Animal Welfare Study in Sheep Transported with Methionine Hydroxy Analog and Dextrose Supplementation

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ABSTRAK

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Transportasi ternak dapat berdampak negatif pada kesejahteraan hewan dan performa setelah transportasi. Penelitian ini bertujuan mengevaluasi efek suplementasi metionin hydroxy analogue (MHA) dan dekstrosa sebelum transportasi pada status kesejahteraan hewan domba lokal. Penelitian menggunakan 27 domba jantan ekor tipis dengan bobot badan rata-rata 21,8±2,0 kg, berusia 10-12 bulan, yang dibagi secara acak dalam tiga perlakuan: domba tanpa transportasi dan pemberian suplemen (K: kontrol); domba yang diangkut dengan sistem transportasi tanpa pemberian suplemen (P1); domba yang diangkut dengan sistem transportasi dan diberi suplementasi aditif (P2: MHA 0,5 g/kg bobot badan dan dekstrosa 0,5 g/kg bobot badan). Domba P1 dan P2 diangkut selama 8 jam menggunakan kendaraan komersial yang biasa digunakan oleh peternak. Hasil penelitian menunjukkan bahwa domba di kelompok P2 secara signifikan memiliki penurunan bobot badan yang lebih rendah dibandingkan dengan P1 dan memiliki waktu pemulihan yang lebih cepat (P<0,05). Domba di kelompok P2 juga menunjukkan tingkah laku pascatransportasi yang lebih baik daripada P1, dan tidak berbeda nyata dibandingkan dengan kelompok kontrol. Kelompok P2 memiliki rasio N/L yang signifikan lebih rendah daripada P1 dan sebanding dengan kelompok kontrol (P<0,05). Domba pada kelompok P2 juga memiliki hormon kortisol dan glukosa darah yang signifikan lebih rendah daripada kelompok P1, yang menunjukkan peningkatan status kesejahteraan domba pada kelompok P2 dan Kontrol. Oleh karena itu, dapat disimpulkan bahwa suplementasi MHA dan dekstrosa sebelum transportasi meningkatkan status kesejahteraan domba selama transportasi.

Kata Kunci: Kesejahteraan Ternak, Dekstrosa, *Methionine Hidroxy Analogue*, Domba, Pengangkutan

ABSTRACT

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Livestock transportation adversely affects animal welfare and performance during subsequent production periods. This research aimed to evaluate the effects of methionine hydroxy analog (MHA) and dextrose supplementations before transportation on the animal welfare status of sheep. The study used 27 thin-tailed male sheep with an average body weight of 21,8±2,0 kg, 10-12 months of age, which were divided randomly into three treatments: sheep without transportation and additional supplement (K: Control); sheep transported without additional supplement (P1); sheep transported with additional supplement (P2: MHA 0.5 g/kg body weight and dextrose 0.5 g/kg body weight). Sheep in groups P1 and P2 were transported for 8 hours using commercial vehicles usually used by local farmers. The results showed that sheep in the P2 group had significantly lower body weight loss than those in the P1 group and faster recovery time (P<0.05). Sheep in the P2 group also demonstrated better post-transportation behavior than those in P1, with no significant difference from the control group. The experimental sheep in the P2 group had a significantly lower N/L ratio than those in P1 and was comparable to the control group (P<0.05). The P2 group also has significantly lower cortisol hormone and blood glucose concentrations than P1, which indicates improved sheep welfare status in P2 and Control groups. Therefore, it can be concluded that supplementation of MHA and dextrose before departure improves sheep's welfare status under transportation.

