

The Impact of an Anti-Weight Loss Booster with Ascorbic Acid and GABA on Thin-Tailed Sheep Following Transportation

G. Husnu^{1,a)}, Panjaitan. I^{1,b)}, Sofiana. A^{1,c)}

¹Department of Animal Husbandry, Politeknik Negeri Lampung, Indonesia

^{a)}Corresponding author : ghoffarhusnu@polinela.ac.id

^{b)}pjtnimel@gmail.com

^{c)}anjarsofiana@polinela.ac.id

Abstract. This study investigates the effects of ascorbic acid and gamma-aminobutyric acid (GABA) supplementation on the physiological responses of thin-tailed sheep subjected to transportation stress. The research was conducted in Lakaran Village, Wonosobo District, Tanggamus, Lampung, involving 20 male thin-tailed sheep divided into four treatment groups: control (R0), ascorbic acid 100 mg/kg (R1), GABA 0.3 g/head (R2), and a combination of ascorbic acid 100 mg/kg + GABA 0.3 g/head (R3). The results indicate that the combination of ascorbic acid and GABA (R3) significantly reduced body weight loss, demonstrating a synergistic effect in alleviating stress compared to single or no supplementation. The respiratory rates in the supplemented groups (R1, R2, R3) showed better stabilization than the control group, with R3 approaching normal levels. Although all treatment groups exhibited increased heart rates and rectal temperatures due to the stressful transport environment, the combination approach proved to be more effective in managing stress. Overall, this study highlights the potential of ascorbic acid and GABA as supplement strategies to enhance livestock welfare during transportation, contributing to better farming practices.

Keywords: Ascorbic acid, body weight loss, GABA, thin-tailed sheep, transportation

INTRODUCTION

Transporting livestock is a common practice in today's farming industry, but it can cause significant stress to the animals. This stress can lead to noticeable changes in their health and performance. For instance, animals may experience increased respiratory and heart rates, fluctuations in body temperature, and even weight loss. Factors like vibrations from vehicles, noise, and unfamiliar environments contribute to this stress.

Research by [1] and [2] highlights the importance of managing stress during transport, as it is crucial for the well-being of the animals and the economic success of livestock operations. For ruminants, such as thin-tailed sheep, stress can lead to higher levels of Reactive Oxygen Species (ROS) and cortisol [3], which are harmful to their health. When ROS levels become too high, it results in oxidative stress, which damages cells [4]. Increased cortisol is also linked to changes in heart rate, respiratory rate, and body temperature, all of which can negatively impact meat quality and overall productivity [5].

To reduce transport stress, farmers typically use methods like providing rest breaks, ensuring a steady supply of water, and minimizing noise. However, these strategies often fall short of addressing the complex ways animals respond to long journeys and changing conditions. This is particularly important because weight loss during transport can lead to financial losses for farmers.

One promising solution is the combination of ascorbic acid (vitamin C) and Gamma-Aminobutyric Acid (GABA) as supplements for sheep during transport. Ascorbic acid is an antioxidant that helps neutralize harmful free radicals, or ROS, which tend to increase under stress. By reducing ROS levels, ascorbic acid helps keep the animal's body balanced and prevents cellular damage during transport [6]. It can be given as an injection or oral supplement, making it a safe option for supporting animal health in stressful situations[7].

On the other hand, GABA works as a calming neurotransmitter that helps reduce excessive activity in the nervous system caused by stress. This allows animals to feel calmer and less anxious [8]. GABA also has the potential to boost antioxidant activity in stressed livestock[9]. Therefore, combining ascorbic acid and GABA is expected to offer enhanced protection against the negative effects of transportation stress. This combination could greatly improve the welfare of animals during transport and help reduce losses for farmers.

