Effect of different transportation period on body weight loss, hematological and biochemical stress responses of sheep

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ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pengaruh durasi transportasi selama 4, 8 dan 12 jam terhadap penurunan bobot badan, profil hematologi dan biokimia darah domba. Sebanyak 24 ekor domba ekor tipis jantan didistribusikan terhadap enam kelompok perlakuan yaitu: kelompok transportasi selama 4, 8 dan 12 jam (T4, T8 dan T12) dan kelompok non-transportasi selama 4, 8 dan 12 jam (NT4, NT8 dan NT12), masing-masing perlakuan terdiri dari 4 ekor domba. Semua domba perlakuan diangkut dengan sebuah mobil pick up dengan tingkat kepadatan 0,28 m^2/ekor sesuai dengan perlakuan. Parameter yang diukur adalah penyusutan bobot badan, profil hematologi dan biokimia darah. Hasil penelitian menunjukkan bahwa peningkatan durasi transportasi nyata menurunkan (P<0,05) bobot badan dan meningkatkan (P<0,05) kadar hematokrit, kortisol, serta glukosa darah. Namun demikian, nilai-nilai tersebut masih berada dalam kisaran normal pada domba. Dapat disimpulkan bahwa domba ekor tipis yang sehat dapat mentolerir efek buruk transportasi sampai 12 jam perjalanan.

Kata Kunci: transportasi, domba, bobot badan, hematologi, biokimia darah

ABSTRACT

The study was conducted to evaluate the duration of road transportation during 4, 8 and 12 hours on body weight loss, hematological and biochemical responses of sheep. Twenty-four male Javanese thin tailed sheep were separated into transported group (T4, T8 and T12) and non-transported group (NT4, NT8 and NT12). Transportation (T) groups were transported for 4, 8 and 12 h in the same open pick up with 0.28 m^2/head of loading density. Body weight, haematological and biochemical blood profiles were determined. The results showed that increasing of transportation duration significantly increased (P<0.05) body weight loss and decreased (P<0.05) hematocrit, cortisol and glucose values in

the blood. However, these values were in the normal range of sheep well-being. In conclusion, transported healthy Javanese thin tailed sheep under good condition without feed and water access up to 12 h can tolerate to adverse effect of transportation without disturbing their welfare.

Keywords: : transportation, sheep, body weight, haematological, biochemical

INTRODUCTION

Unavoidable transportation as a supporting factor in the livestock business is known as one of the common cause of stress. An animal will be experienced of transportation at least once in their lifetime for one of the purposes of selling, slaughtering, fattening or breeding in sheep production systems (Zhong *et al.*, 2011). The handling, uploading, fasting, confinement, vibration, downloading, changing light condition, poor air quality and mixing of unfamiliar group are kinds of potentials stressors during transport that may cause of physiological stress and behavior changes in sheep (Grandin, 1997; Cockram *et al.*, 2000; Saeb *et al.*, 2010; Samimi *et al.*, 2018). Moreover, Maria *et al.* (2003) and Adzitey (2011) reported that duration and distance, loading density, weather and road condition can be listed as some stressors in livestock during transport. Stressed transport has a great effect on animal performance, increased of body weight loss, morbidity and mortality and decreased meat quality, and consequently, may cause considerable economic loses (Ayo and Oladele,1996; Swanson and Morrow, 2001; Fazio and Ferlazzo, 2003). Due to both economic and animal welfare problems, stress during transport has become a big concern in a few decades.

Though, until this time there is no uniform limits on duration of transportation for sheep in some countries. European Union and some developed countries have legislated and limited transportation in animals not more than 8 h without access to water and feed during transportation (European Council, 2005). Increasing the duration of transportation may affect animal welfare through fatigue, dehydration, and metabolic disorders as a result of stress during transportation. However, some previous studies reported that road transportation for 3, 9, 15, 18 and 24 h did not indicate dehydrated or metabolic compromised in 38 kg sheep (Knowles *et al.*, 1995), as well as Fisher *et al.* (2010) that transported sheep for 12, 30 and 48 h. In addition, Cole (1995) concluded that untransported sheep with a good condition can tolerate to feed and water withdrawal for 3 days.

