JOURNAL OF ANIMAL BEHAVIOUR AND BIOMETEOROLOGY

RESEARCH ARTICLE
Published Online: January 8, 2025
https://doi.org/10.31893/jabb.2025001

Effect of dietary supplementation with rumen-protected polyunsaturated fatty acids, protected proteins and organic minerals on the performance and physiological responses of Holstein heifers

Mayasari Novi[a] | **Aeni Wida Nu**[a] | **Ismaya Alip Aksi Kotun**[a] | **Tasripin Didin Supriat**[a] | **Tanuwiria Hidayat Ujang**[a]

[a]Faculty of Animal Husbandry, Universitas Padjadjaran, Jl. Ir. Soekarno KM. 21 Jatinangor Sumedang 022-7796416, Indonesia.

Abstract The aim of this study was to evaluate the effects of dietary supplementation with rumen-protected polyunsaturated fatty acids (PUFAs), protected proteins and organic minerals on the performance and physiological responses of heifers. Twenty Friesian Holstein heifers were individually fed diets of chopped straw and concentrate containing either (i) no added supplement (CON; 5); (ii) 3% added protected protein (T1; n=5); (iii) 3% added protected protein and 2% Ca-PUFAs (T2; n=5); or (iv) 3% added protected protein and 2% Ca-PUFAs + 2% organic minerals (T3; n=5). Experimental data such as performance, physiological response, adaptability coefficient, and heat tolerance coefficient (HTC) data were collected for 3 months. Data were analyzed via ANOVA. The ambient temperatures in the middle of the day and the evening were outside the normal range. The THI value indicates that the experimental animals are under moderate heat stress. The results revealed that heifers in group T3 had greater chest circumferences, body lengths and HTC values than did those in the other treatment groups. There was no significant difference in body weight gain, respiration rate, or heart rate between the treatments. Our study revealed that although the average ambient temperatures at midday and evening were outside the normal range, they did not negatively affect the physiological response or performance of heifers. In conclusion, providing a feed supplement consisting of 3% bypass protein, 2% Ca-PUFAs and 2% organic minerals could serve as a heat stress abatement strategy in heifers under extreme conditions.

Keywords: heifers, feed supplements, physiological status, performance

1. Introduction

The performance and health status of heifers should be well managed and prepared, as heifers play important roles as substitutes for replacement. Therefore, the management of heifers, especially nutritional management, must be given great attention. Most smallholder heifer breeders in Indonesia are still faced with problems such as insufficient feed provision for the maintenance and growth of heifers; thus, the first service conception rate is often reduced. A decline in the first service conception rate of approximately 1% per annum has been reported worldwide (Butler et al., 1995; Royal et al., 2000; Evans et al., 2004). Reproductive inefficiency is a significant financial problem for the dairy industry and for smallholder heifer breeders. The productivity of dairy heifers is strongly influenced by the environment in terms of climatological, nutritional, and managerial factors. Nutrition, particularly energy nutrition, fundamentally impacts reproduction. The low productivity and reproduction of heifers can be caused by the low quality of feed and less optimal climatological conditions. Heat stress is a factor that can affect the performance, reproduction, and physiology of dairy heifers in Indonesia.

The average Friesian Holstein (FH) dairy cow in West Java gave birth to its first calf at the age of 28.88 ± 2.76 months (Makin, 2001). The maintenance of heifers aims to obtain prospective brood stock that is ready to mate, has reached body weights ranging from 300--350 kg or at the age of 15 months, and gives birth for the first time at 24 months of age (Moran, 2012). The body weight of a heifer ready for mating is determined by proper environmental conditions and improved feed quality.

The temperature and humidity are strongly influenced by the altitude at the site. The altitude is inversely proportional to the ambient temperature. High temperature and humidity may cause the release of heat from the body to the

 Received: June 27, 2024 | Accepted: December 13, 2024

environment to be greater for maintenance. Sumedang is an area in West Java that had a dairy cattle population of 3,131 in 2020 (BPS Jabar, 2021). These dairy cows are concentrated in several areas, one of which is in the Pamulihan subdistrict. Tunas Mekar is a group of dairy farmers who are members of KSU Tandangsari located in the Pamulihan subdistrict. The environmental conditions in the Pamulihan area are at an altitude of 750 m above sea level, with an average temperature of 27.83°C. The average humidity is 82.03%, the average wind speed is 0.97 m/s, and the air pressure is 1004.73 mb (BPS Sumedang, 2022). The average temperature and humidity are high enough for the maintenance of dairy cows that they can cause heat stress. The microclimate of an area, such as the air temperature, humidity, wind speed, air pressure and wind direction, affects the physiological status of livestock, such as the heart rate, respiration frequency, and rectal temperature. Physiological status can be used as an indicator of heat stress in livestock.

Nutrition strategies to reduce heat stress can be performed by improving feed with additional feed or feed supplements. The supplementation of protein, fat or organic minerals has been reported to improve the performance, reproduction, and physiological response of dairy cows. Fat supplementation, particularly polyunsaturated fatty acid (PUFA) supplementation, has been reported to positively influence cow reproductive performance (see reviews, Wathes et al., 2007; Staples et al., 1998). Moreover, fish meal supplementation as an additional protein source in feed has been reported to increase body weight gain and reproductive performance (Yendraliza, 2013).

A previous study showed that supplementation with mineral chromium minimized stress in cows exposed to heat stress (Bestari et al., 2011). The objective of this study was to evaluate the effects of dietary supplementation with rumen-protected polyunsaturated fatty acids (PUFAs), protected protein and organic minerals on the performance and physiological responses of heifers.

2. Materials and Methods

2.1. Animals and design of the experiment

In total, twenty Friesian Holstein heifers between 12 and 14 months of age with initial body weights of 200 ± 23 kg (coefficient of variation (CV) <15%) were used in this experiment for 70 days. The research was carried out at the Tunas Mekar Livestock Group at KSU Tandangsari. divided into Mekarbakti. Gunungmanik and Raharja Villages. Pamulihan District. Sumedang Regency. Ten days before the initiation of the trial, heifers were penned in groups of 5 pens, which were assigned randomly to 1 of the 4 treatments, with 1 heifer/pen and 5 pens per treatment.

The experimental design used was a completely randomized design (CRD) with four dietary treatments and five replications so that 20 experimental units were obtained. The research treatments were as follows: CON: 60% Straw + 40% Concentrate (100% Concentrate);T1: 60% Straw + 40% Concentrate (97% Concentrate + 3% Protected Protein); T2: 60% Straw + 40% Concentrate (95% Concentrate + 3% protected protein + 2% Ca- PUFA); and T3: 60% Straw + 40% Concentrate (93% Concentrate + 3% protected protein + 2% Ca- PUFA + 2% Organic Minerals).

