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Effects of Niacin Supplementation on Hematological Profile and Post-Transportation Recovery in Madura Cattle



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Abstract

THIS STUDY investigated the effects of niacin supplementation (1000 mg/head/day) on the recovery rate of Madura beef cattle after long-distance transportation. A total of 14 Madura bulls (body weight 224.64 ± 13.1 kg) were transported for 12 hours (911 km) and observed for 28 days post-transportation. Cattle were evenly divided into two treatments: control (n=7) and niacin supplementation (n=7). The parameters observed were feed intake, blood glucose level, and hematological profile. Data was analyzed using repeated measures, ANOVA, and independent sample t-tests. Niacin supplementation significantly increased (P<0.05) hematocrit at 14 days post-transportation and decreased monocyte counts on day 7. However, niacin did not affect (P>0.05) feed intake, body weight gain, blood glucose, and other hematological parameters. Over time, neutrophil and eosinophil percentages gradually increase (P<0.05). This study concludes that niacin supplementation at 1000 mg/head/day influenced hematological recovery by modulating monocyte and hematocrit levels but did not impact cattle performance and blood glucose levels. Higher supplementation or prolonged supplementation duration is required to enhance post-transportation recovery in Madura cattle.

Keywords: Immune modulation, Indigenous cattle, Niacinamide, Performance, Transport stress.

Introduction

Madura cattle are one of the most widely bred indigenous Indonesian cattle, which is a crossbreed between the bull and Bos indicus or Zebu cattle [1]. Madura cattle are centered on Madura Island, East Java, while most of the beef consumption in Indonesia is still in West Java. The duration of transportation for cattle constitutes a vital element that affects their welfare during the transit process. Transportation durations can be categorized into short (<4 hours), medium (4-8 hours), and long (>8 hours) intervals, each presenting unique challenges and stressors for the animals [2-3]. The condition of livestock post-transport can be affected by many factors, such as the position of livestock in the vehicle, the duration of livestock, the mode of transportation, and the ambient temperature during transportation [4]. The stress caused by this transportation process may change the plasma traits, performance, and physiological response of livestock, indicating stress and energy deficit [5-6]. Takemoto et al. [7] reported a notable shift in

multiple metabolic pathways, such as the citric acid cycle, nicotinate and nicotinamide metabolism, and propionate metabolism in steer calves after transportation. Moreover, hematological profiles, including leukocytes, neutrophils, and lymphocyte counts, are significantly affected by transportation [8]. It has been reported that the circulating blood cells required 28 days to return to normal values, while the full recovery may take longer after long-distance transportation [9-10].

Long-distance transport recovery period and energy metabolism regulatory capacity in beef cattle can be improved by dietary supplementation. Niacinamide or vitamin B3 (niacin) may play a crucial role in energy metabolism, functioning as a coenzyme nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) in numerous redox reactions in livestock [11]. It is involved in both anabolic and catabolic pathways, thereby affecting the synthesis of nucleic acids and lipids, as well as facilitating electron transfer in oxidation-reduction reactions for energy

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production and metabolic processes [12]. Niacinamide affects physiology and performance and plays a role in the metabolic pathways of amino acid and fatty acid synthesis, which are important for cattle growth. Niacinamide can be synthesized by rumen microbes in ruminants. However, only 2.7% of rumen microbes can produce multiple vitamins, including niacin, indicating a shortfall of niacin production under certain conditions [13].

Previously, Jinjiang bull cattle (BW 400 kg) supplemented with niacin at 800 mg/kg showed a potency to mitigate the adverse effects associated with heat stress and optimize the functionality of rumen fermentation as well as the production performance [14]. Alfaro et al. [15] also reported that niacinamide supplementation had a positive effect on swelling response and oxidative stress, two of the negative effects commonly found in livestock during transportation. long-distance Conversely, Flachowsky [16] reported that niacin supplementation at a dosage of 100 mg/kg dry matter or equal to 1 g/head/day when the body weight of the bull is lower than 300 kg, with a poor protein diet, and during the adaptation period is beneficial to improve cattle performance. Few studies have explored the niacin supplementation in native Indonesian cattle under long-distance transportation stress. Thus, this study evaluates the impact of 1000 mg/head/day niacin supplementation on feed consumption, hematology, and blood glucose during the recovery phase of Madura Cattle after 12 hours/911 km of transportation. This study provides valuable insight into how niacin supports posttransport recovery, serving as a reference for supplementation strategies aimed at improving dry matter intake, hematological profile, and blood glucose levels of indigenous Madura cattle. This study supports several Sustainable Development Goals by addressing practical interventions to improve livestock productivity and welfare, namely food security (SDG 2), promoting animal health and well-being (SDG 3), and contributing to more responsible and sustainable livestock production (SDG 12).