The physiological stress during road transportation can be shown in concentration of blood constituents, such as cortisol, glucose, plasma urea and creatine kinase (Krawczel *et al.*, 2007; Fisher *et al.*, 2010). Previous studies that focused on the adverse effects of transportation in sheep with duration 24, 48 and 72 h (Horton *et al.*, 1996; Knowles *et al.*, 1996; Fisher *et al.*, 2010) were conducted in temperate regions and, to the best of our knowledge, there have been lack of studies that reported transportation effects on sheep under hot-humid tropical region. Transporting animal in tropical region with high temperature and relative humidity has been more challenging than that in temperate region (Minka and Ayo, 2012). Therefore, this study was conducted to evaluate effect differing transportation and starvation period on body weight loss and physiological responses of Javanese thick tailed sheep.

MATERIALS AND METHODS

Experimental Animals

This study used 32 healthy Javanese thick tailed male sheep aged 10-12 months and 22-24 kg live weight, in which 24 sheep were used for treatments and 8 sheep served as filler. All sheep were fed with concentrate completed feed and originated from the similar farm, located in Bogor, Indonesia.

Procedure

Two days before the study, 32 sheep were selected and housed in a separate pen on the farm. Sheep were grouped and marked with different colours to distinguish all of the treatments approximately 12 h before the beginning of transportation. Treatments were arranged in a factorial design in which the first factor was transportation (transport [T] and non-transport [NT]) and the second factor was duration (4, 8 and 12 h). In total there were six treatment combinations, i.e., T4, T8, T12, NT4, NT8 and NT12.

Both of transported and non-transported sheep were individually weighed before transportation. All three duration groups in the

transported trial were loaded into open pick-up (Suzuki Mega Carry, made in Japan) with a 2.4 m long x 1.6 m wide x 1.2 m tall compartment, whereas non-tranported stayed at same colony cage. The sheep were transported for 4, 8 and 12 h from 04.00 to 16.00 AM without access to feed and drinking water during transportation. Air temperature (°C) and humidity (%) were recorded during the study using Digital Thermo Hygrometer HTC-1 (Taiwan) which placed in the vehicle and colony cage.

Road transportation occurred mainly on public highways with average speed about 40 km/hour. The T4 sheep were unloaded after 4 h of road transport, and at the same time NT4 sheep were weighed and blood samples were collected. Further, four fillers sheep were loaded on to the vehicle to keep balance of density before the journey for another 8 h. Similarly, T8, NT8, T12 and NT12 sheep were weighed and collected of blood samples immediately after unloaded.

Blood samples (6 ml) from each animal were collected via jugular venipuncture and decanted into two sterile tubes. An amount of 4 ml blood was tranferred into one tube without anticoagulant for serum collection for biochemical measurements. The remaining 2 ml blood was put into another tube with 0.14% anticoagulant (EDTA K3, Onemed Healthy Care, Australia) to determine hematological parameters. The tubes were immediately placed in a box ice and taken to the laboratory for analysis.

Whole blood was used to determine red blood cell (RBC), hemoglobin, hematocrit, mean capsular hemoglobin (MCH), mean capsular volume (MCV), mean capsular hemoglobin concentration (MCHC), white blood cell (WBC), neutrophil (N), lymphocyte (L), monocyte and N/L ratio using an automatic blood analyzer (American Abaxis Vetscan HM5). Whereas, the serum was used to determine total protein (TP), albumin, globulin, aspartate transaminase (AST), alkaline phosphatase (ALP), creatine kinase (CK), blood urea nitrogen (BUN), glucose, calcium, phosphorus and magnesium concentration using an automatic biochemical analyzer (American Abaxis Vetscan VS2). The serum of cortisol value was analyzed using enzyme linked immuno sorbent assay (ELISA) method (DRG Diagnostics GmbH, Germany).

Statistical Analysis

Randomized block design with two factors was used in this study. The first factor was transportation (transported and non-transported) and the second factor was duration (4, 8 and 12 h). Data were analyzed by using analysis of variance with Duncan Multiple Range Test for the post-hoc test. The statistical analysis was performed by using SPSS software version 25.0.

