INFLUENCE OF ROAD TRANSPORT ON THE LEVELS OF ACTH AND CORTISOL IN OUTBRED AND INBRED RABBITS

S. Tanchev, L. Sotirov*, S. Georgieva, D. Hristova, Ts. Koynarski*
Trakia University, Faculty of Agriculture – Stara Zagora, 6000
**Trakia University, Faculty of Veterinary Medicine – Stara Zagora, 6000*
e-mail:dhristova@uni-sz.bg

ABSTRACT

The effects of breed, inbreeding and sex on blood hormones cortisol and ACTH concentrations in outbred and inbred rabbits was studied before and after transportation stress. The results allowed assuming that the response of purebred rabbits from consolidated breeds as well as inbred strains with higher level of homozygosity to transportation stress was within a narrower range in comparison with hybrid forms with higher heterozygosity and hence, broader range of reactivity. Therefore, transportation stress which is often combined with other stress factors posed a higher risk for increased mortality rates to purebred and inbred rabbits than to hybrid forms.

Key words: *inbreeding, cortisol, ACTH, transportation stress, rabbits.*

INTRODUCTION

Livestock husbandry production systems and the trade with breeders are connected with use of different modes of transport. Numerous studies have however reported the harmful effects of transportation and associated activities as loading and unloading on the health and welfare of different animal species and the quality of production (**Sanhour et al., 1989; Ayo and Oladele 1996; Broun et al., 1999; Von Borell 2001; Giovagnol et al., 2002; Kannan et al. 2003; Tanchev et al., 2005; Lambertini et al.; 2006, Bucham Maria et al., 2006; Sporer et al., 2008; Minka and Ayo, 2009; Zhong et al., 2011; Gianmarco et al., 2012**). This determines transportation as a major stress factor of livestock production.

Many studies provide evidence that road transport provokes stress in domestic rabbits (*Oryctolagus cuniculus*). Some of them point at different physiological changes during transportation including changes in corticosteroids (**De la Funte et al., 2007; Vignola et. al., 2008; Gianmarco et al., 2012**) . Others demonstrated that road transport worsened rabbit meat quality (**Maria et al., 2006; Lambertini et al., 2006; Vignola et al., 2008; Mazzone et al.; 2010**).

The effect of the different genetic factors on stress response of animals during transportation is also of interest. The studies in this field in various animal species (mice, swine and fowl) showed that factors as genotype, breed, strain and sex determine a variable degree of sensitivity to transportation stress (**Wallance 1976; Tuli et al. 1995; Marche and Marche 1998; Debut et al., 2003; Faure et al., 2003; Fabrega et al., 2004; Obernier and Baldwin, 2006; Averos et al., 2009; Weschenfelder et al., 2012**).

The purpose of the present study was to establish the effect of road transport on blood ACTH and cortisol in outbred and inbred rabbits.

MATERIAL AND METHODS

Experimental animals.

Depending on the breed, the level of inbreeding (Fx) and the sex, the studied rabbits were divided as followed:

Purebred Californian:
Outbred (Fx=0) – 5 ♂ and 5 ♀ , total - 10 rabbits
Inbred(Fx=0.25) – 4 ♂ and 5 ♀ , total - 9 rabbits
Inbred(Fx=0.375) – 4 ♂ and 4 ♀ , total - 8 rabbits

Crossbreds (Californian × Chinchilla):
Outbred (Fx=0) – 13♂ and 17 ♀ , total - 30 rabbits
Inbred (Fx=0.25) – 6 ♂ and 6 ♀ , total - 12 rabbits
Inbred (Fx=0.375) – 8 ♂ and 7 ♀ , total - 15 rabbits

Hormonal assays.

Blood samples from rabbits (manually fixated) were obtained 1 week before transportation stress and 1 h after the stress from the ear marginal vein in tubes with EDTA. Blood samples were centrifugated at 4 °C for 15 min (1811g). Plasma was stored at -20 °C for subsequent cortisol and ACTH assay. Cortisol quantitation was done with a commercial kit Cortisol RIA, (**Crosling, 1995**). ACTH concentrations were analysed with a commercial RIA kit RIA–mat ACTH, (**Moldon and Yalow, 1980**).

Transportation conditions.