METHODS

This study was conducted in August 2024 in Lakaran Village, Wonosobo District, Tanggamus, Lampung, with a total of 20 male thin-tailed sheep, each weighing between 15 and 25 kg. These sheep were maintained in a controlled environment before the experiment began. They were randomly assigned to four treatment groups:

- R0 (control, water only without any supplements)
- R1 (water + ascorbic acid at a dose of 100 mg/kg body weight)
- R2 (water + GABA at a dose of 0.3 g per sheep)
- R3 (water + ascorbic acid 100 mg/kg + GABA 0.3 g per sheep)

The research employed a completely randomized design (CRD) with five replications for each treatment. During the pre-transport phase, lasting one day before transport, the sheep received their designated treatments orally, and initial measurements were taken, including body weight, respiratory rate, heart rate, and rectal temperature.

One hour prior to transportation, the sheep were given oral supplements. They were then transported for eight hours in a specially designed vehicle to simulate conditions of long-distance transport. After the transport, the same variables were measured again to obtain post-transportation data. The collected data were analyzed using ANOVA to determine the significance of differences among treatment groups, followed by post-hoc tests to assess statistically significant differences.

RESULTS AND DISCUSSION

Body Weight Loss

Based on the data regarding the weight loss in thin-tailed sheep from one day before transportation to just after transport, it is evident that there are variations in weight loss among the treatment groups, as presented in Table 1 below.

Table 1. Weight Loss in Thin-Tailed Sheep from One Day Before Transportation to Immediately After Transportation

Treatment	Body weight loss from one day before to immediately after transportation	
	(%)	(kg)
R0	-4,34 ± 3,09	-0,89 ± 0,62 a
R1	-1,99 ± 3,28	-0,30 ± 0,48 ab
R2	-3,81 ± 1,05	-0,54 ± 0,21 ab
R3	0,37 ± 2,93	0,20 ± 0,30 b

Table 1 shows the highest weight loss in the R0 (Control) group, amounting to $-4.34 \pm 3.09\%$, with an absolute weight decrease of -0.89 ± 0.62 kg. This indicates that sheep not receiving GABA or ascorbic acid supplementation experienced higher stress levels, leading to more significant weight loss. In the R1 group (Ascorbic Acid 100 mg/kg body weight), a weight loss of $-1.99 \pm 3.28\%$ was observed, corresponding to a decrease of -0.30 ± 0.48 kg. Although there was still weight loss, this figure was lower compared to the control group, suggesting that ascorbic acid positively impacts the reduction of oxidative stress caused by transportation. In the R2 group (GABA 0.3 g/head), a weight loss of $-3.81 \pm 1.05\%$ and an absolute decrease of -0.54 ± 0.21 kg occurred. While not as effective as R1, GABA contributed to reducing stress by inhibiting central nervous system activity, thereby alleviating anxiety and physiological stress in the sheep. The R3 group (GABA 0.3 g/head + Ascorbic Acid 100 mg/kg body weight) demonstrated the best results, with a weight gain percentage of $0.37 \pm 2.93\%$ and an absolute increase of 0.20 ± 0.30 kg. This illustrates that the synergistic effects of GABA and ascorbic acid are highly effective in mitigating the impact of transportation stress.

Statistical analysis revealed that the R3 group significantly differed from the other groups. Duncan's post-hoc test indicated that this group had a statistically significant difference at the 95% confidence level, reinforcing the finding that the combination of GABA and ascorbic acid is a more effective approach compared to administering either compound alone [10].

The control group (R0) showed the most significant weight loss compared to the other treatment groups, confirming that transportation stress has a clear impact on animals without supplementation. Meanwhile, treatments with GABA (R2) and ascorbic acid (R1) demonstrated insignificant weight retention compared to one another, indicating that both can independently alleviate the effects of transportation stress, though their combination (R3) resulted in significant differences. This suggests a synergistic effect between GABA and ascorbic acid, where GABA alleviates anxiety from stress, and ascorbic acid reduces oxidative stress, providing more effective protection against weight loss during transportation [10].