Material and Methods

This study was conducted in Pamekasan Regency, Madura Island, Indonesia, and the Laboratory of Meat and Draught Animal Nutrition at the Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia.

Animals and experimental design

Fourteen Madura cattle (age 2 years and $224.64 \pm 13.10 \text{ kg BW}$) were obtained from the auction sale at a local livestock market in Pamekasan Regency, Madura Island, Indonesia. After the auction, cattle were deprived of feed but had unrestricted access to water for approximately 1 hour before being loaded

into a trailer for transportation. The feeding management at the rearing farms was not determined. Before transport, the cattle's body size, including chest circumference and body length, was measured using a measuring tape to estimate the cattle's body weight following the Winter Indonesia equation [17]. The cattle were transported from Pamekasan Regency, Madura Island, to the Faculty of Animal Science, IPB University, Bogor, Indonesia. The transport distance was 911 km, which could be covered within 12 hours. The trailer drove at 40-50 km/h on roads and 90-100 km/h on highways.

After arrival, cattle were housed in individual cages equipped with feeders and drinkers. The cattle were randomly allocated into one of two experimental treatments, i.e., P0 = control (n=7) and P1 = niacin supplementation 1000 mg/head/day (n=7) and subsequently evaluated for 28 days. Unprotected niacinamide was obtained from Jubilant Ingrevia, India. Both groups were given a diet consisting of commercial concentrate and forage (Pennisetum purpureum or rice straw). The nutrient composition of the diets is shown in Table 1. Cattle were fed 3-4% of their body weight, and water was given ad libitum. Feeding was carried out four times, namely: concentrate was given at 08.00 AM and 2.00 PM, whereas forage was given at 10.00 AM and 4.00 PM. The amount of feed offered and refused was recorded daily for each cattle to calculate daily feed intake.

The amount of niacin supplementation at 1000 mg/head/day was based on recommendation for low-body-weight cattle [16]. The niacin was administered daily for 28 days after transportation. A niacin supplement of as much as 1000 mg was dissolved in 10 mL of distilled water; then, it was administered orally using a syringe at 2 hours after morning forage feeding.

Hematology and blood glucose profile

Blood samples were obtained from the jugular vein using vacuum tubes preloaded with ethylenediaminetetraacetic acid (EDTA) after 2 hours of morning feeding weekly. Blood samples were divided into 2 parts for hematology and glucose analysis. Blood samples for hematology analysis were directly used to assess hematocrit, hemoglobin, erythrocytes, and leukocytes and their differentiation following Sastradipradja et al. [18]. Meanwhile, blood samples for blood glucose analysis were centrifuged at 3000 rpm for 15 minutes to obtain plasma. The blood glucose was assessed using a commercial kit (cat. no 112191) from Greiner Diagnostic, following the company's instructions.

Statistical analysis

No priori power calculation was performed due to logistical constraints; sample size was determined by

the available animals (n=7/group). The statistical analysis was performed using IBM SPSS 26.0 (SPSS, Chicago, IL). The data on body weight at post-transportation were analysed using independent sample t-test to compare the effect of niacin supplementation. The data, including dry matter intake of forage and concentrate, blood glucose concentration, and hematology profile, were analyzed with Repeated Measures two-way ANOVA between treatments (control niacin supplementation) compared with the number of days in the experimental period (7, 14, 21, and 28). The effect of treatment on each week (day 7,14,21, and 28) of the experimental period was analysed using an independent sample t-test. A significant result was defined at P<0.05.

