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Research Article
The Effect of Gamma Aminobutyric Acid and Ascorbic Acid on Bali Cattle Physiology During Transport

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Abstract
Background and Objective: Gamma aminobutyric acid (GABA) can relieve stress intensityand ascorbic acid can protect against weight loss. Together, these agents can minimize weight loss and stress during cattle transportation. The objective of this study was to evaluate the effects of ascorbic acid and GABA on the physiology of Bali cattle during transportation. **Materials and Methods:** The study was designed using a completely randomized design of factorial patterns. The first factor was ascorbic acid (0 mg kg^{-1} body weight (bw) and 100 mg kg^{-1} bw dosage) and the second factor was GABA (0, 0.6, 1.2 and 1.8 g $head^{-1}$ dosage). Statistical calculations were performed to determine differences between treatments using Duncan's multiplerange tests. **Results:** Ascorbic acid (100 mg kg^{-1} bb) and GABA (1.2 g $tail^{-1}$) reduced cortisol levels and weight loss ($p<0.05$) during transportation. There were no differences in heart rate, rectal temperature and respiratory rate ($p>0.05$). **Conclusion:** Administration of ascorbic acid and GABA (100 mg kg^{-1} bw and 1.2 g $tail^{-1}$, respectively) reduced blood cortisol levels and protected against body weight loss. Coadministration did not have a significant effect on rectal temperature, respiration, or heart rate.

Key words: Ascorbic acid, bali cattle, body weight loss, cattle transportation, cortisol, gamma aminobutyric acid

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Each year, Nusa Tenggara Timur (NTT), a national cattle warehouse, sends approximately 50,000-60,000 head of cattle to Java[1]. Usually, these Bali cattle are distributed to Jakarta and West Java. Since these regions need to reach 469,000 heads per year, NTT provides approximately 10.66-13.04% of their cattle needs.

Bali cattle are transported from NTT to Java over land and sea, due to to the geographical conditions of the archipelago country of Indonesia. The long journey causes exhaustion and increased stress in the cattle, which can lead to behavioral abnormalities, such as recurrent defecation and urination, changes in hematological factors and in hormones, such as cortisol[2].

Cortisol acts as an immunosuppressant and increased levels due to stress can result in a high susceptibility to infection from various diseases, which is known as transit fever.[3] Usually, cattle affected by transit fever will not eat, causing the cattle to lose weight during transportation. Body weight loss can reach 1112% in cattle transported from Mataram to Java Island.[1] Supplements in the form of drugs, minerals, or vitamins, including gamaaminobutyric acid (GABA) and ascorbic acid, can reduce cortisol levels.

Gammaaaminobutyric acid (GABA) is a major inhibitory neurotransmitter that relieves stress intensity, inhibits spontaneous reaction effects in the central and peripheral nervous system and helps process information in the central nervous system. Several reports have indicated that 40 mg kg^{-1} of dry matter GABA supplementation in dairy cattle can reduce heat stress by reducing rectal temperature and increasing milk production and composition[4]. GABA supplementation of 1.2 g day^{-1} was also shown to increase feed intake and milk protein production and reduce non esterified fatty acids in postpartum dairy cattle[5].

Ascorbic acid has attracted the attention of scientists in recent years due to its ability to reduce stress caused by high temperatures, disease and transport in various animal species. Ascorbic acid has also been shown to reduce body weight loss during transportation. Treatment with a 100 mg bw^{-1} ascorbic acid dose resulted in a body weight loss of 2.4% during Bali cattle transportation[6]. A study of goats administered ascorbic acid orally over 8 h of transportation found that goats given ascorbic acid experienced weight loss of 1.04% compared to a weight loss of 11.9% in goats who were not given ascorbic acid[3]. In addition, pig transportation over land for 8 h causes hypothermia but oral administration of ascorbic acid to 23 pigs (100 mg kg^{-1}) has been shown to prevent hypothermia[7].

GABA and ascorbic acid administration may produce positive effects on livestock but less is known about the effects of their coadministration. This study evaluated the effects of GABA and ascorbic acid supplementation given to Bali cattle during transportation on cortisol levels, weight loss and other physiological features.

MATERIALS AND METHODS

Animals: This experiment included 24 male Balinese cattle, with body weights ranging from 150175 kg and ages ranging from 22.5 years. Prior to transportation, Bali cattle were grouped for a week to promote socialization and to prevent fights. At the beginning of the study, the cattle were weighed and the coefficient of variation was calculated; then, the cattle were selected at random for treatment group.

