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# Comparative studies on some parameters of innate resistance and metabolic profile of sheep and their offspring depending on the ration

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***Abstract:*** *Comparative studies were carried out to evaluate the non-specific resistance and some metabolic profile parameters in Mouton Charollais sheep (n=16) and their offspring (n=10) depending on diet type. It was found out that the compound feed type had various effects on studied parameters. Blood lipids and $T_3$ increased statistically significantly ($p<0.05$) in sheep. In offspring, considerable changes were observed in blood urea, APCA ($p<0.05$), body weight and daily weight gain ($p<0.01$). There were no significant changes in the other parameters tested (lysozyme, total protein, γ-GT and $T_4$).*

**Keywords:** sheep, lipids, urea, $T_3$

**Abbreviations:** APCA — alternative pathway of complement activation, ALAT — alanine aminotransferase, MFU — milk feed units, CP — crude protein, CF — crude fibres, PDI — protein digestible in the intestine, PBV — protein balance value in the rumen, VMP — vitamin mineral premixes

## Introduction

It is acknowledged that numerous intrinsic and exogenous factors could have an impact on microbial growth in the rumen as diet composition, type and quality of feeds, feeding regimen, ambient temperature, rumen pH, ruminal passage rate etc. (Verbic, 2002; Beauchemin et al., 2003; Bowman et al., 2003). During the last 10-15 years, a number of changes related to diet formulation have occurred. The attempts of researchers were focused on the evaluation of effect of exogenous enzymes, vitamin-mineral premixes aimed to increase the availability of feed ingredients (Lee et al., 2003; Shimkus et al., 2005; Bivolarski et al., 2009; Todorova et al., 2009; Varlyakov et al., 2010). Despite that, the studies on the influence of ration composition upon the non-specific resistance and some aspects of the metabolic profile in sheep in Bulgaria are rather few. At present, nutrition specialists and feed producers do not realized fully the importance of these cardinal issues, directly related to animal health and higher rate of quality production. The problem with combining ingredients (milk feed units MFU, crude protein CP, crude fibres CF, protein digestible in the intestine PDI, protein balance value in the rumen PBV) and supplements (enzymes, vitamin mineral premixes) in compound feeds produced in our country remains still open.

In most instances, the adequate amount of feed fully satisfies the needs of sheep with low productivity for mineral substances. The issue with highly productive sheep is however different, as their feeds require obligatorily supplementation with vitamin mineral premixes. Young animals need a larger amount of vitamins and minerals for the proper development of bones, and the regular systemic metabolism. The mineral requirements are influences by the body weight, age, gender, physiological conditions, the type and composition of the ration. The most important macroelements for sheep are calcium, phosphorus, magnesium, sodium, potassium, sulfur and chlorine, and from the trace elements group: copper, zinc, cobalt, selenium, iron, iodine, fluorine and molybdenium (Todorov, 2008). The supplementation of feed with vitamin mineral premixes (VMP) prevents a major deficiency that could provoke disease, poor reproductive and productive performance.

The aim of the present study was to perform comparative investigations on some parameters of the non-specific resistance and metabolic profile in sheep and their offspring related to the composition of the ration.

## Material and methods

The experiment was carried out in the Production Base of the Agricultural Institute in Stara Zagora with 16 pregnant sheep from the French breed Mouton Charollais in the 3-3.5 month of gestation. Animals were divided into 2 groups. Experimental (n=8), fed a compound feed for pregnant sheep ($CF_1$) produced by Norex Agro Ltd and after lambing with compound feed for lactating sheep ($CF_2$) produced by the same manufacturer. Control group (n=8), received a compound feed for pregnant sheep ($CF_3$) and lactating sheep ($CF_4$), produced at the forage manufacturing facility of the Agricultural Institute.