RESULTS

The mean of temperature and relative humadity on the vehicle during journey were 25.20 ±1.98°C and 72.50±17.46%, respectively. While these values at the farm during study period were 28.2±4.57°C and 77.9±16.2%, respectively. The average of THI during transportation was 74.1±2.03 (max. 77.0, min. 70.6) and at the experimental farm was 78.2±4.99 (max. 82.7, min. 70.2).

The mean of body weight loss and haematological profile of sheep are presented in Table 1. Increasing transportation and starvation duration of sheep caused a significant increase in body weight loss, hematocrite, neutrophil percentage and decrease in lymphocite percentage (P<0.05). Transportation and starvation of sheep for 12 h increased body weight loss on arrival compared with for 4 and 8 h (P<0.05).

No difference was found between the 4 and 8 h treatments in the body weight loss. Regarding the haematological values, RBC and hemoglobin tended to increase during 4, 8 and 12 h transportation, but there was no significant in RBC and hemoglobin between 4, 8 and 12 h duration both of transportation and non-transportation treatments. Hematocrite followed the same trend as RBC and hemoglobin, i.e., increased after 8 and 12 h transportation. Transported treatment had higher level of RBC, hemoglobin and hematocrite than drink and water deprivated. MCH, MCV, MCHC and monocyte level were not different between treatments. WBC, percentage of neutrophil and ratio N/L also increased significantly while percentage of lymphosyte decresed (P<0.05) after 4, 8 and 12 h transportation. No differences between before and 4 h were found for hematocrit WBC and ratio N/L both of transport and starvation experiment, similarly with 8 and 12 h duration. Duration of transportation and starvation treatment significantly affected (P<0.05) in haematological respon of sheep, however transport and non transported treatment showed almost similar effects.

Table 1. Least Square Means (±SE) of Body Weight Loss and Haematological Variables in Sheep Subjected or Not to 4, 8 and 12 h Transport

Item	Non-transportation			Transportation			P-value		
	.... hr			.... hr			Treatment	Time	Interaction
	4	8	12	4	8	12			
Body weight loss (%)	2.02±2.04^b	3.30±0.62^{ab}	5.94±1.10^a	2.56±1.61^b	3.47±1.40^{ab}	5.49±1.50^a	0.092	0.056	0.273
RBC (10^6/µl)	9.77±0.39	9.93±0.95	10.06±0.34	9.91±0.52	10.00±0.52	10.29±0.40	0.872	0.243	0.223
Hemoglobin (g/dL)	10.53±0.65	11.00±0.92	11.37±0.96	10.03±0.70	10.50±1.31	10.77±0.35	0.619	0.193	0.739
Hematokrit, %	27.41±1.13^{bc}	28.64±0.82^a	29.63±1.76^{abc}	27.33±1.37^c	28.52±1.42^{abc}	29.41±1.43^{ab}	0.861	0.029	0.630
MCV (fl)	26.35±0.61	27.00±2.65	27.67±2.52	26.67±1.53	25.00±2.65	26.67±0.58	0.597	0.697	0.697
MCH (pg)	10.40±0.10	11.10±0.20	11.13±0.68	10.43±0.95	10.30±0.75	10.47±0.23	0.318	0.208	0.649
MCHC (g/dL)	39.10±2.89	38.40±3.03	38.60±2.59	38.87±1.51	38.83±1.87	38.60±1.17	0.943	0.841	0.893
WBC (10^3/µL)	12.55±2.61	12.88±0.22	12.26±0.48	10.12±0.86	12.19±2.75	10.64±1.92	0.108	0.507	0.738
Neutrophil (10^3/µL)	5.36±1.57^a	5.17±0.74^a	5.70±0.46 *	2.66±0.72^b	4.49±1.87^{ab}	3.93±1.39^{ab}	0.020	0.492	0.442
Lymphocyte (10^3/µL)	7.13±1.08	7.65±0.53	6.50±0.58	7.41±0.22	7.64±0.87	6.66±0.52	0.684	0.076	0.942
Monocyte (10^3/µL)	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01	0.05±0.01	0.186	0.732	0.732
Neutrophil (%)	42.12±4.58^{ab}	40.07±5.00^{ab}	46.47±3.82^a	25.98±4.81^c	35.74±7.27^b	36.20±6.05^{ab}	0.003	0.133	0.243
Lymphocyte (%)	57.37±4.57^{bc}	59.43±5.04^{ab}	53.01±3.87^c	73.42±4.69^a	63.76±7.24^b	63.29±6.02^b	0.003	0.133	0.245
Monocyte (%)	0.51±0.02	0.49±0.04	0.52±0.06	0.60±0.13	0.50±0.03	0.51±0.04	0.531	0.304	0.231
N/L ratio	0.74±0.14^{ab}	0.68±0.15^{ab}	0.88±0.14^a	0.36±0.09^c	0.57±0.18	0.58±0.16	0.004	0.152	0.314

