EFFECT OF AIR AMMONIA ON SOME HEMATOLOGICAL PARAMETERS IN SHEEP SUBJECTED TO IMMOBILIZATION OR SHEARING STRESS

IVAN YANCHEV, MARINA DYAVOLOVA, DIMITAR GUDEV,
PENKA MONEVA, DESISLAVA VELIKOVA
Institute of Animal Science – Kostinbrod

Ammonia has been reported to increase the level of some cytokines (IL-1, IL-4, IL-6, TNF) which modulate hematopoiesis in response to thermal injury (**Kataranovski et al.**, 1999). Atmospheric ammonia was suggested to cause the release of cytokines by alveolar macrophages and neutrophils (**Murata and Horino**, 1999). Systemic hematological and immunological effect in response to atmospheric ammonia has been observed in pigs (**Mitloehner,** 2004).

Studies on ammonia-induced changes in hematological indices were conducted mainly in pigs and poultry (**Curtis et al.**, 1975; **Drummond et al**., 1976; **Watches et al.,** 2004; **von Borell et al**., 2007).

Heat stress exercise was found to augment plasma level of ammonia (**Marino et al.**, 2001). Ammonia in the sheep barn is produced by the decomposition of nitrogenous compounds in manure by urease activity. Its level is relatively high in poor ventilated barns, especially in winter months when barn doors and windows are closed. High ambient temperature was reported to cause a change of hematological parameters which was more pronounced in shorn than in unshorn sheep (**Silva et al.**, 1992).

The process of shearing has been demonstrated to decrease white blood cells count (**Piccione et al**., 2008). **Philips et al.** (2012) did not find any change of hematological parameters in sheep exposed to 4 ammonia concentrations (4, 12, 21 and 34 mg/m^3).

The present knowledge about the effect of naturally occurring ammonia in a sheep barn on hematological parameters of sheep exposed to shearing and immobilization is highly insufficient. Consequently, the aim of the present study was to investigate the effect of naturally occurring ammonia in a sheep barn on some hematological parameters in sheep subjected to shearing or immobilization.

MATERIAL AND METHODS

To achieve our goal we conducted two experiments. The first experiment comprised 14 ewes of the Bulgarian synthetic dairy population (BSDP) allocated into two groups: control, reared in the open air around the clock and experimental, kept indoor during the night. Experimental ewes could choose to stay inside or outside during the day. The experiment was carried out in May at maximum ambient temperature outside 25 ^{0}C. Ammonia concentration in the barn measured in the morning before opening of barn doors reached a level of 60 ppm. Blood samples were taken from jugular vein in the morning when experimental ewes were still in the barn. After shearing all animals were exposed to direct solar radiation for 2 h. The second blood sampling was performed 2 h after shearing.

The second experiment comprised 7 ewes of the BSDP and 7 ewes of the Ile de France breed. Both breeds of ewes were kept indoor during the night and had access to yard area during the day. The experiment was carried out in the end of March at ambient temperature 16 ^{0}C outside and 14.5 ^{0}C inside. Ammonia level in the morning before opening of barn doors reached a level of 60 ppm. The ewes were immobilized by bending the joints of the fore and hind legs through tying tin wires around. Blood samples were taken at 9 ^{00} h, immediately after one hour long immobilization. The following hematological parameters were determined: white blood cell (WBC) count, red blood cell (RBC) count, hematocrit, heterophil to lymphocyte ratio. Total erythrocyte and leukocyte counts were determined by manual haemacytometer chamber count. Hematocrit was measured by the microhaematocrit method. Peripheral blood leukocytes were counted on smears. The smears were stained using May-Grunvald and Gimsa stains (**Lucas and Jambos**, 1961). Four hundred leukocytes including neutrophils, eosinophils, basophils, lymphocytes and monocytes were counted microscopically on a slide.

Sheep were kept in a naturally ventilated brick walled barn with tile roof and small windows on the front wall. The windows were kept closed during the night in winter months and during the experimental period. The animals had free access to meadow hay and tap water. Concentrate mix was given once daily. The results of one factor statistical analysis are expressed as means ± *S.E.M*. and were analyzed by ANOVA.