The experimental rations used were a mixture of 60% rice straw and 40% concentrate on a DM basis and feed supplements consisting of protected protein, Ca-PUFAs and/or organic minerals. The forage was given in the form of chopped rice straw. The concentrate was formulated and processed with a KSU Tandangsari feed mill. The concentrate consists of a mixture of pollard. byproduct cake. Corn gluten feed (CGF). rice bran. coconut cake. soy sauce dregs. molasses. minerals. and calcium. The composition and nutrient content of the concentrates in each treatment are shown in Table 1, and the nutrient content of the experimental rations is shown in Table 2.

Table 1 The composition and nutrient content of the concentrates in each treatment.

	Concentrate (%)			
	K0	K1	K2	K3
Concentrate	100	97	95	93
Protected protein	-	3	3	3
Ca-PUFA	-	-	2	2
Organic Minerals	-	-	-	2
Nutrient content of the concentrate (%)				
Dry Matter	85.44	85.36	85.61	85.80
Ash	10.55	11.20	11.13	11.01
Crude Protein	18.18	18.49	18.16	18.21
Crude Fiber	11.74	11.41	11.30	11.13
Ether Extract	8.76	8.87	9.61	9.63
Non-NitrogenFree Extract	50.78	50.03	49.81	50.03
Total Digestible Nutrien	76.92	77.03	77.45	77.85

Note: K0: Concentrate in treatment COM; K1: Concentrate in treatment T1; K2: Concentrate in treatment T2; K3: Concentrate in treatment T3.

Table 2 Nutrient contents of the experimental rations.

Nutrient Content	Treatments			
	CON	T1	T2	T3
Dry Matter	56.30	56.27	56.37	56.45
Ash	16.07	16.33	16.30	16.26
Crude Protein	9.79	9.92	9.78	9.80
Crude Fiber	25.04	24.91	24.87	24.80
Crude Fat	4.13	4.18	4.47	4.48
Non-Nitrogen Free Extract	44.97	44.67	44.58	44.67
Total Digestible Nutrien	61.40	61.44	61.61	61.77

Note: (i) no added supplement (CON; 5); (ii) 3% added protected protein (T1; n=5); (iii) 3% added protected protein and 2% Ca-PUFAs (T2; n=5) or (iv) 3% added protected protein and 2% Ca-PUFAs + 2% organic minerals (T3; n=5)

2.2. Preparation of protected protein, Ca-PUFAs, and organic minerals

Protected protein, namely, fish meal, was sprayed with a homogeneous tannin solution at a dose of 3% fish meal. Ca-PUFAs. This protected fatty acid is made through a saponification process. with $Ca(OH)_2$ and organic minerals, namely, zinc (Zn), copper (Cu), selenium (Se), and chromium (Cr), fermented with *Saccharomyces cerevisiae* (SC) or *Aspergillus oryzae* (AO).

2.3. Statistical analysis

The data were analyzed via SPSS 26 statistical software. A complete randomized design was used in this study. The data obtained were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test. Significance was assumed at P≤0.05.

2.4. Data collection and measurements

2.4.1. Body weight gain

Body weight gain can be described from the growth of livestock, which is obtained from measurements of chest circumference and body length. Body weight results are obtained from measurements of chest circumference and body length via a rondo tape and then converted via the Winter formula (Santosa, 2008), which is carried out routinely every two weeks. The calculation for estimating body weight was performed via the Winter formula:

$$\text{Body weight (pounds)} = \frac{Chest\ Size^2 (inchi)\ x\ PBody\ Length\ (inchi)}{300}$$

Body weight gain = final body weight–initial body weight

2.5. Body measurement

2.5.1. Chest size

The size or circumference of the chest was measured via a Rondo tape by wrapping it around the back of the hump, as shown in Figure 1 (a) (The National Standardization Agency of Indonesia, 2015). Chest circumference measurements will be carried out regularly every two weeks.

2.5.2. Shoulder height

The shoulder height is measured via a measuring stick by measuring the distance from a flat surface to the highest part of the shoulder behind the hump, as shown in Figure 1 (b) The National Standardization Agency of Indonesia, 2015). Shoulder height measurements are carried out routinely every two weeks.

2.5.3. Body Length

Body length is measured via a measuring stick by measuring the distance from the humerus of the shoulder (tuber humeri) to the end of the sitting bone (tuber ischii), as shown in Figure 1 (c) (The National Standardization Agency of Indonesia, 2015). Body length measurements are carried out routinely every two weeks.

2.6. Physiological response

2.6.1. Heart rate

Heart rate measurement is carried out via a stethoscope near the left axilla bone (left chest) for one minute with a stopwatch (Sulistyowati et al., 2019). Three measurements were taken, and the average was taken.

2.7. Respiration frequency

The respiration frequency is measured by observing the movement in the area between the last rib and flank for one minute via a stopwatch (Sulistyowati et al., 2019). Three measurements were taken, and the average was taken.

2.8. Rectal temperature

Rectal temperature is measured by inserting a digital clinical thermometer into the rectum until the tip touches the rectal mucosa for 1–2 minutes until an alarm sounds from the thermometer (Sulistyowati et al., 2019).

2.9. Temperature humidity index (THI)

The THI value was calculated via the formula of Mader et al. (2006):

$$THI = (0.8 \times T) + [(RH/100) \times (T - 14.4)] + 46.4$$

Note:
THI: temperature humidity index.
Q : Ambient temperature.
RH : Ambient humidity.
The environmental temperature and humidity are measured via a data logger during the physiological status measurement process.

2.10. Heat tolerance coefficient

The heat tolerance coefficient (HTC) was calculated following the Rhoad or Ibéria test according to Araujo et al. (2017):

$$HTC = 100 - [18 \times (RT - 38.60)]$$

where HTC = heat tolerance coefficient. 100 = maximum efficiency in maintaining body temperature at 38.6°C. 18 = constant value. 38.60°C = average rectal temperature of the sheep in the thermal comfort zone. RT = final rectal temperature.

2.11. Adaptability coefficient

The AC was calculated following the Benezra test according to Araujo et al. (2017):

$$AC = RT/38 \pm RR/40 \pm HR/62$$

where AC = the adaptability coefficient of the Benezra test; RT = Rectal temperature (°C); RR = Respiratory rate (breaths/min); 40 = respiratory on heifer; 38 = normal rectal temperature on the heifer; 62 = normal heart rate on the heifer; HR = heart rate.

