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Effects of Road Transportation on Some Physiological Stress Measures in Anpera and Boerka Goats

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ABSTRACT

Transporting livestock is a common practice in agriculture, but it can be a stressful situation for the animals. This stress can lead to physiological responses like live weight loss, changes in blood cortisol levels, and immune suppression. Goats are particularly sensitive to transportation stress and stress-induced diseases. In this study, we investigated the effects of road transport on the physiological condition of 33 goats (13 Anpera goats and 20 Boerka goats) transported for 72 hours over approximately ± 2.000 km. Live weight loss was determined by comparing the body weights of the goats before and after transportation. We measured psychological responses (heart rate, respiratory rate, rectal temperature), blood parameters (Blood urea nitrogen/BUN, creatinine, glucose), cortisol, and hematological parameters. The results showed that transportation caused an average weight loss of 4.05 kg/head (14.22%). Heart rate decreased by 14.71 x/minute after transport, while respiratory rate and rectal temperature increased by 8.44 x/minute and 0.41 units, respectively. The BUN levels increased by approximately 10.92 mg/dL (35%) and glucose levels increased by approximately 30.07 mg/dL (48%). Hematological parameters also showed significant changes, with transportation causing alterations in WBC, RBC, hemoglobin, hematocrit, MCV, and MCH levels. The study also showed an interaction between the sex and breed of goats on BUN, glucose, MCV, MCH. The comparison between Anpera goats and Boerka showed that transportation had significant influence on heart rate, rectal temperature, cortisol, RBC, haemoglobin, RDW. In conclusion, transportation for 72 hours caused stress on goats, leading to weight loss, changes in psychological behavior, and blood parameters, and the breed and sex of the goats played a crucial role in the observed changes.

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Introduction

The practice of transporting live animals, which is unavoidable in animal husbandry, has been acknowledged as a significant contributor to animal stress. Physical, psychological, and climatic factors affect animals that are transported by road. Animals under transportation are exposed to physical stressors such as confined spaces, restricted mobility, limited access to feed and water, and aggressive behaviors when mixed with unfamiliar animals (Miranda-de la Lama *et al.*, 2014; Fernandez-Novo *et al.*, 2020; Kumar *et al.*, 2023). Transportation stress has considerable physiological effects such as increased adrenal cortical activity, decreased immunity, increased morbidity, decrease in meat quality and weight loss and in severe cases, mortality caused by infectious diseases (Saeb *et al.*, 2010; Nikbin *et al.*, 2016; Alcalde *et al.*, 2017; Naldurtiker *et al.*, 2023). As a result, transportation stress has both economic and

animal welfare concerns, has drawn significant attention in recent years.

Anpera and Boerka goats are two different types of goats with different genetic and physiological characteristics. Anpera goats are a cross between male Anglo Nubian goats and female Ettawa grade goats and are primarily used for dairy purposes, while Boerka goats are a crossbred between male Boer goats and local female goats and are mainly used for meat production. Despite these differences, both types of goats are commonly transported for various purposes. Numerous studies have been reported that goat does not endure transportation stress well during long journey (Minka and Ayo 2013; Alcalde *et al.*, 2017). According to Minka and Ayo (2013), prolonged road transportation had negative impacts on the muscles and nervous system of goats. Their findings, recorded in goats transported for 12 hours, were compared to a previous study by Ayo *et al.* (2006) that examined the psychological effects of 3-hour road transportation on goats, while

another study by Kannan *et al.* (2000) did not find any changes in the excitability of Spanish goats after a 2-hour transportation period. Furthermore, a study conducted by Alcalde *et al.* (2017) who reported the level of hormone cortisol was found to be higher during long transportation (lasting 6 hours) compared to short transportation (lasting 2 hours), with levels measuring at 42.4 and 46.9 (ng/mL), respectively.

The effects of transportation stress on goats can also be influenced by intrinsic factors such as sex, breed, and the temperament of the animal, as highlighted in various studies (Hughes *et al.*, 2013; Brown and Vosloo 2017; Biobaku *et al.* 2018; Sowińska *et al.* 2020). There is paucity of literature and information available on the effect of long-distance travelling stress on hormone cortisol, blood biochemical levels, and hematology parameters on these goats in tropic climatic condition. Therefore, the current study was aimed to determine the effects of 72 hours of road transportation on physiological condition of Nubian and Boerka goats and the probable effect of sex in these breeds. The findings from this research for providing better insights in the understanding of the stress dynamics in transported Anpera and Boerka goat that is can be used to develop transport management protocols that are tailored to the specific needs of dairy goats and meat goats and can help to reduce the negative impact of transportation on the welfare of goats in both dairy and meat production systems.

Materials and Methods

This study was performed in April 21st 2021 on 13 clinically healthy Anpera goats (3 male and 10 non-lactating non-pregnant female goats, average body weight of 18.71±1.61 kg, age range: 11-22 month) and 20 clinically healthy Boerka goats (10 male and 10 female, average body weight of 31.48±0.77 kg). The animals were managed under the intensive system at the Goat Research Unit at Research Institute for Animal Production, Bogor and Indonesian Goat Research Institute, Galang. Livestock was carried out 72 hours (three days) road travelling with the distance traveled was ±2.000 km.