RESULTS AND DISCUSSION

White blood cell count tended to be higher (*P*>0.05) in experimental sheep (Fig.1). Previous research with sheep

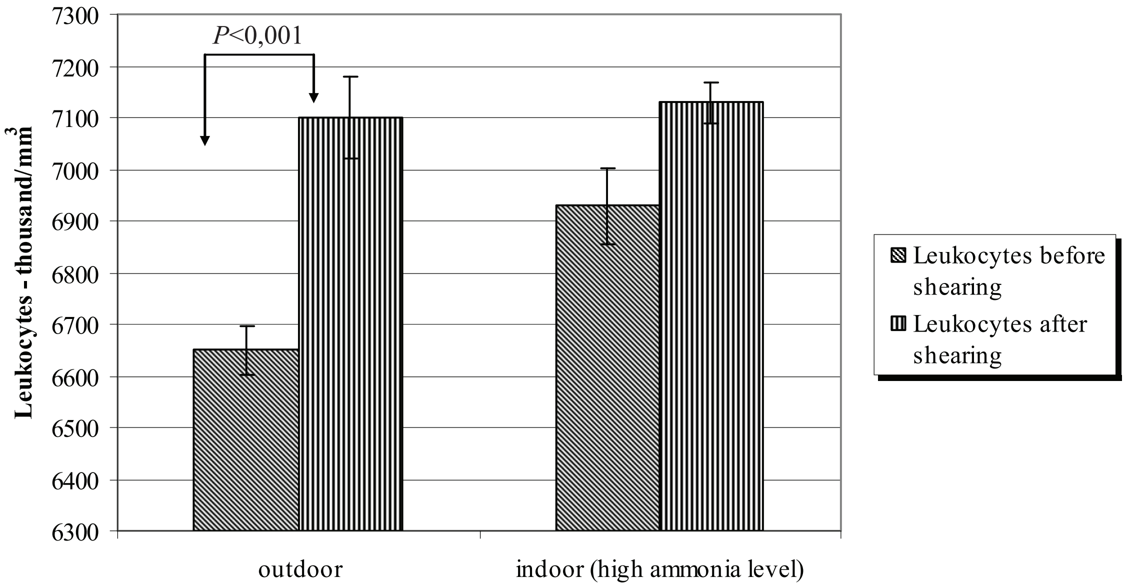

Fig. 1. **White blood cell count following shearing stress in ewes reared indoor or outdoor**

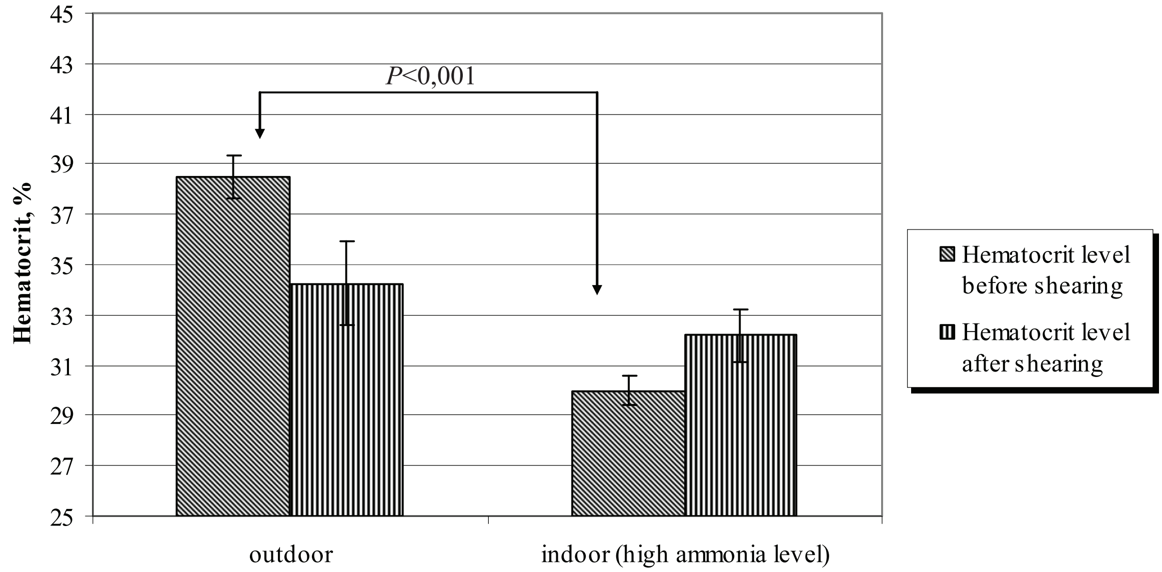

Fig. 2. **Hematocrit level following shearing stress in ewes reared indoor or outdoor**