Key Words: Animal Welfare, Dextrose, Methionine Hydroxy Analogue, Sheep, Transportation

INTRODUCTION

The supply chain for small ruminants (sheep and goats) in Indonesia involves lengthy transportation times and multiple stakeholders. Additionally, facilities and infrastructure remain significant challenges, such as the condition of transportation during the shipment of livestock. In developed countries, the effects of transportation on livestock productivity and performance, welfare, carcass quality, and meat quality have been reviewed by several researchers (Miranda-de la Lama et al. 2014; Rey-Salgueiro et al. 2018). Animal welfare can be evaluated using output indicators expressed by livestock, such as physiological conditions, production/body weight, behavior, and other factors (Broom 2019). However, research on sheep transportation is often conducted in temperate climates, which differ climatologically from tropical climates, using better transportation modes and road infrastructure, generally over shorter distances or travel times. Several researchers have also reported research on goat transportation in hot subtropical areas (Rey-Salgueiro et al. 2018; Biobaku et al. 2018). Other researchers have studied goat transportation in wet tropical areas in Malaysia (Raghazli et al. 2021), but using relatively short transportation times, namely two and six hours of travel. Preliminary studies in Indonesia conducted by Baihaqi et al. (2017) on sheep transportation concluded that the weight loss of livestock during 20 hours of transportation reached 11.59±2.75 percent of the initial weight. This weight loss is still higher than the transportation standards in developed countries such as Australia (5%) (Department of Agriculture and Water Resources 2023). A study by Lendrawati et al. (2020) stated that thin-tailed sheep transported for 12 hours could tolerate the adverse effects of transportation compared to the control group. Nevertheless, animal welfare during transportation in Indonesia has not been extensively studied. Some studies have been conducted to minimize the negative effects of livestock transportation, including providing supplements/additives to livestock before transportation, such as vitamin C (Ahmad Mir et al. 2019), electrolytes (Gupta et al. 2020), Selenium-Methionine and Cromium-Methionine (Mousaie et al. 2014), or molasses (Lendrawati 2020).

Methionine is an essential amino acid that contains a sulfur group and acts as a precursor for carnitine, creatine, cysteine, homocysteine, and succinyl-CoA. Additionally, methionine plays a role in lipid metabolism and activating enzymes related to antioxidants such as methionine sulfoxide reductase A (Blachier et al. 2020). Methionine is also involved in the biosynthesis of glutathione, a crucial molecule for combating oxidative stress (Martínez et al. 2017). Research on the use of methionine in the form of methionine hydroxy analog (MHA) has been applied to reduce heat stress in broiler chickens (Erfani et al. 2021) and dairy cows (Jacometo et al. 2016). Providing methionine as protected MHA is crucial to avoid rumen degradation in ruminant livestock. However, trials of MHA administration in transported sheep are still rare. They provided energy sources before transportation, which is expected to reduce transportation stress. Experiments with protected glucose administration in dairy cows showed improved insulin and plasma glucose concentrations (Sauls-Hiesterman et al. 2020). Based on this information, glucose supplementation in dextrose is expected to increase blood glucose reserves to minimize transportation stress and losses. Dextrose is another name for glucose, a monosaccharide with the chemical formula $C_6H_{12}O_6$ (Townshend et al. 2019). Dextrose is commonly marketed in powder form as a sweetener and nutritional supplement for food and feed. This simple sugar is thought to supply energy to livestock experiencing fatigue. Research conducted by Baihaqi et al. (2022) using methionine or dextrose separately at 0.24 g/body weight improved physiological responses, weight recovery time, and behavior in sheep. However, these studies have not significantly reduced body weight loss after 6 hours of transportation. We hypothesized that combining dextrose and MHA will more effectively reduce stress and body weight loss due to transportation. Based on this information, this research aimed to evaluate the effects of MHA and dextrose administration on the welfare status of local sheep transported under wet tropical climate conditions.

MATERIALS AND METHODS

Materials

The research used 27 male thin-tailed sheep with an average body weight of 21.8±2.0 kg and aged 10-12 months. The materials included protected methionine hydroxy analog (MHA), dextrose, grass feed, and commercial concentrate. The experimental sheep were housed in individual pens size 120x60 cm, and transportation was carried out using a pickup truck commonly used by local farms. Equipment used included sheep weighing scales, a stethoscope, a rectal thermometer, blood sample tubes, and CCTV to record the behaviors of the sheep in pens after transportation.