The GABA supplement used was a fermented extract of various grains that produce active compounds in the form of white powder (Nutrivita Company, USA). The ascorbic acid used was the chemical formula $C_6H_8O_6$ or based on IUPAC systematics, 2 oxoLthreohexono1, 4 lactone 2, 3 enediolin, in the form of a white powder with (Bratachem Bandung). Ascorbic acid was given orally dissolved in 20 mL of water.

Methods: Each of the treatment groups in this study were as follows:

- G0 = GABA Supplement 0 g $head^{-1}$ day^{-1}
- G1 = GABA Supplement 0.6 g $head^{-1}$ day^{-1}
- G2 = GABA Supplement 1.2 g $head^{-1}$ day^{-1}
- G3 = GABA Supplement 1.8 g $head^{-1}$ day^{-1}
- C0 = ascorbic acid 0 mg kg^{-1} bw
- C1 = ascorbic acid 100 mg kg^{-1} BW
- R0 = 0 g $tail^{-1}$ day^{-1}+ascorbic acid = 0 mg kg^{-1} bw
- R1 = 0.6 g $tail^{-1}$ day^{-1}+ascorbic acid = 0 mg kg^{-1} bw
- R2 = 1.2 g $tail^{-1}$ day^{-1}+ascorbic acid = 0 mg kg^{-1} bw
- R3 = 1.8 g $tail^{-1}$ day^{-1}+ascorbic acid = 0 mg kg^{-1} bw
- R4 = 0 g $tail^{-1}$ day^{-1}+ascorbic acid = 100 mg kg^{-1} bw
- R5 = 0.6 g $tail^{-1}$ day^{-1}+ascorbic acid = 100 mg kg^{-1} bw
- R6 = 1.2 g $tail^{-1}$ day^{-1}+ascorbic acid = 100 mg kg^{-1} bw
- R7 = 1.8 g $tail^{-1}$ day^{-1}+ascorbic acid = 100 mg kg^{-1} bw

GABA and ascorbic acid supplements were weighed and diluted with 600 mL of water. GABA and ascorbic acid solutions were incorporated into different "drenching gun" tubes and then administeredorally to livestock 30 min prior to transportation.

Each truck transported 12 tails. After all livestock were secured in to the truck, they drove for 8 h with a 30 min rest. Following arrival, data were collected.

The experiment was conducted using a factorial randomized design consisting of 2 factorials-a combination of GABA with ascorbic acid across 3 replications. Data were analyzed using one way ANOVA followed by Duncan's Multiple Range (DMR) test to determine significant differences between the means. Differences of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The effects of GABA and ascorbic acid supplementation on blood cortisol levels: Table 1 shows that R0 (control) had the highest cortisol levels. Cortisol levels increased by 38.77 ng mL^{-1}. Meanwhile, levels of the R1, R2, R3, R4, R5, R6 and R7 groups ranged from 3.89 ng mL^{-1} (R6) to 6.40 ng mL^{-1} (R3), indicating that ascorbic acid and GABA was able to decrease cortisol levels during transportation. The results of the variance test showed that the single dose factor had no significant effect ($p>0.05$) on the treatment parameters measured in the morning or afternoon. However, the interaction factor between ascorbic acid and GABA was significantly increased ($p<0.05$).

Dosage interactions of ascorbic acid with GABA (up to 100 mg kg^{-1} bw and 1.8 g $tail^{-1}$)reduced blood cortisol levels in Bali cattle during transportation compared to Bali cattle that were not given ascorbic acid with GABA (Table 2 and 3).

A study concluded that cortisol levels of 70 ng mL^{-1} or more are an indicator of stress on livestock[8]. Furthermore, cortisol levels in bulls differ according to the age and gender of the animals. In young males, cortisol level was 67.5 ± 38.4 ng dL^{-1} [9]. Bali cows in the R0 group experienced very high stress levels. However, the other groups showed that ascorbic acid dose and GABA effectively reduced blood cortisol levels during transportation, presumably because the combination of treatments interacted optimally.

Ascorbic acid stimulates and improves spperformance by increasing the production of GABA, which controls neurotransmitters that lower hormone cortisol secretion. GABA inhibition opens chloride ions that are negatively charged, so that the nerve fibers are charged very negatively. Thus, a nerve impulse becomes difficult to deliver through the nerve fibers.

