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The use of non-invasive methods to measure the physiological state of Bali cattle supplemented with herbs and humic acid

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Abstract Morning measurement was not significant difference in rectal, pulse and respiration rate, among Bali cattle with no supplementation (T-0 or Control), or with herbs supplementation (T-1), with herbs supplementation and humic acid 20 g/head (T-2) and with herbs supplementation and humic acid 40 g per head (T-3). Rectal temperature (RT) was correlated with forehead infrared temperature (FIT) which r was 0.515. Noon observation indicated the respiration rate was significantly higher ($P<0.05$) with RT but lower in control animals. Pearson correlation indicated that RT significantly ($P<0.01$) correlated with FIT (r: 0.690); and the correlation with tail base infrared temperature (TBIT) which r was 0.443. During afternoon measurement, the respiration rate was 26-27.6 x/minutes, the pulse rate was 74.9 – 76.88, and the rectal temperature was around 37.77 ± 0.21°C with no significant difference among the treatments. The Thermal Humidity Index (THI) during the experimental periods for all treatments was 78.4-86.87 while the HTC was 2.14-2.30. Supplementation increased the adaptability of experimental animals in this study. In addition, non-invasive urinalysis using a strip (URS-10T) found the absence of blood, protein, ketone, and glucose in either control (T-0) group of Bali cattle with herbs supplementation (T-1) in Bali cattle supplemented with a combination of herbs and humic acid (T-2 and T-3). While positive leucocyte was found in control groups, a small amount of bilirubin and urobilinogen was found in all the treatments. For all the treatments, it was found that specific gravity was 1.015-1.020 whereas, the humic acid supplementation (T-2) had lower urine pH. In conclusion, the addition of herbs and humic acid did not affect the physiological state and the increased adaptability of Bali cattle in humid conditions. Infrared temperature (FIT) can be used to predict the rectal temperature. Test strips can be used to analyze the urine of cattle, however, additional testing is required for clinical diagnosis to reach a more conclusive diagnosis.

Keywords: Oil palm sludge, Infrared temperature, HTC, Dipstic, Urinalysis

Introduction

There has been interested lately in non-invasive methods to observe the physical condition of farm animals. This strategy demonstrated several benefits, including enhanced animal welfare and decreased animal anxiety.

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Conventionally, evaluating the physiological state of these animals required intrusive techniques such as drawing blood. These techniques, however, can cause stress and discomfort in the cattle in addition to being time- and labour-intensive. Non-invasive methods are now a viable alternative to invasive methods for monitoring cattle's health. Core body temperature represents the most vital organs in the body: the brain, liver, and lungs (Idris *et al*., 2021). Rectal, vaginal, tympanic, and rumen temperatures could be used as indirect methods for determining body temperature (Hillman *et al*., 2005) (Idris *et al*., 2021). Rectal temperature is one best standards for evaluating an animal's stress, physiological state, health, and welfare (Falkenberg *et al*., 2014) (Godyń *et al*., 2019). (Goodwin, 1998; Chung *et al*., 2010; Rejeb *et al*., 2016) also mentioned that rectal temperature (RT) has long been regarded as the accepted method for measuring body temperature in veterinary medicine because it corresponds well with the core body temperature. Even though body temperatures vary during the day in different sections of the body (Ribeiro *et al*., 2018).

However, measuring the animal's rectal temperature is a challenging process and causes discomfort to the animal. The method of using thermometers in contact with the rectal mucosa can be hard to carry out, especially for difficult animals, and it may also result in cross-contamination if proper hygiene measures are not implemented. Additionally, there is also the possibility of further rectal injury and discomfort. A simple non-invasive method that can be utilized to measure body temperature is the use of an infrared thermometer (Yadav *et al*., 2017). The employment of an infrared thermometer is a straightforward, non-invasive technique that can be used to assess body temperature. Due to their rapid measurement capabilities and non-intrusive nature, infrared thermometers have significantly increased in popularity. Forehead temperature measurements using infrared technology have become particularly widespread, mainly because of the requirement for fast and convenient screenings, particularly during circumstances like the COVID-19 pandemic.

