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Image of Infrared Thermography and Rectal Temperature of Ewes During Estrus Given Multinutrient Block Supplementation

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ABSTRACT This study aimed to look at infrared thermography images and rectal temperature of ewes during estrus that were given multinutrient block (MNB) supplementation. This study used 16 ewes that had given birth at least once, were in healthy condition, and had a normal reproductive cycle. The feed ingredients used are forage, MNB I (basic MNB), MNB II (MNB I added with Moringa leaves), and MNB III (MNB II plus the mineral Zn). The parameters observed were rectal temperature, microclimate temperature conditions, and infrared thermography images taken. The data from the analysis were tested for significance using ANOVA. Providing MNB supplementation to sheep based on vulva temperature parameters taken from a thermal camera showed a faster estrus response than sheep not given MNB. From the results of the three types of MNB, there was no significant difference in the duration of estrus symptoms. Infrared thermography can be used to strengthen the determination of the estrus phase in ewes.

Keywords: Estrus, MNB, thermography, rectal temperature

INTRODUCTION

Sheep are one of the meat-producing ruminants that have the potential to be developed. This is the reason why breeders are interested in developing sheep farming, especially in Indonesia. The increase in sheep population will undoubtedly increase the need for forage for animal feed. Livestock productivity is influenced by genetics 40% and the environment 60% in the form of feed, temperature and humidity, light intensity, health and others (Kurnianto, 2010).

Additional feed (supplements) are needed by ruminant livestock to fulfil nutritional requirements consisting of protein, energy and minerals (Suyanto et al., 2020). Feed supplements aim to increase more efficient utilization of poor-quality feed (Iskandar et al., 2020). One of the feeds that can be given is multinutrient block (MNB), which is a development of urea molasses block (UMB) (Iskandar et al., 2020). The composition of MNB consists of 50% molasses, 30% corn forage flour, 4% urea, 3% shellfish flour, 3% eggshell flour, 3% salt and 7% bentonite (Iskandar et al., 2020).

On the other hand, *Moringa oleifera* or also called Moringa leaves, is a plant that has the potential to replace vitamin and mineral deficiencies because of its complete nutritional content as animal feed (Agboun et al., 2016; Ahmad et al., 2016; Baptista et al., 2017; Gupta et al., 2012). Furthermore, Fuglie (2001) reported that Moringa leaves contain 27.1% protein and 2050 kcal/kg of metabolic energy. Furthermore (Gopalakrishnan et al., 2016) revealed that Moringa leaves also contain various vitamins, minerals and all essential amino acids. Apart from MNB and Moringa leaves, minerals such as Zinc (Zn) are also needed to increase livestock productivity. Underwood (2001) said that the mineral Zn plays a role in cell growth and division, sexual development, spermatozoa production, embryo formation, pregnancy and activating growth hormone. Zn mineral deficiency will affect the fertility level of livestock because Zn minerals play a role in the maturation process of spermatogenesis and oogenesis (Rostini et al., 2019).

In connection with the provision of supplemental feed to maximize the reproductive performance of ewe, many methods can be used, including to detect the sexual behavior of ewe in estrus, for example, by measuring changes in vaginal temperature, changes in vaginal mucus resistance, LH (Luteinizing hormone) surges, vaginal smears, and use of estrus detection tools (Piccione et al., 2003; Roelofs et al., 2005; Fisher et al., 2008; Murtaza et al., 2020). However, handling livestock when measuring temperature, taking blood samples or inserting an estrus detection probe can cause discomfort and stress in the sheep affecting the actual interpretation of the results. One alternative solution is using of non-invasive diagnostic tools, such as infrared thermography (IRT), with which body surface temperature can be measured quickly and precisely while minimizing discomfort and stress in sheep.

Infrared thermography (IRT) is a non-invasive remote sensing method used to measure changes in heat transfer by detecting changes in body surface temperature so that it can be used as a general indicator of body temperature and stress in livestock (Alsaaod et al., 2014; George et al., 2014; McManus et al., 2016; Nääs et al., 2014; Roberto et al., 2014). The use of IR thermography in livestock production is innovative, cheap, fast, efficient, effective, without radiation exposure, and does not require physical contact, making it possible to read temperature distribution remotely (McManus et al., 2016; Sathiyabarathi et al., 2016). This research is expected to provide information on the use of infrared thermography, a non-invasive method that can detect estrus, especially in ewes.

MATERIALS AND METHODS

This research was conducted from April to May 2023. The research location was carried out at the Research and Entrepreneurship Center of the Animal Husbandry Study Program, Faculty of Agriculture, University of North Sumatra.