3. Results

3.1. Body weight gain and body measurements

The results revealed that the provision of protected protein, Ca-PUFAs, and organic minerals in the complete rations had no significant effect (P>0.05) on the body weight gain or body measurements of the heifers (Table 3). However. heifers in T3 presented the greatest increase in body weight gain, chest circumference, and body length gain. The greatest average body weight gain during the study was obtained in the T3 treatment (34.17 kg), followed by the T2 treatment (32.04 kg). T1 (32.03 kg). and CON (28.26 kg). Moreover, T3 had the greatest chest circumference (6.87 cm), and the smallest value was T1 (5.20 cm). T1 and CON had a difference of 0.33 cm. T1 and T2 have a difference of 1.4 cm. and T1 and T3 have a difference of 1.67 cm. The greatest increase in shoulder height was found in heifers in P3 at 6.60 cm, and the smallest value was found in T2 at 5.00 cm. T2 and CON had differences of 1.20 cm. T2 and T1 have a difference of 1.47 cm. and T2 and T3 have a difference of 1.60 cm. The greatest increase in shoulder height was found in heifers with a T2 of 5.73 cm, and the smallest value was found in heifers with a T3 of 4.47 cm. T3 and CON had a difference of 0.8 cm. T3 and T1 have a difference of 0.2 cm. and T3 and T2 have a difference of 1.26 cm.

3.2. Physiological response

Table 4 shows that there were significant differences in rectal temperature but not respiration frequency or heart rate among the treatments. The results revealed the provision of bypass protein. Ca-PUFAs and organic minerals in the complete rations of heifers reduced (P<0.05) the rectal temperature of heifers in the 5% test in the morning and evening. The lowest renal rectal temperature was produced by T3, and the highest renal temperature was produced by CON. These results indicate that the provision of feed supplements can help maintain the rectal temperature within the normal range in heifers experiencing heat stress.

Table 3 Mean values (mean±SEM) of body weight gain and body weight measurements of heifers between treatments.

Parameter	Treatments				P value
	CON	T1	T2	T3	
Body Weight (kg)	28.26 ± 4.91[a]	32.03 ± 9.76[a]	32.04 ± 5.22[a]	34.17 ± 5.94[a]	0.77
Chest Circumference Gain (cm)	5.53 ± 3.06[a]	5.20±1.78[a]	6.60±2.13[a]	6.87±0.96[a]	0.58
Body Height Gain (cm)	5.27 ± 1.32[a]	4.67 ± 0.78[a]	5.73 ± 2.62[a]	4.47 ± 0.87[a]	0.55
Body Length Gain (cm)	6.20 ± 3.42[a]	6.47 ± 2.88[a]	5.00 ± 2.11[a]	6.60 ±1.85[a]	0.59

Means with different letters (lowercase for rows). within farming systems. differ among themselves (CRD test; P< 0.05) Note: (i) no added supplement (CON; 5); (ii) 3% added protected protein (T1; n=5); or (iii) 3% added protected protein and 2% Ca-PUFAs (T2; n=5) or (iv) 3% added protected protein and 2% Ca-PUFAs + 2% organic minerals (T3; n=5)

Table 4 Mean values (means±SDss) of the microclimate and thermal comfort indices between treatments throughout the da

Variables	Periods of the day			P value	Normal reference range
	07.00	12.00	16.00		
Air Temperature (°C)	21.10±0.52	24.90±1.90	23.24±1.27	<0.01	20–30°C[1]
Air Relative Humidity (%)	96.29±5.99	85.85±10.81	89.57±8.01	0.18	≈60%[1]
THI	69.71±0.55	75.18±1.92	72.83±1.57	<0.01	Until 74[1]

[1] Santos et al. (2021)
Means with different letters (lowercase for rows) differ among themselves (P<0.05).

3.3. Environmental conditions

The tuna Mekar livestock group is one of the livestock groups belonging to the Tandangsari KSU, which is located in the villages of Mekarbakti. Gunungmanik and Raharja. Pamulihan District. Sumedang Regency. The research location is at an altitude of 750 m above sea level (BPS Sumedang Regency, 2022). The temperature and humidity are expressed in terms of the temperature and humidity index or temperature humidity index (THI). The THI value is an indicator of the comfort level of livestock to their environment. Changes in THI values affect the physiological response of dairy cows (Mariana et al., 2016). The average temperature. The humidity and THI values measured during the study are presented in Table 4.

4. Discussion

The response of the rations supplemented with feed was the same as that of the rations that did not supplement the performance variables. The average body weight gain ranged from 403.71–488.14 g/day. The average body weight gain of heifers in the T1 group was the lowest, and that in the T3 group was the highest, even though the difference was not significant. This means that there are no additives that affect body weight gain for better growth. Compared with that of Salman et al. (2014), the body weight of a dairy cow from calf to mating for the first time should increase by at least 500 grams per day.

A high protein content in the diet helps increase the growth of heifers (Yendraliza, 2013). Fish meal, which has a high protein content, is used by ruminants as a source of essential amino acids and a source of nitrogen for the rumen microflora. Thus. By binding fish meal protein with tannins, the protein in the rumen is minimally degraded by rumen microbes such that it is available in the post-Rumen.

The T3 treatment had the greatest effect on increasing body weight gain, chest circumference and body length in heifers. This is due to several factors, such as the environment and feed. Feed quality plays a role in the growth of livestock body length. In the T3 treatment, the presence of protein-enriched Zn minerals improved growth performance and the feed conversion ratio (Suprijati, 2013). The increase in body length is not affected by the fat or thinness of the livestock but is influenced by the body frame (Harmini and Firmansyah, 2016). The T3 treatment resulted in the greatest increase in chest circumference and body length because three supplements make up the feed. Thus, growth will be better than that of the other treatments.

The growth of the body and organs increases in line with the growth of livestock. In addition. Body weight gain is also affected by fat accumulation. The bones in the livestock body, including the leg bones, stop growing when they are experiencing optimum growth. While the ribs can still grow and develop because they are the bones that grow last, they can increase the size of the chest circumference (Pikan et al., 2018).

According to Agil et al. (2016), differences in feed quality can also cause differences in the height of the shoulder. The T2 treatment that resulted in the highest yield contained Ca. According to I Putut (2013), the mineral Ca, in addition to functioning to protect unsaturated fatty acids from rumen biohydrogenation, can also function in bone growth. The increase in shoulder height is related to the growth of the bones of the forelegs. Pradana et al. (2014) reported that the legs of male and female calves aged 0–6 months grow faster than their hips do. This is because the forelegs are more actively moving when the calf is suckling on its mother.

The use of Ca-PUFAs as a form of protected fat does not have a negative impact on the rumen environment; thus, the fermentative digestion process is effective and is useful for avoiding the negative effects of providing fat that exceeds the needs of livestock (Pramono et al. 2018).

The addition of feed supplements had the same effect on respiration frequency and heart rate. The average respiration frequency is known to be within the normal range mentioned by Yani and Purwanto (2006), namely, between 31–48 times per minute. The average heart rate is almost the same as that reported in a study conducted by Sudrajad and Adriarto (2011), which ranged from 46--84 beats per minute. This value is known to be within the range of normal heart rate frequency, as mentioned by Reece et al. (2015), namely, 48–84 beats per minute.