Experimental design

All goats were blood sampled, observed physiological responses (respiration rate, heart rate, rectal temperature) and weighed just before loading onto a truck and after loading to assess shrinkage (72 hours transportation). Blood sample was taken for investigation level of glucose, creatinine, blood urea nitrogen, and hematology. On April 21, 2021, Anpera goats were transported from the Research Institute for Animal Production (-6.213202568975772 °N, 106.84258545464388 °W) and arrived at Bintang Domba Farm (3.4624161559046835 °N, 99.41582770925093 °W), on April 24, 2021 (Figure 1). Meanwhile, Boerka goats were transported from the Indonesian Goat Research Institute (3.417468773161844 °N,

98.88443346783495 °W) on 28th April 2021, and arrived at the Research Institute for Animal Production on 1st May 2021. The goats were transported by trucks on road transportation, and the trucks also crossed the Sunda strait (Banten—Lampung) with a ferry (approximately 2-3 hours). The truck's carrosserie is divided into four compartments using wooden and bamboo blocks, with each compartment measuring approximately 125 cm x 100 cm. Thirteen Anpera goats were transported along with 12 other Anpera goats, but the 12 Anpera goats were not included in the observation of this study. The goats are fed twice a day (morning and afternoon) with elephant grass and field grass, and they are provided with water before and after transportation.

Physiological measurement

Physiological components measured were rectal temperature, respiratory rate, and heart rate. Physiological responses were assessed at the initial location 24 hours prior to loading for transportation, and subsequently measured 1-2 hours upon arrival at the destination. The respiration rate of goats was measured based on the flank movement (x/minute), heart rate (x/minute) was measured using stethoscope, and rectal temperature (°C) was recorded using a rectal thermometer by inserting the thermometer about 1 inch into the rectum until stable.

Blood sample collection

The first samples of blood were taken at the farm before transportation (24 hours before loading, in the afternoon). The second samples were taken after transport or 1-2 hours arrived at the destination. For the Anpera goats, the second blood samples were collected at night, while for the Boerka goats, they were collected in the afternoon. Five milliliter of blood was taken, using a 21-gauge needle, from the jugular vein in each extraction and drawn into EDTA and serum tubes (BD Vacutainer Systems).

Hematological and biochemical serum analysis

Blood samples for haematology and biochemical analysis were analysed at the Clinical Pathology Laboratory, Primate Animal Studies Center, IPB University. The parameters hematological determined were red blood cell count (RBC), white blood cell count (WBC), platelets, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW). The parameters serum biochemical determined were glucose levels (mg/dL), creatinine (mg/dL) and blood urea nitrogen (BUN) (mg/dL).

Blood samples for cortisol analysis were collected in the serum tubes and put in an icebox. It was then centrifuged for 10 minutes at 3000 rpm to separate the serum from other components. The serum was pipetted into clean centrifuge tubes and stored at -20°C until the assay was conducted.

Prior to analysis, the serum was allowed to thaw at room temperature, and hydrochloric acid (HCl) was added to balance the pH to 1.5-2.0. Methylene chloride was then added to the sample and mixed thoroughly using a vortexer. After allowing the mixture to form a separated layer, the lower layer containing the cortisol was pipetted into a clean tube. The extracted cortisol was then dissolved in ELISA buffer and analyzed using a commercial Goat Cortisol ELISA Kit 96T (BT-LAB cat. E0021Go) according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using analysis of variance according to a randomized block design with two factors (sex and breed). The individual goat was considered as the experimental unit. Statistical significance for all the conducted analyses was set at $p < 0.05$, and the means were further tested using Duncan's multiple range test. All statistical analysis were performed using SAS software version 9.0.

Results and Discussion

Physiological response and weight loss

The study investigated the effect of transportation on the live body weight of goats for 72 hours (Table 1). The results showed that transportation had a significant effect ($P < 0.01$) on the live body weight of goats, with a live weight loss of 4.05 kg/head (14.22%) after transportation. However, there was no interaction ($P < 0.05$) between breed and sex on live body weight loss after transportation. The comparison between Anpera goats or dairy type and Boerka goats or meat type groups also showed that transportation had no significant ($P < 0.05$) influence on live body weight loss. Similarly, there was no significant difference detected in the impact of transportation between male and female goats ($P > 0.05$). The loss live body weight of goats in this study was likely due to deprivation of feed and water in transportation. According to Minka and Ayo (2013), the reduction in liveweight of animals during transportation can be attributed to an increase in urination and defecation, which is thought to be triggered by cortisol, as well as loss of body water due to panting and sweating in high temperature and humidity, and prolonged periods without food and water (Kannan *et al.*, 2000). These findings are consistent with a previous study on Boer goats transported by road for 3.5 hours with a density of 0.4 m² and 0.2 m², which showed a loss of live body weight of 0.44-1.36% (Nikbin *et al.*, 2016). The study also reported that the transported animals' group had less carcass shrinkage, about 0.19-0.24 kg/head, than the non-transported animals' group. Likewise, Ambore *et al.* (2009) demonstrated that goats transported for 12 hours over a distance of about 350 km revealed significant decrease about 5.77% in the live body weight than their pre-load weight.

The current study investigated the effect of transportation on the physiological response of goats, and the results showed a significant effect

($P < 0.01$). The heart rate decreased by 14.71 x/minute, while the respiratory rate and rectal temperature increased by 8.44 x/minute and 0.41 units, respectively. There was no interaction between the sex and breed of goats on heart rate, respiratory rate, and rectal temperature ($P > 0.05$). However, a significant difference was found in heart rate and rectal temperature in the main factor breed and sex ($P < 0.05$) between Anpera and Boerka goats as well as between male and female. The study showed that transportation stressors can affect the change of physiological responses of goats. Interestingly, the heart rate was significantly decreased 45.64 x/minute in Anpera goats after transportation but increased 5.40 x/minute in Boerka goats. These findings however contradictory with the reports of Ambore *et al.* (2009) in Osmanabadi goats, who reported an increase in heart rate about 18.7 x/minute after transport for 12 hours over a distance of about 350 km, but similar with Minka and Ayo (2012) in Red Sokoto goats after 12 hours transportation.