Urinalysis is a common laboratory test that has been studied and utilized for a long time. Urine analysis using dipstick is the other non-invasive method to determine health status and physiological change in animals (Afsahi *et al*., 2020). Kwong *et al*. (2013) reported that the pH of urine could be used as a simple method of measuring biochemical markers. However, the need to calibrate the test solution or standard buffer, the use of fresh urine and the need for personal training make the use of pH meters less attractive. Dipsticks, on the other hand, are single-use test strips that may assess several things besides pH, such as protein's existence, glucose, urobilinogen, leucocytes, bilirubin, and nitrite and need far less user training and are less susceptible to prejudice in perception (Kwong *et al*., 2013). Afsahi *et al*. (2020) found a significant relationship between the pH meter and the urine dipstick used to assess the pH of the cattle. Numerous metabolites found in urine can reveal details about the body's current physiologic condition and any disease-related symptoms. In addition, metabolic substances in the urine are affected by dietary treatments. Clarke *et al*.(2020) highlight urine metabolites can be used as a biomarker for nutrient intake.

The unfavourable side effects of modern pharmaceuticals, high input prices, hazardous residues, the resistance of microbes, and the development of cattle farming using organic systems have all contributed to an increase in the use of herbs in livestock production in recent years. Medical herbs can be used as medicines and dietary supplements and have a significant impact on the world's health systems for both people and animals like cattle (Kuralkar and Kuralkar, 2021). The use of herbs as therapeutics and nutritive aids is commonly carried out by farmers in Bengkulu to increase productivity and disease prevention in ruminants (Suteky *et al*., 2020). Herbs contain a secondary metabolic compound that will be excreted mainly through urine after absorption (Acamovic and Brooker, 2005).

Supplementation of organic acids/humic in feed is not only a source of energy but can stimulate (Terry *et al*., 2018). By modifying the gut microbiota and enhancing digestive enzymes, humic acids can enhance rumen digestion and animal performance (digestibility, growth and immune system, and have the ability to absorb and detoxify (Kholif *et al*., 2021), and can maintain microflora balance, and improve nutrient utilization and feed efficiency. There is currently little knowledge about the impact combination of herbs and humic acids on the physiological status and urine analysis. Therefore, the study aimed to determine the connection between the rectal temperature and surface temperature based on an Infrared thermometer Gun.

Materials and methods

This experiment was done in RDP farm Bukit Paninjauan I, Seluma Regency Bengkulu about 30 km from the University of Bengkulu. Twenty Bali cattle were used in this study and were distributed into 4 treatments, 5 Bali cattle in each treatment. All the animals were stanchioned in individual cages and fed with 40% forage + 60% oil palm sludge + 2.5 % tofu waste in individual BW, all fed given was fresh based. The treatments were T-0 (no herbs and no humic acid), T-1 (with herbs supplementation and no humic acid), T-2 (with herbs supplementation and humic acid 20 g/head), and T-3 (with herbs supplementation and humic acid 40 g per head). Herbal supplementation was *Curcuma longa* 0.01 g/kg BW + *Melastoma malabathricum* 0.005 g/kg BW in the form of powder.

The experiment was done in 30 days with 15 days for cattle adaption to diet and management. The cattle had unlimited access to water.

Physiological parameters were performed during 7 days (days 24-30) three times a day on 08.00, 12.00-13.00 and 16.00. By pressing the index and middle fingers against the femoral artery, the pulse rate (x/minute) was measured. The respiration rate (beats per minute) was calculated by enumerating the flank movements. The temperature of the rectum (C) was obtained by entering the rectum with a rectal thermometer and holding it there until stable. Three different body sections had their surface temperatures taken at the same moment as the forehead, thigh, and tail base with the help of an infrared digital thermometer (Medical Infrared Thermometer IT-122-MISOO), and put this thermometer 5 cm away from the desired surface site.