exposed to ammonia concentration from 11 to 34 mg/m^3 over a 12 d duration found no effect of ammonia on WBC count (**Philips et al**., 2012). Similarly, **Gustin et al**. (1994) reported that pigs exposed to atmospheric ammonia of 0, 25, 50 and 100 ppm for 6 days had no change of total WBC count. Shearing caused significant increase of leukocytes in control sheep ($P<0.001$). Our results are not consistent with those of **Silva et al**. (1992) who reported that WBC count declined significantly with rise in environmental temperature from 25 ^{0}C to 46.5 ^{0}C in both unshorn and shorn animals. Also, they found greater difference in the shorn relative to unshorn animals, which they attributed to a process of haemodilution caused by a pronounced peripheral vasodilation, that is compensated via an increase of blood volume due to transfer of water from body tissues into the blood vessels. Consequently, the observed discrepancy between our results and those reported by **Silva et al.** (1992) could be ascribed to a difference in the temperature load experienced by the animals in our and their experimental design. This view is further supported by the reported change of WBC count after shearing that was higher in shorn than in unshorn ewes (**Piccione et al**., 2008). Mild heat stress observed after shearing was accompanied with a rapid shallow panting (**Hales**, 1973) needed to compensate for the lack of insulation, since thermal gradient across 4 cm thick fleece varies between 21 and 40 ^{0}C, whilst thermal gradient in the remaining 5-8 mm

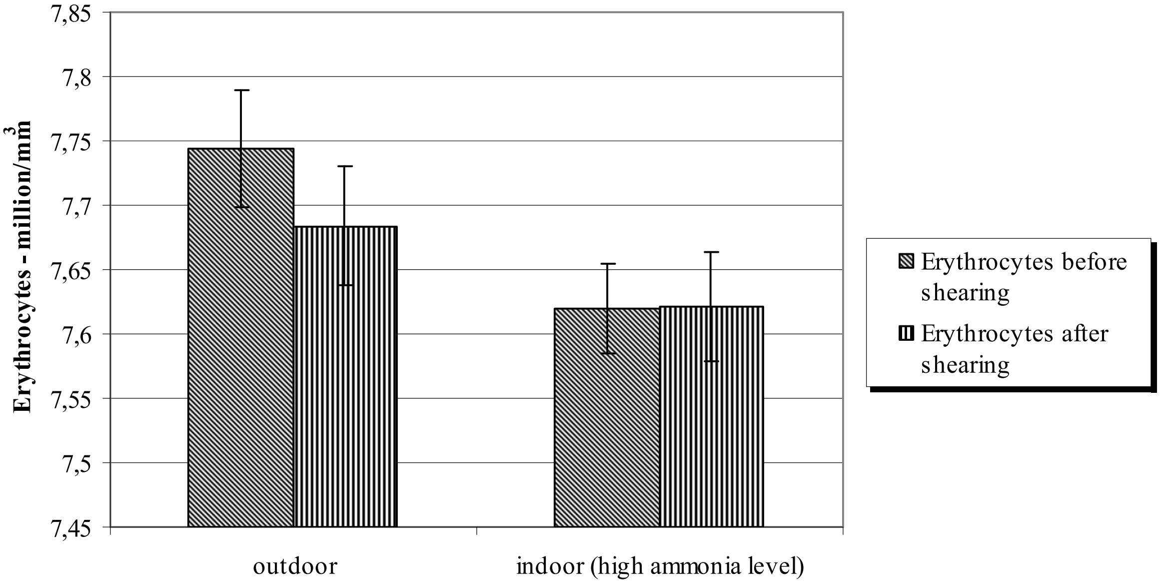

Fig. 3. **Red blood cell count following shearing stress in ewes reared indoor or outdoor**