However, the authors explained there was significant increase in heart rate 20.6-48.4 x/minute after 2-10 hours of journey that the greater increased at 6 hours after transport. Our study also similar with Minka and Ayo (2012) in increasing respiratory rate 19.5-52 x/minute that the the greater increased at 6 hours after transport. The significant ($P < 0.01$) increase in rectal temperatures obtained in the goats were in the normal range of 38.5-40.0°C. In our study, the rectal temperature was increased 0.57 unit in Boerka goat after transportation, but a similar rectal temperature on Boerka goats at before and after transportation. Similarly, Ambore *et al.* (2009) demonstrated that an increase in rectal temperature about 2 unit (°F) after transportation. Minka and Ayo (2012) reported that an increase 1.2 unit (°C) in rectal temperatures after a 12-hour transportation of Red Sokoto goats.

This suggested that changes in heart rate, respiratory rate, and rectal temperature after transport could be attributed to the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic-sympathetic nervous system (Brown and Vosloo 2017; Hong *et al.*, 2019). These physiological responses are thought to be initiated by the physical and psychological stressors that animals encounter during transportation. The activation of the HPA axis leads to the release of stress hormones, cortisol, which mobilize energy and increase cardiovascular and respiratory responses (Brown and Vosloo, 2017). The hypothalamus-sympathetic nervous system recognizes external stressors via signals in the cerebral cortex and secretes catecholamines in the adrenal medulla and brain (Hong *et al.*, 2019), which stimulate the heart to beat faster and cause the bronchial muscles in the lungs to relax. Moreover, transportation stress can cause dehydration, electrolyte imbalances, and acid-base disturbances. Thus, the increase in respiratory rate observed during the same period might be due to the animal's mechanism for eliminating as much heat as possible through enhanced respiratory

vaporization and greater energy requirement associated with the physical activity during transport stress procedures.

Biochemical parameters serum

Effects of transportation on goats' blood biochemical parameters are shown in Table 2. The results, presented in Table 2, showed a significant increase in almost all blood biochemical parameters except creatinine ($P < 0.01$). The study found that BUN levels increased by approximately 10.92 mg/dL (35%) and glucose levels increased by approximately 30.07 mg/dL (48%) after transportation. The study also showed an interaction between the sex and breed of goats on BUN and glucose ($P < 0.01$). In contrast to the findings of Ekiz and Yalcintan (2013), who reported that pre-slaughter stress did not have a significant effect on plasma glucose concentrations in blood among Saanen, Maltese, Gokceada, and Hair Goat Kids breeds, but did have a significant effect on creatinine (CK) concentration. The author was observed that maltese kids had higher plasma CK level than Saanen, Gokceada and Hair Goat kids.

The BUN and glucose levels decreased in male Anpera goats, but not in other groups. In contrast, male Boerka goats showed a significant increase in BUN concentration, while female Boerka goats showed a significant increase in glucose concentration. These findings are in consistent with a previous study by Tajik *et al.* (2016) reported that no significant difference between two sexes of Raini goats in glucose level after transport. However, regardless of sexes, this finding is similar with previous studies, which is increased glucose after transport (Minka and Ayo, 2014; Sowińska *et al.*, 2020; Raghazli *et al.*, 2021). Minka and Ayo (2014) reported that a significant increase in glucose levels on goats after 12 hours transport with total distance about 600 km. Likewise, Sowińska *et al.* (2020) observed increasing level of serum glucose 3.8 mmol/L (100%) in goat kids after transport.

Some studies have reported an increase in glucose levels and a decrease in creatinine levels after transportation (Kumar, 2014; Mir *et al.*, 2018), while others have reported no significant effect on serum glucose levels (Tajik *et al.*, 2016) or creatinine (Raghazli *et al.*, 2021). Indeed, a study also reported an increase in glucose levels and creatinine on suckling goats kids transport 2 and 6 hours (Alcalde *et al.*, 2017). Suggesting that transportation can have varying effects on BUN, glucose, and creatinine levels in goats, depending on the transportation distance, season, nutrition, and breed of goat. In general, transportation tends to cause an increase in glucose, creatinine, and BUN levels in blood, indicating a stress response in the animals. In addition, dehydration and decreased feed intake during transportation can also contribute to changes in these blood parameters. The increase in serum glucose during the post-transportation period may be due to increase in glycogenolysis, stimulated by increased secretions of catecholamine and glucocorticoid

hormones, which are under the control of the sympathetic nervous system (O'Neil, 2019; Paravati *et al.*, 2023). Alcalde *et al.* (2017) reported increased blood CK after 2- and 6-hours transport was detected, compared with initial levels on-farm, as might be expected in animals transported under high summer temperatures. The authors noted that as a response to stress elevation of creatinine concentration, as well as the BUN (Kannan *et al.*, 2000), was assessment of muscular damage during transport.