Research procedure

The experimental protocol of this study was approved by the Institutional Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University, in 2022 (No 031/KEH/SKE/IX/2022). The study used three treatments: first, sheep that were not transported and without additional supplement (K: control); second, sheep transported without additional supplement (P1); and third, sheep transported with additional supplement (P2: MHA 0.5 g/kg body weight and dextrose 0.5 g/kg body weight). Each treatment had nine replicates, with one sheep per replicate. The sheep were adapted to individual pens for 2 weeks before the trial. The experimental sheep in the P2 group were given supplements for five days before transportation. MHA and dextrose were diluted in 100 ml of water and orally administered to the sheep every morning at 07:00 a.m. Sheep in the treatment P1 and P2 were unloaded randomly into the vehicle. The sheep were transported for approximately 8 hours with no rest stop from and to the Small Ruminant Laboratory, Faculty of Animal Science, IPB University.

Body weight loss measurement

The sheep were weighed twice: first, before loading onto the transport vehicle to record the initial body weight, and second, after unloading, to record the final body weight after transportation. Weighing was performed using a hanging scale.

Measurement of physiological responses

The sheep's physiological responses, including heart rate, respiratory rate, and rectal temperature, were measured right after unloading it from the vehicle.

Measurement of blood profiles and metabolites

Blood samples were collected from sheep after unloading from the vehicle using a syringe from the jugular vein in the neck, with a volume of 5 ml. Three milliliters of blood were collected into tubes containing anticoagulants for blood profile analysis, while two milliliters of blood were collected into tubes without anticoagulants for metabolite analysis. The blood profiles included measurements of erythrocytes, hemoglobin, hematocrit, leukocytes, percentage of neutrophils and lymphocytes, as well as the neutrophil/lymphocyte ratio, using a hematology analyzer (MEK-6550J/K Celltax Nihon Kohden). Cortisol levels were measured using a competitive Enzyme-Linked Immunosorbent Assay (ELISA) method based on the procedures provided in the ELISA kit for cortisol hormone (Cat: EIA 1887 DRG, Instrument GmbH, Germany). Meanwhile, glucose levels (mg dL^{-1}) were analyzed using an enzymatic method with KIT number 11219.

Measurement of recovery time

Recovery time was measured by weighing the sheep daily at 06:00 a.m. before feeding. Recovery time is the number of days the sheep need to regain their pre-transportation body weight.

Behavior observation

Behavior was recorded using closed-circuit television (CCTV) recording equipment with two cameras installed on the ceiling of the pen to capture the sheep's activities. Observations were made for five hours after transportation based on Baihaqi et al. (2022). The parameters observed included eating, drinking, standing, walking, and lying/resting behaviors. The frequencies of these behaviors were calculated using CowLog 3.0.2 software (Pastell 2016).

Experimental design and data analysis

This study used a completely randomized design with three treatments (K/control: sheep without transportation and additives supplementation; P1: sheep transported without additives supplementation; and P2: sheep transported with the MHA and dextrose supplementations). Each treatment had nine replicates (9 sheep). The data were analyzed using analyses of variance.

RESULTS AND DISCUSSION

Body weight loss and recovery period of sheep

Body weight loss is an indicator of stress in sheep during transportation. The sheep treated with methionine and dextrose (P2) showed significantly different body weight losses and percentage of body weight losses compared to transported sheep without additives (P1) ($P<0.05$), but not significantly different from the control sheep ($P>0.05$). The data on body weight loss are presented in Table 1.

