

The effects of shearing and ascorbyl palmitate administration on physiological and blood metabolite profile of Padjadjaran sheep under heat exposure treatment

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ABSTRAK

Ascorbyl-6-palmitate (AP) merupakan ester sintetik asam askorbat, telah digunakan sebagai pengawet makanan juga sebagai antioksidan pada kosmetik dan produk terkait lainnya. Penelitian bertujuan untuk mempelajari pengaruh pencukuran dan pemberian ascorbyl palmitate terhadap profil fisiologis domba Padjadjaran yang diberi cekaman panas. Profil fisiologis yang diamati adalah kecepatan respirasi (RR), denyut nadi (PR) dan temperature rektal (RT). Penelitian menggunakan 20 ekor domba Padjadjaran jantan berumur 1 – 1,5 tahun dengan bobot badan 25 hingga 47,5 kg yang diperoleh dari stasiun pembibitan di Purwakarta, Jawa Barat – Indonesia. Perlakuan yang dicobakan terdiri dari dua macam, (1) Pencukuran : tidak dicukur (c_0) dan dicukur (c_1); (2) Pemberian AP; tidak diberi (a_0) dan diberi 400 mg AP (a_1); sehingga perlakuan keseluruhan adalah a_0c_0 , a_0c_1 , a_1c_0 dan a_1c_1 . Cekaman panas diberikan dengan cara penjemuran domba saat temperatur lingkungan mencapai 34°C, 3 jam sehari, 3 hari dalam seminggu. Saat dijemur, setiap domba ditambatkan. Hasil penelitian menunjukkan bahwa pemberian AP dan pencukuran memberikan pengaruh interaksi terhadap frekuensi pernafasan, denyut nadi dan temperatur rektal. Konsentrasi glukosa dan kolesterol dipengaruhi oleh interaksi penambahan AP dengan pencukuran. Disimpulkan bahwa penambahan AP dan pencukuran mengurangi pengaruh negatif cekaman panas berdasarkan profil fisiologis dan metabolit darah domba Padjadjaran. Pencukuran disarankan dilakukan secara berkala.

Kata kunci : Ascorbyl palmitate, cekaman panas, domba, pencukuran

ABSTRACT

Ascorbyl-6-palmitate (AP) was a lipid-soluble synthetic ester of ascorbic acid that has been used as a preservation agent for foods and as an antioxidant in cosmetics and related products. This study aims to investigate the effect of shearing management and ascorbyl palmitate administration on physiological and hematological profiles in under heat load Padjadjaran sheep. The physiological profile involves respiration rate (RR), Pulse rate (PR), and rectal temperature (RT). Twenty rams Padjadjaran 1 – 1,5-

year-old, weighing between 25 to 47.5 kg used from sheep breeding station Purwakarta, West Java - Indonesia. There are two treatments level in this research, (1). Shearing divide into two groups, unshearing (c_0) and shearing (c_1); (2). AP administration also divides two groups, not given (a_0) and given 400 mg each head daily (a_1). The whole treatments were a_0c_0 , a_0c_1 , a_1c_0 and a_1c_1 . Heat exposure application was exposed to sunlight when the sheep exposed, the animal tethered by rope at the neck region. The sheep exposed to the sun when the ambient temperature at least 34°C, 180 minutes each day, three days a week. The result showed that AP administration and shearing have interaction effects on RR, PR and RT. The level of glucose and total protein is affected by interaction effect of AP administration and shearing, while cholesterol affected to shearing. The conclusion, supplementing AP and shearing application could reduce the negative impact of heat stress on physiological and blood metabolite profiles of Padjadjaran sheep. Although the wool of local breed has not economic value, the shearing is regularly needed.