Cortisol

Transportation is known to be a stressful experience for animals and is associated with changes in several physiological parameters, including an increase in cortisol levels. As shown in Table 2, there is significant changes in cortisol concentration were observed after transport in goats in this study. The study found that cortisol levels increased by approximately 80.07 ng/dL (36%) after transportation. Although there was no significant interaction ($P < 0.05$) between breed and sex on cortisol concentration after transportation, different changes were observed in cortisol levels between Anpera and Boerka goats. A significant increase in cortisol concentration was observed in Boerka goats ($P > 0.01$), and male groups also had an increase in cortisol concentration ($P < 0.05$). This is indicated that male goats are more excitable and stress sensitive than female animals and may have higher serum concentrations of cortisol in the same conditions. However, there were few previous studies about the physiological response to transport stress in different sexes. There also have been reported that no significant difference between two sexes in serum concentration of cortisol (Tajik *et al.*, 2016). However, regardless of sexes, previous studies have reported similar findings, with increased cortisol concentration observed in goats after transport (Alcalde *et al.*, 2016; Tajik *et al.*, 2016; Mir *et al.*, 2018; Sowińska *et al.*, 2020). This result is contrary to previous studies, where no differences (Raghazli *et al.*, 2021), were observed in goats prior to and after transport.

Differences between the present study and the previous study could be due to differences in the duration of transportation, age, sex, and breed of goats. Moreover, the adaptation of animals during transportation can lead to fear reduction, thus lowering cortisol secretion in animals (Kannan *et al.*, 2007). Differences between studies could be also due to differences exogenous and endogenous stressors that described by Brown and Vosloo (2017). The increase in cortisol levels caused by handling or transport is thought to be associated with the activation of the hypothalamic-sympathetic nervous system hypothalamic-pituitary-adrenal (HPA) axis. Specifically, during stress, the hypothalamus releases vasopressin (VP) and corticotropin-releasing hormone (CRH), which stimulates the pituitary anterior to secrete adrenocorticotrophic hormone (ACTH). In turn, ACTH stimulates the adrenal cortex to elevate the

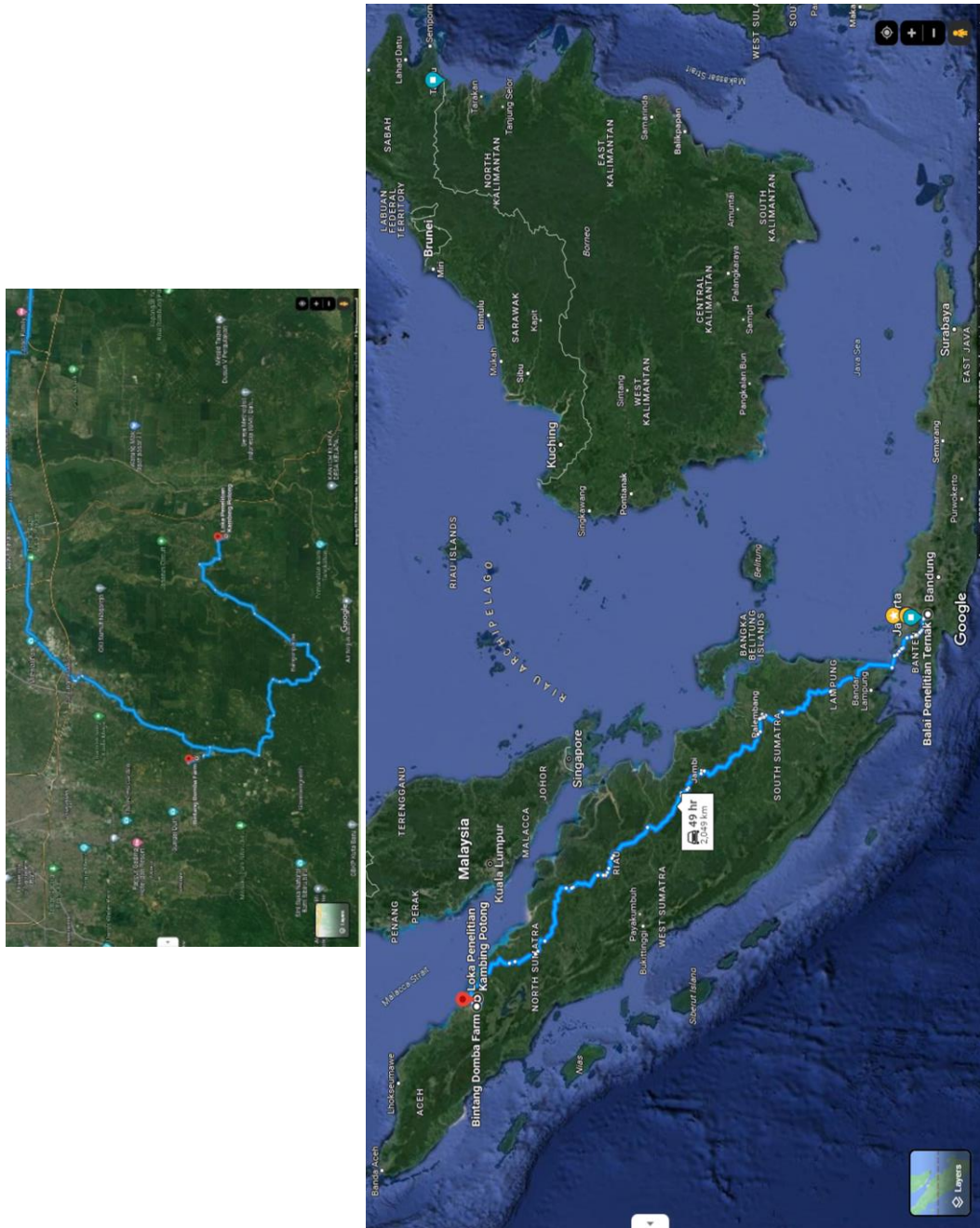


Figure 1. Transportation Route of Anpera and Boerka Goats.