The composition of $CF_1$ included: 15% maize, 21.6% wheat, 25% barley, 15% wheat bran, 20% sunflower meal, 7.5% soybean meal, 2.5% limestone, 0.5% NaCl and 0.3% vitamin mineral premix (VMP) Roche. The nutritive value of $CF_1$ of 11.4% moisture was as followed: 1.07/kg MFU, 15.7% CP, 8% CF, 9.9% PDI, 0,6% PBV, 1% Ca и 0.6% P. The composition of $CF_2$ was: 20% maize, 10% wheat, 16.2% barley, 15% wheat bran, 28% sunflower meal, 7.5% soybean meal, 2.5% limestone, 0.5% NaCl and 0.3% VMP Roche, with nutrition facts (11.2% moisture) 1.03/kg MFU, 20% CP, 9.5% CF,

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11.5% PDI, 2.9% PBV, 1.1% Ca and 0.6% P. $CF_3$ and $CF_4$ contained 30% maize, 46,5% wheat, 20% sunflower meal, 2% limestone, 1% NaCl and 0.5% premix 27K with nutritive value (12.3% moisture) 1.13/ kg MFU, 16.12% CP, 5.3% CF, 0.9% Ca and 0.5% P.

Pregnant ewes received, apart daily pasture, 300 g compound feed whereas lactating ewes received 1 kg grass hay, 1,5 kg silage and 500 g compound feed.

Blood for analysis was obtained from v. jugularis twice prior to lambing at 20-day intervals and 4 times after lambing (at lambing, and by post parturient days 14, 28, and 42).

Investigations on some parameters of innate resistance, metabolic profile and fattening capacity depending on the ration type were performed with offspring of experimental sheep. For this purpose, 10 lambs were selected and divided into 2 groups: experimental (n=5), born from experimental sheep and fed a starter feed for lambs ($CF_5$), produced by Norex Agro Ltd and control (n=5), born from control dams and fed a starter feed ($CF_6$), produced at the forage manufacturing facility of the Agricultural Institute. $CF_5$ contained 38.7% maize, 10% wheat, 20% barley, 19% sunflower meal, 8.7% soybean meal, 2.7% limestone, 0.5% NaCl and 0.4% VMP Roche, with nutritional value (11.4% moisture): 1.25/kg MFU, 17% CP, 6.9% CF, 11.3% PDI, 0.9% PVB, 1.1% Ca and 0.5% P. $CF_6$ was composed by 16.5% maize, 20% oat, 25% bran, 20% sunflower meal, 5% soybean meal, 10% alfalfa hay, 1% dry skimmed milk, 1.5% limestone, 0.5% NaCl and 0.5% premix 26, with nutritional value (11.7% moisture): 0.91/ kg FU, 18.8% CP, 12.7% CF, 0.9% Ca and 0.6% P. Apart breast-feeding, lambs received compound feed ad libitum.

Blood was sampled 3 times from the jugular vein of both groups of lambs at the age of 30, 45 and 60 days. During the trial, at 10-day intervals, lambs were individually weighed. The activity of lysozyme in sheep and their offspring was done by the method of Lie (1985), whereas the alternative pathway of complement activation activity was assayed per Sotirov(1986). Blood serum glucose, lipids, total protein, urea, ALAT and γ-GT were determined with commercial kits on a biochemical analyzer (BA-88 Mindray – China) in the clinical laboratory of the Faculty of Veterinary Medicine.

The concentration of triiodothyronine ($T_3$) and thyroxine ($T_4$) in blood was assayed by radioimmunoassay (RIA) with commercial kits of Immunotech, Prague, Czech Republic. The reproducibility of results according to manufacturer's specifications, were as followed:

For Total $T_3$ RIA kit, cat № IM 1699: intraassay coefficient of variation 3.3%; interassay coefficient of variation 8.6%; sensitivity 0.1 nmol/l; measurement range– 0.1-12 nmol/l.

For $T_4$ RIA kit, cat № 1447: intraassay coefficient of variation 5.1%; interassay coefficient of variation 8.6%; sensitivity – 13 nmol/l; measurement range– 13-400 nmol/l. intraassay 3.3%.