Keywords: Ascorbyl palmitate, heat stress, shearing, sheep

INTRODUCTION

Indonesia has high both ambient temperatures and solar radiation during the year. This condition was a limiting factor and unfavorable for livestock covered with thick hair like sheep. Hair was one of the inhibitors of sheep's heat dissipation and has the potential to cause heat stress. In Indonesia, the sheep farmer has not been done shearing regularly because the local sheep are not the type of producing wool which economic-value. Hair is one of the inhibitors of sheep's heat dissipation and has the potential to cause heat stress. Shearing increases the heat exchange between the body of the animal to the environment so that the release of heat without compensation for heat production (Aleksiev, 2008). The traits of animal behavior are early indicators of adaptation and response to environmental alterations ((De *et al.*, 2017). The previous researcher reported that heat stress affects some metabolite concentrations of blood such as glucose, cholesterol, and total protein.

L-ascorbic acid (vitamin C) was an antioxidant that high solubility in water, so it was easily degraded, very sensitive to Oxygen, light, and heat (Dong and Wang, 2016). Ascorbyl-6-palmitate (AP) was a lipid-soluble synthetic ester of ascorbic acid. AP has been used as an antioxidant in cosmetics and related products (Austria *et al.*, 1997). Food and Drug Administration (FDA) status of AP acknowledged as safe due to the apparent lack of known toxicity. There was a rising interest in possible beneficial effects of AP, linked mostly to its antioxidant capacity, in various areas of research, including to animals.

The objective of this research was to investigate the shearing management and AP

supplementation in the control of heat stress in Padjadjaran sheep.

MATERIALS AND METHODS

Animals and Feeding Management

Twenty rams Padjadjaran 1 – 1,5-year-old, weighing between 25 to 47.5 kg used in the present study came from sheep breeding station at Purwakarta – West Java - Indonesia. The animal was offered a mixture of concentrates (30 %) and forages (70 %) as much as 3.5% dry matter of body weight. The concentrates feed has 13% of protein and 65-70% Total Digestible Nutrient (TDN). Drinking water serves an ad-libitum

Chemicals

Ascorbyl-6-palmitate (99.9% pure) was purchased from **Shandong Zhi Shang Chemical Co.Ltd**, China.

Experimental Procedure

The study was conducted based on experimental methods designed Factorial Randomized Block Design. There were two treatments level in this research, (1) shearing divide two-class, un-shearing (c_0) and shearing (c_1); (2) AP administration also divides two-class, not given (a_0) and given 400 mg each head daily (a_1). The whole treatments were a_0c_0 , a_0c_1 , a_1c_0 and a_1c_1 . Heat shock application was exposed to sunlight. When the sheep were exposed, the animal tethered by rope at the neck region. The sheep exposure to the sun when the ambient temperature at least 34°C.

Heat exposure procedure. Equipment and procedure of heat exposure modified from Indu *et al* 2015. All animals entered into a simple chamber made from bamboo and wood, size 150

x 100 x 100 cm in length, width, and tall, respectively. Each chamber filled in 5 rams appropriate to treatment. The duration of heat expose was 180 mins per day, three days a week.

Blood collection. Collecting blood done every week after heat exposure application. Blood was collected by venipuncture using vacutainer tubes with EDTA from *vena jugularis*. Separated blood plasm use centrifuge at 3500 rpm for 10 minutes, the plasm entered to Eppendorf tube, stored at -20°C until analysis (Indu *et al.*, 2015).

Parameters

Respiration rate. The respiration rate was recorded by counting flank movements per minute from a non-obstructive distance (Al-Azzawi *et al.*, 2017).

Pulse Rate. The pulse rate was measured by a modification of Al-Azzawi *et al.* (2017) fingered the pulses of the *vena femoralis* for one minute.

Rectal Temperature. Rectal temperature measured by inserting a digital thermometer into the rectum as deep as 3 cm, hold it until makes a beep (Al-Azzawi *et al.*, 2017).

Blood Metabolite. The blood determine were total protein, glucose and cholesterol. The examination of all variables using spectrophotometer method with reagent kit produced DiaSys (according to manufacturer's instructions of DiaSys System GmbH, Holzheim, Germany).

Statistical Analysis. The obtain data which included both physiological blood metabolites were analyzed by ANOVA (Steel and Torrie, 1997). Data calculated using Minitab software. The level of significance was set at $P < 0.05$.