The rabbits were transported by truck in two runs in metal cages at a distance of 100 km via the second-class road network, with an average speed of 60 km/h. The cage dimensions (cm) were 84×60×40 (length×width×height). Four rabbits were housed per cage. The transportation took place in two consecutive days in early June (8-10 a.m.) at ambient air temperature between 26 ^{o}C and 28 ^{o}C. The experimental rabbits weighed 3-4 kg and were 4-6 months of age.

Statistical analysis.

Experimental data were processed by dispersion analysis, using additive models with fixed effects. Depending on the number of analysed variable, either ANOVA (analysis of one variable) or MANOVA (analysis of multiple variables) were used. The level of statistical significance was $p<0.05$.

Multiple between-group comparisons were performed with the Least Significant Difference test (LSD test) at a level of significance $p< 0.05$.

RESULTS

The results from the analysis on the influence of all factors on the levels of cortisol and ACTH before and after stress are presented in Table 1. A statistically significant influence on the examined parameters was exercised by the rabbits' breed, the level of inbreeding and the sex, as well as the general interaction of the origins and the level of inbreeding. No significant influence was detected with the remaining combinations of the factors.

Table 1. Influence of the factors - breed, degree of inbreeding and sex on levels of cortisol and ACTH in rabbits.

Factors influence	Wilks Lambada	Raos R	df 1	df 2	p-level
Breed	0.862003*	3.32184*	4*	83*	0.014172*
Level of inbreeding	0.682401*	4.36876*	8*	166*	0.000083*
Sex	0.611433*	13.18665*	4*	83*	0.000001*
Breed+ level of inbreeding	0.742174*	3.33603*	8*	166*	0.001445*
Breed+ sex	0.936476	1.40752	4	83	0.238655
Level of inbreeding+ sex	0.909458	1.00839	8	166	0.431684

df – degree of freedom
* – statistical significant influence

Figures 1 and 2 show the blood levels of cortisol and ACTH depending on the rabbits' breed. A clearly expressed tendency for higher levels of the two hormones was observed in the inbred rabbits.

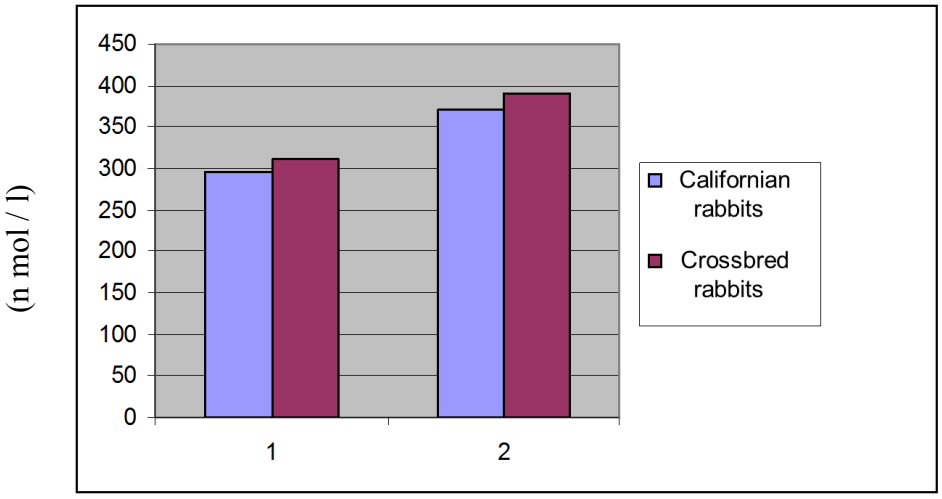

Figure 1. Cortisol levels depending on the breed of rabbits.

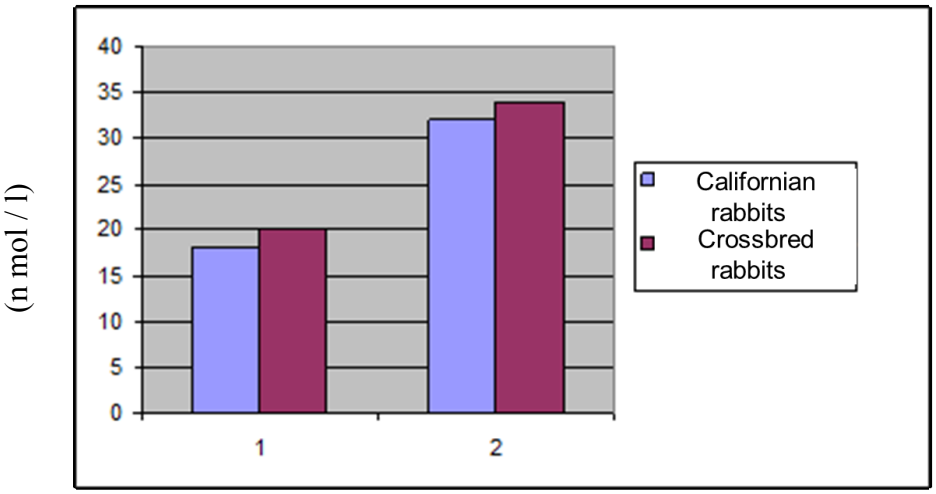

Figure 2. ACTH levels depending on the breed of rabbits.