Respiration frequency is influenced by several factors, including body size, age, muscle movement, environmental temperature, pregnancy, and livestock health status (Reece et al., 2015). The mean values of respiratory frequency in the morning. afternoon and evening observations were 38.51 ± 2.71. 38.89 ± 3.16 and 38.09 ± 4.36. The average THI values in the morning. afternoon and evening observations were 69.71 ± 0.55. 75.18 ± 1.92 and 72.83 ± 1.57. The results of the Pearson correlation analysis revealed that there was no correlation ($P>0.05$). $r=0.059$) between respiratory frequency and THI. These results are in line with research conducted by Mariana et al. (2016), who reported that THI did not cause significant changes in respiratory frequency. The environmental factors that directly affect respiration frequency are microclimates such as temperature and humidity, which are reflected in the THI value. Changes in THI values affect the respiration frequency of heifers. The increase in the frequency of respiration during the day was caused by an increase in the temperature of the cage environment. The high ambient temperature causes the thermoregulation process to increase. Amir et al. (2017) stated that if the heat load received is too high. The evaporation heat dissipation process becomes active. This can be seen from the increase in respiration frequency.

An increase in heart rate can be caused by several factors, such as temperature, air humidity, wind speed and light intensity (Suprayogi et al., 2019). The mean heart rate frequency in the morning. The average number of afternoon and evening observations was 61.41 ± 5.75. 64.50 ± 8.17 and 60.94 ± 9.19. The average THI value from the morning, afternoon and evening observations was 69.71 ± 0.55. 75.18 ± 1.92 and 72.83 ± 1.57. THI values in the afternoon and evening indicate that dairy cows are experiencing mild stress. This is in accordance with the opinion of Moran (2005), who stated that the ideal THI value for dairy cows is less than 72 if the THI value exceeds 72. FH dairy cows experience mild stress ($72 \leq THI \leq 79$). moderate stress ($80 \leq THI \leq 89$) and severe stress ($90 \leq THI \leq 97$). The results of the correlation analysis between heart rate frequency and the THI score via the Pearson correlation method revealed that the correlation was negative or that there was no correlation ($P>0.05$). $r=0.09$).

These results indicate a balance of metabolism through the peripheral circulation as an effort to balance the release of body heat (Reece et al., 2015). The environmental factors that directly affect heart rate frequency are microclimates such as temperature and humidity, which are reflected in the THI value. Changes in THI values affect the heart rate frequency of heifers. An increased heart rate occurs during the day. This is caused by an increase in temperature in the cage environment. The increase in heart rate is affected by the heat load received by the body as a result of an increase in ambient temperature (Astuti et al., 2015). An increase in heart rate occurs as a result of an increase in ambient temperature, which affects blood vessels. namely, the capillaries. To expand the capacity of the blood vessels (vasodilation), an adjustment in the rate of blood flow occurs. This vasodilation signals that the hypothalamus orders the heart to pump more blood throughout the body (Amir et al., 2017).

As shown in Table 4, the lowest rectal temperature was produced by T3, and the highest rectal temperature was produced by CON. The average rectal temperature was almost the same as that reported in a study conducted by Mariana et al. (2018). which ranges from 37.4 ± 0.65–38.3 ± 0.46°C. This value is lower than the normal range reported by Schutz (2005), who reported that the normal range for the rectal temperature of a dairy cow is 38.20–39.10°C.

The mean values of rectal temperature in the morning. afternoon and evening observations were 37.79 ± 0.19. 38.34 ± 0.26 and 38.41 ± 0.14. The average THI values in the morning. afternoon and evening observations were 69.71 ± 0.55. 75.18 ± 1.92 and 72.83 ± 1.57. The results of the correlation analysis between rectal temperature and THI values via the Pearson correlation method revealed that the correlation was positive ($P<0.05$). $r=0.533$). The results of this analysis are in accordance with the findings of Mariana et al. (2016), who reported that there is a positive correlation between rectal temperature and THI. Rejeb et al. (2016) reported that rectal temperature is an indicator of the body's heat balance response to the environment.

The environmental factors that directly affect rectal temperature are microclimates such as temperature and humidity, which are reflected in the THI value. Changes in THI values affect the rectal temperature of heifers. The THI values during the afternoon and evening observations indicated that the cattle experienced mild heat stress. In cows that experience heat stress, body temperature increases to maintain body heat balance. Rectal temperature is very important in determining heat stress and livestock adaptation processes (Rejeb et al 2018). Under conditions of mild stress. An increase in body temperature can balance the release of heat. Therefore, the process of heat release does not involve an increase in heart rate or respiration rate.

An increase in rectal temperature in the afternoon can be caused by feeding in the afternoon. This increase occurred because of an increase in body metabolic heat. because the heiferd had just consumed feed (Suherman, 2014). Livestock produce heat in the body in an effort to produce the energy needed for survival. The heat generated is important for maintaining body temperature and a high metabolic rate in dairy cows so that they continue to produce normally. The heat gain from feed energy increases the heat load in the livestock body (Suherman and Purwanto, 2020). Body temperature is represented by rectal temperature. Rectal temperature affects 86% of the body temperature (Amir et al., 2017).

The impact of heat stress is an increase in the body temperature of cells, which causes changes in the levels of intracellular proteins. This triggers cells to release heat shock protein (HSP) to prevent cell death (Biutifasari, 2022). HSPs protect proteins and organelles from damage during cellular stress. This mechanism is very important for maintaining homeostasis in the body during heat stress (Cartwright et al., 2022). Bypassing protein supplementation in feed can increase the amount of protein that can be digested by the body. Because tannin–protein bonds are difficult for rumen microbes to degrade, the availability of protein in the postrumen tract increases (Tanuwiria and Hidayat, 2019).

Cattle experiencing heat stress require additional energy to increase heat dissipation from the body (Suherman et al., 2013). Supplementation with Ca-PUFAs in feed can increase the availability of essential fatty acids in the post-Rumen tract. This occurs because the Ca–oil bond is stable at the rumen pH, and the Ca–oil bond is released at the acidic pH of the abomasum. This makes it possible for more fatty acids to be absorbed by the small intestine (Pramono et al., 2013).

Microminerals play many roles in the body's metabolic processes. Zinc (Zn) plays a role in the function of several enzymes and metabolic processes related to carbohydrate and protein metabolism (Imanto et al., 2018). Copper (Cu) is a mineral that plays a role in metabolic processes. hemoglobin formation and physiological processes in the body (Arifin, 2007). Heat stress conditions can reduce feed intake. Reduced feed intake causes the concentration of insulin in the body to decrease so that it affects metabolic processes (Mousavi et al., 2019). The mineral chromium (Cr) plays an important role in glucose metabolism (Lashkari et al., 2018). Cows that experience heat stress have increased glucose metabolism, which causes a decrease in the Cr concentration in the body. Thus, Cr supplementation is needed in feed (Pechova and Pavlata, 2007). In line with research conducted by Shan et al. (2020), giving the mineral Cr in organic form to dairy cows experiencing heat stress can reduce the rectal temperature. According to Bestari et al. (2011), the use of Cr minerals can minimize stress. The need for Cr when cattle experience stress increases with decreasing body temperature. Stabilizing the heart rate and increasing the respiratory frequency will have a positive effect on livestock production performance.

