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Research Article

Evaluating Green Concentrate on Physiological and Metabolic Blood Profile of *Friesian Holstein* Cows in Negative Energy Balance

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Abstract | Negative energy balance (NEB) is common in early lactation dairy cows, significantly impacting the performance and metabolism of *Friesian Holstein* (FH) dairy cows. Feeding green concentrate is an effective strategy to prevent NEB due to its high protein content and palatability. This study aims to assess the effects of green concentrate on the physiological status and metabolic blood profile of dairy cows. Utilizing a completely randomized design (CRD), the study included three treatments with six replications: Commercial Concentrate (CC), Green Concentrate Formulation-1 (GCF1), and Green Concentrate Formulation-2 (GCF2). Key indicators for evaluating the impact of green concentrate on reducing NEB in dairy cows encompassed physiological parameters (such as body temperature, respiratory rate, and heart rate), heat tolerance (including heat tolerance coefficient, HTC, and Benezra coefficient, BC), hematological aspects (like red blood cells, RBC, white blood cells, WBC, hemoglobin, and hematocrit), liver function tests (such as aspartate transaminase, AST, alanine aminotransferase, ALT, and alkaline phosphatase, ALP), and blood urea levels. The results indicated that FH dairy cows fed green concentrate improved significantly ($p<0.05$) in physiological values, including body temperature, RBC count, hemoglobin, hematocrit, AST, and ALP levels. Furthermore, highly significant effects ($p<0.01$) were observed in heart rate and blood urea levels. These evaluation indicators demonstrated that the cows receiving green concentrate had enhanced nutritional intake, positively influencing their physiological status and metabolic blood profile. This research suggests that farmers can utilize green concentrate as an effective measure to mitigate NEB and as a cost-effective substitute for commercial concentrates, ultimately contributing to the improved welfare of dairy farmers.

Keywords | *Friesian holstein*, Green concentrate, Metabolic blood profile, Negative energy balance, Physiological, Metabolic blood profile

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INTRODUCTION

Friesian holstein dairy cows exhibit varying performance across different lactation phases. After calving (postpartum), these cows often experience NEB, which leads to reduced performance and health. This phenomenon occurs when the energy required for postpartum recovery and milk production exceeds the energy obtained from feed intake (Mekuariaw, 2023; Jóźwik *et al*., 2012). During NEB, energy reserves in lipids, glycogen, and protein are mobilized into Beta-Hydroxybutyrate (BHB) to meet these energy requirements (Xu *et al*., 2018). These events increase the work of the liver and potentially lead to fat accumulation in the liver (ketosis) (Xu *et al*., 2020; Zhang *et al*., 2020). NEB can occur up to 140 days postpartum (Harde *et al*., 2019; Fenwick *et al*., 2008). The incidence of NEB is strongly influenced by feed quality, feed digestibility, and prepartum body condition (Mekuariaw, 2023). Feeding nutrient-rich feed can accelerate the transition to a positive energy balance (Permana *et al*., 2020). In addition, protein intake can accelerate the postpartum recovery process, as one protein molecule can be converted into 40 ATP, which is an energy source (Gibson et al., 2019).

To effectively reduce NEB, it is essential to utilize concentrates with high protein and energy content (Suranindyah *et al*., 2020). Presently, the supply of concentrate feed is primarily reliant on commercial sources. However, commercial concentrates are costly, uneconomical, and often unavailable. Therefore, there is an urgent need to identify alternative sources of concentrates. One promising alternative is green concentrate, which is an effective feed strategy due to its high protein content and palatability. Green concentrates are typically derived from leguminous plants, which offer various advantages (Ako *et al*., 2023). Among these legumes are indigofera (*Indigofera zollingeriana*) and gliricidia (*Gliricidia sepium*). Indigofera boasts a crude protein content of 30.5%, crude fiber at 19.0%, and carbohydrates at 36.6%, along with several phytonutrients, including condensed tannins, saponins, and flavonoids (Gunun *et al*., 2022). In contrast, gliricidia contains a crude protein level of 24.22%, crude fiber at 38.2%, crude fat at 2.8% (Winarti, 2016) and mineral content, including calcium at 2% and phosphorus at 0.21% (Soleh *et al*., 2022).