Table 1 shows that P2 sheep experienced improvements in mitigating body weight loss, which is confirmed by data showing no significant difference from control sheep ($P>0.05$) without transportation. The transportation process can cause stress in sheep, which in turn can lead to body weight loss. The level of body weight loss varies depending on the duration of transportation and other factors. Body weight loss can also be influenced by several factors, including body heat expenditure through sweating, respiration, urination, and defecation, which sheep perform to maintain homeostasis (Xu et al. 2023). The supplementation of MHA and dextrose improved the condition of transported sheep by reducing body weight loss due to transportation., and this demonstrates that the combined supplementation of MHA and dextrose has a better effect than separate supplementation, as observed in previous studies (Baihaqi et al. 2022). MHA can prevent oxidative stress and plays a role in lipid metabolism by activating enzymes related to antioxidants (Elango 2020). Furthermore, methionine mitigates the negative impacts of heat stress on protein degradation by upregulating genes associated with protein synthesis and downregulating those involved in protein breakdown, thereby enhancing protein retention (Erfani et al. 2021). Methionine is an essential amino acid crucial for protein synthesis and methylation processes and as a precursor for cysteine synthesis (Martínez et al. 2017). However, methionine is often degraded by rumen microorganisms, reducing its availability for absorption in the small intestine. MHA, a more stable form of methionine, resists microbial breakdown in the rumen, ensuring a higher bioavailability of methionine for the animal (Solis-Cruz et al. 2022). This stability is particularly beneficial during transportation when sheep experience reduced feed intake and increased metabolic demands. Meanwhile, dextrose is a glucose monosaccharide that can be an energy source during transportation. Supplementing with dextrose ensures a readily available energy source, helping to maintain metabolic functions and prevent energy deficits that can lead to weight loss and weakened body condition. Therefore, combining MHA and dextrose can reduce stress and minimize weight loss during sheep transportation.

Transportation can cause body weight loss in livestock. The recovery period is the period needed for sheep to regain their initial body weight before transportation. Table 1 shows that sheep in the P2 treatment had a faster recovery time to their initial body weight than P1 sheep (P<0.05); this may be due to the higher daily weight gain in sheep in the P2 treatment compared to those in the P1 group. Methionine is an essential amino acid animals require for average growth and health. Animals must obtain methionine through their diet as they cannot synthesize this substance in their bodies. Methionine is one of the limiting amino acids for protein synthesis in growing ruminants (Wu et al. 2022). Protein synthesis in ruminant livestock produces peptides, amino acids, and ammonia (NH3). Protein is essential for animals, and a deficiency in amino acids can prevent protein synthesis, causing other amino acids to be converted into energy substrates or fats. The daily weight gain in sheep receiving MHA treatment is higher because damaged body tissues from transportation can be repaired more quickly. Therefore, the increased methionine amino acid in MHA sheep contributes to daily weight gain in sheep. Another factor is the administration of dextrose, a source of energy that livestock needs. Dextrose supplementation indicates that the energy stored by livestock is sufficient to support body weight gain. Energy sources such as rumen-protected sugar can alter the microbial diversity in the rumen, influencing both the fermentation pattern and microbial metabolism (Wang et al. 2021). The improvements observed with MHA and dextrose administration indicate that the welfare status of the sheep improves compared to those without additive administration.

Physiological responses of sheep

Physiological responses are indicators of stress and animal welfare in livestock. These can be measured through respiratory rate, heart rate, and rectal temperature. Physiological responses are reactions of livestock to various environmental changes. The physiological responses of the sheep in this study are shown in Table 2.

Table 2 shows that the P2 treatment resulted in significantly lower respiratory rates and heart rates compared to P1 (P<0.05) and was not significantly different from the control treatment (P>0.05). Transportation can induce stress in sheep, as evidenced by elevated heart and respiratory rates. The response is

Table 1. Body weight loss and recovery period of transported sheep among treatments

Variable	Control	P1	P2
Initial body weight (kg)	21.09±1.6[a]	22.43±2.1[a]	22.07±2.3[a]
Final body weight (kg)	20.45±1.5[a]	21.02±2.1[a]	21.28±2.7[a]
Weight loss (kg)	0.62±0.3[b]	1.41±0.3[a]	0.79±0.2[b]
Weight loss (%)	3.04±1.3[b]	6.33±1.5[a]	3.62±0.9[b]
Recovery time (days)	3.22±1.2[b]	5.55±1.1[a]	3.33±1.3[b]

Values within the same row followed by different letters are significantly different (P<0.05). Control= sheep that were not transported and without additives; P1= sheep transported without additives; P2= sheep transported with supplementation of MHA at 0.5 g/kg body weight and dextrose at a dose of 0.5 g/kg body weight