RBC: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cells;
N/L ratio: neutrophil-lymphocyte ratio
a,b,c: different letters represent significant differences between treatments

Transported sheep had higher cortisol levels than non-transported after 4, 8 and 12 h transportation (Table 2). Transportation during 8 h had higher of cortisol value than 4 h and 12 h, while transport during 4 h and 12 h showed almost similar in cortisol (28.50 ± 2.79 and 26.53 ± 2.97 ng/ml). Cortisol level remain constant in non-transport treatment during 4, 8 and 12 h. Glucose, AST and creatine kinase followed the same trend of cortisol, i.e., increased significantly after 4, 8 and 12 h of transportation ($P<0.05$). Albumin, globulin, ALP and BUN values were similar between transported and non-transported treatments during 4, 8 and 12 h. Calcium, phosphorus and magnesium were quite consistent both for transported and non-transported groups.

DISCUSSION

The main results in the current study were that increase transportation and starvation duration on Javanese thick tailed resulted in increasing of body weight loss, hematocrite, percentage of neutrophil, N/L ratio, cortisol, glucose, total protein, but clinically these effect for any duration fell within normal ranges. The new information from this study would suggest that transportation and starvation of sheep under hot-humid tropical environment until 12 h did not disturb animal health. Sheep showed more stress and dehydration after 8 h transport or starvation that caused by higher temperature and humadity during transport or on the farm. Villarroel et al. (2011) reported that suddenly changing in temperature and humidity during road transport can affect animal welfare. Furthermore, Silanikove (2000) stated that THI as used to determine condition of environment can effect heat stress in animal: ≤74, comfort; 75-78, alert stress; 79-83, excess stress; ≥84, emergency.

In our result, THI values fell within the alert category for transport and almost danger for starvation on the farm treatment. Heat stress and dehydration as an effect of high temperature can stimulate evaporative heat loss by panting and sweating in the animal (Caufield et al., 2014). Similar to Miranda-de la Lama et al. (2011) also found that temperature and humadity rise quickly in the truck after loading. In contrast in this study temperature during transport lower than temperature on farm during 4, 8 and 12 h period (25.20 and 28.20 °C).

Body weight loss during transportation as a result of metabolic and dehydration disorders associated with animal welfare greatly affects livestock production (Fisher et al., 2010). The magnitude of body weight lost in the present study was 2.56, 3.4, 5.49% and 2.02, 3.30, 5.94% for the 4, 8 and 12 h transport and starvation respectively. There was no diffrences in body weight loss between transported and starvation sheep in this study may caused by both of treatment deprived of feed and water for 4, 8 and 12 h. Knowles (1998) also stated that decreasing body weight of animal depend on duration of animals suffering from lack of food and water.

Body weight loss in our result lower than studied by Zhong et al. (2011) that during 8 h of road transportation of Ujumqin sheep may lose 9.04% of body weight. Furthermore, Knowles et al. (1995) recorded that transport of sheep with body weight 38 kg during 3, 9, 15, 18 and 24 h in temperature of environment below of 20° C were adapted to the journey and clinically not suffered for dehydration or metabolic disorder. Moreover, Cole (1995) also reported that healthy untransported sheep tolerated with food and water deprivation for 3 day, similarly in transported sheep under good conditions (Knowles et al., 1996).

