R. Bras. Zootec., 49:e20200035, 2020
https://doi.org/10.37496/rbz4920200035

Ruminants
Full-length research article

Revista Brasileira de Zootecnia

*Brazilian Journal of Animal Science*
e-ISSN 1806-9290
www.rbz.org.br

# Effect of dietary supplementation with β-hydroxy-β-methylbutyrate on stress parameters in goat kids

**Janina Sowińska**[1], **Stanisław Milewski**[2], **Dorota Witkowska**[1*], **Katarzyna Ząbek**[2], **Jan Miciński**[2], **Anna Wójcik**[1], **Tomasz Mituniewicz**[1]

[1] University of Warmia and Mazury, Faculty of Animal Bioengineering, Department of Animal and Environmental Hygiene, Olsztyn, Poland.
[2] University of Warmia and Mazury, Faculty of Animal Bioengineering, Department of Sheep and Goat Breeding, Olsztyn, Poland.

***Corresponding author:**
dorota.witkowska@uwm.edu.pl

**Received:** March 6, 2020
**Accepted:** June 9, 2020

**How to cite:** Sowińska, J.; Milewski, S.; Witkowska, D.; Ząbek, K.; Miciński, J.; Wójcik, A. and Mituniewicz, T. 2020. Effect of dietary supplementation with β-hydroxy-β-methylbutyrate on stress parameters in goat kids. Revista Brasileira de Zootecnia 49:e20200035.
https://doi.org/10.37496/rbz4920200035



**ABSTRACT** - The objective of this study was to determine the effect of dietary supplementation with β-hydroxy-β-methylbutyric acid (HMB) on live weight loss and selected blood parameters in goat kids after transportation to the slaughterhouse. The study was performed with goat kids that were weaned at 30 days of age and divided into two groups (GK): a control group and an experimental group whose diet was supplemented with HMB (at 50 mg $kg^{-1}$ of BW) for 60 consecutive days. At the end of the 90-day rearing period, the animals were fasted for 12 h (with access to water) and were transported to the slaughterhouse in the following morning. Blood for analysis was sampled before transportation (BST1) and after unloading in the slaughterhouse (BST2). The animals were weighed on the same dates to determine live weight loss. Red blood cell counts (RBC), white blood cell counts (WBC), hemoglobin concentration (HGB), hematocrit (HCT), neutrophil to lymphocyte (N:L) ratio, and cortisol and glucose concentrations were determined in the sampled blood. The experimental goat kids were characterized by lower weight loss after transportation. Group of kids and BST did not induce variations in RBC, WBC, HGB, and HCT. Cortisol concentration was affected by both GK and BST. Cortisol levels increased after transportation in both groups, but this parameter was significantly higher in the control than in experimental animals at BST2. Glucose levels and the N:L ratio did not differ significantly between GK, but glucose concentration and the N:L ratio were higher at BST2 than at BST1 in both groups. The experimental goat kids were characterized by lower weight loss and lower cortisol concentration after transportation, which could point to the efficacy of HMB in boosting immunity and alleviating transportation stress in goat kids.

**Keywords:** body, goat, stress, supplementation

## 1. Introduction

During pre-slaughter handling, animals are exposed to considerable stress related to fasting, loading onto vehicles, duration of transportation, transportation conditions, unloading in the slaughterhouse, conditions in the slaughterhouse, and waiting time before slaughter (Kannan et al., 2003; Kadim et al., 2006; Terlouw et al., 2008; Minka and Ayo, 2010b; Miranda-de la Lama et al., 2010; Akin et al., 2018).

Numerous handling operations are performed within a short period and are a source of stress, which decreases immunity and disrupts homeostasis in animals (Kannan et al., 2003; Sowińska et al., 2006;

Kadim et al., 2007; Minka et Ayo, 2007; De la Fuente et al., 2010, 2012; Adenkola and Ayo, 2010). These stressors increase susceptibility to infection, contribute to body weight loss, and compromise meat quality (Kannan et al., 2002; Kadim et al., 2006, 2007; Ferguson and Warner, 2008; Nikbin et al., 2016; Yalcintan et al., 2018).

Considerable research has been devoted to minimizing stress responses in various animal species through the use of pharmaceuticals, herbs, yeast, seaweeds, minerals, and vitamins (Ali and Al-Qarawi, 2002; Young et al., 2003; Galipalli et al., 2004; Kannan et al., 2007a,b; Minka and Ayo, 2007, 2013; Ali et al., 2006; Ferguson and Warner, 2008; Ambore et al., 2009; Sowińska et al., 2016, 2017). However, not all pharmaceuticals and preparations, in particular antidepressant drugs, can be administered to livestock due to the safety requirements imposed on animal products (Ali and Al-Qarawi, 2002; Young et al., 2003; Ali et al., 2006).

β-hydroxy-β-methylbutyrate (HMB) is an oxidation product of α-ketoisocaproic acid, which is produced primarily in muscle, liver, and fat tissue from the amino acid leucine (Brosnan and Brosnan, 2006). Leucine plays an important role for the organism, participating in the processes of protein metabolism and inhibiting the processes of their breakdown occurring during effort. Leucine activates the signaling factor of rapamycin (mTOR) in mammals to promote protein synthesis in skeletal muscle and adipose tissue. It is also the main regulator of mTOR-sensitive feed intake responses to high-protein diets. Meanwhile, leucine regulates blood glucose levels by promoting gluconeogenesis and helps maintain lean mass in a hypocaloric state. It is also beneficial for animal nutrition (Li et al., 2011).