Thermal Humidity Index (THI), Heat tolerance coefficient, and Adaptability coefficient

THI= (1,8 x T + 32) – [(0.55-0.0055 x RH) x (1.8 x T -26)] was based on (Thompson and Dahl, 2012) which T= air temperature and RH= Relative Humidity.

HTC-Benezra = (RT/38,33) + (RR/22); where RT: Rectal Temperature RR: Respiration Rate (Benezra, 1954)

AC1 : RT/38.33 ± RR/22

The adaptability coefficient 2 (AC2) was obtained by adding the HR to the preceding calculation to boost the test's detection capabilities.

AC2 : RT/38.33 ± RR/22 ± HR/74

Urinalysis: Urine was collected directly into a 20-mL clean container and avoided contact with cattle bodies, urine samples were analysed directly after collection. Reagen Strip for Urinalysis (URS-10T - Henso Medical, Hangzhou Co., Ltd.) was used in this analysis. The reagent area of the strip was put into the urine sample and waited for the time specified by the manufacturer. To analyze the test strip and compared it to the given colour reference grid, hold it horizontally. Because different researchers may interpret squares with colours that fall between the negative and lowest positive values differently, urine analysis performed with reagent strips might be subjected.

Results

There was a highly significant difference ($P<0.05$) in the air temperature, the relative humidity, and the Temperature Humidity Index (THI) at three different periods of observation in the morning, noon, and afternoon (Table 1).

Table 1. The average and range of air temperature, relative humidity, and THI

	Temperature (ºC)		Relative humidity (%)		THI	
Morning	27.50^{a}	(26.0-29.5)	89.90^{a}	(85.0-92.0)	80.17^{a}	(77.88-82.40)
Noon	33.40^{c}	(32.0-35.0)	70.00^{c}	(59.0-86.0)	86.39^{c}	(83.69-89.13)
Afternoon	29.70^{b}	(28.0-32.0)	81.40^{b}	(72.0-92.0)	82.60^{b}	(79.31-85.78)

All-day measurements revealed that there was an effect of supplementation ($P<0.05$) on respiration rate and rectal temperature, as seen in Table 2. The treatment affected rectal temperature in the morning and noon observation.

Table 2. Physiological value of cattle under herbs and humic acid

Measurements	Treatments					
	T-0	T-1	T-2	T-3	SEM	P
Morning						
Respiration rate (x/minutes)	27.6^{a}	25.2^{b}	26.0^{ab}	25.4^{b}	0.35	<0.05
Pulse rate (x/minutes)	74.5	74.8	76.3	76.2	0.51	>0.05
Rectal temperature (ºC)	36.9^{a}	37.1^{ab}	37.3^{b}	37.1^{ab}	0.06	<0.05
Forehead IT (ºC)	33.4	33.5	33.7	33.7	0.05	>0.05
Tail base IT (ºC)	32.9^{a}	33.1^{b}	33.0^{ab}	32.7^{ab}	0.08	<0.05
Thigh IT (ºC)	33.2	33.3	33.1	32.7	0.11	>0.05
Noon						
Respiration rate (x/minutes)	29.2^{a}	26.6^{b}	28.2^{ab}	26.8^{b}	0.35	<0.05
Pulse rate (x/minutes)	74.9	76.0	76.8	75.3	0.61	>0.05
Rectal temperature (ºC)	37.3^{a}	37.7^{b}	38.0^{b}	37.8^{b}	0.07	<0.05
Forehead IT (ºC)	35.7	35.8	36.2	36.4	0.10	<0.05
Tail base IT (ºC)	35.3	35.5	35.2	35.2	0.10	>0.05
Thigh IT (ºC)	35.6	35.6	35.5	34.8	0.09	>0.05
Afternoon						
Respiration rate (x/minutes)	27.6^{a}	25.6^{b}	26.4^{b}	26.6^{b}	0.26	<0.05
Pulse rate (x/minutes)	74.6	76.3	74.9	77.2	0.05	>0.05
Rectal temperature (ºC)	37.7	37.7	37.9	37.8	0.05	>0.05
Forehead IT (ºC)	35.4	35.4	35.3	35.3	0.14	>0.05
Tail base IT (ºC)	35.5^{a}	35.4^{a}	35.0^{b}	35.0^{b}	0.08	<0.05
Thigh IT (ºC)	35.2^{a}	35.3^{a}	35.1^{a}	34.5^{b}	0.11	<0.05

IT: Infrared Temperature.