Results

Feed intake and daily body weight gain

Niacin supplementation did not significantly affect (P>0.05) the concentrate and forage dry matter (DM) intake throughout the experiment (Figure 1). However, the DM intake of concentrate and forage was gradually increased (P<0.001) over time. Post long-distance transportation, cattle undergo metabolic recovery and microbial balance within the first two weeks [10]. Our study found a significant increase (P<0.05) in forage intake in weeks 3 and 4 after transportation compared to week 1, while a concentrate intake was significantly increased (P<0.05) in weeks 2, 3, and 4 compared to week 1 post-transportation.

Both treatment groups experienced a 21% weight loss following transportation (Table 2), primarily due to dehydration, feed intake reduction, and increased metabolic energy expenditure [19]. However, niacin supplementation had no significant effect (P>0.05) on body weight recovery of cattle after 28 days post-transportation (Table 2).

Blood glucose and hematology profile

Niacin supplementation did not significantly alter (P>0.05) blood glucose levels of Madura cattle compared to the control group, but it changed significantly (P<0.01) throughout the experiment (Figure 2A).

Niacin supplementation increased (P<0.05) haematocrit percentage on day 14 post-transportation (Figure 2C), but no treatment effect (P>0.05) was detected on haemoglobin, haematocrit, and erythrocyte counts (Figure 2B, 2C, 2D) throughout the experiment. Both treatment groups showed a significant decline (P<0.05) in haemoglobin, hematocrit, and erythrocyte counts throughout the experiment.

Niacin supplementation significantly decreased (P<0.05) total leukocyte counts, and changes (P>0.01) throughout the experiment (Figure 2E).

By day 7, total leukocytes were lower (P<0.05) in the niacin group compared to the control. Further, total leukocytes in both groups increased (P<0.05) on day 14 before returning to the baseline on days 21 and 28.

Throughout the 28-day recovery period, Madura cattle exhibited significant leukocyte responses that reflect the progression from acute stress to immune adaptation. Niacin supplementation did not change (P>0.05) the percentage of lymphocytes (Figure 3), and it was consistently within the normal range throughout the experiment, indicating that adaptive immune capacity was maintained despite transportation stress. In this study, basophil levels showed variation (P>0.05)by supplementation and over time, suggesting minimal involvement in early stress Supplementation of niacin did not change (P>0.05) the percentage of neutrophils and basophils (Figure 3). Over time, neutrophil and eosinophil percentages increased (P<0.01). Neutrophil significantly percentages gradually increased from day 7 to 28, compared to baseline, while the percentage of eosinophil's started to increase on days 14 and 21 before returning to baseline. These results reflect the innate immune system's sustained engagement in homeostatic re-establishment following the acute phase of stress.

Monocytes, key mediators of inflammation, declined sharply (P<0.01) by day 14 compared to baseline. Niacin supplementation at mg/head/day accelerated the decline (P<0.05) of monocytes, which was significantly lower on day 7. did not influence (P>0.05)neutrophil/lymphocyte (N/L) ratio, while it rose progressively (P<0.001) from day 7 to 28. This result indicates a shift in the innate immune system that was more dominant during the recovery process after transportation.

Discussion

Transportation stress can adversely affect cattle welfare by triggering physiological stress responses, anxiety, reducing dry matter intake, and inducing body weight loss, leading to significant economic losses for producers [20-22]. In this study, both forage and concentrate intake did not change due to niacin supplementation. Our results are in line with Zou et al. [14] that niacin supplementation up to 800 mg/kg did not alter the feed intake of cattle. While niacin may alleviate the stress symptoms, it does not directly imply changing the feed intake [23].

In this study, daily forage and concentrate intake gradually increased after the first week post-transportation. This result is consistent with [24], who reported a significant decrease in feed intake during the first week of post-transport, followed by a progressive increase and more stable levels in the subsequent weeks. The gradual increase in feed

intake may also indicate an adaptation process since the Madura cattle in this study were previously fed only fresh forage and rice straw. These findings suggest that while niacin supplementation at 1000 mg/head/day may alleviate stress symptoms, it does not directly influence forage or concentrate DM intake in Madura cattle.

