the emotional reactivity and the sensibility to stress in dairy ewes showed that 32 animals (26.5%) were with Calm temperament (C); 60 animals (51.3%) - with Nervous temperament (N) and 26 animals (22.2%) - with Intermediate temperament (I).

MEASUREMENT OF LYSOZYME CONCENTRATIONS

Blood serum lysozyme concentrations were determined according the method of LIE [16]. Twenty ml of 2% agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0.07 M Na$_2$HPO$_4$ and NaH$_2$PO$_4$, pH = 6.2) were 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 again prepared in veronal-veronal Na buffer: 80 µL of 1% rabbit erythrocyte suspension were added to each well. After incubation for 1 hour at 37°C, samples were centrifuged at 150 g for 3 min at room temperature (25°C). Thereafter, 150 µL of each supernatant were removed and placed in flat-bottomed plates for measurement of optic density at 540 nm by “Sumal-PE2” ELISA reader (Karl Zeiss, Germany). The final APCA activity (measured in CH50 - calculations made on the base of 50% complement-induced haemolysis of applied erythrocytes) and lysozyme concentrations were calculated using special computer programs developed in the Trakia University.

Statistical analysis

Data were analysed using the fixed effect MANOVA model (Program Statistica, Statsoft, Inc., USA) and evaluated through the following formula:

$Y_{ij} = m + a_i + e_{ij}$ where $Y_{ij}$ is the observation value of the investigated trait, $m$ the population mean, $a_i$ the breed effect and $e_{ij}$ the random errors. Differences were considered as significant when $p$ values were less than 0.05.

Results

Table I shows the lysozyme concentrations in dairy ewes from a different temperament type. The highest lysozyme concentrations were observed in sheep with calm (C) temperament ($0.300 \pm 0.032$ mg/mL), whereas they were lowered

<table>
<thead>
<tr>
<th>Temperament type</th>
<th>Number of animals</th>
<th>Mean ± SE</th>
<th>VC%</th>
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</thead>
<tbody>
<tr>
<td>Calm (C)</td>
<td>32</td>
<td>0.300 ± 0.032***</td>
<td>59.7</td>
</tr>
<tr>
<td>Intermediate (I)</td>
<td>26</td>
<td>0.230 ± 0.032</td>
<td>70.4</td>
</tr>
<tr>
<td>Nervous (N)</td>
<td>60</td>
<td>0.173 ± 0.013</td>
<td>57.8</td>
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Table I. — Lysozyme concentrations (mg/mL) in dairy ewes from various temperament types. SE - standard error; VC - variation coefficient. *** p<0.001.

in sheep with intermediate (I) temperament type ($0.230 \pm 0.032$ mg/mL). The lowest lysozyme activities were obtained in ewes from the nervous (N) temperament type ($0.173 \pm 0.013$ mg/mL). The difference between C and N ewes was statistically significant (p<0.001). The data evidenced that the calm temperament type was related to the highest lysozyme concentrations, while lysozyme concentrations were markedly reduced in nervous animals despite the high varia-
tions among individuals (coefficients of variation between 59.7 and 70.4%).

Table II presents the influence of the temperament type upon APCA activities. The highest values were measured in the calm temperament type (144.97 ± 4.35 CH50), followed by the intermediate temperament type (131.99 ± 3.50 CH50) and the nervous temperament type (130.18 ± 2.87 CH50). Again, the differences between the C and N types were statistically significant (p<0.05) but the coefficients of variation were lower (13.5(17.1 %) than those of lysozyme concentrations.

<table>
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<tbody>
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</tr>
<tr>
<td>Nervous (N)</td>
<td>60</td>
<td>130.18 ± 2.87</td>
<td>17.1</td>
</tr>
</tbody>
</table>

Table II. — Influence of temperament type upon APCA activity (CH50). SE - standard error; VC - variation coefficient. **p<0.01.

Discussion

The presented data suggested that ewes from the calm temperament type exhibited a higher innate resistance with high lysozyme and APCA concentrations compared to the nervous type. So these animals could be useful for studying with more accuracy relations between the resistance to stress and immune defense to infections.

Several authors reported that increases of blood cortisol concentrations were partially responsible for decreases of lysozyme concentrations during stress [10, 17, 19]. PANANELLI et al. [18] explained the mechanisms of this decrease by the glucocorticoid-mediated inhibition of the lysozyme gene transcription. They reported that the minimum blood concentrations of dexamethasone or cortisol for inhibiting lysozyme synthesis should be 1 mmol/L. By contrast, acute and short-time stress challenges induced elevations of lysozyme synthesis instead of decreases [24]. Because glucocorticoid concentrations were probably not or weakly modified during such stimuli and were kept below 1 mM, they did not alter lysozyme synthesis. It is probably the case for “calm” ewes, whereas in “nervous” animals, the initial cortisol concentrations would be already elevated, so an additional stress would induce repression of the synthesis of lysozyme and complement globulins. This hypothesis will be verified in further investigations.

FEVOLDEN et al. [9] studied the resistance against furunculosis and vibriosis in rainbow trouts. They reported that trouts with low sensitivity to stress were more resistant to furunculosis whereas those with high sensitivity to stress were more resistant to vibriosis. According to these facts, lysozyme is an important element of the defense in stress-sensitive trouts because the sensitivity to stress is related to high cortisol concentrations, that in over-threshold concentrations had an inhibiting effect.

LECHOWSKI et al. [15] challenged 15 pigs with various stresses and observed that during immobilisation, the serum lysozyme concentrations increased. This was interpreted as mobilisation of the natural systemic mechanisms of defence. Similar results were obtained by DEMERS and BAYNE [2]. They induced stress in rainbow trouts by removing them out of the water and after that, determined the lysozyme, cortisol and adrenaline concentrations. According to the authors, the acute and short-time stress increased the cellular and humoral components of innate immunity, while the chronic, continuous stress could result in immunosuppression. RADZIKOWSKA et al [20] have previously shown that low faecal worm egg counts were observed more frequently in sheep with quieter temperament and that higher body weights were associated also with better temperaments.

As a conclusion, the increased sensitivity to stress decreased blood serum lysozyme and complement concentrations in dairy ewes and could contribute to the development of sensitivity to infections.

References

10. — GONCHAROV A I. : Nonspecific immunity factors in calves in relation to the influence of the type high nervous activity on the functional components of innate immunity, while the chronic, continuous stress could result in immunosuppression. RADZIKOWSKA et al [20] have previously shown that low faecal worm egg counts were observed more frequently in sheep with quieter temperament and that higher body weights were associated also with better temperaments.
15. — LECHOWSKI R., SAWOSZ E., KLUCINSKI W., J. CHACHU-