Research findings revealed that without supplementation heat tolerance and adaptability coefficient were decreased (Table 3). A correlation of r = 0.515 was found between the rectal temperature and the forehead IT. When observed at noon, the respiration rate of the control animals was considerably lower (P<0.05) and their respiration rate was higher (P<0.05) compared to those of the treatment animals. The tail base IT showed a substantial (P<0.01) Pearson association with Forehead IT (r: 0.690) and Rectal Temperature (r: 0.443).

Table 3. Heat tolerance coefficient and adaptability coefficient

Measurements	Treatments					
	T-0	**T-1**	**T-2**	**T-3**	**SEM**	**P**
Morning						
HTC	2.22^{a}	2.11^{a}	2.15^{ab}	2.12^{b}	0.01	<0.05
AC1	2.50^{a}	2.40^{b}	2.45^{ab}	2.42^{ab}	0.06	<0.05
AC2	3.51	3.42	3.48	3.44	0.01	>0.05
Noon						
HTC	2.30^{a}	2.19^{b}	2.27^{ab}	2.20^{b}	0.01	<0.05
AC1	2.17^{a}	2.08^{b}	2.12^{ab}	2.09^{ab}	0.04	<0.05
AC2	3.18	3.11	3.16	3.11	0.01	>0.05
Afternoon						
HTC	2.24^{a}	2.15^{b}	2.19^{ab}	2.20^{ab}	0.01	<0.05
AC1	2.19^{a}	2.10^{b}	2.14^{ab}	2.15^{ab}	0.05	<0.05
AC2	3.19	3.13	3.15	3.19	0.01	>0.05

Urine analysis showed that all experimental cattle had negative value on glucose, blood, protein, nitrite, and ketone bodies (Table 4). The result from the other parameter (Leucocytes, Urobilinogen, and Bilirubin) is seen in the Table 4.

Table 4. Urinalysis of cattle under herbs and humic acid supplementation

	Leukocytes				**Urobilinogen**				**Bilirubin**			
	T-0	**T-1**	**T-2**	**T-3**	**T-0**	**T-1**	**T-2**	**T-3**	**T-0**	**T-1**	**T-2**	**T-3**
Neg	3	5	5	5	4	3	3	3	2	5	4	4
1+	1	0	0	0	1	2	2	2	3	0	1	1
2+	1	0	0	0	0	0	0	0	0	0	0	0
3+	0	0	0	0	0	0	0	0	0	0	0	0

	T-0	T-1	T-2	T-3
pH	7.00	7.00	6.70	6.70
USG	1.018	1.015	1.019	1.020
Glucose	-	-	-	-
Blood	-	-	-	-
Protein	-	-	-	-
Nitrite	-	-	-	-
Ketone Bodies	-	-	-	-

USG: Urine Specific Gravity

Discussion

This research was conducted in June-July 2023 with the air temperature average is 27.5ºC in the morning and 33.4ºC at noon. Air temperature in this study is slightly high compared to our previous research in 2021 which was 24-27.7ºC (Dwatmadji *et al*., 2021). Based on data from Meteorology, Climatology, and Geophysical Agency the average temperature during that month was 26.7ºC. Indonesia's air temperature anomalies in July 2023 was the 4th highest anomalous value throughout the observation period since 1981. Throughout the testing period, the THI was 77.88-89.13 and the relative humidity ranged from 70-89.9% (59-92%). The temperature-humidity index (THI) was formulated as a gauge of animal comfort. THI has been developed in a lot of studies using the following hot stress thresholds in cattle: when THI is less than 68 cattle are in the comfort zone, THI between more than 68 to less than 72 cattle in moderate discomfort, while discomfort (72 < THI < 75), alertness (75 < THI < 79), cattle is in danger when the THI 79 < or < 84), and emergency (THI > 84). However, this calculation is mainly used for cattle in temperate regions, therefore the thresholds of the comfort of Bali cattle could be different. According to Ørskov (2007) reported that cattle in tropical regions have better adaptability, and at least 40% of the energy gained from the food consumed must be primarily lost as evaporative heat.