Numerous researchers have demonstrated that dietary supplementation with β-hydroxy-β-methylbutyric acid (HMB), a leucine derivative with immunostimulatory properties, has a positive effect on defense mechanisms, improves performance in animals (Gatnau et al., 1995; Papet et al., 1997; Peterson et al., 1999; Puchajda-Skowrońska et al., 2006; Wiąz et al., 2010; Wójcik et al., 2014; Ząbek et al., 2016), and is a safe compound (Rathmacher et al., 2004). Previous reports in animals are very inconsistent with respect to HMB doses and supplementation duration. The predominant HMB doses used in pigs were 15 mg $kg^{-1}$ of BW (Flummer et al., 2012) and 50 mg $kg^{-1}$ of BW (Tatara et al., 2007); in cattle, 20,5 mg $kg^{-1}$ of BW (Van Koevering et al., 1993); but in sheep, higher than 100 mg $kg^{-1}$ of BW (Tatara, 2008).

The results of the above studies encouraged the authors to investigate whether the immunostimulant could also minimize stress responses in goat kids during pre-slaughter handling and transportation to the slaughterhouse. Therefore, the objective of this study was to determine the effect of dietary supplementation with HMB on live weight loss and selected blood parameters in goat kids after transportation to the slaughterhouse.

## 2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (protocol No. 18/2013). The study was conducted in Olsztyn, Warmia and Mazury, Poland (53°46' North latitude, 20°30' East longitude, and altitude of 128 m).

The study was performed with 24 single-born male Alpine goat kids that were weaned at 30 days of age and divided into two equal groups: a control group and an experimental group. After weaning, both groups were administered identical diets, composed of the milk replacer (throughout the entire rearing period) and after 30 days of experiment, the following feed was introduced: cielak complementary feed mixture (Wipasz, Olsztyn) and grass haylage. Milk replacer was administered throughout the whole period by the following scheme: days 1-10 of experiment – 4200 mL/animal/day; days 11-20 of experiment – 2800 mL/animal/day; days 21-30 of experiment – 1900 mL/animal/day; and days 31-60 of experiment – 500 mL/animal/day. When the animals reached the 60th day of age, they began to receive extra grass haylage and complementary feed mixture, according to the NRC (2007), for goats with a daily average gain of 100 g. During the experiment, the amount of administered feed and leftovers was controlled. Chemical composition was determined with standard methods (AOAC, 2005). Based on the results, the amount of nutrients ingested by goat kids was determined for both groups throughout the experimental period (Table 1).

The diets of experimental goats were additionally supplemented with HMB (Metabolic Technologies Inc. Ames, IA, USA), administered with the supplementary feed mix at 50 mg $kg^{-1}$ of BW. In the available scientific studies, there are no results for goats; therefore, in our study, we applied the most frequently dosage used in other animal species. The animals were kept in boxes in accordance with the guidelines included in the animal protection act, and under the constant veterinarian supervision.

At the end of the 90-day rearing period, the animals were fasted for 12 h (with access to water) and were transported to the slaughterhouse in the following morning. The kids were transported in a standardized vehicle for animal transportation. During transportation, temperature and humidity inside the vehicle were measured with LB-520 thermo-hygrometers (Lab-EL, Poland). The duration of transportation and the conditions inside the stock crate are presented in Table 2. Goat kids were transported to the slaughterhouse on a windless day (in June) with ambient temperature of 14.3 °C and relative air humidity of 61% at 6 h. Vehicle speed, temperature, and humidity inside the vehicle during the 90 min of transportation were adequate. The space allowance per animal inside the vehicle was consistent with the provisions of Council Regulation (EC) No. 1/2005 of 22 December 2004 on the protection of animals during transportation and related operations.

The animals were weighed before and after transportation to the slaughterhouse to determine live weight loss. Blood was sampled from the jugular vein before transportation (BST1) and after transportation (BST2) to determine stress responses. Hematological analyses were performed to determine red blood cell counts (RBC), white blood cell counts (WBC), hemoglobin concentration (HGB), hematocrit (HCT), and leukocyte profile (leukogram). Basic parameters were determined in the Sysmex hematology analyzer. A differential analysis of leukograms was performed by Pappenheim blood smear staining, and the results were used to calculate the neutrophil to lymphocyte ratio (N:L). Cortisol and glucose concentrations in the blood serum were determined in biochemical analyses with a Cobas Integra 800 analyzer (Roche, Rotkreuz, Switzerland). Cortisol levels were measured with a competitive enzyme immunoassay kit with an anti-cortisol polyclonal antibody (Elycsys Cortisol, Roche, Rotkreuz, Switzerland). Glucose levels were measured with an enzymatic reference method involving hexokinase. Blood parameters were determined in a specialist laboratory according to the Analytical Quality Control procedure (Chapter 2: Laboratory diagnostics).

**Table 1 -** Total nutrient intake per group in the investigated period (30 days)

| Specification | Group | |
|---|---|---|
| | Control | Experimental |
| Dry matter (kg) | 228.58 | 230.00 |
| Feed unit for meat production | 0.29 | 0.29 |
| Crude protein (kg) | 27.01 | 27.17 |
| PDIN (kg) | 26.74 | 26.91 |
| PDIE (kg) | 27.01 | 27.17 |
| Crude fiber (kg) | 40.82 | 41.08 |

PDIN, PDIE - protein digestible in the small intestine when rumen fermentable nitrogen or energy, respectively.