Table 1. Body weight and physiological response of Anpera and Boerka Goat during transportation 72 hours

Parameter	Transportation	Means	SEM	P value	Anpera Goat		Boerka Goat		P value	Sex		P value
					P		P			P		
					Male	Female	Male	Female		Male	Female	
Body weight (kg)	Before	28.48 ^a	1.61	**	27.07	17.68	30.91	37.26	NS	19.85	34.09	24.30
	After	24.43 ^b	1.34		24.77	15.42	26.32	31.44	NS	17.58	28.88	23.16
	Variation	-4.05	-0.27		-2.26	-2.30	-5.82	-4.59		-2.27	-5.20	-3.42
Heart rate (x/minute)	Before	117.49 ^a	2.62	**	122.00	125.13	107.20	118.80	NS	124.41 ^a	113.00 ^b	116.17 ^a
	After	102.79 ^b	4.04		76.00	79.60	110.40	126.40		78.77 ^c	118.40 ^{ab}	95.00 ^b
	Variation	-14.71	1.42		-45.53	-46.00	7.60	3.20		-45.64	5.40	-21.17
Respiratory rate (x/minute)	Before	44.28 ^b	1.28	**	51.78	39.00	46.80	44.80	NS	41.95	45.80	42.90
	After	52.73 ^a	2.16		50.67	49.60	51.20	58.00		49.85	54.60	53.68
	Variation	8.44	0.89		10.60	-1.11	13.20	4.40		7.90	8.80	7.50
Rectal temperature (°C)	Before	39.31	0.06	**	39.20	39.13	39.55	39.26	NS	39.15 ^b	39.41 ^b	39.35 ^{bc}
	After	39.72	0.08		39.27	39.34	39.82	40.13		39.32 ^b	39.98 ^a	39.58 ^b
	Variation	0.41	0.03		0.21	0.07	0.87	0.27		0.17	0.57	0.23

**) P>0.01; *) P>0.05; NS= non-significant (P>0.05).

Table 2. Cortisol and blood parameters of Anpera and Boerka Goat during transportation 72 hours

Parameter	Transportation	Means	SEM	P value	Anpera Goat		Boerka Goat		P value	Sex		P value
					P		P			P		
					Male	Female	Male	Female		Male	Female	
BUN (mg/dL)	Before	30.42 ^b	2.24	**	42.17 ^a	28.75 ^{bc}	20.68 ^c	38.31 ^{ab}	**	31.85 ^b	29.5 ^b	33.53 ^b
	After	41.35 ^a	2.09		27.4 ^{bc}	36.92 ^{ab}	49.08 ^a	42.22 ^a		34.72 ^b	45.65 ^a	39.57 ^{ab}
	Variation	10.92	-0.15		-14.77	8.17	28.40	3.91		2.88	16.16	6.04
Creatinine (mg/dL)	Before	1.01	0.05	NS	1.08	0.66	1.24	1.12	NS	0.75	1.18	0.89
	After	1.09	0.04		1.14	0.86	1.20	1.19		0.92	1.19	1.02
	Variation	0.07	-0.01		0.07	0.20	-0.04	0.07		0.17	0.01	0.13
Glucose (mg/dL)	Before	61.61 ^b	2.47	**	63 ^c	59.2 ^c	66.1 ^c	59.1 ^c	**	60.08 ^b	62.60 ^b	59.15
	After	91.68 ^a	6.93		49.33 ^c	61.78 ^c	97.8 ^b	128.89 ^a		58.67 ^b	112.53 ^a	95.33
	Variation	30.07	4.46		-13.67	2.58	31.70	69.79		-1.41	49.93	36.18
Cortisol (ng/mL)	Before	222.37 ^b	16.35	**	227.02	313.50	143.24	218.10	NS	291.88 ^a	180.67 ^b	263.29 ^a
	After	302.45 ^a	14.13		226.82	315.53	313.83	301.97		293.36 ^a	307.9 ^a	308.4 ^a
	Variation	80.07	-2.22		-20	2.04	170.59	83.88		1.48	127.23	45.11

BUN = Blood urea nitrogen, **) P>0.01; *) P>0.05; NS= non-significant (P>0.05).

Table 3. Hematological parameters of Anpera and Boerka Goat during transportation 72 hours