Ethical Approval

All rams were maintained under standard environmental conditions with ad libitum feed and water. The animals were treated humanely during the whole period of the experimental study, and the work was considered by the institutional Research Ethics Committee Padjadjaran University No. 1157/UN6.KEP/EC/2019 on ethical standards in animal experimentation.

RESULTS

Environmental condition

The site of research was located at the Laboratory of Animal Breeding and Biometric – Animal Husbandry Faculty - Padjadjaran

University (6° 55'23.2 "S 107°46'12.2"E). The range of temperature minimum in housing is 20.9 – 23.2°C, and a maximum 29.2 – 33.5°C. The range of minimum relative humidity 44 - 65% and a maximum of 78 – 99%. Environmental condition measure by digital thermo-hygrometer.

Physiological Profiles

Physiological profiles, respiration rate (RR), pulse rate (PR), and rectal temperature (RT) before and after heat shock application present in Table 1.

The RR before heat exposure around the normal range 25.13 ± 6.93 to 39.60 ± 24.79 breath per minute. ANOVA showed that shorn has a significant effect ($P < 0.05$) on RR in the first and second weeks after heat shock, while AP administration in the sixth week. In the fifth week, AP and shearing have an interaction effect on RR. Shearing treatment has a positive effect (low) to RR. Treatment a_1c_1 tends a positive effect on RR. In fifth-week, combination of AP administration and shearing (a_1c_1) leads to sheep able to control heat stress at a high level, other treatment at a heavy level. It indicates these factors' synergism to control heat stress.

According to the scale stress level developed by (Silanikove 2000b), the stress level in this research is presented in Table 2. Before heat shock, about 85%, 5%, and 10% of animal in no stress condition, low level, and medium-high stress level, respectively. Until the sixth-week heat shock, the animal stress level increase, from 1st week until 5th week, 25%, 30%, 20%, 15%, and 15% ram status in no stress.

The pulse rate range before heat load application is 53.86 ± 4.78 to 56.8 ± 1.40 beats per minute. ANOVA showed that in the second week, unshorn able to control pulse rate ($P < 0.05$), and in the fourth and sixth-week measurement occur an interaction ($P < 0.05$) between shearing and AP administration.

Before heat shock, the average rectal temperature 38.4 – 38.54°C. Variation above and below normal is a measure of the animal's ability to resist environmental stress factors. Until the fourth week and sixth week, AP and shearing no significant effect on rectal temperature. A fifth week AP and shearing interact to affect rectal temperature, without combination effect of AP and shearing (only shear or only AP, a_1c_0 and a_0c_1) have a good effect on rectal temperature.

Table 1. Average \pm SE of Respiratory Rate (RR), Pulse Rate (PR), and Rectal Temperature (RT) of Padjadjaran Sheep