**Table 2 -** Duration and conditions inside the transportation vehicle

| Parameter | Range |
|---|---|
| Duration of transportation | 1.5 h |
| Vehicle speed | 55-65 km/h |
| Stocking density | 0.3 $m^2$/animal |
| Air temperature | 15.6-16.3 °C |
| Relative humidity | 70.2-76.8% |

The animals were handled during transportation, and blood samples were collected by the same team of experienced professionals. The duration of handling operations, including herding, weighing, and blood sampling in the goat farm and in the abattoir did not exceed 45 s.

The results were processed statistically in the Statistica 13.0 PL program (StatSoft Inc., Tulsa, OK, USA). Live weight loss and blood parameters were analyzed ($\bar{x}$±SD). The results were validated by two-way (GK × BST) or one-way (GK or BST) ANOVA. The differences among groups of kids (GK) or blood sampling time (BST) were estimated using the following model:

$$Y_{ij} = \mu + Y_i + e_{ij},$$

in which $Y_{ij}$ is the observation of dependent variable, μ is the overall mean, $Y_i$ is fixed effect of supplementation = ($Y_i = \mu_i - \mu$), $\mu_i$ is the mean for the i group, and $e_{ij}$ is the random residual error.

Fixed effect of supplementation (GK) and blood sampling time (BST) and their interaction were included in the model. The model used was:

$$Y_{ij} = \mu + S_i + T_j + (S \times T)_{ij} + e_{ij},$$

in which $Y_{ij}$ is the observation of dependent variable, μ is the overall mean, $S_i$ is the effect of HMB supplementation (GK), $T_j$ is the effect of blood sampling time (BST), $(S \times T)_{ij}$ is the interaction term of GK and BST, and $e_{ij}$ is the random residual error.

## 3. Results

During the 60 days of the study, no great differences in the feed intake of the kids were recorded (Table 1). The experimental goats consumed no more than 0.62% DM and, consequently, no more crude protein and crude fiber. It may be thus assumed that it was comparable in both groups.

No significant differences in body weights were observed between groups before transportation and after unloading in the slaughterhouse (Table 3). Body weights were somewhat higher in animals whose diets were supplemented with HMB, but the noted difference was not significant. Live weight loss after transportation (kg, %) was significantly lower in the experimental group than in the control group.

In the group of hematological parameters, RBC, WBC, HBC, and HCT values did not differ significantly between GK or BST (Table 4).

Cortisol levels were significantly affected by both GK and sampling dates. Transportation led to a significant increase in cortisol concentration in both groups, but it was significantly higher in the control than in the experimental group, in which the diet was supplemented with HMB (Table 4).

Serum glucose concentration did not differ significantly between groups. The above parameter increased significantly after transportation in both groups. A similar pattern of changes was observed in the N:L ratio (Table 4).

**Table 3** - Body weight of control and experimental group of kids and body weight losses during pre-slaughter transportation (mean±SD)

| Indices | Group of kids | |
|---|---|---|
| | Control | Experimental |
| Body weight before transportation (kg) | 15.78±2.30 | 16.64±2.76 |
| Body weight after transportation (kg) | 15.25±2.27 | 16.25±2.63 |
| Body weight loss during transportation | | |
| kg | 0.53±0.16x | 0.39±0.14y |
| g kg$^{-1}$ | 33.7±8.9x | 23.6±9.7y |

x,y - means within rows followed by different letters are different at P≤0.05.

**Table 4 -** Effect of group of kids and blood sampling time on blood parameters (mean±SD)

| Blood parameter | Group of kids (GK) | | | | Significance P-value | | |
|---|---|---|---|---|---|---|---|
| | Control (CG) | | Experimental (EG) | | | | |
| | CG-BST1 | CG-BST2 | EG-BST1 | EG-BST2 | GK | BST | GK × BST |
| RBC ($10^{12}$·L$^{-1}$) | 2.61±0.51 | 2.90±0.47 | 2.42±0.48 | 2.72±0.48 | NS | NS | NS |
| WBC ($10^{9}$·L$^{-1}$) | 14.49±2.42 | 17.01±3.21 | 13.58±3.40 | 16.08±5.27 | NS | NS | NS |
| HGB (mmol·L$^{-1}$) | 6.23±0.48 | 6.54±0.58 | 5.96±0.66 | 6.25±0.75 | NS | NS | NS |
| HCT (L·L$^{-1}$) | 0.86±0.18 | 0.96±0.17 | 0.79±0.17 | 0.89±0.18 | NS | NS | NS |
| N:L | 0.62±0.11B | 1.29±0.45A | 0.83±0.17D | 1.18±0.30C | NS | ** | NS |
| Cortisol (mmol·L$^{-1}$) | 45.54±13.80B | 136.62±36.16AY | 35.88±15.18D | 97.70±14.35CZ | ** | ** | ** |
| Glucose (mmol·L$^{-1}$) | 3.76±0.62B | 7.56±1.91A | 4.03±0.53D | 7.06±1.75C | NS | ** | NS |

BST - blood sampling time; BST1 - before transportation; BST2 - after transportation; RBC - red blood cell; WBC - white blood cell; HGB - hemoglobin; HCT - hematocrit; N:L - neutrophil to lymphocyte ratio; NS - not significant.
A,B - CG between BST1 and BST2, P≤0.01; C,D - EG between BST1 and BST2, P≤0.01; Y,Z - BST2 between CG and EG, P≤0.01.
** Statistical significance at P≤0.01.