Table 2. Physiological responses of transported sheep among treatments

Variable	Control	P1	P2
Respiration rate (minutes^{-1})	110.91±8.6[b]	130.31±6.1[a]	113.07±4.3[b]
Heart Rate (minutes^{-1})	53.51±2.5 [b]	61.24±2.3[a]	54.52±2.1[b]
Rectal temperature (^{0}C)	39.40±0.10[a]	39.51±3.34[a]	39.70±2.25[a]

Values within the same row followed by different letters are significantly different (P<0.05). Control= sheep that were not transported and without additives; P1= sheep transported without additives; P2= sheep transported with supplementation of MHA at a dose of 0.5 g/kg body weight and dextrose at a dose of 0.5 g/kg body weight attributed to heat stress and the animals' attempts to dissipate body heat via respiration and blood circulation. Several studies have also documented increased heart and respiratory rates due to transportation (Navarro et al. 2020; Zhang et al. 2020). Supplementation of protected methionine in ruminants can reduce heat stress and improve the efficiency of thermoregulation (Davidson et al. 2024); this suggests that administering MHA and dextrose contributes to enhancing the welfare status of transported sheep.

Blood profile and metabolites of sheep

The hematological blood profile of the treated sheep can be seen in Table 3. Table 3 shows significant differences in neutrophil, lymphocyte profiles, and the neutrophil-to-lymphocyte (N/L) ratio ($P<0.05$). The N/L ratio is considered an indicator of stress levels in livestock (Carbillet et al. 2019). This study reveals significant differences between the control and treatment groups (P1 and P2). Transported sheep showed a significant increase in neutrophils and a decrease in lymphocytes, leading to a higher N/L ratio. These findings are consistent with the study by Marcato et al. (2021), which reported that transported calves experienced an increased proportion of neutrophils and a decreased proportion of lymphocytes in the blood.

The increase in neutrophils occurs because animals release cortisol in response to stress, triggering neutrophilia and increasing the number of neutrophils in the blood. The next step is lymphopenia, a decrease in blood lymphocytes, leading to an increased N/L ratio in animals experiencing transportation stress (Pascual-Alonso et al. 2017). In sheep receiving dextrose and methionine, an improvement in the N/L ratio was observed compared to the untreated group, indicating the positive impact of these treatments on transported sheep, with N/L values not significantly different from the control group (Table 3).

Blood metabolites of sheep

Blood metabolites, including glucose, creatinine, total protein, and cortisol hormone levels, are animal welfare indicators. This study's glucose and cortisol hormone profiles showed significant differences ($P<0.05$). The blood metabolite profiles are presented in Table 4. Table 4 shows that transported sheep receiving additives had significantly lower glucose and cortisol hormone profiles compared to sheep that did not receive additives ($P<0.05$). Sheep in P2 treatment also did not significantly differ from the control group's glucose or cortisol hormone levels ($P>0.05$), indicating improvement in sheep welfare status in P2. An increase in cortisol levels indicates stress in livestock. The increase in cortisol in P1 suggests that these sheep experienced more stress than the control and P2 groups, in line with the findings of Pascual-Alonso et al. (2017), which reported a doubling of blood cortisol levels in transported sheep. Stress in sheep leads to increased cortisol levels. Cortisol stimulates gluconeogenesis, the breakdown of glycogen in body tissues into glucose needed for energy, which can result in elevated blood glucose levels, as observed in this study. This observation is supported by Xiao et al. (2024), who also found that increased glucose levels were associated with elevated cortisol hormone levels.

Behavior of sheep

Behavior is a key indicator of animal welfare. Animals with good welfare status will express normal behavior. In this study, sheep behavior was observed for five hours after transportation. The behavioral data are presented in Table 5.