Another impact of heat stress is that it can increase lipid peroxidation. This is associated with the production of large amounts of free radicals that cause oxidative stress in livestock. This can initiate peroxidation of polyunsaturated fatty acids (Sutedjo, 2016). The increase in free radicals can be overcome by adding antioxidant compounds to the feed. The increased activity of antioxidant enzymes is considered a protective response to oxidative stress (Altan et al., 2013). The mineral Zn is an important component of the cell structure that acts as an antioxidant and protects the body from lipid peroxidation (Widhyari, 2012). Selenium (Se) plays a role in homeostasis, and selenium metabolism functions as an antioxidant (Neve. 2002).

HTC and AC can be used to identify the adaptability levels of an individual animal in response to various environmental conditions. Compared with those in the other treatments, the H3 treatment resulted in greater HTC and lower AC, especially at midday and evening. These changes were possibly an effect of additive protein bypass and fat and mineral supplementation in T3. The HTC of heifers in T3 was high in the morning (Table 5) when the air and rectal temperatures were low. The HTC values at midday and evening were lower than those in the morning, when the THI values at midday and evening were above 72. The decrease in the AC value of heifers in T3 at midday and afternoon occurred because the supplemental air temperature during both times was high. The results indicated that the provision of feed supplements in the T3 diet could increase the adaptability of heifers under extreme conditions. The results of the present study indicate that the heifers in this study had better adaptability because of the combination of protected protein, Ca PUFAs and organic minerals.

Table 5 Mean values (means±SDss) of the heat tolerance coefficient (HTC) and adaptability coefficient (AC) of heifers between treatments throughout the day.

Periods of the day	Heat Tolerance Coefficient (HTC)				p value
	CON	T1	T2	T3	
07.00	109.22 ± 5.7[a]	115.55 ± 5.5[b]	114.98 ± 2.56[b]	118.86 ± 2.36[b]	0.02
12.00	101.51 ± 3.61[a]	106.48 ± 4.15[a]	104.03 ± 2.94[a]	106.91 ± 4.37[a]	0.13
16.00	99.93 ± 4.37[a]	104.25 ± 2.02[ab]	104.25 ± 3.27[ab]	105.33 ± 1.57[b]	0.05
	Adaptability Coefficient (AC)				
07.00	0.97 ± 0.008[a]	0.97 ± 0.0079[a]	0.97 ± 0.0037[a]	0.96 ± 0.003[b]	0.02
12.00	0.99 ± 0.005[a]	0.98 ± 0.006[a]	0.98 ± 0.004[a]	0.98 ± 0.006[a]	0.13

16.00	0.99 ± 0.006ᵃ	0.98 ± 0.003ᵇ	0.98 ± 0.0046ᵇ	0.98 ± 0.002ᵇ	0.05

Means with different letters (lowercase for rows) within farming systems differ among themselves (ANOVA; P<0.05). HTC = heat tolerance coefficient, AC = adaptability coefficient. Note: (i) no added supplement (CON; 5); (ii) 3% added protected protein (T1; n=5); (iii) 3% added protected protein and 2% Ca-PUFAs (T2; n=5) or (iv) 3% added protected protein and 2% Ca-PUFAs + 2% organic minerals (T3; n=5)

5. Conclusions

Heifers fed complete rations supplemented with 3% bypass protein, 2% Ca-PUFAs and 2% organic minerals were able to reduce the negative effects of heat stress; thus, heifers could maintain their growth performance and physiological parameters.

Further study of these supplements in a large number of heifers is needed to help us understand the complex relationships between feeding supplements and their interactions with environmental variables and physiological parameters.

Acknowledgment

Thank you to the Academic Leadership Grant Universitas Padjadjaran through Ujang Hidayat Tanuwiria with contract number 2203/UN6.3.1/PT.00/2022, which has funded this research.

Ethical Considerations

The Ethical Committee of Universitas Padjadjaran, Bandung, Indonesia, approved the protocol of this experiment with registration number 0718070998.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

This research has been funded by Academic Leadership Grant Universitas Padjadjaran through Ujang Hidayat Tanuwiria with contract number 5413/UN6.3.1/PT.00/2024.

References

Adawiah T. Sutardi T. Toharmat W. Manalu N. Ramli. Tanuwiria UH (2007) *Responses to Supplementation of Mineral Soap and Organic Minerals and Roasted Soybeans on Fermentability Indicators in Sheep Rumen. Media Peternakan*. 30(1):63-70.

Agil MLB. Salman. Indrijani H (2016) *Identification of Characteristics and Body Size of Lactation Fries Holland Cattle in Bogor Livestock Business Area*. Student EJournal. 5(4): 1-12.

Ako A (2012) *Tropical Dairy Animal Science 2nd*. Bogor: IPB Press.

Altan O. Pabuccuoglu A. Altan A. Konyalioglu S. Bayraktar H (2013).*Effect of heat stress on oxidative stress. lipid peroxidation and some stress parameters in broilers. British Poultry Science*. 44(4):545-550. http://dx.doi.org/10.1080/00071660310001618334

Amir A., Purwanto B.P., Permana IG (2017) *Response of Dairy Cattle Thermoregulation to Different Feed Energy. Jurnal Ilmu dan Teknologi Peternakan*. 5(2):72-79. https://doi.org/10.20956/jitp.v5i2.3069

Andaka G (2009) *Optimization of Peanut Oil Extraction Process with N-Hexane Solvent. Jurnal Teknologi.* 2(1):80-88.

Arifin Z (2007) *The Importance of Copper (Cu) Minerals in Animal Bodies in Relation to Diseases. Wartoza*. 17(2):93-99.

Astuti A. Erwanto. Santosa PE (2015) *Effect of Forage-Concentrate Administration on Physiological Response and Performance of Simmental-breed Cattle. Jurnal Ilmiah Peternakan Terpadu*. 3(4):201-207. http://dx.doi.org/10.23960/jipt.v3i4.p%25p

Atrian P. Shahryar HA (2012) *Heat Stres in Dairy Cows (A Review). Res in Zoology.* 2:31-37. http://dx.doi.org/10.5923/j.zoology.20120204.03

Badan Pusat Statistik Jabar (2021) *Populasi Hewan Ternak (ekor) Wilayah Jawa Barat 2018-2020. https://jabar.bps.go .id/indicator/158/255/1/populasi-hewan-ternak-.html.* Accessed on 9 February 2023

Badan Pusat Statistik Kabupaten Sumedang (2022) *Kabupaten Sumedang dalam Angka 2021. https://sumedangkab .bps.go.id.* Accessed on 7 March 2023

Badan Pusat Statistik. Kabupaten Sumedang. 2020. *Kabupaten Sumedang Dalam Angka Sumedang Regency in Figures 2021. ISSN: 0215-4269*. BPS Kabupaten Sumedang. Sumedang

Becker CA. Woolums AR. Stone AE (2020) *Recognizing Heat Stres in Dairy Cattle: How to Visually Record Respiration Rate.* Mississippi State University.