The results of this study support the hypothesis that the combination of GABA and ascorbic acid can offer better protective effects against the impact of transportation stress in thin-tailed sheep. GABA, as an inhibitory neurotransmitter, plays a role in reducing excessive excitability in the brain, which typically occurs during stress [11]. This reduction in anxiety can potentially decrease extreme stress responses, such as increased respiratory and heart rates, often correlated with body weight loss. Previous research by [11] showed that GABA administration in animals led to reduced cortisol levels, the primary stress hormone, which correlates with decreased weight loss.

Furthermore, ascorbic acid functions as a potent antioxidant, mitigating the oxidative stress induced by transportation processes [12]. As outlined by [13], the use of ascorbic acid in reducing stress is attributed to its antioxidant properties, specifically its ability to eliminate the effects of oxidative stress. Oxidative stress is a significant factor contributing to cellular damage during transportation, ultimately leading to weight loss. Ascorbic acid helps maintain cellular integrity and repair oxidative damage, thereby supporting post-transportation immune stability [13].

The combination of these two compounds (GABA and ascorbic acid) produces a synergistic effect, where GABA mitigates physiological stress components while ascorbic acid alleviates oxidative damage. Research by [9] supports these findings, demonstrating that GABA can enhance the antioxidant performance of ascorbic acid in heat-stressed ruminants. This aligns with research [14] indicating that GABA administration can improve nutrient digestibility, growth performance, and antioxidant status in beef cattle experiencing heat stress.

Respiratory Rate

Based on observations, the average respiratory rate in the control group (R0) was 39.60 ± 7.79 breaths per minute, which was considerably higher compared to the treatment groups that received supplements. The R1 group (water + ascorbic acid) and R2 group (water + GABA) recorded respiratory rates of 35.60 ± 4.78 and 37.20 ± 4.38 breaths per minute, respectively. However, the R3 group, which received a combination of ascorbic acid and GABA, showed the lowest respiratory rate at 34.40 ± 3.58 breaths per minute (**Table 2**).

Table 2. Average Respiratory Rate of Thin-Tailed Sheep After Transportation

Treatment	Average Respiratory Frequency (Times/minute)
R0	$39,60 \pm 7,79$ ns
R1	$35,60 \pm 4,78$ ns
R2	$37,20 \pm 4,38$ ns
R3	$34,40 \pm 3,58$ ns

The recorded normal respiratory rate was 29.75 ± 3.15 breaths per minute [15], providing a baseline for assessing the treatment effects on livestock health. The control group (R0) exhibited a much higher respiratory rate of 39.60 ± 7.79 breaths per minute. This increase indicates a heightened stress response to transportation, aligning with findings by [16], which state that transport stress often triggers an increase in respiratory rates in livestock. The elevated respiratory rate in the control group reflects a physiological response to stress induced by transportation. When animals experience stress, the sympathetic nervous system within the hypothalamic-pituitary-adrenal axis is activated, releasing cortisol into the bloodstream [17][18]. Increased cortisol levels can weaken the animal's immune response [19] and often lead to respiratory irregularities. Thus, the respiratory rate in the control group exceeding normal limits can be understood as a clear indicator of negative stress responses. Supplementation with GABA and ascorbic acid significantly improved respiratory rates, particularly in the R3 group, which received both

supplements. GABA, as an inhibitory neurotransmitter, plays a crucial role in reducing anxiety and stabilizing stress responses [20]. Studies suggest that GABA can decrease sympathetic nervous system activity, thereby reducing excessive respiratory rates. Further, [21] confirms that GABA not only lowers anxiety but also enhances respiratory function by relieving muscle tension in the airways. Ascorbic acid, or vitamin C, has strong antioxidant properties. Oxidative stress caused by transportation can damage lung tissue and worsen respiratory conditions [22]. By reducing oxidative stress, ascorbic acid helps maintain respiratory health, leading to normalized respiratory rates. Research [22] shows that ascorbic acid can improve lung function in mammals under oxidative stress, supporting our findings of closer-to-normal respiratory rates in the R3 group.