Materials

The tools used in this research were a thermal imaging camera, portable pocket weather meter, digital thermometer, cage, tissue, cotton, plastic gloves, syringe, and scales. The materials used were 16 ewes, forage, multinutrient blocks (MNB), Moringa leaves, mineral Zn (ZnO), and Prostaglandin-F2α (PGF2α) injection from the Lutalyse brand.

Experimental design

This research used a Completely Randomized Experimental Design (CRD) with 4 treatments and 4 replications. This study used 16 ewes that had given birth at least once, were in healthy condition, and had a normal reproductive cycle. The feed ingredients used are forage, MNB I (basic MNB), MNB II (MNB I added with Moringa leaves), and MNB III (MNB II plus the mineral Zn). The MNB supplement composition e used in this research is shown in Table 1.

Table 1. Composition of Multinutrient Block (MNB) feed supplements

Raw material	MNB I (%)	MNB II (%)	MNB III (%)
Molasses	40	35	35
Rice Bran	40	35	35
Bentonite	7	6	6
Clam Shell Flour	6	5	5
Urea	4	4	4
Salt	3	2.5	2.5
Moringa leaves	0	12.5	12.5
Zn minerals	0	0	45 ppm

The ewes that have been selected are placed in pens, each of which contains one lamb. The first treatment (P0) was given forage, the second treatment (P1) was given forage and MNB I feed, the third treatment (P2) was given MNB II forage and feed, and the fourth treatment (P3) was given MNB III forage and feed. Each type of MNB is given 500 grams/head/day.

Research procedure

Before treatment, the sheep underwent environmental adaptation for 14 days. Treatment feeding was carried out for 4 weeks. Feed consumption was measured based on the difference between the amount of ration given and the remaining ration during the study and expressed in units of grams/head/day. After 4 weeks, all ewes were synchronized in estrus using the hormone PGF2α (Lutalyse) at a dose of 10 mg/head (2 ml) intramuscularly, twice injected with an interval of 11 days following the procedure carried out by Hidayah et al., (2022). Next, observation and examination of estrus are carried out on ewes. Observations were made after the second Lutalyse injection (H0) until 6 days after the second Lutalyse injection (H6). Observations were carried out three times a day, namely morning (07:00), afternoon (12:00), and afternoon (17:00).

Research Variables

Environmental Temperature Observation

Observations of environmental conditions include microclimate conditions consisting of environmental temperature (°C) and air humidity (%) around the research cages, which are also observed and recorded using a temperature measuring device/weather meter (Perez Marquez et al., 2019). Thermal Humidity Index (THI) of sheep livestock was analyzed based on Kohli et al. (2014) to determine the stress level of the livestock. THI is calculated using the following formula:

$$THI = 1.8\ Ta - (1 - RH)(Ta - 14.3) + 32$$

Information:

THI : Thermal Humidity Index
Ta : Temperature in the cage (°C)
RH : Average humidity (%)

Research Variables

Infrared Thermography Image Capture

Infrared thermal (IRT) images were taken on the labia vulva and vestibule vagina of each animal using a Flir One Pro brand thermal-imaging camera. Pictures were taken three times at a distance of 50 cm (Hovinen et al., 2008), 100 cm (Deak et al., 2019) and 150 cm (Sykes et al., 2012).

Rectal Temperature Measurement

Rectal temperature measurements are carried out using a digital thermometer, namely by inserting the thermometer into the sheep's rectum and waiting until the device beeps. The value displayed on the thermometer after that time is a reflection of the animal's rectal temperature (Suprayogi, 2013). The results obtained will be tabulated in the table provided.

Data analysis

The data from the analysis were tested for significance using ANOVA, and if the results were significantly different, then they would be tested further using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Nutrient Content of Multinutrient Block (MNB) Supplementation Feed

The results of the proximate analysis of the multinutrient block (MNB) supplementation feed used in this research can be seen in the table below.

Table 2. Nutrient Content of Multinutrient Blocks (MNB) used in research

Sample Type	DM (%)	Ash (%)	CP (%)	Fiber (%)	CF(%)
MNB I	85.50	21.51	20.51	14.06	5.12
MNB II	84.66	20.25	24.55	13.31	6.03
MNB III	80.59	19.93	22.73	13.77	6.18

Information: DM = Dry Matters; CP=Crude Protein;CF =Crude Fat; Laboratory of Nutrition and Animal Feed, Andalas University (2023).