With regard to the cortisol levels, the differences between the groups were not significant. In the levels of ACTH before and after stress, the trend was preserved, however the established differences were significant.

The level of inbreeding had a varying influence on blood cortisol before and after stress – Figure 3.

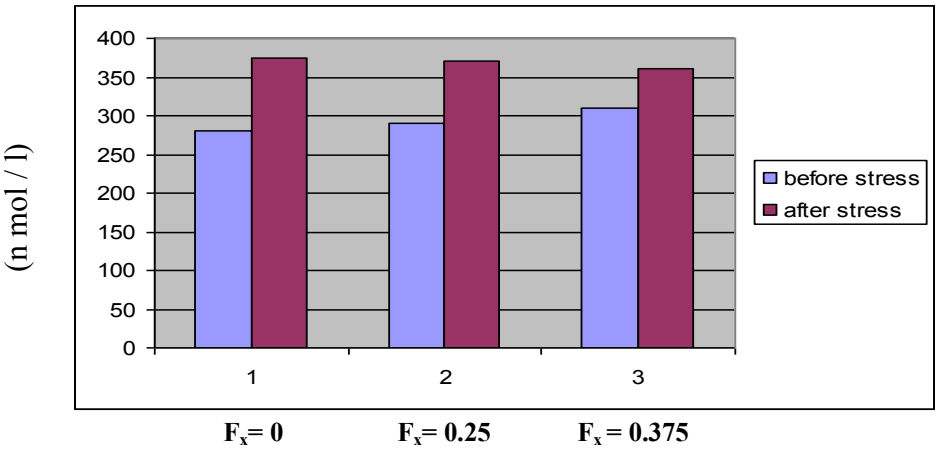

Figure 3. Cortisol levels depending on inbreeding level of rabbits.

Before stress, a slight parallel increase in cortisol levels along with increase in the level of inbreeding was found. Regardless of the fact that the increase in the mean values of cortisol from Fx = 0 towards Fx = 0.375 was apparently not greater, the difference between the groups of outbred and inbred rabbits with Fx = 0.375 before stress was significant. On the other hand, after stress we established a relatively high level of cortisol values without the presence of considerable differences between the groups, depending on the level of inbreeding. There was a slight decrease in the values as the inbreeding levels increased, yet the differences were not significant.

The trend in blood ACTH concentrations was different from that observed for cortisol (Fig. 4.)

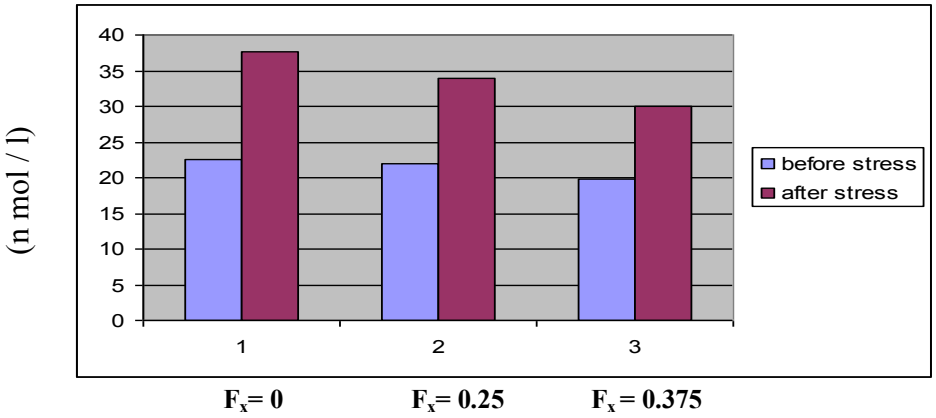

Figure 4. ACTH levels depending on the inbreeding level of rabbits.