Parameter	Treatment	Before Heat Shock	The Week after Heat Exposure					
			1	2	3	4	5	6
RR (Breath per minutes)	a ₀ C ₀	38.06 \pm 15.42	58.2 \pm 10.49 ^{ab}	58.2 \pm 14.41 ^{ab}	70.4 \pm 31.41 ^a	79.6 \pm 16.04 ^a	140.2 \pm 12.71 ^{bc}	138.00 \pm 10.36 ^b
	a ₀ C ₁	39.60 \pm 24.79	51.8 \pm 24.66 ^{ab}	51.8 \pm 20.84 ^{ab}	74.0 \pm 34.19 ^a	57.0 \pm 15.52 ^a	131.00 \pm 7.00 ^b	141.6 \pm 9.52 ^b
	a ₁ C ₀	26.20 \pm 6.83	68.4 \pm 19.96 ^b	75.2 \pm 27.74 ^b	68.8 \pm 21.76 ^a	62.4 \pm 34.43 ^a	150.2 \pm 17.46 ^c	126.00 \pm 18.70 ^a
	a ₁ C ₁	25.13 \pm 6.93	38.6 \pm 11.80 ^a	41.6 \pm 11.45 ^a	47.0 \pm 21.20 ^a	55.2 \pm 18.41 ^a	112.0 \pm 14.38 ^a	124.8 \pm 9.54 ^a
PR (beath per minutes)	a ₀ C ₀	53.86 \pm 4.78	58.6 \pm 5.94 ^{ab}	52.5 \pm 3.70 ^a	63.2 \pm 10.35 ^a	65.4 \pm 2.07 ^b	57.8 \pm 10.61 ^a	61.2 \pm 3.03 ^a
	a ₀ C ₁	56.4 \pm 3.64	57.4 \pm 1.67 ^a	61.4 \pm 5.85 ^c	64.6 \pm 7.89 ^a	61.8 \pm 4.38 ^a	64.2 \pm 10.68 ^a	67.8 \pm 3.42 ^{bc}
	a ₁ C ₀	55.8 \pm 5.89	66.0 \pm 2.44 ^c	53.2 \pm 6.61 ^a	59.6 \pm 5.85 ^a	58.8 \pm 7.72 ^a	63.6 \pm 6.18 ^a	68.8 \pm 3.89 ^c
	a ₁ C ₁	56.8 \pm 1.40	61.4 \pm 3.57 ^b	57.4 \pm 3.84 ^b	57.8 \pm 7.52 ^a	64.4 \pm 2.19 ^{ab}	66.4 \pm 3.64 ^a	63.0 \pm 6.20 ^{ab}
RT (°C)	a ₀ C ₀	38.46 \pm 0.24	39.22 \pm 0.31 ^a	38.84 \pm 0.26 ^a	39.12 \pm 0.14 ^a	38.84 \pm 0.26 ^a	39.90 \pm 0.50 ^{ab}	39.86 \pm 0.48 ^a
	a ₀ C ₁	38.4 \pm 0.15	39.32 \pm 0.52 ^a	38.84 \pm 0.18 ^a	39.30 \pm 0.25 ^a	38.84 \pm 0.18 ^a	39.46 \pm 0.11 ^a	39.42 \pm 0.32 ^a
	a ₁ C ₀	38.54 \pm 0.28	39.4 \pm 0.14 ^a	39.04 \pm 0.15 ^a	39.58 \pm 0.21 ^a	39.04 \pm 0.15 ^a	39.7 \pm 0.36 ^a	39.70 \pm 0.27 ^a
	a ₁ C ₁	38.46 \pm 0.13	39.8 \pm 1.31 ^a	38.82 \pm 0.33 ^a	39.38 \pm 0.64 ^a	38.82 \pm 0.33 ^a	40.0 \pm 0.30 ^{ab}	40.00 \pm 0.74 ^a

a₀C₀ = no ascorbyl palmytate administration – unshear, a₀C₁ = no ascorbyl palmytate administration shearing, a₁C₀ = ascorbyl palmytate administration – unshear, a₁C₁ = ascorbyl palmytate administration – shearing, Hb = Haemoglobin, RBC = Red Blood Cell, PCV=Packed Cell Volume, WBC= White Blood cells. Means within the same row with different superscripts are significantly different (P<0.05).

Table 2. The Stress Level in Padjadjaran Sheep

Respiration Rate	Stress Level	Before Heat Shock	Week					
			1	2	3	4	5	6
----- % -----								
< 40 breaths/ min ¹⁾	No stress	85	25	30	20	15	15	0
40 – 60 breaths/ min ¹⁾	Low	5	40	40	40	45	35	0
61 - 80 breaths/ min ¹⁾	Medium – high	10	20	20	20	20	35	0
81 – 120 breaths/ min ¹⁾	High	0	15	10	5	5	10	25
121 – 200 breaths/ min ²⁾	Heavy	0	0	0	15	15	5	75
> 200 breaths/ min ¹⁾	Severe	0	0	0	0	0	0	0

¹ Scale adapted from Silanikove (Silanikove, 2000)

² Own study

Blood Metabolite Profile

Blood metabolite profiles, glucose, cholesterol, and total protein present in Table 3. According to the average value, antioxidant AP affect glucose concentration ($P < 0.05$). In the third and fourth week, no significant effect of treatment on glucose. In the fifth week antioxidant effect ($P < 0.05$) to glucose, while in the sixth week there is an interaction effect ($P < 0.05$) to glucose.