Heart Rate

The observed effects of the combined GABA and ascorbic acid supplements on the heart rate of thin-tailed sheep after transportation are shown in Table 3 below.

Table 3. Average Heart Rate of Thin-Tailed Sheep Post-Transportation

Treatment	Average Heart Rate (Times/minute)
R0	103,40 ± 4,93 a
R1	110,80 ± 8,40 ab
R2	118,60 ± 5,94 b
R3	119,60 ± 4,09 b

Heart rate measurements in thin-tailed sheep highlight a significant physiological response to transportation stress. The control group (R0) recorded a heart rate of 103.40 ± 4.93 beats per minute, notably above the normal range (75.50 ± 5.45 bpm) [15]. This increase indicates sympathetic nervous system activation as a stress reaction, where hormones like adrenaline are released to prepare the body for a challenging situation [23]. Transportation stress can trigger a "fight or flight" response, raising heart rate and blood flow to support physical readiness [23].

In the treatment groups, R1 (water + ascorbic acid) and R2 (water + GABA) recorded heart rates of 110.80 ± 8.40 and 118.60 ± 5.94 bpm, respectively. While supplementation aims to reduce stress effects, these values suggest that physiological stress responses persisted, likely due to environmental conditions and the variety of pressures the animals experienced during transport [24]. Interestingly, group R3, given a combination of ascorbic acid and GABA, showed the highest heart rate, at 119.60 ± 4.09 bpm. This suggests that while a synergistic effect of both supplements was expected, prolonged transportation stress continued to impact the animals' physiological response.

In summary, although ascorbic acid and GABA supplementation showed potential in mitigating stress effects, further research is necessary to optimize dosages and combinations, as well as to enhance transportation management to improve animal welfare overall.

Rectal Temperature

The rectal temperature data for thin-tailed sheep after transportation is detailed in Table 4 below.

Table 4. Average Rectal Temperature of Thin-Tailed Sheep Post-Transportation

Treatment	Average Rectal Temperature (°C) after Transportation
R0	39,48 ± 0,11
R1	39,76 ± 0,68
R2	39,76 ± 0,22
R3	40,18 ± 0,54

The measurements of rectal temperature in thin-tailed sheep reveal significant changes due to transportation stress. The data recorded a rectal temperature of 39.48 ± 0.11 °C in the control group (R0), indicating that this temperature is above the normal range (38.85 ± 0.25 °C), R1 (water + ascorbic acid) and R2 (water + GABA) showed rectal temperatures of 39.76 ± 0.68 °C. Meanwhile, the R3 group (water + ascorbic acid + GABA) exhibited the highest temperature at 40.18 ± 0.54 °C. This increase is likely attributed to the extreme heat conditions during the study. Previous research has indicated that high ambient temperatures can lead to elevated rectal temperatures in sheep during transportation [25]. Overall demonstrates that while the supplements provided show potential in supporting animal welfare, a more comprehensive management approach and further research are needed to determine effective doses and combinations to maintain optimal body temperatures in animals during transport.

CONCLUSIONS

The study demonstrated that supplementation with ascorbic acid and GABA effectively mitigated the negative effects of transportation stress in thin-tailed sheep. Among the treatment groups, the combination of ascorbic acid and GABA (R3) exhibited the most significant reduction in body weight loss, highlighting the synergistic effects of these supplements in alleviating stress. In terms of respiratory rate, groups receiving supplementation (R1, R2, and R3) showed better stabilization compared to the control group (R0), with group R3 approaching normal respiratory conditions.

Heart rate and rectal temperature measurements indicated elevated values across all treatments, which is expected given that the sheep were in an uncomfortable situation during transportation. Overall, this research indicates that the combination of ascorbic acid and GABA presents a more effective strategy for managing transportation stress compared to single supplementation or no supplementation.