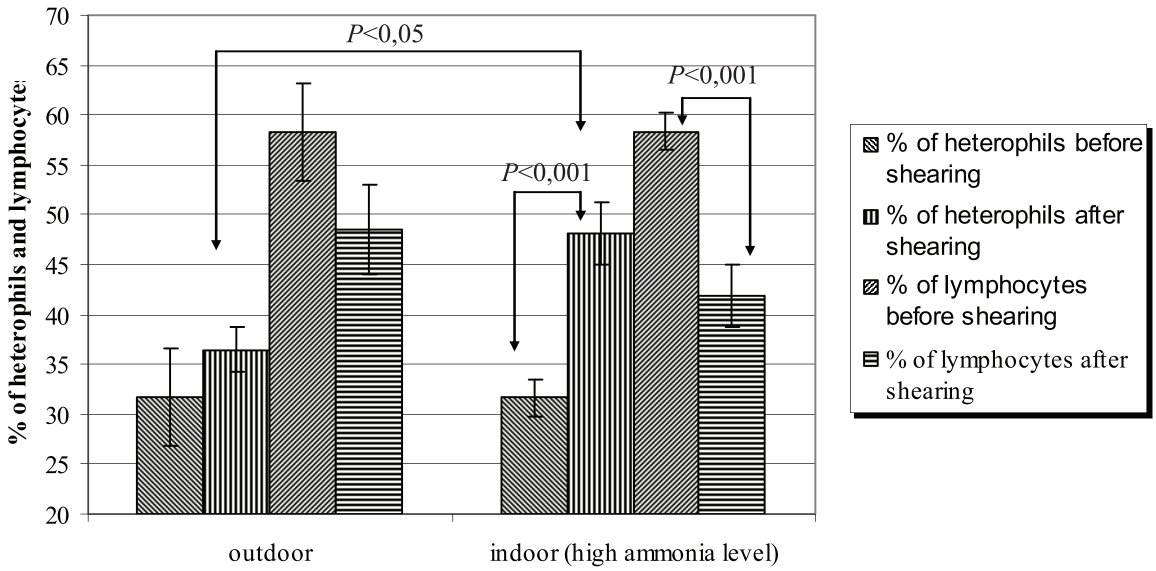

Fig. 4. **Effect of ammonia on heterophils and lymphocytes percentage in sheep subjected to shearing stress**

wool after shearing is reduced to 4 ^{0}C (**Mackfarlane**, 1966). Besides, even mild heat stress was reported to cause regional distribution in blood flow (**Hales**, 1973). We assume that the controversial results concerning WBC count could be due to interaction between the effects of heat and glucocorticoids on WBC.

Glucocorticoids are known to increase WBC count through demargination of heterophils from the endothelial lining of the blood vessels into the general circulation (**Pharmacology Weekly**, 2009). Consequently, higher level of glucocorticoids could counteract the reduction of WBC count induced by heat.

Shearing resulted in a slight increase of WBC count in experimental sheep which did not reach level of significance. There is no available literature concerning the effect of ammonia on shearing – induced change of WBC count. Earlier finding that ammonia stimulated nitric oxide synthesis (**Monfort et al**., 2007) provides a possible explanation for the observed effect of ammonia on WBC, since NO was found to take part in thermoregulation via its vasodilatory effect (**Mills et al**., 1997).

Hematocrit level before shearing was significantly lower ($P<0.001$) in the experimental than in the control group (Fig. 2). This result is consistent with the reported increase of body temperature with increase of atmospheric ammonia from 16 to 54 ppm in broilers kept under 32 ^{0}C (**Yahav**,