Shearing generally affect cholesterol, in third week glucose affect antioxidant, while shearing affect glucose in the fourth week. In the fifth and sixth week, no significant effect of treatment on glucose.

According to the average, shearing and AP administration no different effect on total protein. Both in the third and fourth week, shearing has significance to total protein, in the fifth week there are interaction effects of treatment on total protein; in the sixth week total protein no affected by treatment.

DISCUSSION

In sheep, the RR normal 25 – 30 breath per minute (Indu *et al.*, 2014; Veerasamy Sejian *et al.*, 2010). RR was an indicator of heat load and heat stress in animals (Okourwa, 2015). RR before heat exposure in current research classified no stress. McManus *et al.* (2016) stated that according to RR, there was heat stress level classification, no stress: up to 40 breath per minutes, low stress: 40 – 60; middle stress: 61 –

80; high stress: 81 – 120, very high stress: 121 – 193, and severe heat stress: more than 193 breath per minutes. Increasing RR showed that animals endeavor to the maintenance of normal body temperature in a way increasing heat exhausting through breath evaporation (Shaji *et al.*, 2017). When heat shock at 28.95°C, RR of shorn and unshorn of sheep no significantly different (Taha *et al.*, 2018). Higher RR is a physiological response to increasing oxygen delivery to the lungs, which could improve heat production and, subsequently, elevate body temperature (Habibu *et al.*, 2017).

In sheep farming, shearing was a routine activity, helpful thermogenesis inductor (Al-Ramamneh *et al.*, 2011). Shearing effect on the oxidative parameter and change the homeostasis balance (Piccione *et al.*, 2011). In the sixth week, APs significant effect ($P < 0.05$) decrease RR compared to non-AP.

The previous study stated that range pulse rate in sheep 98.5 – 103,6 pulse per minutes (Seixas *et al.*, 2017), 70 – 80 pulses per minutes and increase to 83 pulses when exposing to heat load (Al-Haidary *et al.*, 2012), 90 – 107 pulses (Wojtas *et al.*, 2014). Another researcher report in the 15th first day after shearing, the pulse rate significantly increase, then decrease gradually (Casella *et al.*, 2016). However, in the first week, the pulse rate in non-AP administration treatment lower than administration AP, which means that in case AP does not need yet to control heat stress, reinforce by RR in this session categorized as low

Table 3. Average \pm SE of Glucose, Cholesterol and Total Protein of Padjadjaran Sheep

Treatment	Week after Heat Exposure				Average
	3 rd	4 th	5 th	6 th	
Glucose					
a ₀ c ₀	66.85 \pm 7.54 ^{ns}	62.29 \pm 13.18 ^{ns}	99.21 \pm 30.05 ^b	70.22 \pm 13.11 ^b	74.64 \pm 7.55 ^b
a ₀ c ₁	53.35 \pm 5.38 ^{ns}	63.34 \pm 5.79 ^{ns}	96.61 \pm 54.48 ^b	140.22 \pm 93.01 ^c	83.06 \pm 31.32 ^b
a ₁ c ₀	44.01 \pm 30.29 ^{ns}	56.56 \pm 6.99 ^{ns}	53.37 \pm 13.23 ^a	63.04 \pm 12.85 ^{ab}	62.97 \pm 21.78 ^a
a ₁ c ₁	57.45 \pm 34.28 ^{ns}	57.6 \pm 6.06 ^{ns}	46.90 \pm 9.38 ^a	54.19 \pm 24.23 ^a	52.82 \pm 14.81 ^a
Cholesterol					
a ₀ c ₀	243.38 \pm 103.34 ^b	97.58 \pm 89.91 ^a	113.95 \pm 46.60 ^{ns}	97.60 \pm 39.38 ^{ns}	135.21 \pm 48.48 ^a
a ₀ c ₁	246.03 \pm 117.22 ^b	207.11 \pm 102.81 ^b	106.09 \pm 34.98 ^{ns}	94.69 \pm 13.73 ^{ns}	153.13 \pm 37.8 ^b
a ₁ c ₀	102.49 \pm 43.19 ^a	108.38 \pm 21.93 ^a	83.17 \pm 22.69 ^{ns}	98.84 \pm 37.32 ^{ns}	98.22 \pm 10.50 ^a
a ₁ c ₁	97.35 \pm 20.86 ^a	126.31 \pm 19.52 ^b	139.87 \pm 33.45 ^{ns}	98.77 \pm 26.33 ^{ns}	110.75 \pm 17.44 ^b
Total Protein					
a ₀ c ₀	4.25 \pm 1.33 ^a	6.48 \pm 1.02 ^b	7.01 \pm 0.76 ^b	5.59 \pm 0.64 ^{ns}	5.79 \pm 0.38 ^{ns}
a ₀ c ₁	6.10 \pm 0.48 ^b	6.21 \pm 0.34 ^a	5.38 \pm 0.52 ^a	5.63 \pm 0.63 ^{ns}	5.83 \pm 0.43 ^{ns}
a ₁ c ₀	4.29 \pm 0.74 ^a	5.17 \pm 1.31 ^b	5.21 \pm 0.54 ^a	5.07 \pm 2.28 ^{ns}	5.08 \pm 1.03 ^{ns}
a ₁ c ₁	5.45 \pm 0.55 ^b	3.07 \pm 2.63 ^a	7.39 \pm 0.52 ^b	5.29 \pm 1.22 ^{ns}	5.47 \pm 0.96 ^{ns}