Table 5 indicates that sheep receiving the MHA and dextrose supplement exhibited a higher percentage of standing behavior compared to the control ($P<0.05$), which suggests that the MHA and dextrose supplementation

Table 3. Haematological blood profiles of transported sheep among treatments

Variables	Control	P1	P2
Haematocrit (%)	32.96 ± 2.7^{a}	34.98 ± 5.4^{a}	33.46 ± 5.6^{a}
Erythrocytes (10^5/dL)	10.22 ± 0.1^{a}	10.75 ± 1.5^{a}	9.84 ± 2.0^{a}
Haemoglobin (g/dL)	8.98 ± 0.7^{a}	9.41 ± 1.1^{a}	8.92 ± 1.4^{a}
Leukocytes (10^3/dL)	21.02 ± 6.5^{a}	29.01 ± 12.1^{a}	26.17 ± 6.4^{a}
Neutrophils (%)	63.66 ± 8.7^{a}	77.55 ± 9.4^{b}	72.11 ± 8.2^{ab}
Lymphocytes (%)	33.72 ± 4.1^{b}	22.00 ± 3.2^{a}	27.44 ± 7.5^{ab}
N/L Ratio	2.08 ± 0.8^{a}	4.42 ± 1.5^{b}	3.30 ± 1.4^{ab}

Values within the same row followed by different letters are significantly different ($P<0.05$). Control= sheep that were not transported and without additives; P1= sheep transported without additives; P2= sheep transported and supplemented with MHA at 0.5 g/kg body weight and dextrose at a dose of 0.5 g/kg body weight dextrose

Table 4. Blood metabolites profiles of transported sheep among treatments

Variable	Control	P1	P2
Glucose (mg/dl)	85.93±17.02[b]	119.30±26.34[a]	87.21±8.27[b]
Creatinine (mg/dl)	2.43±0.91[a]	1.60±0.87[a]	1.68±0.05[a]
Total protein (g/dl)	6.80±0.72[a]	6.13±1.19[a]	6.23±1.04[a]
Cortisol Hormone (ng/mL)	5.27±3.70[b]	16.78±3.67[a]	7.13±2.19[b]

Values within the same row followed by different letters are significantly different (P<0.05). Control= sheep that were not transported without additives; P1= sheep transported without additives supplementation; P2= sheep transported supplemented with MHA at 0.5 g/kg body weight and dextrose at a dose of 0.5 g/kg body weight

Table 5. Percentage of sheep behaviour during five hours after transportation among treatments

Behavior	Control	P1	P2
Eating	22.21±2.4[a]	16.92±4.3[a]	24.52±1.7[a]
Drinking	3.41±3.1[a]	6.07±2.6[a]	5.27±1.3[a]
Standing	39.17±2.5[a]	29.4±3.1[b]	40.02±4.7[a]
Walking	13.04±2.2[a]	7.92±1.1[b]	9.17±2.1[ab]
Resting	22.16±4.6[b]	39.68±5.3[a]	21.02±2.3[b]

Values within the same row followed by different letters are significantly different (P<0.05). Control= sheep that were not transported and without additives; P1= sheep transported without additives; P2= sheep transported with supplementation of MHA at 0.5 g/kg body weight and dextrose at a dose of 0.5 g/kg body weight

supplementation positively affects the energy levels and physical condition of the sheep. Consequently, P2 sheep could stand for longer periods compared to P1 sheep. It is hypothesized that P1 sheep lost more energy and experienced greater stress during transportation, resulting in a shorter standing duration than the control and P2 groups. MHA may act as an antioxidant to prevent transportation stress (Del Vesco et al. 2015), while dextrose provides additional energy to minimize energy loss during transportation. The resting behavior of P1 sheep was significantly higher than the control and P2 groups (P<0.05), indicating that P1 sheep expended more energy during transportation and required more rest afterward. Transportation stress can induce stress in sheep, and stressed sheep require longer rest periods to recover their energy. Resting behavior serves to conserve energy used by the animal (Pascual-Alonso et al. 2017). Post-transportation rest effectively lowers cortisol levels and helps animals recover from travel stress (Pascual-Alonso et al. 2017).

CONCLUSION

The supplementation of MHA and dextrose to sheep transported for 8 hours effectively reduced body weight loss, shortened recovery time, decreased respiratory and heart rates, and lowered blood cortisol and glucose levels. Furthermore, the sheep with these supplements exhibited better behavior than those without additives. These findings indicate that sheep receiving MHA and dextrose supplementation before transportation have a better welfare status than those without additives.

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