Berman A (2008) *Increasing Heat Stres Relief Produced by Coupled Coat Wetting. and Forced Ventilation. J. Dairy Sci.* 91(12):4571-4578. https://doi.org/10.3168/jds.2008-1175

Biutifasari V (2022) *Heat Shock Protein (HSP) 70 in Apoptosis and Inflammation. Surabaya Biomedical Journal*. 1(3):213-220. https://doi.org/10.30649/sbj.v1i3.35

Blezinger SB. Hutcheson DP. Chirase NK. Mies WL (1992) *Effect of Supplementation Trace Mineral Complexes on Rectal Temperature. Feed Intake. and Body Weight Change with Infectious Bovine Rhinotracheitis Virus Challenged Feedlot Cattle. J. Anim. Sci.* 70(1):301.

Cahyani RD. Nuswantara LK. Subrata A (2012) *Effect of Soybean Flour Protein Protection with Mangrove Leaf Tannins on Ammonia. Undegraded Protein and Total Protein Concentrations in Vitro. Animal Agricultural Journal.* 1(1):159–166.

Cartwright SL. Schmied J. Livernois A. Mallard BA (2022) *Effect of In-vivo heat challenge on physiological parameters and function of peripheral blood mononuclear cells in immune phenotyped dairy cattle. Veterinary Immunology and Immunopathology.* 246:1-8. https://doi.org/10.1016/j.vetimm.2022.110405

Cozler YL. Peyraud JL. Troccon (2008) *Effect of Feeding Regime. Growth Intensity and Age at First Insemination on Performances. and Longevity of Holstein Heifers Born during Autumn. Livestock Sci.* 124:72-81. http://dx.doi.org/10.1016/j.livsci.2008.12.011

Fidler AP. Vandevender K (1981) *Heat Stress in Dairy Cattle. Agriculture and Natural Resources. https://www.uaex .edu/publications/pdf/fsa3040.pdf.* Accessed on 17 December 2022

Fielder SE (2015) *Normal Rectal Temperature Ranges.https://www.merckvetmanual.com/special-subjects/reference-guides/ normal-rectal-temperature-ranges.* Accessed on 27 March 2023

Harmini. Firmansyah. MA (2016) *Display of Bali Cattle Given Forage and Addition of Supplements (Silage and Minerals) on Malan Island*. Katingan. Sains Peternakan. 14(2): 42-49. http://dx.doi.org/10.20961/sainspet.14.2.42-49

Heraini D. Purwanti BP. Suryahadi (2016) *Comparison of Environmental Temperature and Dairy Cattle Productivity Using the Stochastic Frontier Approach (Case Study in Smallholder Animal Husbandry of KUTT Suka Makmur). Jurnal Sains Terapan Edisi IV*. 6(1): 16-24.

I Putut S (2013) *Animal Nutrition Needs*. Fakultas Kedokteran Hewan. Universitas Udayana. Denpasar. 74-5805 https://doi.org/10.24198/jsdh.v2i1.35887

Imanto NY. Harjanti DW. Hartanto R (2018) *Blood Glucose and Milk Lactose Levels in Dairy Cattle With Herbal Supplements and Proteinic Minerals. Jurnal RISET Agribisnis & Peternakan*. 3(2):22-30.

Jackson PGG. Cockcroft PDG (2002) *Clinical Examination of Farm Animals*. Blackwell Publishing Company. USA.

Junaidi M. Novita. Cut I. Dzarnisa (2016) *Study of Physiological Conditions of Peranakan Dairy Cattle. Fries Holl and (PFH) on People's Farm in Suka Mulya Village. Lembah Seulawah District. Aceh Besar District. Aceh Province. Jurnal Ilmiah Mahasiswa Pertanian Unsyiah.* 1(1):709-718.

Kargar S. Mousavi F. Karimi-Dehkordi S. Ghaffari MH (2018) *Growth Performance. Feeding Behavior. Health Status. and Blood Metabolites of Environmentally Heat-loaded Holstein Dairy Calves Fed Diets Supplemented with Chromium. Jurnal Dairy Sci.* 101:9876-9877. https://doi.org/10.3168/jds.2017-14154

Kaufman JD. Saxton AM. Rius AG (2018) *Short communication: Relationships among temperature-humidity index with rectal. udder surface. and vaginal temperatures in lactating dairy cows experiencing heat stress. J. Dairy Sci*. 101:6424–6429. https://doi.org/10.3168/jds.2017-13799

Kumar S. Ajeet BVK. Meena K (2011) *Effect og Heat Stres in Tropical Livestock and Different Strategis for its Amelioration. https://cyberleninka.ru/article/n/effect-of-heat-stres-in-tropical-livestock-and-different-strategies-for-its-ameliora tion.* Accessed on 9 March 2023.

Kurnia F. Suhardiman M. Stephani L. Purwadaria T (2012) *The Role of Nano-Minerals as Feed Additives to Increase Productivity and Quality of Livestock Products. WARTAZOA.* 22(4):187-193.

Lashkari S. Habibian M. Jensen SK (2018) *A Review on the Role of Chromium Supplementation in Ruminant Nutrition—Effects on Productive Performance. Blood Metabolites. Antioxidant Status, dan Immunocompetence. Biological Trace Element Research. https://doi.org/10.1007/s12011-018-1310-5*

Mader TL. Davis S. Brand TB (2006) *Environmental Factors Influencing Heat Stres in Feedlot Cattle. J. Animal Science*. 84:712-719. https://doi.org/10.2527/2006.843712x

Mahmud A. Busono W. Surjowardojo P. Tribudi YA (2020) *Milk Production of Holstein Friesian (FH) Dairy Cattle in Different Lactation Periods. Jurnal Ilmu dan Teknologi Peternakan.* 8(2):79-84. https://doi.org/10.20956/jitp.v8i2.10132

Makin M (2001) *Performance Comparison of Milk Production and Reproductive Properties of Sahiwal Cross Dairy Cattle with Holland Fries. Jurnal Bionatura.* 3(3): 173-184.