Pulse rate and respiration rate act as early warning signs of worsening heat stress and offer a simple way for non-invasive as well as remote evaluation of the heat stress reaction. Our result showed that the respiration rate in cattle without supplementation was significantly higher than in the supplementation groups. However, the respiration rate in this experiment was still in normal condition for Bali cattle 20-34 beats/minute (Aritonang *et al*., 2017; Dwatmadji *et al*., 2021 Aditia *et al*., 2017).

Rectal temperature tends to lower in non- supplementation group but still in the normal range (Dwatmadji *et al*., 2021 and Putra *et al*., 2019). Table 2 shows that rectal temperature is low in the morning and then rises during noon. The core body temperature varies significantly during the day (Sarangi, 2018). According to Kubkomawa *et al*. (2015), the animals' energy expenditure to escape the heat throughout the day may be the cause of the daytime temperature increase. The temperature of an animal is truly determined by the equilibrium between heat released from the body as well as heat generated by muscle activity and the body's basic metabolism (Kubkomawa *et al*., 2015).

Throughout the study, all skin temperature (forehead, thigh, and tail base) measures were lower than rectal temperature. The average skin temperature in the morning was 33.2 ºC increased at noon to 35.6 ºC and it coincided with the increasing air temperature from 27.5ºC to 33.4ºC. Soerensen and Pedersen (2015) mentioned that skin temperature dropped at low ambient temperatures while rising temperatures caused skin temperature increases faster in a warmer temperature.

Pearson correlation showed that Rectal Temperature (RT) was correlated with forehead IT (r: 0.515) in morning observation, and RT significantly ($P<0.01$) correlated with forehead IT (r: 0.690). Also, the RT had the correlation with tail base IT (r:0.443) in noon evaluation and no correlation was found during afternoon observation. It seems the skin temperature is incredibly dependent on the site of observation. Yadav *et al*. (2017) said skin temperature differs depending on anatomical location. After comparing the non-supplemented and supplemented cattle, it was found that the HTC and Adaptability Coefficient 1 (AC1) differed significantly ($P<0.05$) (Table 3). These measurements can be used to determine an individual animal's degree of adaptation in response to different environmental variables (Araujo *et al*., 2017).

The findings also showed that AC1 is in the range of 2.08-2.5. Kumar (2023) in their research comparing the adaptability of cross-breed cows found that the AC1 was 2.2-2.5 depending on the season. As the normal value of HTC and AC1 were 2, it seems that supplemented cattle in this experiment had better adaptability than control.

Generally, no or a little bilirubinuria was excreted by a healthy animal, and normal urine does not contain any blood, protein, glucose, nitrite, or ketone (Parrah *et al*., 2013), and value of 1.020–1.045 is the normal urine specific gravity (USG) (Afsahi *et al*., 2020). Through assessment of cattle urine using a urine dipstick and a pH meter, it was found that the pH value of urine had relatively similar value: dipstick (6.7-7) and a pH meter (7.19-8.44). An excellent correlation was reported by Constable *et al.* (2019) in which urine pH of Holstein-Friesian cows' had a strong correlation when using Multistix-SG urine dipstick and pH paper (Hydrion). The findings revealed that urine pH in cattle supplemented with humic acid tend to be more acidic. Mavangira *et al*. (2010) showed that diet can have short-term effects on urine pH. This experment showed that using dipstick can be used for urine analysis, but for clinical analysis additional testing is necessary to provide a more conclusive diagnosis. In conclusion, test strips can be used to evaluate cattle urine, and forehead IT can be used to predict rectal temperature.

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