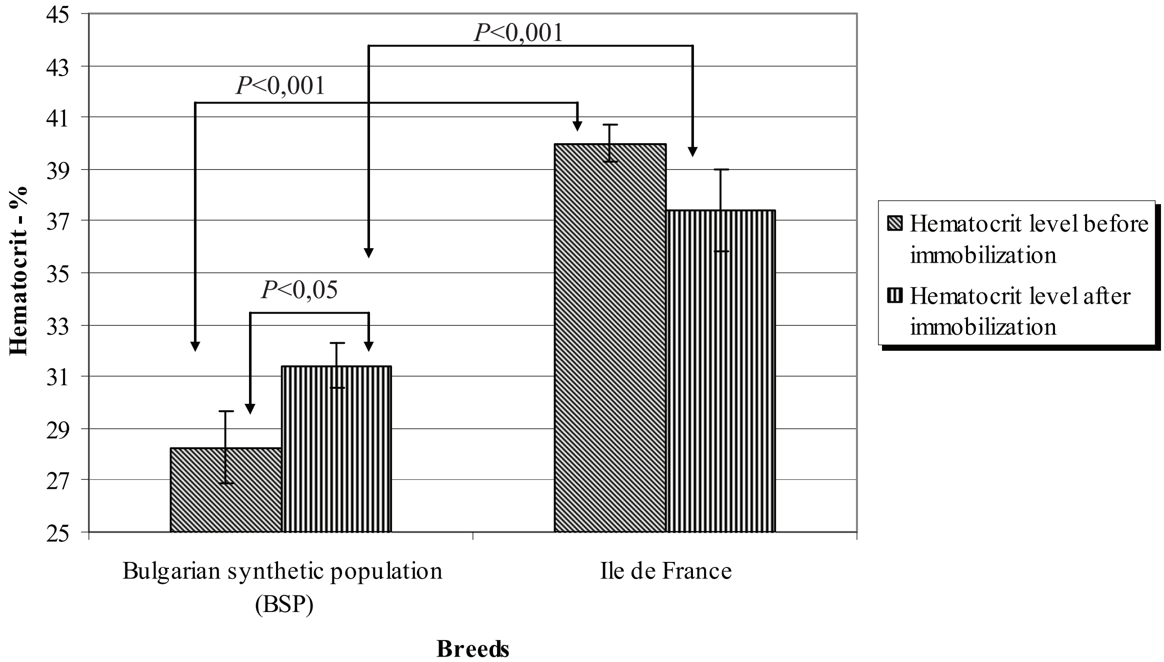

Fig. 5. **Hematocrit level following immobilization in ewes of the Bulgarian synthetic population, and Ile de France breeds kept indoor**

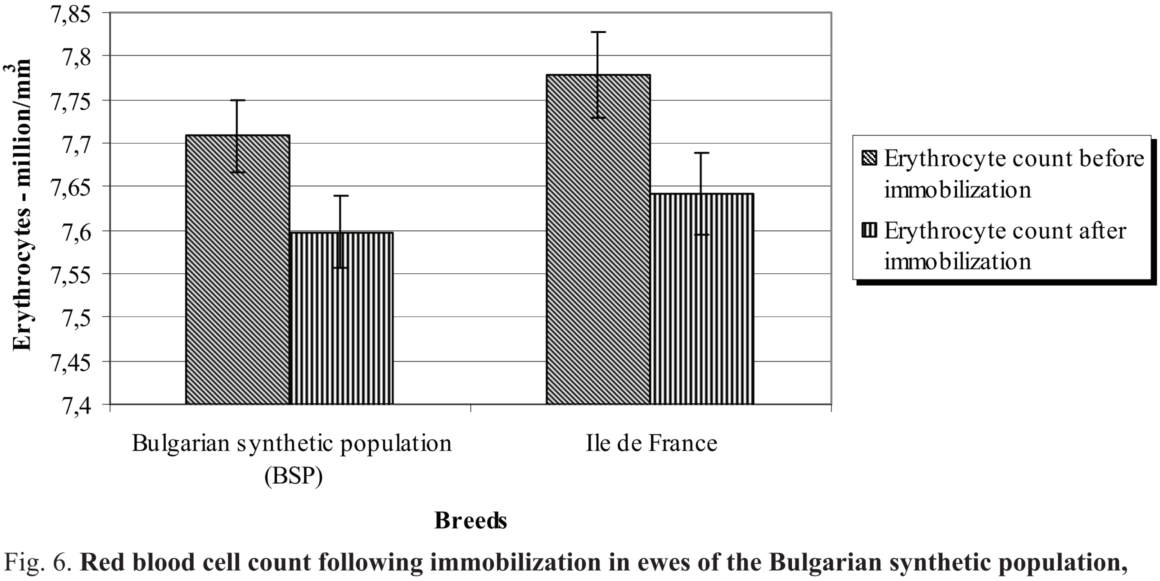

Fig. 6. **Red blood cell count following immobilization in ewes of the Bulgarian synthetic population, and Ile de France breeds kept indoor**

2004) and suggests that the observed decline of hematocrit in the experimental ewes was most probably influenced by ammonia - induced increase of body temperature. It has been reported that a rise in core temperature of only 0.5 ^{0}C causes a peripheral vasodilation (**Eckert et al.**, 1988) which is accompanied by hemodilation.