Data were statistically processed with Statistica v. 6.1. (StatSoft Inc., 2002). Means and standards errors of means were determined with descriptive statistical methods. The effect of biologically active substances on tested parameters in the different groups was determined by ANOVA and the post hoc LSD test.

## Results and discussion

The results from laboratory investigation of blood biochemical parameters in Mouton Charollais ewes are presented in Table 1 and 2. Regardless of the different composition of compound feeds given to both groups, there were no significant changes in studied parameters before lambing. Experimental sheep exhibited higher blood $T_4$ and APCA concentrations whereas all other indices were with higher values in controls. Yet, the differences were not

**Table 1.** Blood glucose, lipids, total protein, urea, ALAT and γ-GT (mean±SEM) in Mouton Charollais sheep before and after lambing (* p<0.05)

| Group | Glucose nmol/l | Lipids mg/dl | Total protein g/l | Urea mmol/l | ALAT U/l | γ-GT U/l |
|---|---|---|---|---|---|---|
| Before lambing | | | | | | |
| Experimental | 2.41±0.11 | 163.26±17.56 | 78.76±1.47 | 4.65±0.22 | 34.52±3.71 | 31.58±2.82 |
| Control | 2.54±0.12 | 168.13±12.27 | 80.07±1.62 | 4.65±0.24 | 39.26±2.31 | 38.63±2.51 |
| After lambing | | | | | | |
| Experimental | 2.94±0.18 | 231.18±10.66 | 76.71±2.12 | 11.43±0.72 | 13.23±1.58 | 32.63±3.65 |
| Control | 2.71±0.13 | 203.36±7.84* | 79.54±2.33 | 9.82±0.75 | 13.73±2.35 | 32.19±3.54 |

**Table 2.** Blood $T_3$, $T_4$, lysozyme and alternative pathway of complement activation (APCA) activity (mean±SEM) in Mouton Charollais sheep before and after lambing (* p<0.05)

| Group | $T_3$ nmol/l | $T_4$ nmol/l | Lysozyme µg/ml | APCA $CH_{50}$ |
|---|---|---|---|---|
| Before lambing | | | | |
| Experimental | 1.84±0.13 | 63.86±2.80 | 0.29±0.05 | 151.21±4.73 |
| Control | 1.45±0.12* | 59.50±6.60 | 0.30±0.05 | 142.18±6.69 |
| After lambing | | | | |
| Experimental | 1.68±0.14 | 74.78±6.60 | 0.19±0.02 | 170.00±10.39 |
| Control | 2.22±0.21* | 78.72±7.16 | 0.25±0.03 | 176.39±13.17 |

statistically significant suggesting that the supplementation of a different compound feed to the diet of pregnant sheep did not have a considerable effect on blood glucose, total protein, urea, enzymes ALAT and γ-GT, $T_4$, and innate resistance parameters (lysozyme and APCA). Similar to these data were reported in studies on innate resistance parameters (Bivolarski and Sotirov, 2001) and blood biochemistry in sheep (Binev et al., 2006). The deviations in some of these parameters could be attributed to the different sheep breed.

We have observed a statistically significant increase in blood plasma $T_3$ concentration in experimental ewes compared to controls – 1.84±0.13 nmol/l (Table 2), vs 1.45±0.12 nmol/l, respectively ($P<0.05$). The opposite trend in plasma $T_3$ levels was exhibited by lactating ewes – 1.68±0.14 nmol/l in the experimental group and 2.22±0.21 nmol/l in controls ($P<0.05$). A statistically significant difference was established in mean lipids in bloods which were higher in experimental sheep (231.18±10.66 ng/dl) than in controls 203.36±7.84 ng/dl ($P<0.05$).