## 4. Discussion

In the current experiment, live weight loss after transportation (Table 3) was significantly lower in the experimental group than the control group. The experimental kids were heavier and characterized by significantly lower weight loss.

Numerous authors have demonstrated that transportation to the slaughterhouse and the accompanying handling operations are considerable stressors that decrease live body weight. During transportation, animals lose weight due to dehydration and loss of energy required for the maintenance of homeostasis (Knowles, 1995). Many researchers have evaluated body weight loss in young ruminants in view of the animals' age and breed, duration of transportation, and space allowances inside the vehicle (Kannan et al., 2000, 2007a,b; Kadim et al., 2006; Kadim et al., 2007; Nikbin et al., 2016; Akin et al., 2018; Yalcintan et al., 2018), but their findings were inconclusive. In a study by Kannan et al. (2000), live weight loss in 15-month-old goats was not affected by stocking density and reached 9.8-10.2% after 2.5 h of transportation. In contrast, Nikbin et al. (2016) reported higher live weight loss (1.36%) in 12-month-old goats after 3.5 h of transportation at higher stocking density compared with lower stocking density (0.44%). In the work of Akin et al. (2018), differences in stocking density did not influence live weight loss during short transportation (45 min), whereas significantly higher weight loss was noted after longer transportation (3 h) at higher stocking density.

Live weight loss during transportation to the slaughterhouse should be minimized to increase profits, and many researchers have analyzed the efficacy of various supplements that enhance defense mechanisms and decrease weight loss in animals (Kannan et al., 2007b; Minka and Ayo, 2007, 2013; Minka et al., 2009; Sowińska et al., 2017). The results of their studies were also ambiguous. The addition of seaweed extract to the diets of goats of two breeds did not affect live weight loss after 6 h of transportation, but significant differences in the evaluated parameter were noted between breeds (Kannan et al., 2007b). Sowińska et al. (2017) found no significant differences in the body weights of young rams fed beta-glucan, determined after 80 min of transportation. According to other authors, the administration of ascorbic acid (vitamin C) minimized live weight loss in goats. Minka and Ayo (2007) observed significantly lower weight loss (1.04%) in the group of goats supplemented with vitamin C than in the control group (11.9%) after 8 h of transportation. Lower live weight loss was also reported in goats that received ascorbic acid (1.6 and 5.7%) before a 12-h-long transportation (Minka et al., 2009). Ambore et al. (2009) demonstrated that herbal preparations (Restobal liquid, Stresomix premix) were effective in reducing transportation-related live weight loss in goats. In the present study, the experimental kids were characterized by significantly lower weight loss after transportation, which suggests that dietary supplementation with HMB results in positive effects.

Transportation is one of the greatest stressors in livestock rearing. The severity of stress is determined not only by handling operations before transportation and slaughter (Knowles et al., 1998; Fisher et al., 2005), but also by other factors that cause physiological changes in animals (Hartung, 2003; Minka and Ayo 2007). The values of RBC, WBC, HGB, and HCT did not differ significantly between blood sampling times (Table 4), which is consistent with the results of many studies. Ekiz et al. (2012) did not observe significant differences in the hematological parameters of lambs after 75 min of transportation in comparison with base levels. Bórnez et al. (2009) also reported an absence of significant variations in RBC, HCT, and HGB values in 30-day-old and 70-day-old lambs after 30 min of transportation. Ali et al. (2006) did not observe significant differences in HCT, HGB, or WBC in lambs after 2 h of transportation, and Miranda-de la Lama et al. (2011) found similar values of RBC, WBC, and HCT in lambs after 3 h of transportation.

Stress stimulates the hypothalamic-pituitary-adrenal axis to release cortisol from adrenal glands, which is why cortisol concentration in the blood is regarded as a reliable indicator of stress (Ferguson and Warner, 2008; Minka and Ayo, 2010b; Zimerman et al., 2013). Numerous authors have reported an increase in the serum cortisol levels of transported animals (Knowles, 1995; Nwe et al., 1996; Kannan et al., 2000, 2003; Kadim et al., 2006; Sowińska et al., 2006; Miranda-de la Lama, 2010; Yalcintan et al., 2018).

Kannan et al. (2000) noted a significant increase in goat cortisone levels after 2.5 h of transportation, regardless of stocking density. In another study, cortisol concentration increased after 2 h of transportation in Alpine goats from all age groups (Kannan et al., 2003). A considerable increase in the cortisol levels of male goats, regardless of breed, was also reported after 2 h of transportation by Kadim et al. (2006). In the current study, cortisol concentration increased significantly in 90-day-old male Alpine goats after 1.5 h of transportation. The above findings confirm that even short transportation is a source of considerable stress for animals.