ACKNOWLEDGMENTS

The author acknowledges Lampung State Polytechnic for funding and Metro Aqikah Farm for providing sheep to support the research

REFERENCES

- [1] T. Grandin, "On-farm conditions that compromise animal welfare that can be monitored at the slaughter plant," *Meat Sci.*, vol. 132, no. January, pp. 52–58, 2017, doi: 10.1016/j.meatsci.2017.05.004.
- [2] R. Y. Acharya, P. H. Hemsworth, G. J. Coleman, and J. E. Kinder, "The Animal-Human Interface in Farm Animal Production: Animal Fear, Stress, Reproduction and Welfare," *Animals*, vol. 12, no. 4, pp. 1–14, 2022, doi: 10.3390/ani12040487.
- [3] A. Y. Kassab and A. A. Mohammed, "Ascorbic Acid Administration As Anti-Stress Before Transportation of Sheep," *Egypt. J. Anim. Prod.*, vol. 51, no. 1, pp. 19–25, 2014, doi: 10.21608/ejap.2014.93664.
- [4] O. E. Oke *et al.*, "Oxidative stress in poultry production," *Poult. Sci.*, vol. 103, no. 9, 2024, doi: 10.1016/j.psj.2024.104003.
- [5] P. A. Gonzalez-Rivas, S. S. Chauhan, M. Ha, N. Fegan, F. R. Dunshea, and R. D. Warner, "Effects of heat stress on animal physiology, metabolism, and meat quality: A review," *Meat Sci.*, vol. 162, p. 108025, 2020, doi: 10.1016/j.meatsci.2019.108025.
- [6] N. S. Minka and J. O. Ayo, "Physiological and behavioral responses of goats to 12-hour road transportation, lairage and grazing periods, and the modulatory role of ascorbic acid," *J. Vet. Behav. Clin. Appl. Res.*, vol. 8, no. 5, pp. 349–356, 2013, doi: 10.1016/j.jveb.2013.01.001.
- [7] K. T. Biobaku, T. O. Omobowale, A. O. Akeem, A. Aremu, N. Okwelum, and A. S. Adah, "Use of goat interleukin-6, cortisol, and some biomarkers to evaluate clinical suitability of two routes of ascorbic acid administration in transportation stress," *Vet. World*, vol. 11, no. 5, pp. 674–680, 2018, doi: 10.14202/vetworld.2018.674-680.
- [8] K. Kim and H. Yoon, "Gamma-Aminobutyric Acid Signaling in Damage Response, Metabolism, and Disease," *Int. J. Mol. Sci.*, vol. 24, no. 5, 2023, doi: 10.3390/ijms24054584.
- [9] J. Cheng, N. Zheng, X. Sun, S. Li, J. Wang, and Y. Zhang, "Feeding rumen-protected gamma-aminobutyric acid enhances the immune response and antioxidant status of heat-stressed lactating dairy cows," *J. Therm. Biol.*, vol. 60, pp. 103–108, 2016, doi: 10.1016/j.jtherbio.2016.06.011.
- [10] G. Husnu, A. Rochana, K. A. Kamil, and E. Setyowati, "The Effect of Gamma Aminobutyric Acid and Ascorbic Acid on Bali Cattle Physiology During Transport," *Pakistan J. Nutr.*, vol. 18, no. 12, pp. 1133–1138, 2019, doi: 10.3923/pjn.2019.1133.1138.
- [11] Y. H. Li *et al.*, "Effect of g-aminobutyric acid on growth performance, behavior and plasma hormones in weaned pigs," *Can. J. Anim. Sci.*, vol. 95, pp. 165–171, 2015, doi: 10.4141/CJAS2013-148.
- [12] G. E. Tresia, A. F. Trisiana, and B. Tiesnamurti, "Effects of Road Transportation on Some Physiological Stress Measures in Anpera and Boerka Goats," *Bul. Peternak.*, vol. 47, no. 3, p. 142, 2023, doi: 10.21059/buletinpeternak.v47i3.83317.
- [13] J. O. Ayo, N. S. Minka, and M. Mamman, "Excitability scores of goats administered ascorbic acid and transported