It is known that thyroid hormones ($T_3$ and $T_4$) are the most important modulators of metabolic rate (Christian and Trenton, 2003) that enhance the growth, development and adaptation of animals (Tomov et al., 1989). The relationships observed in plasma $T_3$ during the pregnancy and the lactation provide evidence for the possible effect of two factors. On one side, this is the different composition of received feed and one the other – the physiological status of ewes. The independent effect of each of these factors could be determined more precisely only in additional studies as triiodothyronine concentrations are known to be also influenced by the season, age, gender etc.

It should be pointed out that when pre- and post-lambing periods were compared, blood lipids, urea, $T_4$ and APCA increased after the parturition. The opposite tendency was observed for ALAT activities that were reduced more than twice after lambing (Table 1 and 2).

The effect of long-term feeding a vitamin mineral premix on blood lipids showed that despite the significant difference, obtained values were within the reference range and thus, excluded the possibility of damage of lipoprotein membranes and proved the preserved integrity of hepatocytes and optimization of the function of the liver as universal systemic biochemical laboratory. In this connection, the statistically significant reduction in blood ALAT activity in both groups and the insignificant decrease in blood γ-GT also deserve some attention. Their activity increases when destruction of the liver parenchyma is present. Although lowered, their activities were within the physiological range and therefore, did not support the possibility for liver tissue alteration consequently to long-term feeding rations with a different composition.

Tables 3 and 4 present data from blood analyses of lambs from the experimental and control groups. In experimental lambs, blood urea was considerably higher 10.28±0.41 nmol/l vs 8.10±0.49 nmol/l ($P<0.05$). A similar tendency was observed in APCA activity, which was statistically significantly higher in controls ($P<0.05$) (Table 4). Therefore, the different composition of the ration had an effect on the innate resistance of lambs. For the other tested parameters, the values in both groups were very similar, as observed in respective dams. An exception was mean ALAT activity that was 40.06±9.20 U/l in experimental lambs and 24.89±3.83 U/l in controls, although the difference was not significant.

Mean body weight of experimental lambs was 22.60±0.99 kg vs 18.70±0.81 kg ($P<0.01$) in controls. A similar trend was observed in the daily weight gain: 377±17 g in experimental lambs and 312±5 g in controls ($P<0.01$). These results allowed us to affirm that the compound feed produced by Norex Agro Ltd was better utilized and contributed to a more intensive growth rate in lambs.

**Table 3.** Blood glucose, lipids, total protein, urea, ALAT and γ-GT (mean±SEM) in Mouton Charollais lambs (* $p<0.05$)

| Group | Glucose nmol/l | Total protein g/l | Urea mmol /l | ALAT U/l | γ-GT U/l |
|---|---|---|---|---|---|
| Experimental | 4.23±0.16 | 53.31±2.16 | 10.28±0.41 | 40.06±9.20 | 63.77±4.25 |
| Control | 3.79±0.15 | 56.05±2.91 | 8.10±0.49* | 24.89±3.83 | 59.90±2.61 |

**Table 4.** Blood $T_3$, $T_4$, lysozyme and APCA activity (mean±SEM) in Mouton Charollais lambs (* $p<0.05$)

| Group | $T_3$ nmol/l | $T_4$ nmol/l | Lysozyme µg/ml | APCA $CH_{50}$ |
|---|---|---|---|---|
| Experimental | 2.51±0.17 | 79.53±7.16 | 0.15±0.03 | 171.14±4.81 |
| Control | 2.43±0.12 | 75.27±5.20 | 0.14±0.03 | 190.44±4.43* |

## Conclusion

In conclusion, it could be affirmed that feeding of ewes and their offspring with compound feed with a different composition had a variable effect on innate resistance and metabolic profile parameters. It was found out that statistically significant changes occurred in blood lipids and T3 in sheep and in blood urea, APCA, body weight and daily weight gain in offspring. There were no significant changes in the other parameters tested (lysozyme, total protein, γ-GT and $T_4$). Having in mind that Mouton Charollais sheep are reared in Bulgaria for the last 7-8 years, the research in this field should be continued. This way, the optimal diet formulation for sheep aimed at increasing their productivity and improving their physiological status could be found.

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