The proximate analysis results shown in Table 6 indicated that the highest ash content was in MNB I feed, then MNB II and the lowest was in MNB III. According to Wulandari et al. (2015) good ash content in animal feed should not exceed 15%. The ash content value of MNB feed in this study was higher than the standard. This indicates that the MNB feed supplementation in this study had high mineral content. According to Sudarmadji and Bambang

(2003) the ash content in feed is related to the mineral content contained in the feed. The higher the ash content, the higher the mineral content. Fulfilment of mineral needs for livestock should not be too high because minerals and vitamins are needed by the body in small quantities (Wulandari et al., 2015). Therefore, the value of ash content in feed must be in accordance with the established standards for animal feed requirements.

The highest PK value is in MNB II feed, then MNB III and the smallest is in MNB I feed. Meanwhile, the highest LK content is in MNB III feed and the smallest is in MNB I feed. The PK and LK values in this research feed are in accordance with SNI 2019, namely for a minimum PK content of 10% and a maximum LK content of 7%. The crude fiber content in each type of MNB feed in this study was less than 18%. According to Rudiah (2011), the crude fiber content for goats and sheep in concentrate feed is less than 18%. The higher the crude fiber content in the ration, the lower the digestibility of the ration and will reduce the dry matter consumption of the ration.

Moringa leaves have a fairly high protein value so they can be used as a good additional feed for ruminants (Popalayah and Afa, 2017). Apart from that, according to Witariadi et al., (2011) Moringa leaf flour contains complete nutrients such as protein, vitamins and minerals. The results of research from Toleng et al. (2010) stated that Balinese cows given Moringa leaves came into estrus more quickly postpartum than those without Moringa leaves. Furthermore, Suyanto et al., (2020) stated that giving Moringa leaves as additional nutritional feed to female livestock can maximize the performance of reproductive organs.

Micro minerals such as Zn are important in maintaining livestock fertility and fertility (Widhyari et al., 2015). The mineral content of Zn in ruminant feed is generally relatively low, so many incidents of Zn deficiency are found in ruminant livestock (Tintin et al., 2019). Multinutrient block (MNB) feed supplementation is an additionalfeed for ruminant livestock to overcome the low quality of basal feed from small-scale or traditional livestock. It is hoped that the complementary feed provided can meet the nutritional needs of livestock to increase their productivity.

Microclimatic Conditions of the Research Environment

Microclimate is the environmental conditions in the cage that can directly influence the livestock life. The results obtained from measuring microclimatic conditions in the research environment are shown in Table 3.

Table 3. Temperature and Humidity of the Environment Around the Research Cage

Time	Ambient Temperature (°C)			Humidity (%)			THI
	Average	Min	Max	Average	Min	Max	
7:00	25.67 ± 1.08	24.00	28.00	80.44 ± 3.90	70.00	88.00	75.98
12:00	32.44 ± 1.46	29.00	34.00	67.06 ± 4.48	58.00	78.00	85.18
17:00	30.11 ± 1.13	27.00	32.00	69.50 ± 3.05	60.00	72.00	81.38

Air temperature and humidity are factors that are interrelated and play a role in determining the comfort level of livestock. Tropical climate countries have relatively high average daily temperatures, namely 24-34°C (Yani and Purwanto, 2006). The average environmental temperature for morning, afternoon and evening is 25.67 ± 1.08 °C, respectively 32.44 ± 1.46°C and 30.11 ± 1.13°C. The results of the average temperature measurements during the study in Table 3 show that the average temperature in the morning, afternoon and evening is still at a comfortable temperature for livestock.

The average humidity in the cage is 80.44 ± 3.90% for the morning, 67.06 ± 4.48% for the afternoon, and 69.50 ± 3.05% for the afternoon. According to Sodiq and Abidin (2010), the relative humidity for sheep and goats to grow is 60-80%. The average value for cage humidity during the day and evening is still within normal limits. However, the humidity in the morning is slightly above normal limits. High air humidity can be caused by several factors, including insufficient water availability in the cage, wind speed blowing in the cage and air temperature as a controlling factor for evaporation (Nuriyasa, 1991).

Thermal Humidity Index (THI) is one indicator to assess the potential for heat stress from the environment to livestock (Sejian et al., 2018). Based on Table 8 above, it can be seen that the THI value in the morning is 75.98, in the afternoon, it is 84.41, and in the afternoon it is 81.38. Hamdan et al. (2018) stated that the THI value is normal if <74, 75-78 is alert status, 79-83 is dangerous status, and >84 is very dangerous. Nienaber and Han (2007) reported that ruminants will begin to regulate their body homeostasis if the THI is more than 74, as indicated by an increase in respiration rate. The heat stress experienced by livestock can affect their physiological conditions, including heart rate, respiratory rate and rectal temperature (Nardone et al., 2010). According to Gaughan et al. (2008), the value of a temperature index is difficult to determine stress in livestock because the value of a THI depends on the surrounding environmental conditions and the condition of the livestock itself.