As the level of inbreeding increased, the levels of ACTH before and after stress went down. Before stress, the differences between the groups were not substantial and were insignificant, yet after stress the outbred rabbits surpassed their inbred peers, with the differences being statistically significant. The mean values of blood cortisol and ACTH in female rabbits were statistically significantly greater than the corresponding values in male individuals (Fig. 6.). Quite expectedly, the levels of both hormones after stress were significantly higher than those before stress in both sexes (Fig. 5. and 6.).

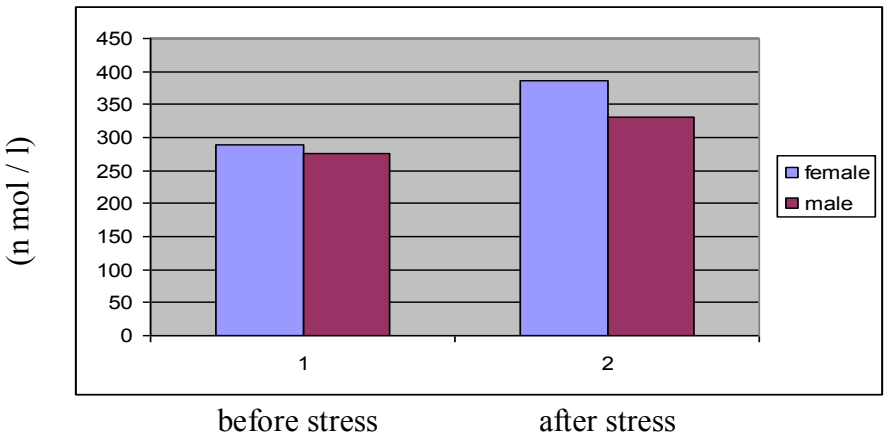

Figure 5. Cortisol levels depending on the sex of the rabbits.

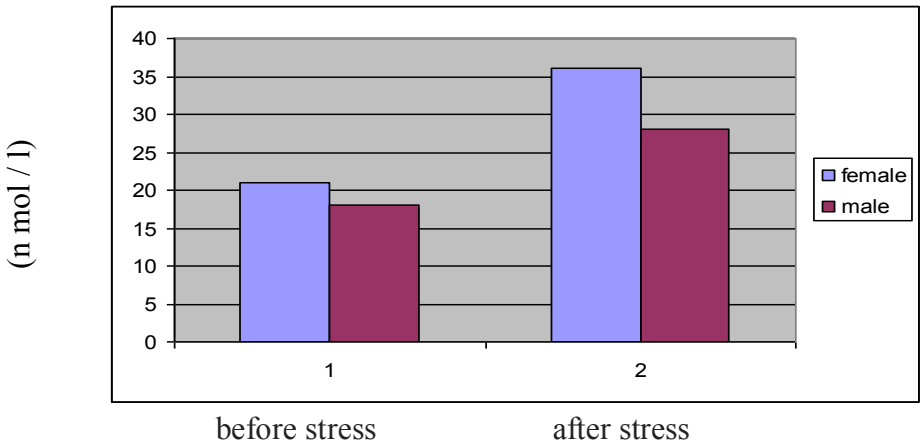

Figure 6. ACTH levels depending on the sex of the rabbits.

DISCUSSION

The analyses of the results indicated that road transportation is a stress factor, which leads to an increase in the levels of ACTH and cortisol in outbred as well as inbred groups of rabbits. This had been established by a number of other authors as well, while performing studies on cortisol, corticosterone, and ACTH before and after road transport of outbred rabbits and species of outbred animals (**Obernier and Baldwin, 2006; Vignola et al., 2008; Adenkola and Ayo, 2009; Aye et al., 2011; Giammarco et al., 2012**).

In the current study, the comparative analyses were more important, as they showed the influences of the factors breed, level of inbreeding and sex on blood ACTH and cortisol as criteria for stress after transportation.

All these factors had a significant effect on ACTH, expressed through a considerable increase of its values after transportation, compared to the hormone's level before the transportation of the rabbits. An important element was the fact that the increase in the level of inbreeding caused a decrease in the ACTH levels. The differences between purebred and inbred groups were significant with regard to this parameter. Regarding the breed factor, the results indicated that both before and after stress, the Californian rabbits had lower values of ACTH compared to crossbred rabbits, which is a criterion for differences in the range of their response to transportation stress.