Hematocrit level in the control ewes tended to decrease after shearing (P>0.05) while in the experimental ewes it remained unchanged (Fig. 2). **Philips et al**. (2012) did not find any effect on hematocrit in sheep exposed to 4 ammonia concentrations. (4, 12, 21 and 34 mg/m^3). **Patterson et al**. (1995) reported that exposure to heat stress increased hematocrit level. The observed trend towards a decline of hematocrit in the control ewes after shearing could be attributed to the opposite effects of heat and plasma cortisol level on hematocrit value. Shearing is known to increase plasma cortisol which on its turn could counteract downregulatory effect of heat on hematocrit level. However, in our previ-

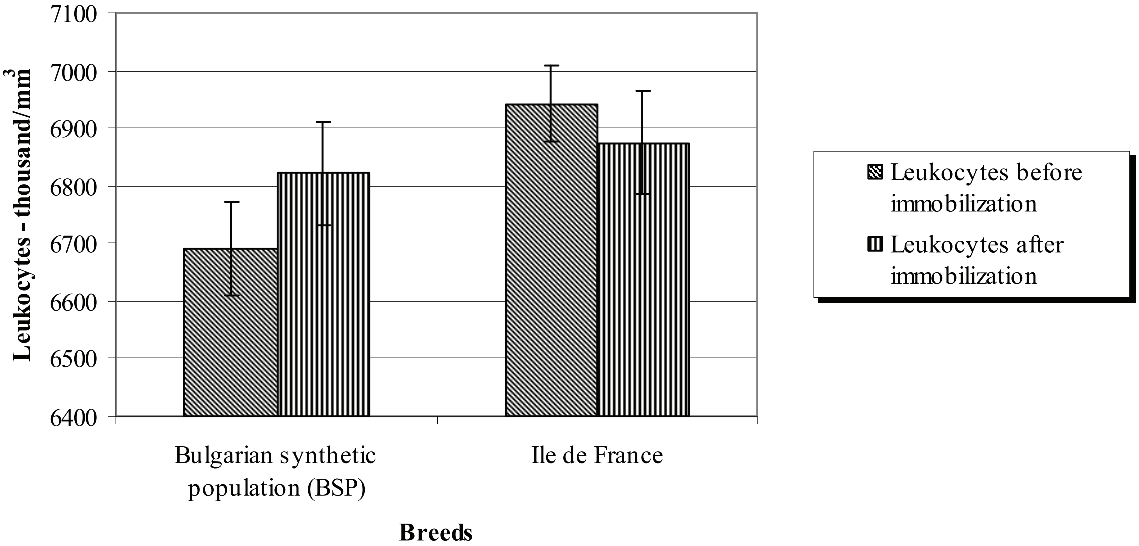

Fig. 7. **White blood cell count following immobilization in ewes of the Bulgarian synthetic population, and Ile de France breeds kept indoor**

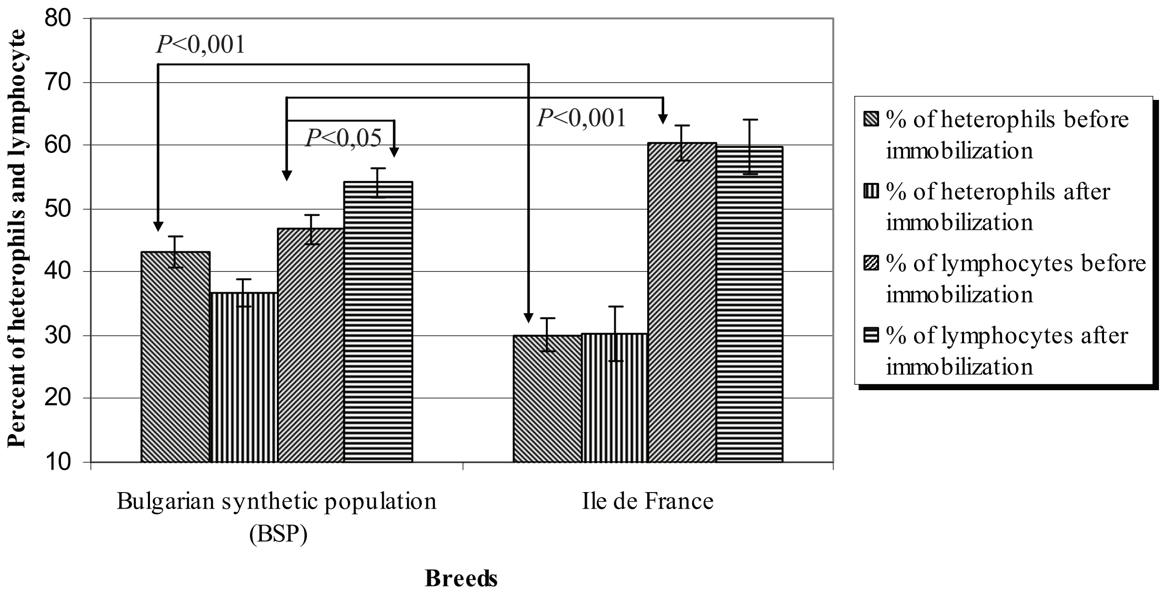

Fig. 8. **Effect of immobilization on heterophils and lymphocytes percentage in ewes of the Bulgarian synthetic population, and Ile de France breeds kept indoor**