Rectal Temperature

Rectal temperature is a good indicator to describe the internal temperature in the animal's body. Skin surface temperature, rectal temperature and body temperature increase with increasing environmental temperature. Rectal temperature also shows the effects of environmental stress on sheep (Dikmen et al., 2012). The results obtained from measuring the rectal temperature of sheep can be seen in Table 4.

Table 4. Effect of Multinutrient Block on Sheep Rectal Temperature (°C)

Days to-	Observation Time (hours)	Treatment			
		P0	P1	P2	P3
0^{ns}	7:00	38.45±0.37	38.38±0.17	38.13±0.43	38.50±0.14
	12:00	38.65±0.37	38.60±0.14	38.48±0.29	38.70±0.18
	17:00	38.48±0.26	38.58±0.96	38.45±0.25	38.58±0.22
1^{ns}	7:00	38.83±0.55	38.78±0.31	39.03±0.21	39.35±0.50
	12:00	39.13±0.29	38.93±0.15	39.13±0.96	39.10±0.42
	17:00	39.03±0.36	38.78±0.26	39.10±0.08	39.10±0.42
2^{ns}	7:00	38.85±0.51	39.08±0.26	38.68±0.17	38.60±0.37
	12:00	39.20±0.55	38.83±0.67	38.53±0.47	38.60±0.47
	17:00	39.15±0.51	38.83±0.72	38.38±0.39	38.40±0.41
3^{ns}	7:00	38.35±0.74	38.60±0.32	38.53±0.38	38.48±0.22
	12:00	38.50±0.50	38.83±0.43	38.73±0.36	38.60±0.20
	17:00	38.33±0.49	38.63±0.39	38.63±0.30	38.50±0.00
4^{ns}	7:00	38.40±0.36	38.45±0.31	38.50±0.18	38.63±0.96
	12:00	38.58±0.36	38.60±0.14	38.65±0.10	38.63±0.22
	17:00	38.45±0.25	38.68±0.17	38.68±0.96	38.60±0.26
5^{ns}	7:00	38.49±0.36	38.45±0.31	38.50±0.18	38.63±0.96
	12:00	38.58±0.36	38.60±0.14	38.65±0.10	38.63±0.22
	17:00	38.45±0.25	38.68±0.17	38.68±0.96	38.60±0.26
6^{ns}	7:00	38.45±0.37	38.38±0.17	38.13±0.43	38.50±0.14
	12:00	38.65±0.37	38.60±0.14	38.48±0.29	38.70±0.18
	17:00	38.48±0.26	38.58±0.96	38.45±0.25	38.58±0.22

Note: The results of analysis of variance did not show significant differences (P>0.05).

The results of variance analysis showed that the treatment did not have a significant effect (P>0.05) on sheep rectal temperature. The absence of any influence on the sheep's rectal temperature during the course of the study shows that neither the control nor the addition of supplement feed significantly changed the condition of the sheep's rectal temperature. This is in accordance with research conducted by Stachurska et al., (2023), that there is no difference in rectal temperature during or outside the estrus phase.

Rectal temperature is a body temperature that can be used to measure heat tolerance in livestock, including the process of adding and losing body heat. Marai et al. (2007) stated that the normal rectal temperature of sheep is 38.8°C to 39.9°C. Rectal temperature is considered an index of body temperature even though there are temperature variations in several parts of the animal's body at different times of the day (Sarangi, 2018). Purnamasari et al., 2018 state that sheep are livestock that have good capabilities in homeothermal processes. This means that sheep can maintain their bodies in a balanced state by removing excess heat from their bodies when exposed to high temperatures. The sheep in this study have adapted to the surrounding environmental conditions so that their physiology is still in normal condition at a THI above average.

Infrared Thermography Image Capture

Infrared thermography is able to monitor changes in temperature and infrared which have an impact on physiological status related to reproduction (Pamungkas et al., 2020). The results of measuring sheep temperature using a thermal camera can be seen in the picture and table below. The graph above illustrate the temperature conditions of the vulva region of ewe sheep taken using a thermal camera.