Makkar HPS (2003) *Effects and fate of tannins in ruminant animals. adaptation to tannins. and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Research.* 49:241–256. https://doi.org/10.1016/S0921-4488(03)00142-1

Mariana E. Hadi DN. Agustis NQ (2016) *Physiological Response and Milk Quality of Holstein Friesian Dairy Cattle in the Long Dry Season in the Highlands. Jurnal Agripet.* 16(2):131-139. https://doi.org/10.17969/agripet.v16i2.5888

Mariana E. Sumantri C. Astuti DA. Anggraeni A. Gunawan A (2018) *Microclimates. Thermoregulation and Productivity of Holstein Friesians at Different Altitudes. Jurnal Ilmu dan Teknologi Peternakan Tropis*. 6(1):70-77. http://dx.doi.org/10.33772/jitro.v6i1.5617

Miller BEI (1973) *Sumposium on Nitrogen utilization by the Ruminant. Proc. Nutr. Soc*. 32:79-84.

Moran J (2005) *Tropical Dairy Farming: Feeding Management for Small Holder Dairy Farmers in the Humid Tropics*. Landlinks Press. USA. https://doi.org/10.1071/9780643093133

Moran J (2012) *Managing High Grade Dairy Cows in the Tropics. Australia (AU):* Csiro Publishing. http://dx.doi.org/10.1071/9780643107892

Mousavi F. Dehkordi SK. Kargar S. Bakhtiari MK (2019) *Effects of dietary chromium supplementation on calf performance. metabolic hormones. oxidative status. and susceptibility to diarrhea and pneumonia. Animal Feed Science and Technology*. 248:95–105. http://dx.doi.org/10.1016/j.anifeedsci.2019.01.004

National Research Council [NRC] (2001) *Nutrient Requirement of Diary Cattle. Update 2001*. National Academy Press. Washington. D.C. https://doi.org/10.17226/9825

Neve J (2002) *Selenium as a `Nutraceutical': How To Conciliate Physiological and Supra-Nutritional Effects for an Essential Trace Element. Curr. Opin. Clin.Nutr. Metab. Care.* 5:659–663. https://doi.org/10.1097/00075197-200211000-00008

Newbold CJ (1995) *Microbial Feed Additives for Ruminants. Botechoology in Animal Feeds and Animal Feeding*. 259-278. http://dx.doi.org/10.1002/9783527615353.ch13

Novianti J. Purwanto BP. Atabani A (2013) *Physiological Response and Milk Production of FH Dairy Cattle on Elephant Grass (Pennisetum purpureum) with Different Cutting Sizes. Jurnal Ilmu Produksi dan Teknologi Peternakan.* 1(3): 138-146.

Nurdin E (2011) *Dairy Cow Management*. Graha Ilmu. Yogyakarta.

Nuriyasa IM (2017) *Livestock Adaptation*. Fakultas Peternakan Universitas Udayana.

Pechova A. Pavlata L (2007) *Chorium as an Essential Nutrient: A Riview. Veterinarni Medicina.* 52(1):1-18. http://dx.doi.org/10.17221/2010-VETMED

Pikan S. Tahuk PK. Sikone HY (2018) *Display of Body Weight. Linear Body Size. Age and Body Condition Score of Bali Cattle Slaughtered at the RPH of Kefamenanu City. Journal Of Animal Science*. 3(2):21-24. https://doi.org/10.32938/ja.v3i2.288

Pradana IMYW. Sampurna IP. Suatha IK (2014) *Growth Dimensions of Body Height of Bali Calves. Buletin Veteriner Udayana*. 6(1): 81-85.

Pramono A. Kustono DT. Putro PP. Danayanta E. Hartadi H (2013) *Protection Evaluation of Calcium Soap as Feed Supplement Based on Digestibility of Dry Matter. Organic Matter Digestibility and In Vitro pH in Rumen and Post Rumen. Jurnal Sains Peternakan.* 11(2):70-78. https://doi.org/10.20961/sainspet.v11i2.4828

Pramono A. Yusuf A. Widyawati SD. Hartadi H (2018) *The Effect of Protected Fat Supplementation on Nutrient Consumption and Digestibility of Holstein Friesian Dairy Cattle. Sains Peternakan*. 16(1): 34-39. https://doi.org/10.20961/sainspet.v16i1.19700

Qisthon A. Hartono M (2019) *Physiological Responses and Heat Resistance of Boerawa Goats and Ettawa Crossbreeds on Stable Microclimate Modification through Fogging. Jurnal Ilmiah Peternakan Terpadu*. 7(1):206 – 211. http://dx.doi.org/10.23960/jipt.v7i1.p206-211

Reece WO. Ericson HH. Goff JP. Uemura EE (2015) *Duke's Physiology of Domestic Animals. Ed ke-13.* Willey Blackwell. London (GB).

Rejeb M. Sadraoui R. Najar T. M'rad MB (2016) *A complex interrelationship between rectal temperature and dairy cow's performance under heat stress conditions. Journal Animal Science*. 4:24–30. http://dx.doi.org/10.4236/ojas.2016.61004

Salman LB. Sumantri C. Noor RR. Saefuddin A. Talib C (2014) *Growth Curve of Fries Hollands Dairy Cattle from Birth to Age of First Marriage with Logistic Mathematical Model. Jurnal Informatika Pertanian*. 23(1):75 – 84.

Santosa U (2008) *Manage Cattle Farm Professionally.* Penebar Swadaya. Jakarta.

Sastrosupadi A (2000) *Practical Experiment Design in Agriculture Sector.* Kanisius. Yogyakarta.

Scaletti RW. Trammell DS. Smith BA. Harmon RJ (2003) *Role of Dietary Copper in Enhancing Resistance to Escherichia coli Mastitis. J. Dairy Sci.* 86:1240-1249. https://doi.org/10.3168/jds.s0022-0302(03)73708-4

Schutz KE. Rogers AR. Cox NR. Tucker CB (2009) *Dairy cows prefer shade that offers greater protection against solar radiation in summer: shade use. behavior. and body temperature. Appl Anim Behav Sci.* 116:28-34. https://doi.org/10.3168/jds.2010-3608

Sejian V. Gaughan JB. Bhatta R. Naqvi SMK (2016) *Impac of Climate Change on Livestock Productivity.* Boadening Horizons. India.

Setiawan AA. Erwanto. Hartono M. Qisthon A (2021) Effect of Manipulation of Stable Climate Through Fog on Physiological Responses and Heat Resistance of Sapera Goats and Ettawa Breeds*. Jurnal Riset dan Inovasi Peternakan.* 5(1): 64-69.

Shan Q. Ma FT. Jin YH. Gao D. Li HY. Sun P (2020). *Chromium Yeast Alleviates Heat Stres by Improving Antioxidant and Immune Function in Holstein Mid-lactation Dairy Cows. Animal Feed Science and Technology.* 269:1-9. https://doi.org/10.1016/j.anifeedsci.2020.114635

Silva TH. Guimaraes I. Menta PR. Fern danes L. Paiva D. Ribeiro TL. Celestino ML. Saran Netto A. Ballou MA. Machado VS (2021) *Effect of InjecTabel Trace Mineral Supplementation on Peripheral Polymorphonuclear Leukocyte Function. Antioxidant Enzymes. Health. and Performance in Dairy Cows in Semi-Arid Conditions. Journal of Dairy Science.* 105(2).