Parameter	Transportation	Means	SEM	P value	Anpera Goat			Boerka Goat			Breeds		Sex		P value
					Male	Female	P value	Male	Female	P value	Anpera	Boerka	Male	Female	
WBC (10 ⁹ /μL)	Before	16.47 ^b	0.81	**	17.13	16.38	12.80	20.02	16.55	16.41	13.8 ^b	18.2 ^a	20.58 ^a	21.73 ^a	*
	After	21.27 ^a	0.93		21.90	19.63	20.18	23.82	20.15	22.00	20.58 ^b	21.73 ^a	20.15	22.00	NS
	Variation	4.81	0.12		4.77	3.25	7.38	3.80	3.60	5.59	3.60	3.53	3.60	5.59	
RBC (10 ⁶ /μL)	Before	13.21 ^a	0.27	**	12.77	11.88	13.30	14.60	12.08 ^b	13.95 ^a	13.18 ^a	13.24 ^a	13.26 ^a	11.62 ^b	**
	After	12.27 ^b	0.35		13.50	13.19	10.03	13.22	13.26 ^a	11.96 ^b	10.83 ^b	13.21 ^a	13.26 ^a	11.62 ^b	**
	Variation	-0.95	0.08		0.73	1.32	-3.28	-1.38	1.18	-2.33	-2.35	-0.03	-2.35	-0.03	
Haemoglobin (g/dL)	Before	9.57 ^b	0.21	*	8.93	8.53	9.52	10.84	8.62 ^b	10.18 ^a	9.38	9.69	9.35	10.43	NS
	After	10.01 ^a	0.17		10.00	10.19	9.16	10.67	10.15 ^a	9.92 ^a	9.35	10.43	9.35	10.43	NS
	Variation	0.44	-0.04		1.07	1.66	-0.36	-0.17	1.52	-0.26	-0.03	0.75	-0.03	0.75	
Haemato-crit (%)	Before	24.86 ^b	0.84	**	22.67	21.26	23.78	30.21	21.58	27.00	23.52	25.74	23.52	25.74	NS
	After	27.73 ^a	1.05		24.43	24.21	25.38	34.60	24.26	29.99	25.16	29.41	25.16	29.41	NS
	Variation	2.87	0.21		1.77	2.95	1.60	4.39	2.68	3.00	1.64	3.67	2.68	3.00	
MCV (fL)	Before	14.07 ^b	1.29	**	17.53 ^{bc}	17.88 ^{bc}	17.88 ^{bc}	5.4 ^d	17.80 ^b	11.64 ^c	17.80	11.64	17.80	11.64	NS
	After	20.66 ^a	1.31		11.87 ^c	16.24 ^{bc}	25.41 ^a	22.96 ^{ab}	15.23 ^{bc}	24.19 ^a	22.28	19.60	22.28	19.60	NS
	Variation	6.59	0.02		-5.67	-1.64	7.53	17.56	-2.57	12.55	4.48	7.96	4.48	7.96	
MCH (pg)	Before	5.64 ^b	0.53	**	7.10 ^{ab}	7.20 ^{ab}	7.22 ^{ab}	2.07 ^c	7.18 ^{ab}	4.65 ^c	7.19	4.64	8.33	7.16	NS
	After	7.62 ^a	0.46		5.13 ^b	7.05 ^{ab}	9.29 ^a	7.27 ^{ab}	6.61 ^b	8.28 ^a	8.33	7.16	8.33	7.16	NS
	Variation	1.98	-0.07		-1.97	-0.15	2.07	5.20	-0.57	3.64	1.14	2.53	1.14	2.53	
MCHC (g/dL)	Before	50.62	11.69	NS	39.97	40.32	78.56	36.16	40.24	57.36	69.65	38.24	37.69	36.82	NS
	After	37.16	1.05		41.50	42.47	365.5	31.16	42.25	33.86	37.69	36.82	37.69	36.82	NS
	Variation	-13.45	-10.64		1.53	2.15	-42.01	-5.00	2.01	-23.51	-31.96	-1.42	-31.96	-1.42	
Platelet (10 ⁹ /μL)	Before	677.00	53.89	NS	484.33	792.40	707.78	591.70	721.31	646.68	651.92	692.05	992.00	877.00	NS
	After	922.30	67.07		640.00	1027.60	1097.60	726.40	938.15	912.00	992.00	877.00	992.00	877.00	NS
	Variation	245.30	13.17		155.67	235.20	389.82	134.70	216.85	265.32	340.08	184.95	340.08	184.95	
RDW (%)	Before	21.09	0.50	NS	19.40	20.99	19.71	23.07	20.62 ^{ab}	21.39 ^{ab}	19.64 ^b	22.03 ^{ab}	16.58 ^c	23.92 ^a	NS
	After	21.03	1.10		21.70	23.93	15.05	23.91	23.42 ^a	19.48 ^b	16.58 ^c	23.92 ^a	16.58 ^c	23.92 ^a	NS
	Variation	-0.06	0.60		2.30	2.94	-4.66	0.84	2.79	-1.91	-3.05	1.89	-3.05	1.89	
MPV (fL)	Before	3.48	0.20	NS	4.00	3.53	3.04	3.72	3.64	3.38	3.26	3.63	3.26	3.77	NS
	After	3.35	0.31		3.83	3.06	2.36	4.48	3.24	3.42	2.70	3.77	3.24	3.42	NS
	Variation	-0.13	0.12		-0.17	-0.47	-0.68	0.76	-0.40	0.04	-0.56	0.15	-0.56	0.15	
PDW (%)	Before	10.96	0.27	NS	9.90	10.87	11.12	11.22	10.65	11.17	10.84	11.05	12.08	11.28	NS
	After	11.59	0.41		11.63	12.24	12.21	10.31	12.10	11.26	12.08	11.28	12.08	11.28	NS
	Variation	0.63	0.13		1.73	1.37	1.09	-0.91	1.45	0.09	1.24	0.23	1.24	0.23	

BC= white blood cells; RBC= red blood cells; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; JW= Red blood cell distribution width; MPV= Mean platelet volume; PDW= platelet distribution width; ***) P>0.01; *) P>0.05; NS= non-significant (P>0.05).

secretion of glucocorticoids, mainly cortisol, for the body to deal with stressor(s). This mechanism has been reported in several studies, including those by Zimmerman *et al.* (2013), Brown and Vosloo (2017), Hong *et al.* (2019), and Cockrem (2013). The cortisol is important in regulating stress and immune response and reinstates homeostasis (Hulbert and Moisés, 2016). Furthermore, Hu *et al.* (2020) found the different degrees of stomach walls damage, with the change of expression levels of heat shock protein 27 (HSP27), heat shock protein 70 (HSP70) and heat shock protein 90 (HSP90), occurred after goats' transportation for 2 and 6 hours.