Table 5. Results of temperature measurements with a thermal camera (°C) distance 50 cm

Days to-	Observation Time (hours)	Treatment			
		P0	P1	P2	P0
0ns	7:00	37.43±0.17	37.38±0.17	37.30±0.18	37.50±0.14
	12:00	37.68±0.96	37.60±0.14	37.48±0.96	37.70±0.18
	17:00	37.53±0.50	37.50±0.14	37.45±0.13	37.58±0.22
1	7:00	37.83±0.17[a]	38.43±0.17[b]	38.28±0.17[b]	38.43±0.17[b]
	12:00	38.13±0.19[a]	38.70±0.14[b]	38.50±0.08[b]	38.53±0.10[b]
	17:00[ns]	38.18±0.50	38.50±0.14	38.28±0.17	38.28±0.10
2	7:00	38.70±0.22[b]	38.33±0.26[a]	38.43±0.17[ab]	38.63±0.17[ab]
	12:00[ns]	38.78±0.13	38.63±0.19	38.70±0.14	38.85±0.06
	17:00	38.75±0.17[b]	38.30±0.37[a]	38.50±0.14[ab]	38.65±0.13[ab]
3[ns]	7:00	37.43±0.22	37.35±0.29	37.43±0.17	37.60±0.29
	12:00	37.58±0.17	37.58±0.22	37.58±0.17	37.78±0.34
	17:00	37.40±0.14	37.38±0.15	37.43±0.13	37.60±0.42
4[ns]	7:00	37.60±0.29	37.38±0.22	37.45±0.34	37.45±0.34
	12:00	37.78±0.34	37.63±0.13	37.68±0.33	37.68±0.33
	17:00	37.60±0.42	37.45±0.21	37.45±0.31	37.45±0.31
5[ns]	7:00	37.30±0.18	37.25±0.13	37.38±0.17	37.23±0.26
	12:00	37.48±0.10	37.55±0.10	37.60±0.14	37.35±0.24
	17:00	37.45±0.13	37.30±0.22	37.50±0.16	37.25±0.13
6[ns]	7:00	37.23±0.26	37.38±0.17	37.20±0.14	37.38±0.17
	12:00	37.35±0.24	37.60±0.14	37.70±0.14	37.60±0.14
	17:00	37.25±0.13	37.50±0.16	37.48±0.46	37.50±0.16

Note: Numbers in rows followed by different letters showed significant differences ($P<0.05$); ns = not significantly different ($P>0.05$).

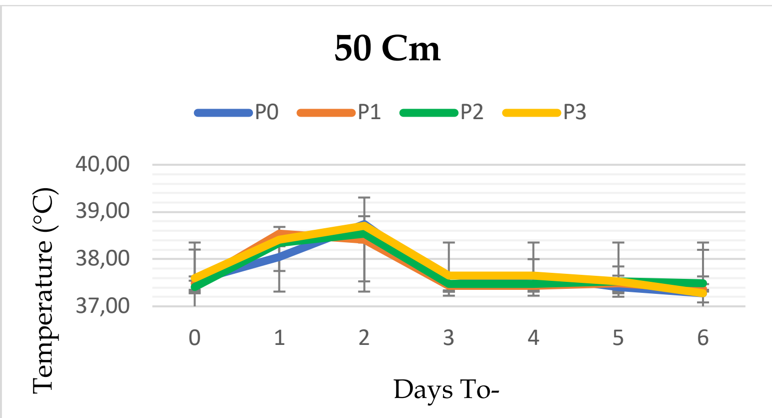

Figure 1. Sheep temperature graph taken using a Thermal Flir One camera.

Based on Table 5 above, the results of temperature observations using a thermal camera at a distance of 50 cm show that the average temperature for P0, P1, P2, and P3 increased on the first day after injection. Temperature on the first day after PGF2α injection in treatments P1, P2, and P3. The average temperature value for each treatment P1, P2, and P3 is 38.43±0.17 °C, 38.28±0.17 °C, And 38.43±0.17 °C. The increase in temperature in these three treatments occurred in the morning. Furthermore, for the P0 treatment, temperature increased on the second day after injection PGF2α with values 38.70±0.22 °C.

The use of infrared thermography (IRT) allows non-invasive recording of animal body temperature in the form of infrared radiation emitted using infrared thermography (Santoso et al., 2023). The advantage of an infrared thermal camr measuring surface temperature is that there is no need for contact between the tool and the animal's body because this tool is a non-contact technology, so it can minimize stress on the animal (Santoso et al., 2023).

CONCLUSION

Providing multinutrient block feed (MNB) has a real influence on the onset of estrus in ewes. Infrared thermography as a non-invasive method can be used to strengthen the determination of estrus response in ewes.

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