Glucose is a source of energy that is rapidly depleted under exposure to stress (Broom et al., 1996), which is why serum glucose concentration is also regarded as an indicator of transportation stress in animals (Kannan et al., 2000, 2003; Minka and Ayo, 2010a). Research has demonstrated that an increase in blood glucose levels is preceded by an increase in cortisol concentration (Sanhouri et al. 1992). Cortisol plays a very important role in gluconeogenesis because it stimulates the liver to convert fat and protein to indirect metabolites. These metabolites are ultimately converted to glucose as a source of energy (Saeb et al., 2010). Numerous researchers have demonstrated that glucose is a reliable indicator of stress in animals. Glucose levels increased in response to the stress induced by 1.5-2.5 h (Kannan et al., 2000, 2003; Rajion et al., 2001; Ali et al., 2006; Sowińska et al., 2016, 2017), 6 h (Nwe et al., 1996; Galipalli et al., 2004; Kannan et al., 2007b), as well as 12 h of transportation (Minka and Ayo, 2010a). In the present experiment, a significant increase in the serum glucose levels of goat kids transported to the slaughterhouse also indicates that glucose is a useful parameter for evaluating stress responses in animals.

Many studies have also evaluated the impact of transportation stress on the N:L ratio in livestock. Stress triggers the release of corticoids, which increase neutrophil counts and decrease lymphocyte counts in the leukogram (Stanger et al., 2005). A significant increase in the N:L ratio was observed regardless of the duration of transportation (1.5-12 h) (Nwe et al., 1996; Kannan et al., 2000, 2007a; Rajion et al., 2001; Galipalli et al., 2004; Minka and Ayo, 2007; Minka et al., 2009; Sowińska et al., 2016, 2017). In the current study, the N:L ratio in goat kids also increased significantly after transportation to the slaughterhouse.

Various methods for minimizing stress and its negative effects on animal health have been evaluated in the literature. The efficacy of various preparations, including seaweed (*Ascophyllum nodosum*) extract (Galipalli et al., 2004; Kannan et al., 2007a,b), xylazine (Ali et al., 2006), ascorbic acid (Minka and Ayo, 2007, 2010a; Minka et al., 2009), and dried yeast (*Saccharomyces cerevisiae*) (Sowińska et al., 2016, 2017), has been evaluated in animals. In the works of Galipalli et al. (2004) and Kannan et al. (2007a), seaweed (*Ascophyllum nodosum*) extract did not lower cortisol concentration, glucose levels, or the N:L ratio in goats after transportation. Dietary supplementation with dried brewer's yeast (Sowińska et al., 2016) and beta-glucan (Sowińska et al., 2017) did not cause significant differences in cortisol levels between control and experimental lambs, but it significantly lowered blood glucose and the N:L ratio after transportation.

Ali et al. (2006) showed a significantly higher HCT and lymphocyte counts and significantly lower cortisol and glucose levels in the group of goats which received xylazine before the 2-h transportation compared with the control group. The efficacy of ascorbic acid supplementation before transportation, in particular under exposure to high temperature and high humidity, was demonstrated in a series of experiments by Minka and Ayo (2007, 2013) and Minka et al. (2009). In the present study, goat kids fed diets supplemented with HMB were characterized by significantly lower cortisol concentration after transportation. The evaluated supplement had no significant effect on glucose levels or the N:L ratio, but glucose concentration after transportation increased 1.7-fold in the experimental group and 2-fold in the control group. The N:L ratio increased 1.4- and 2.1-fold in the respective groups, which indicates that HMB is a promising feed additive.

## 5. Conclusions

The results of this study demonstrated that the supplementation of goat kid diets with β-hydroxy-β-methylbutyrate (at 50 mg $kg^{-1}$ of BW) can alleviate stress and its consequences on the animals' health. The analyzed immunostimulant, a leucine derivative, has a beneficial influence on immune function and reduces stress responses evaluated based on blood parameters and live weight loss.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: J. Sowińska and S. Milewski. Data curation: J. Sowińska, K. Ząbek, J. Miciński and A. Wójcik. Formal analysis: J. Sowińska and T. Mituniewicz. Investigation: J. Sowińska, S. Milewski, D. Witkowska, K. Ząbek, J. Miciński, A. Wójcik and T. Mituniewicz. Methodology: J. Sowińska, D. Witkowska, K. Ząbek and T. Mituniewicz. Project administration: S. Milewski. Software: T. Mituniewicz. Supervision: J. Sowińska and D. Witkowska. Writing-original draft: J. Sowińska and D. Witkowska.

## References

Adenkola, A. Y. and Ayo, J. O. 2010. Physiological and behavioural responses of livestock to road transportation stress. A review. African Journal of Biotechnology 9:4845-4856.

Akin, P. D.; Yilmaz, A. and Ekiz, B. 2018. Effects of stocking density on stress responses and meat quality characteristics of lambs transported for 45 minutes or 3 hours. Small Ruminant Research 169:134-139. https://doi.org/10.1016/j.smallrumres.2018.08.009

Ali, B. H. and Al-Qarawi, A. A. 2002. Evaluation of drugs used in the control of stressful stimuli in domestic animals: A review. Acta Veterinara Brno 71:205-216. https://doi.org/10.2754/avb200271020205

Ali, B. H.; Al-Qarawi, A. A. and Mousa, H. M. 2006. Stress associated with road transportation in desert sheep and goats, and the effect of pretreatment with xylazine or sodium betaine. Research in Veterinary Science 80:343-348. https://doi.org/10.1016/j.rvsc.2005.07.012

Ambore, B.; Ravikanth, K.; Maini, S. and Rekhe, D. S. 2009. Haematological profile and growth performance of goats under transportation stress. Veterinary World 2:195-198.