Sordillo LM. Aitken SL (2009) *Impact of Oxidative Stres on the Health and Immune Function of Dairy Cattle. Vet. Immunol. Immunopathol.* 128:104–109. https://doi.org/10.1016/j.vetimm.2008.10.305

Sudrajad P. Adiarto (2011) *The Effect of Heat Stress on the Milk Production Performance of Holstein Friesian Cattle at the Baturraden Dairy Cattle Superior Cattle Breeding Center.*Seminar nasional Teknologi Peternakan dan Veteriner.

Suherman D. Purwanto BP. Manalu W. Permana IG (2013) *Model for Determination of Critical Temperature in Dairy Cattle Based on Production Capability and Feed Management. Jurnal Sain Peternakan Indonesia.* 8(2):121-138. https://doi.org/10.31186/jspi.id.15.2.200-211

Suherman D. Purwanto BP (2020) *Estimation Model of Upper Critical Temperature in Heifers Based on Feed Management. Jurnal Sain Peternakan Indonesia.* 15(2):200-211. https://doi.org/10.31186/jspi.id.15.2.200-211

Suherman D (2014) *Effects of Feeding Timing and Energy Level on Heat Stress Based on Rectal Temperature and Fries Holland Heifer Skin. Jurnal Sain Peternakan Indonesia*. 9(2):117- 129. http://dx.doi.org/10.31186/jspi.id.9.2.117-129

Sulistyowati E. Suherman D. Badarina I. Mujiharjo S. Fanhar S (2019) *Physiological Response of Lactating Holland Fries Cows Given Ration with Concentrate Containing Durian Peel (Durio zibethinus) Fermented Pleorotus ostreatus. Jurnal Sain Peternakan Indonesia*. 14(1):101-112. http://dx.doi.org/10.31186/jspi.14.1.101-112

Suprayogi A. Ihsan K. Ruhyana AD (2019) *Physiological Value of Dried Dairy Cattle in Pangalengan: Hematology. Heart Rate. Respiration Frequency. and Body Temperature. Jurnal Ilmu Pertanian Indonesia (JIPI). Oktober 2019*. 24(4):375-381. https://doi.org/10.18343/jipi.24.4.375

Suprijati (2013) *Organic Zinc as Ruminant Feed Additive. WARTAZOA*. 23(3):142-157.

Suryani H. Zain M. Jamarun N. Ningrat RWS (2015) *The Role of Direct Fed Microbials (DFM) Saccharomyces cerevisiae and Aspergillus oryzae on Ruminant Livestock Productivity: Review. Jurnal Peternakan Indonesia*. 17(1):27-37.

Susanti E. Ladjin N. Qadrini L. Adoe VS. Supratman. Arina F (2021) *Textbook of Statistics for University*. Penerbit Adab. Indramayu

Sutardi T (1980) *Foundation of Nutrition Science*. Departemen Ilmu dan Makanan Ternak Fakultas Peternakan. Institut Pertanian Bogor. Bogor.

Sutedjo H (2016) *Physiological Impact of Heat Stress on Livestock. Jurnal Nukleus Peternakan*. 3(1):93-105. https://doi.org/10.35508/nukleus.v3i1.791

Tampubolon D. Sukmiwati M. Sumarto (2018) *Chemical Characteristics and Amino Acid Profiles of Sembilang Fish Meal (Paraplotosus albilabris) with Different Handling Methods. Worst Fishery Periodic*. 46(1):11-18.

Tanuwiria UH. Budiman A. Susilawati I. Mushawwir A. Solihat I (2017) *Protection of Fishmeal Protein by Tannin Extract of Calliandra callothyrsus Leaves on Fermentability. Undegraded Dietary Protein. Dry Matter and Organic Matter Digestability In Vitro.* Proceedings of the 5th International Seminar of Animal Nutrition and Feed Sciences. Mataram.

Tanuwiria UH. Budinuryanto DC. Darodjah S. dan Putranto WS (2011) *Study on the Preparation of Mineral-Oil Complexes and the Effects of Its Use in Rations on Fermentability and Digestibility (in vitro). Jurnal Ilmu Ternak.* 10(1):32-38.

Tanuwiria UH. Hernaman I. Susilawati I. Ayuningsih. B (2018) *Increasing Production and Reproductive Performance of Dairy Cattle through Feed Nutrition Engineering. Laporan Kemajuan. Riset Academic Leadership Grant (ALG).* Universitas Padjadjaran.

Tanuwiria UH. Hidayat R (2019) *Effect of Tannin Level on Protein Protection of Golden Snail Flour (Pomacea canaliculata) on Fermentability and Digestibility in vitro. Jurnal Ilmu Ternak.* 19 (2):122-130. https://doi.org/10.24198/jit.v19i2.25730

Tanuwiria UH (2007) *Protection of Fish Meal by Various Tannin Sources and Their Effect on Fermentability and Digestibility (In Vitro). Jurnal Agrol.* 14(1):56-60.

Weiss WP (2003) *Selenium Nutrition of Dairy Cows: Comparing Responses to Organik and Inorganik Selenium Forms*. *https://en.engormix.com/dairy-cattle/articles/selenium-nutrition-of-dairy-cows-t33391.html.* Accessed on 9 March 2023

.Wibawa AAPP (2016) *Mineral and Water Metabolism.* Fakultas Peternakan. Universitas Udayana.

Widhyari SD (2012) *The Role and Impact of Zinc (Zn) Deficiency on the Immune Response System. WARTOZA*. 22(3):141-148.

Widyawati R. Mussa ORPA. Pratama MDW. Roeswandono (2020) *Comparison of Fat Content and Specific Gravity of Friesian Holstein (FH) Milk in Bendul Merisi. Surabaya (Lowland) and Nongkojajar. Pasuruan (Highland). Jurnal Vitek Bidang Kedokteran Hewan.* 10:15-19. https://dx.doi.org/10.30742/jv.v10i0.47

Wu ZZ. Peng WC. Liu JX. Xu GZ. Wang DM (2021). *Effect of Chromium Methionine Supplementation on Lactation Performance. Hepatic Respiratory Rate and Anti-Oxidative Capacity in Early-Lactating Dairy Cows. the International Journal of Animal Biosciences.* 15:1-7. https://doi.org/10.1016/j.animal.2021.100326

Yani A. Purwanto BP (2006) *Effect of Microclimate on Physiological Responses of Holland Fries Cattle and Environmental Modifications to Increase Their Productivity. Media Peternakan.* 29 (1):35-46.