- during hot-dry conditions,” *J. Vet. Sci.*, vol. 7, no. 2, pp. 127–131, 2006, doi: 10.4142/jvs.2006.7.2.127.
- [14] K. Guo *et al.*, “Improving effects of dietary rumen protected γ -aminobutyric acid additive on apparent nutrient digestibility, growth performance and health status in heat-stressed beef cattle,” *Anim. Sci. J.*, vol. 89, no. 9, pp. 1280–1286, 2018, doi: 10.1111/asj.13053.
- [15] S. Agik, D. A. Astuti, F. Satrija, and Suprianto, “Physiological Status of Sheep Reared Indoor System Under The Tropical Rain Forest Climatic Zone,” *Anim. Prod. Sustain. Agric. Trop.*, p. 66, 2006.
- [16] A. F. Trisiana, A. Destomo, and F. Mahmilia, “Pengangkutan ternak : Proses , kendala dan engaruhnya pada ruminansia kecil (transportation of animal : process , challenge and the effect on small ruminant),” *WARTAZOA*, vol. 31, no. 1, pp. 43–53, 2021, doi: <http://dx.doi.org/10.14334/wartazoa.v31i1.2512> Pengangkutan.
- [17] I. Zulkifli *et al.*, “Physiological responses in goats subjected to Road Transportation under the hot, humid tropical conditions,” *Int. J. Agric. Biol.*, vol. 12, no. 6, pp. 840–844, 2010.
- [18] A. Y. Adenkola and J. O. Ayo, “Physiological and behavioural responses of livestock to road transportation stress: A review,” *African J. Biotechnol.*, vol. 9, no. 31, pp. 4845–4856, 2010.
- [19] P. D. Warriss, “The transport of animals: A long way to go,” *Vet. J.*, vol. 168, no. 3, pp. 213–214, 2004, doi: 10.1016/j.tvjl.2003.10.002.
- [20] P. Brambilla, J. Perez, F. Barale, G. Schettini, and J. C. Soares, “GABAergic dysfunction in mood disorders,” *Mol. Psychiatry*, vol. 8, no. 8, pp. 721–737, 2003, doi: 10.1038/sj.mp.4001362.
- [21] K. A. Yamada, W. P. Norman, P. Hamosh, and R. A. Gillis, “Medullary ventral surface GABA receptors affect respiratory and cardiovascular function,” *Brain Res.*, vol. 248, no. 1, pp. 71–78, 1982, doi: 10.1016/0006-8993(82)91148-9.
- [22] A. C. Carr and B. Frei, “Vitamin C absorption and dietary allowance,” no. March, pp. 1086–1107, 2018.
- [23] E. H. von Borell, “The biology of stress and its application to livestock housing and transportation assessment,” *J. Anim. Sci.*, vol. 79, no. E-Suppl, p. E260, 2001, doi: 10.2527/jas2001.79e-supple260x.
- [24] V. Deiss *et al.*, “Can emotional reactivity predict stress responses at slaughter in sheep?,” *Appl. Anim. Behav. Sci.*, vol. 119, no. 3–4, pp. 193–202, 2009, doi: 10.1016/j.applanim.2009.03.018.
- [25] Lendrawati, R. Priyanto, M. Yamin, A. Jayanegara, W. Manalu, and Desrial, “Respon Fisiologis dan Penyusutan Bobot Badan Domba Lokal Jantan terhadap Transportasi dengan Posisi Berbeda dalam Kendaraan,” *J. Agripet*, vol. 19, no. 2, pp. 113–121, 2019, doi: <https://doi.org/10.17969/agripet.v19i2.14877>.