AOAC - Association of Official Analytical Chemists. 2005. Official method of analysis 18th ed. AOAC International, Washington, DC.

Bórnez, R.; Linares, M. B. and Vergara, H. 2009. Haematological, hormonal and biochemical blood parameters in lamb: Effect of age and blood sampling time. Livestock Science 121:200-206. https://doi.org/10.1016/j.livsci.2008.06.009

Broom, D. M.; Goode, J. A.; Hall, S. J. G.; Lloyd, D. M. and Parrott, R. F. 1996. Hormonal and physiological effects of a 15-hour road journey in sheep: Comparison with the responses to loading, handling and penning in the absence of transport. British Veterinary Journal 152:593-604. https://doi.org/10.1016/S0007-1935(96)80011-X

Brosnan, J. T. and Brosnan M. E. 2006. Branched-chain amino acids: enzyme and substrate regulation. Journal of Nutrition 136:207S-211S. https://doi.org/10.1093/jn/136.1.207S

De la Fuente, J.; Sánchez, M.; Pérez, C.; Lauzurica, S.; Vieira, C.; González de Chávarri, E. and Díaz, M. T. 2010. Physiological response and carcass and meat quality of suckling lambs in relation to transport time and stocking density during transport by road. Animal 4:250-258. https://doi.org/10.1017/S1751731109991108

De la Fuente, J.; González de Chávarri, E.; Sánchez, M.; Vieira, C.; Lauzurica, S.; Díaz M. T. and Pérez, C. 2012. The effects of journey duration and space allowance on the behavioural and biochemical measurements of stress responses in suckling lambs during transport to an abattoir. Applied Animal Behaviour Science 142:30-41. https://doi.org/10.1016/j.applanim.2012.08.010

Ekiz, B.; Ekiz, E. E.; Kocak, O.; Yalcintan, H. and Yilmaz, A. 2012. Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs. Meat Science 90:967-976. https://doi.org/10.1016/j.meatsci.2011.11.042

Ferguson, D. M. and Warner, R. D. 2008. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? Meat Science 80:12-19. https://doi.org/10.1016/j.meatsci.2008.05.004

Fisher, A. D.; Steward, M.; Duganzich, D. M.; Tacon, J. and Matthews, L. R. 2005. The effects of stationary periods and external temperature and humidity on thermal stress conditions within sheep transport vehicles. New Zealand Veterinary Journal 53:6-9. https://doi.org/10.1080/00480169.2005.36461

Flummer, C.; Kristensen, N. B. and Theil, P. K. 2012. Body composition of piglets from sows fed the leucine metabolite β-hydroxy β-methyl butyrate in late gestation. Journal of Animal Science 90:442-444. https://doi.org/10.2527/jas.53923

Galipalli, S.; Gadiyaram, K. M.; Kouakou, B.; Terril, T. H. and Kannan, G. 2004. Physiological responses to preslaughter transportation stress in Tasco-supplemented Boer goats. South African Journal of Animal Science 34(Supplement 1): 198-200.

Gatnau, R.; Zimmerman, D. R.; Nissen, S. L.; Wannemuehler, M. and Ewan, R. C. 1995. Effects of excess dietary leucine and leucine catabolites on growth and immune responses in weanling pigs. Journal of Animal Science 73:159-165. https://doi.org/10.2527/1995.731159x

Hartung, J. 2003. Effects transport on health of farm animals. Veterinary Research Communications 27:525-527. https://doi.org/10.1023/B:VERC.0000014212.81294.78

Kadim, I. T.; Mahgoub, O.; Al-Kindi, A.; Al-Marzooqi, W. and Al-Saqri, N. M. 2006. Effects of transportation at high ambient temperatures on physiological responses, carcass and meat quality characteristics of three breeds of Omani goats. Meat Science 73:626-634. https://doi.org/10.1016/j.meatsci.2006.03.003

Kadim, I. T.; Maghoub, O.; AlKindi, A. Y.; Al-Marzoqui, W.; Al-Saqri, N. M.; Almaney, M. and Mahmoud, I. Y. 2007. Effects of transportation at high ambient temperatures on physiological responses, carcass and meat quality characteristics in two age groups of Omani sheep. Asian-Australasian Journal of Animal Sciences 20:424-431. https://doi.org/10.5713/ajas.2007.424

Kannan, G.; Terrill, T. H.; Kouakou, B.; Gazal, O. S.; Gelaye, S.; Amoah, E. A. and Samaké, S. 2000. Transportation of goats: effects on physiological stress responses and live weight loss. Journal of Animal Science 78:1450-1457. https://doi.org/10.2527/2000.7861450x

Kannan, G.; Terrill, T. H.; Kouakou, B.; Gelaye, S. and Amoah, E. A. 2002. Simulated preslaughter holding and isolation effects on stress responses and live weight shrinkage in meat goats. Journal of Animal Science 80:1771-1780. https://doi.org/10.2527/2002.8071771x

Kannan, G.; Kouakou, B.; Terrill, T. H. and Gelaye, S. 2003. Endocrine, blood metabolite and meat quality changes in goats as influenced by short-term, preslaughter stress. Journal of Animal Science 81:1499-1507. https://doi.org/10.2527/2003.8161499x