Hematological parameters

The effects of transportation on various hematological parameters in goats were studied, and the results are presented in Table 3. The study found that transportation had a significant effect on several hematological parameters, including white blood cells (WBC), red blood cell (RBC), haemoglobin, haematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Specifically, there was an increase approximately $4.8 \times 10^3/\mu\text{L}$ (29%) WBC, 0.44 g/dL (4.5%) haemoglobin, 2.87% haematocrit, 6.59 fL MCV, 1.98 pg MCH after transportation, meanwhile RDC count decreased approximately $0.95 \times 10^6/\mu\text{L}$ (7%). There was no interaction between the sex and breed of the goats, except for MCV and MCH. However, a significant difference was found in RDC, haemoglobin, and RDW in the main factor breed, and WBC and RDC in the main factor breed sex. The MCHC, platelet, MPV, PDW were similar among goats and not significant changed after transport ($P > 0.05$).

The present study's findings were consistent with previous studies reporting an increase in haemoglobin, haematocrit, RBC and WBC level in goats after transportation (Raghazli *et al.*, 2021; Mir *et al.*, 2018), which is thought due to the spleen contraction caused by the release of catecholamine secretion (Zhen *et al.*, 2013). Furthermore, Ekiz *et al.* (2012) reported that animals with severe dehydration normally shown to have high haemoglobin, haematocrit, total protein and red blood count concentration. However, Ragzhali *et al.* (2021) found that there was no significant difference in the levels of haemoglobin and haematocrit in goats after a 2-hour journey, but a significant difference was observed after a 6-hour journey. This may be due to differences in the duration of transportation, age, and breed of goats, which are known to affect these hematological parameters. The increase in RBC concentration due to transportation in goats is also depicted by study of (Kumar, 2014) who reported that the RBCs values increased after 8 h of transportation and attained the pre-transportation level after 24 h of post-transportation. Furthermore, Ekiz *et al.* (2012) reported that animals with severe dehydration normally shown to have high haemoglobin, haematocrit, total protein and red blood count concentration.

Conclusions

It can be concluded that transportation for a duration of 72 hours caused stress on the goats, leading to weight loss, changes in psychological behavior, and blood parameters. The dramatic increases in cortisol, glucose, and BUN observed in the goats subjected to the effects of road transportation suggest that these parameters may be reliable indicators of stress in Anpera and Boerka goats. The breed and sex of the goats played a crucial role in the observed changes. The study found that the transportation caused an average weight loss of 4.05 kg/head (14.22%), and the heart rate decreased by 14.71 x/minute, while the respiratory rate and rectal temperature increased by 8.44 x/minute and 0.41 units, respectively. The cortisol levels increased by approximately 80.07 ng/dL (36%) and blood parameters showed an increase in BUN levels by approximately 10.92 mg/dL (35%) and glucose levels by approximately 30.07 mg/dL (48%). The hematological parameters also showed significant changes, with transportation causing alterations in the WBC, RBC, haemoglobin, haematocrit, MCV, and MCH levels. These findings demonstrate the importance of considering the breed and sex of the animals in transportation studies and highlight the need for measures to mitigate the stress experienced during transportation.

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References

- Alcalde, M. J., M. D. Suárez, E. Rodero, R. Álvarez, M. I. Sáez, and T. F. Martínez. 2017. Effects of farm management practices and transport duration on stress response and meat quality traits of suckling goat kids. *Animal* 11: 1626-1635. <https://doi.org/10.1017/S1751731116002858>
- Ambore, B., K. Ravikanth, S. Maini, and D. S. Rekhe. 2009. Haematological profile and growth performance of goats under transportation stress. *Vet. World* 5: 195-198.
- Ayo, J.O., N. S. Minka, and M. Mamman. 2006. Excitability scores of goats administered ascorbic acid and transported during hot dry conditions. *J. Vet. Sci.* 7: 127-131.