Our results are in line with Luo et al. [25], who found niacin supplementation up to 640 mg/kg did not show a significant effect on body weight and feed intake of growing cattle. Despite this, niacin plays a crucial role in growth performance and feed efficiency by maintaining the rumen environment and enhancing nutrient digestibility, particularly crude protein utilization [25-26]. Additionally, niacin regulates lipid metabolism, improving energy availability, while also promoting anti-inflammatory and antioxidant properties, and vasodilation, which can be beneficial for post-transportation recovery [14-15]. Though the niacin supplementation improved the inflammatory responses transportation in the current study, the energy requirements and metabolism disruptions may counteract these advantages, resulting in no changes in average daily gain (ADG). Given its influence on energy metabolism, higher supplementation levels or prolonged supplementation periods may be necessary to enhance cattle recovery and growth. This study concludes that 1000 mg/head/day may not be sufficient for significant body weight recovery, and future research should explore optimal dosages and supplementation durations to maximize niacin function on cattle performance post-transportation.

The transportation process is energetically demanding, as evidenced by alterations in certain blood metabolites [27]. Blood glucose, a key metabolic stress indicator in cattle, fluctuates in response to catecholamine and cortisol release during transportation stress, which increases insulin activity and glucose uptake, while it reduces fat mobilization [28-30]. In this study, the blood glucose levels remained within the normal range (45-75 mg/dL) [31], aligning with Luo et al. [23], who reported no change in serum glucose with niacin supplementation up to 640 mg/kg. This suggests that cattle were not in a negative energy balance or at risk of ketosis, as niacin deficiency correlated with glucose metabolism and the incidence of ketosis [32].

The blood glucose levels declined during the first two weeks (day 7 and 14), followed by an increase in later weeks, reflecting the physiological response to stress-induced energy deficits. Initially, transportation stress induces stress hormone releases such as cortisol and adrenaline, which elevate blood glucose by stimulating glycogenolysis and gluconeogenesis [33]. However, as homeostasis is restored, glucose levels decline due to increased energy expenditure, reduced feed intake, and glycogen depletion, which may be observed in

several days following transportation [34-35]. Additionally, dehydration and prolonged feed withdrawal cause energy deficit, contributing to low blood glucose. The subsequent increase in glucose levels in later weeks suggests metabolic recovery as feed intake stabilizes. Despite the role of niacin as a precursor for NAD and NADP in energy metabolism, the supplementation of 1000/mg/head/day was insufficient to significantly impact the post-transport recovery, suggesting that higher dosages may be required to enhance metabolic adaptation and support post-transport recovery.

The haemoglobin, haematocrit, and erythrocyte count observed in this study were within the normal range for cattle, as reported by Marjory et al. [36]. During transport, the cattle went through a fasting process leading to dehydration and stress-induced physiological responses. The stress may increase cortisol levels, which may affect haematological parameters [37-39]. Previously reported transportation stress induced a temporarily reduced body weight and altered blood volume, red blood cell turnover, and erythropoiesis in cattle [40-41]. The increase of haematocrit at day 14 after transportation suggests that niacin can support haematological recovery by enhancing metabolic response and reducing oxidative stress [42]. However, previous research suggested that the effects of niacin are doseand time-dependent, indicating higher doses or prolonged supplementation may be required to sustain the haematological improvements [32].

In this study, leukocyte counts were within the normal range for cattle $(4.4\text{-}10.8 \times 10^3)$ as reported by Marjory et al. [36]. The early increase of total leukocytes in the control group reflects the acute stress response to transportation, due to cortisol-mediated immune activation [43]. The significantly lower leukocyte counts in the niacin-supplemented group on day 7 suggested that niacin may impair the acute stress-induced leukocyte surge through its anti-inflammatory and antioxidant properties. Previously reported, niacin inhibits leukocyte myeloperoxidase release and activity, which is essential for reducing oxidative stress and inflammation [44].

Neutrophils mobilized rapidly from bone marrow in response to cortisol and stress-induced immune activation [21]. The sustained elevation until day 28 suggests an ongoing immune modulation process, possibly linked to tissue repair and homeostasis restoration [10]. Meanwhile, eosinophils are more sensitive to stress hormones and typically decline under acute stress but increase later as the immune balance is restored [45]. The delayed rise in eosinophil percentage at day 14 suggests that the immune system prioritizes restoring neutrophils before eosinophils, reflecting their secondary role in post-stress immune recovery [21].