Kannan, G.; Saker, K. E.; Terrill, T. H.; Kouakou, B.; Galipalli, S. and Gelaye, S. 2007a. Effect of seaweed extract supplementation in goats exposed to simulated preslaughter stress. Small Ruminant Research 73:221-227. https://doi.org/10.1016/j.smallrumres.2007.02.006

Kannan, G.; Terrill, T. H.; Kouakou, B. and Galipalli, S. 2007b. Blood metabolite changes and live weight loss following brown seaweed extract supplementation in goats subjected to stress. Small Ruminant Research 73:228-234. https://doi.org/10.1016/j.smallrumres.2007.02.010

Knowles, T. G. 1995. A review of post-transport mortality among younger calves. Veterinary Record 137:406-407. https://doi.org/10.1136/vr.137.16.406

Knowles, T. G.; Warris, P. D.; Brown, S. N. and Edwards, J. E. 1998. Effects of stocking density on lambs being transported by road. Veterinary Record 142:503-509. https://doi.org/10.1136/vr.142.19.503

Li, F., Yin, Y.; Tan, B.; Kong, X. and Wu, G. 2011. Leucine nutrition in animals and humans: mTOR signaling and beyond. Amino Acids 41:1185. https://doi.org/10.1007/s00726-011-0983-2

Minka, N. S. and Ayo, J. O. 2007. Physiological responses of transported goats treated with ascorbic acid during the hot-dry season. Animal Science Journal 78:164-172. https://doi.org/10.1111/j.1740-0929.2007.00421.x

Minka, N. S. and Ayo, J. O. 2010a. Serum biochemical activities and muscular soreness in transported goats administered with ascorbic acid during the hot-dry season. European Journal of Translational Myology - Basic Applied Myology 1:193-203.

Minka N. S. and Ayo J. O. 2010b. Physiological responses of food animals to road transportation stress. African Journal of Biotechnology 9:6601-6613.

Minka, N. S. and Ayo, J. O. 2013. Physiological and behavioral responses of goats to 12-hour road transportation, lairage and grazing periods, and the modulatory role of ascorbic acid. Journal of Veterinary Behavior 8:349–356. https://doi.org/10.1016/j.jveb.2013.01.001

Minka, N. S.; Ayo, J. O.; Sackey, A. K. and Adelaiye, A. B. 2009. Assessment and scoring of stresses imposed on goats during handling, loading, road transportation and unloading, and the effect of pretreatment with ascorbic acid. Livestock Science 125:275-282. https://doi.org/10.1016/j.livsci.2009.05.006

Miranda-de la Lama, G. C.; Rivero, L.; Chacón, G.; Garcia-Belenguer, S.; Villarroel, M. and Maria, G. A. 2010. Effect of the pre-slaughter logistic chain on some indicators of welfare in lambs. Livestock Science 128:52-59. https://doi.org/10.1016/j.livsci.2009.10.013

Miranda-de la Lama, G. C.; Monge, P.; Villarroel, M.; Olleta, J. L.; Garcia-Belenguer, S. and María, G. A. 2011. Effects of road type during transport on lamb welfare and meat quality in dry hot climates. Tropical Animal Health and Production 43:915-922. https://doi.org/10.1007/s11250-011-9783-7

NRC - National Research Council. 2007. Nutrient requirements of small ruminants. Sheep, goats, cervids and New World camelids. 6th ed. National Academy Press, Washington, DC.

Nikbin, S.; Panandam, J. M. and Sazili, A. Q. 2016. Influence of pre-slaughter transportation and stocking density on carcass and meat quality characteristics of Boer goats. Italian Journal of Animal Science 15:504-511. https://doi.org/10.1080/1828051X.2016.1217752

Nwe, T. M.; Hori, E.; Manda, M. and Watanable, S. 1996. Significance of catecholamines and cortisol levels in blood during transportation stress in goats. Small Ruminant Research 20:129-135. https://doi.org/10.1016/0921-4488(95)00781-4

Papet, I.; Ostaszewki, P.; Glomat, F.; Obled, C.; Faure, M.; Bayle, G.; Nissen, S.; Arnal, M. and Grizad, J. 1997. The effect of high dose of 3-hydroxy-3-methylbutyrate on protein metabolism in growing lambs. British Journal of Nutrition 77:885-896. https://doi.org/10.1079/BJN19970087

Peterson, A. L.; Qureshi, M. A.; Ferket, P. R. and Fuller, J. C. 1999. Enhancement of cellular and humoral immunity in young broilers by the dietary supplementation of β-hydroxy-β-methylbutyrate. Immunopharmacology and Immunotoxicology 21:307-330. https://doi.org/10.3109/08923979909052765

Puchajda-Skowrońska, H.; Siwicki, A. K.; Wójcik, R. and Pudyszak, K. 2006. Effects of 3-hydroxy-3-methylbutyrate (HMB) on selected performance indices and non-specific defense mechanisms in geese. Medycyna Weterynaryjna 62:89-92.