a₀c₀ = no ascorbyl palmytate administration – unshear, a₀c₁ = no ascorbyl palmytate administration shearing, a₁c₀ = ascorbyl palmytate administration – unshear, a₁c₁ = ascorbyl palmytate administration – shearing. Means within the same row with different superscripts are significantly different (P<0.05).

heat stress. The higher PR in the stressed animals dissipate more heat to its surroundings by increasing the blood flow to their body surfaces (Shaji *et al.*, 2017).

Rectal temperature is an indicator of heat storage in an animal's body and usually be used to assess the adversity of the thermal environment (Ganaie *et al.*, 2013). Rectal temperature, respiration rate, and heart rate reflect the degree of stress imposed on animals (Ganaie *et al.*, 2013). Antioxidant administration no effect on RT (Chauhan *et al.*, 2014), a combination of minerals and vitamin E (Sejian *et al.*, 2014), melatonin application (Bouroutzika *et al.*, 2020) may reduce RT, dose 100 mg kg⁻¹ body weight in goat able to control heat stress. Vitamin C administration in 500 mg/ kg feed highly significant decrease in RR and PR, but no

significant effect on TR (Al-Azzawi *et al.*, 2017). Ascorbic acid supplementation 50 and 100 mg/ kg body weight significantly decrease RR, PR and RT compare to control without supplementation (Khan and Konwar, 2015).

Compared to previous research, the glucose, cholesterol and total protein concentration grazed sheep are 43.25 \pm 1.36 mg/dL, 56.64 \pm 1.68 mg/dL and 7.14 \pm 0.12g/dL respectively (Badakhshan and Mirmahmoudi, 2016). Glucose and cholesterol concentration of sheep supplemented curcumin 900 mg per sheep, housed at 33.32°C are 3.81 and 3.3 mmol/ L respectively (Jiang *et al.*, 2019). Glucose, cholesterol and total protein concentration of lambs which free access to water lower than limited access (Vosooghi-Postindoz *et al.*, 2018). Singh *et al.* (2016) report that the total protein concentration of Indian indigenous sheep

at heat stress condition higher than normal, while cholesterol and glucose concentration oppositely. In goat during summer, ascorbic acid supplementation 50 and 100 mg/ kg body weight significantly increase plasma total protein compare to 0 mg, while on glucose no difference (Khan and Konwar, 2015). In current study, during heat exposure application, sheep tethered, they are no chance to escape to shady.

CONCLUSION

It could be concluded, supplementing AP and shearing application could reduce the negative impact of heat stress on physiological and blood metabolite profiles of Padjadjaran sheep. Although the wool of local breed has not economic value, the shearing regularly needed.

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