ous investigation with cows (**Gudev et al**., 2004) subjected to transport from farm to climatic chamber and exposed to heat (35 ^{0}C) we found quick decline of transport-induced increase of cortisol within 30 min following the exposure to heat. This finding was explained with an overwhelming inhibitory effect of heat load on cortisol production aimed at decreasing adrenal response to the psychological component of heat stress, thus reducing endogenous heat production, caused by the calorigenic effect of the stress hormones (**Yousef and Johnson**, 1967). It is noteworthy to point that blood samples were taken 2 h after shearing when heat load might have overwhelmed shearing-induced increase of plasma cortisol since shearing was reported to increase plasma cortisol level (**Mears et al**., 1999). Our previous experiment have indicated that exposure of locally bred shorn sheep to direct solar radiation caused sharp increase of rectal temperature and respiratory rate (**Alexiev et al**., 2003) These data are in favor of our view that hematocrit level after shearing depends on the strength of the heat load which predetermines the extent of the overwhelming power of heat on adrenal function (decline of cortisol and catecholamines production in any particular experimental design). Furthermore the effect of ammonia on hematocrit level before shearing could also be influenced by ambient temperature. In our previous investigation with rabbits reared under low or high ambient temperature (in press) we found opposite effects of ammonia on rectal temperature. Atmospheric ammonia resulted in an increase of rectal temperature at ambient temperature of 24 ^{0}C and a decrease at ambient temperature below 10 ^{0}C. Consequently, ammonia in our present experiment might have contributed to the observed decline in basal hematocrit level by its effect on thermoregulation. Most probably ammonia modulated body temperature via its stimulatory effect on nitric oxide production (**Monfort et al**., 2002). Nitric oxide was reported to stimulate heat dissipation via increased sweating (**Mills et al**., 1997). It is well documented that heat stress increases reactive oxygen species production (**Del Vesco et al**., 2013). It has been proposed that uncoupling proteins which are known to take part in thermogenesis may play a role in the elevation of mitochondrial reactive oxygen species (**Mujahid et al**., 2009). The authors reported an increase in mitochondrial membrane potential and respiration rate in chicken under heat stress. These data give support of our view that the rate of heat production due to shearing and the lack of fleece led to increase of heat production to the extent that heat load overcame heat dissipating effect of ammonia. The adverse effect of heat could be further aggravated by ammonia-induced production of NO which belongs to family of chemicals collectively called reactive nitrogen species. Erythrocyte count before and following shearing was similar to both groups (Fig. 3) and suggests that the observed decline of basal hematocrit level in experimental ewes was not due to hemodilution. **Roller** (1967) reported sustained water loss by erythrocytes in cattle given solution of urea and water directly into rumen .Also, oxidative stress alters plasma membrane redox system in red blood cells (**Pandey and Rizvi**, 2011). Therefore, the lower basal hematocrit level in experimental ewes against the background of unchanged erythrocyte count was most probably due to a loss of erythrocyte volume, caused by ammonia.

The percentage of heterophils and lymphocytes in the control ewes remained unchanged 2 h after the shearing (Fig. 4). Heterophil to lymphocyte ratio has long been recognized as an indicator of stress (**Maxwell,** 1993). Also, it was demonstrated that hormones released by adrenal glands in response to stress augmented heterophil to lymphocyte ratio (**Dhabhar et al**., 1995). Our result is not consistent with the reported increase of plasma cortisol level after shearing (**Mears et al.**, 1999). In our previous study we mentioned above plasma cortisol level declined within 30 min after exposure of cows to heat stress (**Gudev et al**., 2004). Therefore, it could be assumed that the initial increase of plasma cortisol during and after shearing was downregulated by additional influx of heat to the animals after the shearing, leading to decline of plasma cortisol level within 2 h after shearing as judged by the unchanged heterophil to lymphocyte ratio. Sheep shearing resulted in an increase in the percentage of heterophils (P<0.001) and decline in lymphocyte percentage (P<0.01) in the experimental ewes (Fig. 4). This result indicates that ammonia modified adrenal response to shearing. The altered pattern of heterophils and lymphocytes after shearing could be due to the modulatory effect of ammonia on NO synthase activity (**Swamy et al.**, 2005). Gaseous ammonia was found to increase neutrophil percentage during transport stress (**Philips et al**., 2010). Besides, nitric oxide has also been reported to be effective as a central modulator of temperature regulation (**Gerstberger**, 1999). The mechanism of action of ammonia remains to be elucidated.