Rajion, M. A.; Saat, I. M.; Zulkifli, I. and Goh, Y. M. 2001. The effects of road transportation on some physiological stress measures in goats. Asian-Australasian Journal of Animal Sciences 14:1250-1252. https://doi.org/10.5713/ajas.2001.1250

Rathmacher, J. A.; Nissen, S.; Panton, L.; Clark, R. H.; May, P. E.; Barber, A. E.; D'Olimpio, J. and Abumrad, N. N. 2004. Supplementation with a combination of beta-hydroxy-beta-methylbutyrate (HMB), arginine, and glutamine is safe and could improve hematological parameters. Journal of Parenteral and Enteral Nutrition 28:65-75. https://doi.org/10.1177/014860710402800265

Saeb, M.; Baghshani, H.; Nazifi, S. and Saeb, S. 2010. Physiological response of dromedary camels to road transportation in relation to circulating levels of cortisol, thyroid hormones and some serum biochemical parameters. Tropical Animal Health and Production 42:55-63. https://doi.org/10.1007/s11250-009-9385-9

Sanhouri, A. A.; Jones, R. S. and Dobson, H. 1992. Effects of xylazine on the stress response to transport in male goats. British Veterinary Journal 148:119-128. https://doi.org/10.1016/0007-1935(92)90103-8

Sowińska, J.; Brzostowski, H.; Tański, Z. and Lisowska, J. 2006. Stress reaction of lambs to weaning and short transport to slaughterhouse regards to the breed and age. Medycyna Weterynaryjna 62:946-948.

Sowińska, J.; Tański, Z.; Milewski, S.; Ząbek, K.; Wójcik, A.; Sobiech, P. and Illek, J. 2016. Effect of diet supplementation with the addition of *Saccharomyces cerevisiae* upon stress response in slaughter lambs. Acta Veterinaria Brno 85:177-184. https://doi.org/10.2754/avb201685020177

Sowińska, J.; Milewski, S.; Tański, Z.; Witkowska, D.; Ząbek, K.; Sobiech, P. and Mituniewicz, T. 2017. The effect of dietary supplementation with β-1,3/1,6-D-glucan on stress parameters and meat quality in lambs. Journal of Animal and Feed Sciences 26:18-25. https://doi.org/10.22358/jafs/68050/2016

Stanger, K. J.; Ketheesan, N.; Parker, A. J.; Coleman, C. J.; Lazzaroni, S. M. and Fitzpatrick, L. A. 2005. The effect of transportation on the immune status of *Bos indicus* steers. Journal of Animal Science 83:2632-2636. https://doi.org/10.2527/2005.83112632x

Tatara, M. R.; Śliwa, E. and Krupski, W. 2007. Prenatal programming of skeletal development in the offspring: Effects of maternal treatment with β-hydroxy-β-methylbutyrate (HMB) on femur properties in pigs at slaughter age. Bone 40:1615-1622. https://doi.org/10.1016/j.bone.2007.02.018

Tatara, M. R. 2008. Neonatal programming of skeletal development in sheep is mediated by somatotrophic axis function. Experimental Physiology 93:763-772. https://doi.org/10.1113/expphysiol.2007.041145

Terlouw, E. M. C.; Arnould, C.; Auperin, B.; Berri, C.; Le Bihan-Duval, E.; Deiss, V.; Lefèvre, F.; Lensink, B. J. and Mounier, L. 2008. Pre-slaughter conditions, animal stress and welfare: current status and possible future research. Animal 2:1501-1517. https://doi.org/10.1017/S1751731108002723

Wiąz, M.; Bratos, M.; Kaczmarek, S. and Rutkowski, A. 2010. Application of the beta-hydroxy-beta-methylbutyrate (HMB) acid in broiler chicken feeding. Rocznik Naukowe Polskiego Towarzystwa Zootechnicznego 6:101-108.

Wójcik, R.; Małaczewska, J.; Siwicki, A. K.; Miciński, J. and Zwierzchowski, G. 2014. The effect of β-hydroxy-β-methylbutyrate (HMB) on selected parameters of humoral immunity in calves. Polish Journal of Veterinary Sciences 17:357-359. https://doi.org/10.2478/pjvs-2014-0049

Van Koevering, M. T.; Gill, D. R.; Smith, R. A.; Owens, F. N.; Nissen, S. and Ball, R. L. 1993. Effect of β-hydroxy-β-methylbutyrate on the health and performance of shipping-stressed calves. Oklahoma State University Research Reports 312-316.

Yalcintan, H.; Akin, P. D.; Ozturk, N.; Avanus, K.; Muratoglu, K.; Kocak, O.; Yilmaz, A. and Ekiz, B. 2018. Effect of lairage time after 2 h transport on stress parameters and meat quality characteristics in Kivircik ewe lambs. Small Ruminant Research 166:41-46. https://doi.org/10.1016/j.smallrumres.2018.07.007

Young, J. F.; Stagsted, J.; Jensen, S. K.; Karlsson, A. H. and Henckel, P. 2003. Ascorbic acid, α-tocopherol, and oregano supplements reduce stress-induced deterioration of chicken meat quality. Poultry Science 82:1343-1351. https://doi.org/10.1093/ps/82.8.1343

Ząbek, K.; Wójcik, R.; Milewski, S.; Małaczewska, J.; Tański, Z. and Siwicki, A. K. 2016. Effect of β-hydroxy-β-methylbutyrate acid on meat performance traits and selected indicators of humoral immunity in goats. Japanese Journal of Veterinary Research 64:247-256. https://doi.org/10.14943/jjvr.64.4.247

Zimerman, M.; Domingo, E.; Grigono, G.; Taddeo, H. and Willems, P. 2013. The effect of pre-slaughter stressors on physiological indicators and meat quality traits on Merino lambs. Small Ruminant Research 111:6-9. https://doi.org/10.1016/j.smallrumres.2012.12.018