White blood cell commonly has been used to access animal health. Paul et al. (2008) reported that rising cortisol levels as stressors response in animals affected haematological, increase in neutrophil counts and decrease in lymphocytes. However, our current study found that the number of neutrophil, lymphocyte and neutrophil : lymphocyte ratio were not influenced by transportation only but also by starvation. This result agreed with Fisher et al. (2010) that N:L ratio increased after 12, 30 and 48 h transport.

A significant increase in neutrophil, in the N/L ratio and decrease lymphocyte was found during 4, 8 and 12 h both of transportation and starvation treatment. Non transported trial showed greater N/L ratio than transported sheep. The reason for this result might be due to non transport treatment that keep on farm heat stress as a result of increasing of ambient temperature, consequently for this result not transported sheep more stressed than transported trial.

Increasing of total protein, albumin and hematocrit usually are used as hydration indicator in animal. In our case, only hematocrit were increased significantly as an effect of transportation and starvation during 4, 8 and 12 h periods. Hematocrit on transported and starvation

Table 2. Least Square Means (±SE) of Physiological Stress Variables in Sheep Subjected or Not to 4, 8 and 12 h Transport

Item	Non-transportation			Transportation			P-value		
	4 hr	8 hr	12 hr	4 hr	8 hr	12 hr	Treatment	Time	Interaction
Cortisol (ng/ml)	6.28±0.30^d	11.22±0.46^c	6.71±1.27^{cd}	28.50±2.79^b	35.27±4.51^a	26.53±2.97^b	< 0.001	0.003	0.429
Glucose (mg/dl)	83.18±8.01^{ab}	57.78±0.95^c	66.14±4.33^c	75.13±5.28^b	43.63±4.66^d	89.73±4.45^a	0.836	<0.001	<0.001
TP (g/dl)	6.60±0.46	7.43±0.55	7.17±0.35	6.47±0.21	6.53±0.59	7.30±0.61	0.267	0.097	0.131
Albumin (g/dl)	4.07±0.38	4.47±0.40	4.47±0.38	4.07±0.38	4.27±0.67	4.57±0.25	0.893	0.515	0.784
Globulin (g/dl)	2.57±0.45	2.63±0.12	2.73±0.15	2.57±0.45	2.20±0.26	2.67±0.35	0.308	0.264	0.331
ALP (U/L)	143.33±5.03^a	103.33±17.93^b	101.00±15.13^b	85.67±22.59^b	136.83±43.48^{ab}	110.67±33.71^{ab}	0.712	0.665	0.038
AST (U/L)	92.33±14.74	106.67±60.12	104.67±41.24	94.67±21.08	144.67±60.12	126.33±39.31	0.808	0.435	0.755
CK(U/L)	186.63±35.60^b	208.33±25.54^{ab}	189.00±46.81^b	223.33±52.18^{ab}	267.00±25.94^a	185.33±38.76^b	0.499	0.099	0.785
BUN (mg/dl)	9.67±4.62	10.33±1.58	12.00±1.73	9.33±2.89	10.33±3.79	10.67±2.31	0.693	0.566	0.918
Ca (mg/dl)	8.57±0.55	9.27±0.57	9.27±0.38	9.37±0.32	9.43±0.86	9.23±0.25	0.117	0.355	0.296
P(mg/dl)	9.23±2.06	8.73±0.74	9.10±1.30	7.60±3.48	5.58±1.59	8.00±2.07	0.230	0.993	0.978
Mg (mg/dl)	2.90±0.17	2.93±0.15	2.90±0.20	2.90±0.20	2.93±0.12	3.00±0.12	0.746	0.918	0.896

TP, total protein; ALP, alkaline phosphatase; AST, aspartate transaminase; CK, creatinin kinase; BUN, blood urea nitrogen; Ca, calcium; P, phosphor; Mg, magnesium
a,b,c: different letters represent significant differences between treatments

for 8 and 12 h generally being greater than 4 h periods. However, these levels clinically did not exceed the normal limits that recognized for sheep.