- Biobaku K. T., T. O. Omobowale, A. O. Akeem, A. Aremu, N. Okwelum, and A. S. Adah. 2018. 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*. 11: 674-680.
- Brown, E. J. and A. Vosloo. 2017. The involvement of the hypothalamopituitary-adrenocortical axis in stress physiology and its significance in the assessment of animal welfare in cattle. *Onderstepoort J. Vet. Res.* 84: 1-9. doi:10.4102/ojvr.v84i1.1398.
- Cockrem, J. F. 2013. Individual variation in glucocorticoid stress responses in animals. *Gen. Comp. Endocrinol.* 181: 45-58. https://doi.org/10.1016/j.ygcen.2012.11.025.
- Ekiz, B., E. E. Ekiz, O. Kocak, H. Yalcintan, and A. Yilmaz. 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 Sci.* 90: 967-976. https://doi.org/10.1016/j.meatsci.2011.11.042.
- Ekiz, E. E. and H. Yalcintan. 2013. Comparison of certain haematological and biochemical parameters regarding pre-slaughter stress in Saanen, Maltese, Gokceada and Hair Goat kids. *J. Fac. Vet. Med. Istanbul Univ.* 39: 189-196.
- Fernandez-Novoa, A., S. Pérez-Garnelo, A. Villagrà, N. Pérez-Villalobos, and S. Astiz. 2020. The effect of stress on reproduction and reproductive technologies in beef cattle: A review. *Animals* 10: 1-23. doi:10.3390/ani10112096.
- Hong, H., E. Lee, I. H. Lee, S. Lee. 2019. Effects of transport stress on physiological responses and milk production in lactating dairy cows. *Asian-Australas J. Anim. Sci.* 32: 442-451.
- Hu, W., T. Ye, Y. Yang, B. Liu, and W. Zheng. 2020. Effects of transport stress on pathological injury and expression of main heat shock proteins in the caprine stomach. *BMC Vet. Res.* 16: 347. https://doi.org/10.1186/s12917-020-02569-z
- Hughes, H. D., J. A. Carroll, N. C. B. Sanchez, and J. T. Richeson. 2013. Natural variations in the stress and acute phase responses of cattle. *Innate Immunity* 20: 888-96. https://doi.org/10.1177/1753425913508993
- Hulbert, L. E. and S. J. Moisé, 2016. Stress, immunity, and the management of calves. *J. Dairy Sci.* 99: 1-18. https://doi.org/10.3168/jds.2015-10198.
- Kannan, G., H. Terrill, B. Kouakou, O. S. Gazal, S. Gelaye, E. A. Amoah, and S. Samake. 2000. Transportation of goats: effects on physiological stress responses and liveweight loss. *J. Anim. Sci.* 78: 1450-1457.
- Kannan, G., K. E. Saker, T. H. Terrill, B. Kouakou, S. Galipalli, and S. Gelaye. 2007. Effect of seaweed extract supplementation in goats exposed to simulated preslaughter stress. *Small Ruminant Res.* 73: 221-227. https://doi.org/10.1016/j.smallrumres.2007.02.006.
- Kumar, K. 2014. Effect of transportation stress on physiological performance and immune function of the goats. Karnal: MVSc, National Dairy Research Institute.
- Kumar, P., A. A. Abubakar, M. A. Ahmed, M. N. Hayat, U. Kaka, M. Pateiro, A. Q. Sazili, L. C. Hoffman, and J. M. Lorenzo. 2023. Pre-slaughter stress mitigation in goats: Prospects and challenges. *Meat Sci.* 195 (109010).
- Minka, N. S. and J. O. Ayo. 2012. Assessment of thermal load on transported goats administered with ascorbic acid during the hot-dry conditions. *Int. J. Biometeorol.* 56: 333-41. https://doi.org/10.1007/s00484-011-0437-2
- Minka, N. S. and J. O. Ayo. 2013. 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.* 8: 349-356.
- Minka, N. S. and J. O. Ayo. 2014. Effects of transportation positions and orientations on muscular damage of goats transported by road for 12 h during the hot-dry conditions. *J. Vet. Med. Anim. Health* 6: 75-82.
- Mir, N. A., A. Ashutosh, S. A. Shergojry, S. A. Wani, and F. A. Sheikh. 2018. Effect of induced transportation stress in goats supplemented with vitamin C and jaggery during hot dry season. *Biol. Rhythm Res.* 1-11. doi:10.1080/09291016.2018.1452591.
- Miranda-de la Lama, G. C., M. Villarroel, and G. A. María. 2014. Livestock transport from the perspective of the pre-slaughter logistic chain: a review. *Meat Sci.* 98: 9-20. https://doi.org/10.1016/j.meatsci.2014.04.005.
- Naldurtiker, A., P. Batchu, B. Kouakou, T. H. Terill, G. W. McCommon, and G. Kannan. 2023. Differential gene expression analysis using RNA-seq in the blood of goats exposed to transportation stress. *Nature* 13: 1984. https://doi.org/10.1038/s41598-023-29224-5.
- Nikbin, S., J. M. Panandam, and A. Q. Sazili. 2016. Influence of pre-slaughter transportation and stocking density on carcass and meat quality characteristics of Boer goats. *Ital. J. Anim. Sci.* 15: 504-511.
- O'Neil, H. A. 2019. A review on the involvement of catecholamines in animal behaviour. *S. Afr. J. Anim. Sci.* 49: 1-8. http://dx.doi.org/10.4314/sajas.v49i1.1
- Paravati, S., A. Rosani, and S. J. Warrington. 2023. Physiology, Catecholamines. [Updated 2022 Oct 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK507716/

- Raghazli, R., O. Azalea-Hani, U. Kaka, A. A. Abubakar, J. C. Imlan, H. Hamzah, A. Q. Sazili, and Y. Goh. 2021. Physiological and electroencephalogram responses in goats subjected to pre-and during slaughter stress. *Saudi J. Biol. Sci.* 28: 6396–6407.
- Saeb, M., H. Baghshani, S. Nazifi, and S. Saeb. 2010. Physiological response of dromedary camels to road transportation in relation to circulating levels of cortisol, thyroid hormones and some serum biochemical parameters. *Trop. Anim. Health Prod.* 42: 55-63.
- Sowińska, J., S. Milewski, D. Witkowska, K. Ząbek, J. Miciński, A. Wójcik, and T. Mituniewicz. 2020. Effect of dietary supplementation with β -hydroxy- β -methylbutyrate on stress parameters in goat kids. *Rev. Bras. de Zootec.* 49: e20200035. <https://doi.org/10.37496/rbz4920200035>.
- Tajik, J., S. Nazifi, and R. Eshtraki. 2016. The influence of transportation stress on serum cortisol, thyroid hormones, and some serum biochemical parameters in Iranian cashmere (Raini) goat. *Vet. Arhiv.* 86: 795-804.
- Zhen, S., Y. Liu, X. Li, K. Ge, H. Chen, C. Li, and F. Ren, 2013. Effects of lairage time on welfare indicators, energy metabolism and meat quality of pigs in Beijing. *Meat Sci.* 93: 287–291. <https://doi.org/10.1016/j.meatsci.2012.09.008>.
- Zimerman, M., E. Domingo, G. Grigono, H. Taddeo, and P. Willems, P. 2013. The effect of pre-slaughter stressors on physiological indicators and meat quality traits on Merino lambs. *Small Rumin. Res.* 111: 6-9. <https://doi.org/10.1016/j.smallrumres.2012.12.018>.