When the brain experiences an abundance of stress, cortisol, norepinephrine, or epinephrine (adrenaline) levels increase. To neutralize excessive cortisol and adrenaline, the brain produces neurotransmitters, one of which is GABA, which has an inhibitory effect on the nervous system. GABA is composed of glucose, pyruvate and glutamine compounds[10]. GABA produces a calming effect and can alleviate symptoms of depression by stimulating the production of endorphins, resulting in a comfortable effect both physically and mentally. GABA also opens Cl channels and causes hyperpolarization, which inhibits delivery of an action potential, resulting in sedative and anesthetic effects.

Effects of GABA and ascorbic acid supplementation on weight loss: In Table 4, there was a decrease in body weight following transportation. The highest change in body weight

Table 1: Average cortisol levels before and after transportation

Treatments		Cortisol levels		
		Difference (Ng mL^{-1})	Morning (Ng mL^{-1})	Afternoon (Ng mL^{-1})
R0	Mean	106.36[a]	145.13[a]	38.76
	SD	68.43	110.23	42.93
R1	Mean	40.41[b]	46.33[b]	5.92
	SD	42.73	5.50	48.22
R2	Mean	35.42[b]	41.12[b]	5.70
	SD	1.57	3.88	2.33
R3	Mean	29.90[b]	36.30[b]	6.40
	SD	17.05	10.99	8.06
R4	Mean	13.51[b]	20.69[b]	7.18
	SD	4.81	3.48	8.19
R5	Mean	46.87[b]	52.70[b]	5.82
	SD	3.39	5.41	8.76
R6	Mean	46.59[b]	50.48[b]	3.89
	SD	29.82	6.91	23.16
R7	Mean	50.94[b]	56.00[b]	5.06
	SD	16.41	12.28	4.25
Total	Mean	46.25	56.09	9.84
	SD	36.93	48.86	23.55
		$p<0.05$	$p<0.05$	$p>0.05$

SD: Standard Deviation

was found in the R0 (control) group, which was 14.67 kg and the lowest change was in the R6 group (0.33 kg dose). Variations in weight loss during transportation occurred due to the influence of variations in the dose of ascorbic acid and GABA. The more a dose of ascorbic acid and GABA were given, the lower decrease in body weight or the percentage of weight loss.

The effect of the ascorbic acid factor was significantly increased ($p<0.05$), as well as the effect of the GABA factor and the combination of both ($p<0.01$), on body weight loss during transportation.

There was no significant difference in body weight loss across R1, R2 R3, R4, R5, R6 and R7 groups; however, the seventh treatment was different from treatment R0 (control) (Table 5). A significant decrease in body weight was observed in Bali cattle that were not given ascorbic acid and GABA (R0), compared to all other treatment groups.

Table 6 shows the weight loss difference compared to control group. Administration of ascorbic acid and GABA [100 mg kg^{-1} body weight and 1.2 g head^{-1} (R6)] reduced body weight loss ($p<0.05$) compared to control group (R0), indicating that the combination reduced body weight loss of the Bali cattle during transportation. This effect was possibly due to the ability of ascorbic acid to stimulate aminobutyric acid (GABA) production in the brain. Administration of ascorbic acid at a higher dose makes GABA more effective in reducing cortisol production, so the impact of body weight loss due to transportation is decreased[2].

Effects of GABA and ascorbic acid on rectal temperature, respiratory rate and heart rate: The dose factor had no significant effect ($p>0.05$) on the additional parameters measured. Ascorbic acid (up to a dose of 100 mg kg^{-1} bw) and GABA administration (up to 1.8 mg kg^{-1} bw) did not have a negative effect on rectal temperature, respiration, or heart rate, presumably because the cattle were able to cope with external stresses so these parameters were within the normal range (Table 7). However, in all treatment groups, respiratory frequency was above the normal range before and after transportation. The presence of respiratory frequency may be due to the sudden effect of physical activity in the form of

Table 2: Cortisol differences prior to transportation (morning)

Treatments		N	Subset for alpha = 0.05: 1	2
Duncan[a]	R4	3	13.5100	
	R3	3	29.9067	
	R2	3	35.4200	
	R1	3	40.4100	
	R6	3	46.5933	
	R5	3	46.8733	
	R7	3	50.9400	
	R0	3		106.3633
	Significance		0.216	1.000

[a]Means of groups in homogeneous subsets

Table 3: Cortisol differences after transportation (afternoon)

Treatments		N	Subset for alpha = 0.05: 1	2
Duncan[a]	R4	3	20.6900	
	R3	3	36.3067	
	R2	3	41.1233	
	R1	3	46.3333	
	R6	3	50.4833	
	R5	3	52.7000	
	R7	3	56.0000	
	R0	3		145.1300
	Significance		0.346	1.000