A similar trend was observed with regard to the influence of the studied factors on cortisol. In this case, the levels of the hormone after transportation stress increased in both groups, with the heterogeneous animals (crossbred rabbits) exhibiting higher blood hormonal values and a wider range of variation. The differences between the groups, however, were not significant. With regard to the influence of inbreeding, however, there was a clear reverse trend, which shows that the increase in the level of inbreeding was related to decreased level of cortisol after transportation stress. This means that for both hormones the increase in inbreeding leads to a narrowing in the range of the rabbits' response to stress during and after transport.

After analysis of the results, two things could be noted – first, the increase in the extent of inbreeding led to a gradual reduction in the blood levels of ACTH and cortisol after transportation stress, and second, the levels of these hormones in purebred rabbits were lower after transportation and varied within a narrower range than the concentrations in crossbreds. This gave us reason to assume that the increase of inbreeding levels leads to a narrower range of rabbits' response to transportation stress. On the other hand, the comparative analysis showed that despite the implementation of inbreeding, crossbred animals preserved their heterogeneity over a longer period, which guaranteed them a broader range of reactivity, compared to purebred Californian rabbits.

The studies performed by other authors on the influence of various genetic factors in the formation of the stress response in different animal species are conflicting. For example, in a study on the influence of transportation on the stress reaction of laboratory mice and wild mice, **Wallance (1976)** established hat the different genotypes reacted in different ways.

Another study on the variations of blood cortisol and ACTH in pigs from three genotypes, subjected to vibrations (hh – halothane positive and two different halothane negative lines – HHA and HHB) showed that genotype-related differences were not observed in the variations in the levels of the two hormones (**Perremans et al., 2001**).

Differences in the response to transportation stress in two lines of broilers was reported by **Debut et al. (2003). Faure et al. (2003)** established differences in the behaviour and changes of the hormone corticosterone in two species of ducks, which were subjected to stress through contacts and manipulations by the serving staff. The same authors found out that in most cases the levels of corticosterone and other behavioural reactions in inter-species hybrids had approximately intermediate values.

CONCLUSIONS

Our results gave us reason to assume that purebred rabbits from consolidated breeds, as well as rabbits from inbred lines, which were characterised by a higher degree of homozygosity had a narrower range of response to transportation stress, compared to the hybrid forms, which exhibited a higher heterozygosity and a broader range of reactivity. This means that under transportation stress, which is often combined with other stress factors, purebred and inbred rabbits are at greater risk of high mortality rates, compared to hybrid forms.

REFERENCES

1. **Adenkola AY, Ayo JO**. 2008. Effect of road transportation on erythrocyte osmotic fragility of pigs administered ascorbic acid during the harmattan season in Zaria, Nigeria, J Cell Anim Biol; 3: 004–008.
2. **Averos X, Herranz A, Sanches R, Gozalves LF**. 2009. Effect of the duration of commercial journeys between rearing farms and growing finishing farms on the physiological stress response of weaned piglets. Livest Sci; 122: 339–344.
3. **Ayo JN, Minka S, Ldoga ES.** 2011. Ameliorative effects of ascorbic acid on rectal temperature, excitability score and live weight of rabbits transported by road. Afr J Biotechnol; 10: 9978–9984.
4. **Brown SN, Knowles TG, Edwards JE, Warriss PD**. 1999. Behavioural and physiological responses of pigs to being transported for up to 24 hours followed by six hours recovery in lairage. Vet Rec; 145: 421–426.
5. **Buckham M, Sporer KR, Weber PS, Burton JL, Earey B, Crowe MA**. 2006.Transportation of young beef bulls alters circulating physiological parameters that may be effective biomakers of stress. J Anim Sci; 86: 1325–1334.
6. **Crosling J**. Standartization of hapten immunoprocedures total cortisol. Scand. J Clin Lab. 1995; 53, 216: 3–41.
7. **Debut M, Berri C, Baeza E, Sellier N, Arnould C, Guemene D, Jehl N, Boutten B, Jego Y, Beaumond C, Le Bihan-duval E.** 2003. Variation of chicken technological meat quality in relation to genotype and pres laughter stress conditions. Poult Sci; 82: 1829–1838.
8. **De la Fuente J., M.T Diaz., M Ibanez, Gonzales DE E. Chavarri.** 2007. Physiological response of rabbits to heat, cold, noise and mixing in the context of transport. Anim. Welfare, 16: 41-47.
9. **Fabrega E, Manteca X, Font J, Gispert M, Carrion D, A. Velarde Ruiz-De-La-Torre JL, Diestre A.** 2004. A comparison of halothane homozygous negative and positive pietrain sire lines in relation to carcass and meat quality and welfare traits. Meat Sci; 66: 777–787.
10. **Faure JM, Val-Laillet D, Guy G, Bernadet MD, Guemen D.** 2003. Fear and stress reactions in two species of duck and their hybrid. Horm Behav; 43: 568–572.
11. **GIanmarco M, Vignola G, Mazzone G, Fuzaro I, Lambertini L.** 2012. Haemotological parameters as indicators of transport stress in rabbits. In: Proceedings of the 10th World Rabbit Congress. Sharm El-Sheikh, Egypt: WRC; 1033–1037.
12. **Giovagnoli G, MARINUCCI MT, BOLLA A, BORGHESE A.** 2002. Transport stress in horses: An electromyographic study on balance preservation. Livest Prod Sci; 73: 247–254.
13. **Kannan G, Kouakou B, Tterril LH, Gelaye S.** 2003. Endocrine, blood metabolite, and meat quality changes in goats as influenced by short-term, preslaughter stress. J Anim Sci; 81: 1499–1507.
14. **Lambertini L, Vignola G, Badiani A, Zaghini G, Forrnigoni A.** 2006. The effect of journey time and stocking density during transport on carcass and meat quality in rabbits. Meat Sci; 72: 641–646.