Monocytes play a critical role in early immune activation and inflammation, which are rapidly mobilized in response to stress [45-46]. The significant decline within the first 7 days suggests that niacin supplementation at 1000 mg/head/day accelerates inflammatory resolution through its antiinflammatory and antioxidant properties, which reduce oxidative stress and inflammatory signaling in monocytes and macrophages [47]. This aligns with van de Wouw et al. [48], who reported that niacin facilitates the return of monocyte levels to baseline by mitigating oxidative stress and limiting excessive immune activation. As the calves recover from transportation stress, the impact of niacin on monocytes stabilized, leading to no difference in monocyte numbers after the initial period [7].

Our results highlight the complex immune modulation process following transportation stress, where monocytes respond rapidly, modulated by niacin, followed by a more gradual response in neutrophils and eosinophils as part of the longer-term adaptation to transport stress. Our study indicates niacin supplementation at a dosage of 1000 mg/head day accelerates monocyte reduction but does not affect other leukocyte significantly suggesting higher dosages or prolonged supplementation might needed to influence the broader immune response of transported cattle.

Conclusion

In conclusion, niacin supplementation at 1000 mg/head/day contributed to early hematological

responses, such as a decrease in total leukocytes and monocyte percentage, and a gradual increase in neutrophil and eosinophil, as well as increased hematocrit levels at two weeks post-transportation. However, the niacin supplementation at 1000 mg/head/day was insufficient to improve feed intake, body-weight recovery, and blood glucose over 28 days. Further research with higher supplementation levels or prolonged supplementation duration with additional physiological biomarkers should be explored to define practical supplementation strategies to enhance overall cattle performance post-transportation.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The Directorate of Management approved the experimental procedure for Research and Innovation Permit and Scientific Authorities, National Research and Innovation Agency of Indonesia (ethics approval number; 214/KE.02/SK/12/2023).

TABLE 1. Chemical composition of feed (%DM)

Nutrient	Rice straw	Elephant grass (Pennisetum purpureum)	Concentrate
Ash*	18.43	16.61	3.92
Crude protein (CP)*	8.32	9.75	10.77
Ether extract (EE)*	2.65	1.48	3.08
Crude fiber (CF)*	29.32	29.46	14.60
Nitrogen-free extract (NFE)*	41.28	42.70	67.63
Total digestible nutrients (TDN)	46.95 ^a	52.21 ^b	50.21 ^c

^{*} NIRS analysis by Animal Logistics Indonesia Netherlands Laboratory (2023) $^aTDN = -17.2649 + 1.2120 \text{ CP} + 0.8352 \text{ NFE} + 2.4637 \text{ EE} + 0.4475 \text{ CF} [49], <math>^bTDN = -21.7656 + 1.4284 \text{ CP} + 1.0277 \text{ NFE} + 0.4867 \text{ CF} + 1.2321 \text{ EE} [49], <math>^cTDN = 40.2625 - 0.1379 \text{ CF} + 1.1903 \text{ EE} + 0.4228 \text{ NFE} + 0.1969 \text{ CP} [49].$

TABLE 2. Effects of niacin supplementation on the body weight of Madura cattle during the post-transportation recovery period

Parameters	Pre-	Post-transportation				
	transportation BW (kg)*	Day-0 BW (kg)	Day-28 BW (kg)*	Weight loss post- transportation (%)	ΔDay-0 and Day- 28 BW (kg)	P- value
Control	289.65 ± 24.02	226.71 ± 14.19	295.64 ± 22.59	-21.73	68.92 ± 17.32	0.064
Niacin	282.99 ± 27.45	222.57 ± 12.66	274.34 ± 14.11	-21.35	51.77 ± 16.27	0.004

^{*} Body weight estimation uses the Winter formula in Ni'am et al. [17]; Day-0 was designated as the day the animals arrived at the experimental facility, BW = body weight.