[a]Means of groups in homogeneous subsets; Harmonic mean sample size: 3.000

Table 4: Average body weight before and after transportation

Treatments	Body weight	Morning (kg)	Afternoon (kg)	Weight loss (kg)	Weight los (%)
R0	Mean	236.00	221.33	14.670	6.230
	SD	21.00	21.45	5.030	2.260
R1	Mean	226.33	221.00	5.330	2.360
	SD	5.03	6.55	2.080	0.930
R2	Mean	217.67	216.33	1.330	0.620
	SD	10.01	11.01	1.520	0.737
R3	Mean	223.00	221.33	1.670	0.770
	SD	27.71	28.30	1.150	0.580
R4	Mean	224.33	221.00	3.330	1.410
	SD	19.00	16.00	3.050	1.270
R5	Mean	217.67	214.33	3.330	1.500
	SD	6.50	3.51	3.050	1.360
R6	Mean	202.00	201.67	0.330	0.160
	SD	1.00	.57	0.577	0.280
R7	Mean	231.33	226.67	4.670	2.030
	SD	7.09	10.40	5.033	2.240
Total	Mean	222.29	217.96	4.330	1.890
	SD	15.90	14.44	5.020s	2.150

SD: Standard deviation

Table 5: Weight loss across treatment groups

		Subset for alpha = 0.05	
Transportation	N	1	2
R6	3	0.33	
R2	3	1.33	
R3	3	1.67	
R4	3	3.33	
R5	3	3.33	
R7	3	4.67	
R1	3	5.33	
R0	3		14.67
Significance		0.100	1.000

Table 6: Weight loss difference compared to group R0

Control (I)	Treatments (J)	Mean difference (IJ)
RO	R1	8.000
	R2	12.000
	R3	11.667
	R4	10.000
	R5	10.000
	R6	13.000*
	R7	8.667

Table 7: Physiological changes during transportation

	Treatments	Rectal temperature morning	Rectal temperature afternoon	Respiratory rate morning	Respiratory rate afternoon	Heartrate morning	Heart rate afternoon
RO	Mean	38.70	38.77	55.00	51.67	63.33	70.67
	SD	0.40	0.05	11.53	15.69	7.23	6.02
R1	Mean	38.60	38.97	56.67	55.33	69.67	64.67
	SD	0.43	0.47	2.51	1.52	7.37	10.50
R2	Mean	38.77	38.53	57.33	48.00	64.33	68.33
	SD	0.20	0.20	4.16	6.08	4.72	2.51
R3	Mean	39.00	38.57	58.67	44.67	74.33	75.67
	SD	0.36	0.15	4.72	11.01	5.50	2.08
R4	Mean	38.83	38.77	60.67	47.67	73.33	73.67
	SD	0.32	0.15	5.68	6.02	5.50	10.78
R5	Mean	38.53	38.57	51.00	48.33	73.00	66.00
	SD	0.50	0.11	3.60	6.65	3.60	10.00
R6	Mean	38.70	38.53	47.67	45.67	77.67	64.00
	SD	0.17	0.20	8.50	6.11	9.01	5.29
R7	Mean	38.27	38.53	61.67	59.67	67.67	66.67
	SD	0.32	0.05	4.50	7.02	2.51	9.50
Total	Mean	38.68	38.65	56.08	50.13	70.42	68.71
	SD	0.36	0.23	6.94	8.59	6.96	7.70

SD: Standard deviation

restraint, forced withdrawal and censure. The range of heart rate, respiratory rate and rectal temperature were 6070, 1216. and 3739°C, respectively[10,11].

This experiment showed that a combination of GABA and abscorbic acid can be used by farmers to reduce the impact of transport stress, such as elevated blood cortisol levels and decreased body weight of Bali cattle during transportation and to reduce the financial losses caused by transportation.

CONCLUSION

Administration of ascorbic acid and GABA (100 mg kg^{-1} bw and 1.2 g $tail^{-1}$, respectively) reduced blood cortisol levels and protected against body weight loss. Coad ministration did not have a significant effect on rectal temperature, respiration, or heart rate.

SIGNIFICANCE STATEMENT

This study identified a possible synergistic effect of GABA and ascorbic acid administration on reducing body weight loss of Bali cattle during transportation. This study may help researchers determine appropriate combinations of agents for optimal effects. Thus, this combination of agents and possibly other combinations, may be explored further.

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