15. **Marche M, Marche G.** 1998. Meat quality traits and muscle characteristics in high or low fear lines of Japanese quails (Coturnix japonica) subjected to acute stress. Brit Poult Sci; 39: 372–378.

16. **Maria GA, Buy T, Liste G, Villaroel G, Safiudo M, Olleta L.** 2006. Effects of transport time and season on aspects of rabbit meat quality. Meat Sci; 72: 773–777.

17. **Mazzone G, VIgnola G, Gianmarco M, Manetta AC, Lambertini L.** 2010. Effects of loading methods on rabbit welfare and meat quality. Meat Sci; 85: 1, 33–39.

18. **Minka NS, Ayo JO.** 2009. Physiological responses of food animals to road transportation stress. Afr J Biotechnol; 8: 7415–7427.

19. **Moldon R, Yalow R.** 1980. Artefacte in radioimmunoassay of ACTH in tissue extracts in plasma. Horm. Metal Res; 12, 3: 105–110.

20. **Obeernier JA, Baldwin RL.** 2006. Establishing an appropriate period of acclimatization following transportation of laboratory animals. ILAR J ; 47: 364–369.

21. **Perremans S, Randall J, Rormbouts G, Decuypere E, Geers R.** 2001. Effect of whole-body vibration in the vertical axis on cortisol and adenocorticotropic hormone levels in piglets. J Anim Sci; 79: 9751–981.

22. **Sanhouri AA, Jones RS, Dobson H.** 1991. Pentobarbitone inhibits the stress response to transport in male goats. Brit Vet J; 147: 42– 48.

23. **Tanchev S.** Experimental studies upon some phenotype and genetic effects of inbreeding in multiparous mammalian species (*Oryctolagus cuniculus, Sus scrofa domesticus*). DSc, Trakia University, Stara Zagora, Bulgaria, 2006.

24. **Tuli J, Smith JA, Morton DB.** 1995. Stress measurements in mice after prasnportation. Lab Anim; 29: 132–138.

25. **Vignola G, Gianmarco M, Mazzone G, Angelozzi G., Lambertini L**. 2008. Effects of loading method and crate position on the truck on some stress indicators in rabbits transported to the slaughterhouse. In: Proceedings of the 9th World Rabbit Congress. Verona, Italy: WRC; 1257–1261.

26. **Von Borell EH.** The biology of stress and its application to livestock housing and transportation assessment. J Anim Sci 2001; 79: 260–267.

27. **Wallance ME.** 1976. Effects of stress due to deprivation and transport in different genotypes of house mouse. Lab Anim; 10: 335–347.

28. **Weschenfelder J, Sander C, Kluge M, Kirkby KC, HImmerich H.** 2012. The influence of cytokines on wakefulness regulation: clinical relevance, mechanisms and methodological problems. Psychiatr Danub; 24: 112-126.

29. **Zhong RZ, Tu HW, Zhou DW, Sunt HX, Zhao CS.** 2011. The effects of road transportation on physiological responses and meat quality in sheep differing in age, J Anim Sci; 89, 11: 3742–3751.