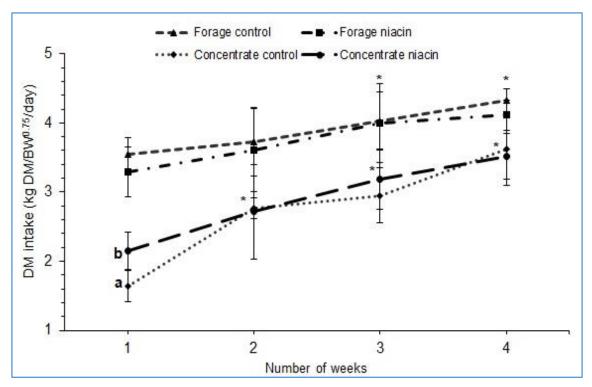


Fig. 1. Effects of niacin supplementation on daily dry matter intake of Madura cattle during the post-transportation recovery period. DM = dry matter; BW = body weight. * Significant differences (P<0.05) were observed in comparison to week 1. Significant differences between treatments at the designated week (P<0.05).

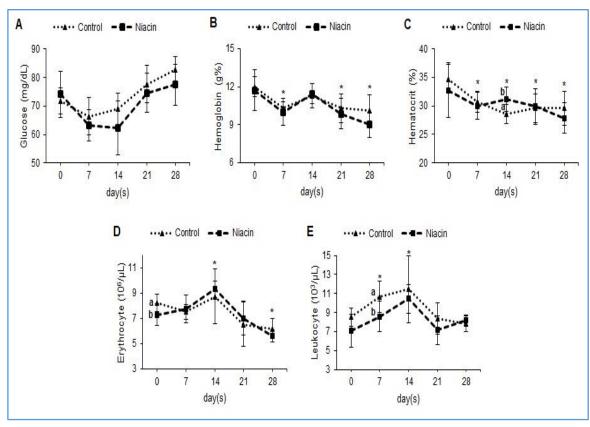


Fig. 2. Effects of niacin supplementation on blood glucose (A), hemoglobin (B), hematocrit (C), total erythrocyte counts (D), and total leukocyte counts (E) of Madura cattle during the post-transportation recovery period. Day-0 was designated as the day the animals arrived at the experimental facility. *Significant difference (p<0.05) in comparison to day 0; a,b Significant difference (p<0.05) between the treatments.

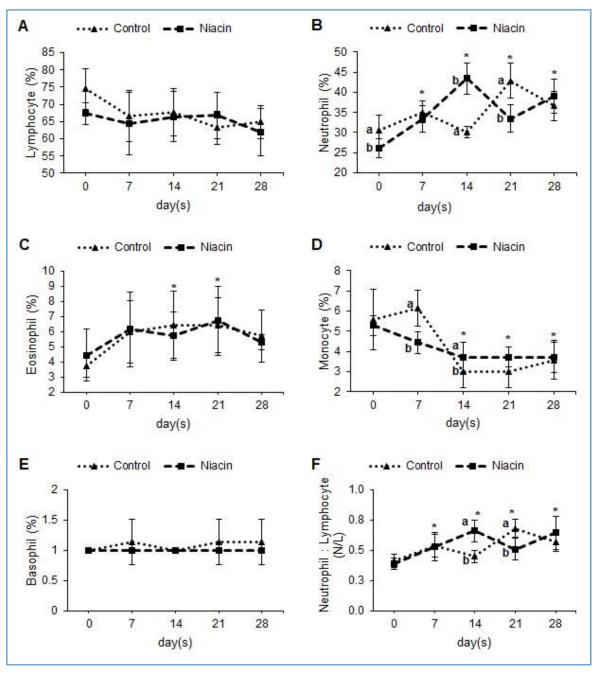


Fig. 3. Effects of niacin supplementation on the percentage of lymphocyte (A), neutrophil (B), eosinophil (C), monocyte (D), basophil (E), and neutrophil-lymphocyte ratio (F) of Madura cattle during the post-transportation recovery period. Day-0 was designated as the day the animals arrived at the experimental facility. * Significant difference (p<0.05) in comparison to day 0; a,b Significant difference (p<0.05) between the treatments.

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