Cortisol commonly is used to determine physiological stress response of the transported sheep (Krawezel *et al.*, 2007). Previous studies showed that cortisol level tend to increase during and following transported sheep (Ekiz *et al.*, 2013). In addition, serum cortisol level in transport sheep increases at the beginning, post loading and back to basal level after few hours of journey (Broom *et al.*, 1996; Cockram *et al.*, 1997). In our case, cortisol reached the peak after 8 h transport (35.27 ng/dl), while after 12 h transport cortisol level similar with after 4 h transport (26.53 and 28.50 ng/dl). Consistent with Pascual-Alonso *et al.* (2016) reported that cortisol levels were twice the pre-transport after 4 h transport and two times greater than coltrol. The results of glucose are consistent with the measured cortisol levels.

Our results showed that serum glucose was influenced by interaction between treatment and length transport of the sheep. Glucose serum in transport group increased in 4 h period, decreased in 8 h, and increased after 12 h transport; however, this variable did not change in non transport sheep for any duration. Higher serum glucose is an indicator of metabolic response to stress in animals (Moberg, 1985; Ali *et al.*, 2006). Moreover, Knowles *et al.* (1995) stated that increasing plasma glucose because of response to initial stress and it will be decrease for up to 24 h as a metabolic exhaustion marker. Miranda-de la Lama *et al.* (2012) have shown that increasing of glucose during stress is started by an increasement of cortisol levels as an effect gluconeogenic.

In contrast with current study, greater glucose concentration was found after 12 h transport, while the greater cortisol level was showed after 8 h transport. The reason for this may be that transport during 8 h more stressed for the sheep because their start to expose heat stress as a result of increasing of temperature. Moreover, glucose reserves begin to run out after 8 h of transportation and energy needs obtained from the breakdown of body fat and protein, so that glucose levels rise again after 12 transportation. This phenomenon was supported by blood urea nitrogen levels that greater after 12 h of transportation. Some studies agree with our explanations that rising blood urea nitrogen (BUN) during transport indicated that limited glucose reserves, therefore body fat and protein have to break down to obtain energy during stress condition (Fisher *et al.*, 2010).

Transport and starvation trial have similar effect in blood urea nitrogen concentration, that tend to increase with increasing duration of trial. Transport for 4, 8 and 12 h duration in this study were not seriously stressed for Javanese thick tailed, but deprivation of feed and water caused changing in some blood metabolites, like BUN, total protein and glucose concentration. These changes could be affected to food deprivation, more pronounced in 12 h, because of increased duration of transport or starvation as compared with 4 and 8 h periods. Moreover, Montane *et al.* (2002) stated that increase of blood urea nitrogen in transported sheep may cause by physical exercise during transport, effect of glucocorticoids on protein catabolism and food deprivation.

Creatinine kinase became necessary variable to describe injuries and exhaustion that encountered in sheep during transport (Kent, 1997; Tadich *et al.*, 2009; Fisher *et al.*, 2010). Our result showed that CK activity continues to increasing with increasing duration of the journey. Transport for 12 h showed the higher CK activity than 4 and 8 h periods, whereas CK activity in not transport group tend to remain. Gregory (1998) indicated that increasing of CK activity as a result of physical damage encountered during handling, loading and slaughter. In addition, Fisher *et al.* (2010) reported that concentration of creatine kinase raised at arrival. However, increased creatine kinase in this study relatively small and was similar to the studies of Fisher *et al.* (2010) and Parrot *et al.* (1998), which transported sheep for 48 h and 31 h, respectively.

CONCLUSIONS

Increasing road transportation and starvation duration for Javanese thick tailed up 4, 8 and 12 h resulted in reduced body weight, increased cortisol, glucose and hematocrit. However, these results are acceptable for animal well-being. This finding indicates that transported healthy Javanese thick tailed under good condition in hot-humid tropical environment up to 12 h can tolerate to adverse effect of transportation without disturbing their welfare. In addition, transported sheep of more than 8 h should be fed and watered to decrease the body weight loss.

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