Immobilization, unlike shearing resulted in significant increase (P<0.05) of hematocrit level in the ewes of the BSDP (Fig. 5). It is widely known that shearing is more powerful stressor than immobilization. The lack of significant increase in hematocrit level after shearing was probably due to the removal of the fleece along with the fact, that shearing was conducted outdoor, while immobilization took place indoor. Basal level of hematocrit was significantly higher (P<0.001) in Ile de France ewes than in the ewes of the BSDP. The observed difference in the basal hematocrit level could be attributed to presence of breed difference. High values of hematocrit (ranging from 30 to 35%) in Ile de France ewes were reported by **Almeida et al.** (2012). Hematocrit level in Ile de France ewes tended to decline after the immobilization, yet it remained significantly higher (P<0.01) than in BSDP ewes. Erythrocyte count before and following immobilization was similar in both breeds of ewes (Fig. 6) despite the higher hematocrit level in the Ile de France breed. These data support our assumption mentioned above that ammonia affects erythrocyte size.

White blood cell count in both breeds of ewes was not influenced significantly by the immobilization (Fig. 7). There was a trend towards increased total leukocyte numbers after immobilization in BSDP ewes which was similar to that observed after shearing.

The percentage of heterophils tended to be lower while the percentage of lymphocytes increased (P<0.05) after immobilization in the ewes of BSDP breed (Fig. 8) despite the fact that immobilization elevates plasma cortisol level in sheep (**Al-Qarawi**, 2005). Stress is known to increase heterophil to lymphocyte ratio. Therefore, the observed opposite effect of immobilization on heterophil to lymphocyte ratio may be attributed to the effect of ammonia.

There were no significant differences in heterophil and lymphocyte percentages after immobilization in the ewes of the Ile de France breed. However, basal percentage of heterophils was lower (P<0.01) and basal percentage of lymphocytes was higher (P<0.01) than those in the BSDP ewes. We assume that the observed breed differences in basal heterophil and lymphocyte percentage were due to breed differences rather than to the effect of ammonia although we cannot rule out a possible existence of breed difference in the sensitivity to ammonia.

CONCLUSION

Ammonia decreased hematocrit level and modified heterophil percentage and white blood cells count in response to shearing.

There was significant breed difference in the sensitivity to ammonia as judged by heterophil percentage and hematocrit before and after immobilization.

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I. Yanchev, M. Dyavolova, D. Gudev, P. Moneva, D. Velikova
Institute of Animal Science- Kostinbrod

SUMMARY

The aim of the present study was to investigate the effect of ammonia on some hematological parameters in sheep subjected either to shearing or immobilization stress. To achieve our aim, we conducted two experiments. In one experiment, sheep of the Bulgarian synthetic population were allocated into 2 groups - control and experimental. Control animals were kept indoor with the barn door closed during the night. Ammonia range in the morning, before opening the barn doors reached as high value as 60 ppm. Maximum ambient temperature outside was 25^0C. The second experiment examined the effect of immobilization on the same hematological indices in two breeds of sheep (Bulgarian synthetic population and Ile de France breeds) reared indoor under high ammonia level (up to 68 ppm during the night).

Experimental sheep kept under high ammonia level had higher percentage of heterophils after shearing as compared to the control group. Basal level of hematocrit was significantly lower in the experimental group relative to the control group against the background of unchanged red blood cell count. Also, white blood cell count in the control group, unlike that in the experimental group, increased significantly after shearing.

Immobilization under high ammonia level did not cause any change in the investigated hematological parameters in both breeds of sheep. However, Ile de France breed had higher basal and stress-induced levels of hematocrit and lower basal percentage of heterophils relative to Bulgarian synthetic dairy sheep. It is concluded that ammonia modified adrenal response to shearing as judged by the change of heterophil to lymphocyte ratio.

Key words: *heterophils, lymphocytes, ammonia, sheep, immobilization, shearing*