Previous research has shown that providing a 50% green concentrate can enhance milk production (Ako *et al*., 2023). Additionally, the inclusion of indigofera meal in the diet of goats was found not to significantly affect their hematological values (Muda *et al*., 2021). Furthermore, incorporating indigofera as a filler in urea multi-nutrient molasses blocks has been reported to improve the performance of postpartum Bali cattle and increase their pregnancy rates (Utamy *et al*., 2020). Various physiological and metabolic blood profiles can be assessed to evaluate the effect of green concentrate on dairy cows and its potential to reduce NEB. These include physiological status indicators (such as body temperature, respiratory rate, and heart rate), heat tolerance (including HTC and BC), hematological aspects (like RBC, WBC, hemoglobin, and hematocrit), liver function tests (such as AST, ALT, and ALP), and blood urea levels.

MATERIALS AND METHODS

Experimental Site

The research was conducted in Dusun Penette, Cendana Village, located in the Cendana District of Enrekang Regency, South Sulawesi Province. Hematological value testing was performed at the Dika Health Clinic in Sidrap Regency, South Sulawesi, while liver function and blood urea testing occurred at the Makassar Health Laboratory Center.

Ethical Approval and Animal Welfare

This research has obtained ethical approval for using research animals from the Animal Ethics Committee of the Faculty of Veterinary Medicine at Udayana University, under certificate number (B/184/UN14.2.9/2024). The dairy cows involved in this study were treated in accordance with established animal welfare standards and were placed in clean and comfortable pens with adequate air circulation. Feeding was provided to meet the specific needs of the animals, and water was made available ad libitum. No anesthesia methods or procedures that could potentially cause deformities in the dairy cows were utilized during the study. Blood sampling was conducted by placing the cows in a handling cage to ensure their calmness before drawing blood from the jugular vein. This process was executed with care to minimize stress for the dairy cows. A 3 ml sample was collected using a vacutainer needle, placed in an EDTA tube, and stored in a cooler box for preservation.

Experimental Design

The study utilized a completely randomized design (CRD) comprising three treatments and six replications, categorized as follows: Commercial Concentrate (CC), Green Concentrate Formulation-1 (GCF1), and Green Concentrate Formulation-2 (GCF2). The CC is a commercially available concentrate commonly used by farmers, sedangkan GCF1 dan GCF2 merupakan konsentrat yang diformulasikan khusus yang mengandung tepung indigofera dan gamal sebagai sumber protein. Pada GCF1 mengandung 10% Indigofera dan 20% gamal, sedangkan pada GCF2 mengandung mengandung 5% Indigofera dan 15% gamal.

Experimental Diet

Both commercial concentrates and green concentrate formulations have nutritional content in accordance with the needs of lactating dairy cows based on guidelines set by the NRC (2001). The green concentrate is formulated to contain 16% crude protein and a minimum of 70% Total Digestible Nutrients (TDN). The raw materials for these formulations are derived from by-products of various industries. This formulation mixed ground corn, bran, sorghum, and molasses as primary energy sources. Ground corn is sourced from sorted out at factories, which, although no longer utilized in the industry due to its incomplete form, retains the same essential nutrients as whole corn. The sorghum used is sorted from the cereal crop in Maros. Bran, a by-product of the rice milling process, is included for its relatively high energy content. Additionally, molasses, a by-product of the sugar processing industry, serves as an effective energy source. The protein sources in this formulation include coconut cake meal, which is a by-product of copra processing into coconut oil, as well as indigofera and gliricidia meal, both of which are excellent protein sources. Detailed feed composition is provided in Table 1 below.

Table 1: Composition of green concentrate formulation.

Feedstuff (%)	CC	GCFI	GCF2
Commercial Concentrate	100	0	0
Indigofera meal	0	15	5
Gliricidia meal	0	25	15
Sorghum	0	16	16
Bran	0	13	23
Coconut cake meal	0	0	20
Ground corn	0	30	20
Molasses	0	1	1
Total	100	100	100

CC: Commercial Concentrate; **GCF1:** Green Concentrate Formulation-1; **GCF2:** Green Concentrate Formulation-2.

The commercial concentrate feed was sourced from CV. Sarana Nutrisi Sembilan, marketed under the brand name Rumfeed. The formulation for the green concentrate consisted of ingredients such as indigofera, gliricidia, sorghum meal, ground corn, coconut cake meal, molasses, and bran. The preparation of indigofera and gliricidia meal commenced with harvesting the plants, which were then dried using a dehydrator for 7 hours at a temperature of 70°C. The raw materials were ground into meals using a disc mill. The nutritional content of commercial concentrate and green concentrate used can be seen in Table 2 as follows.

Research Implementation

The study involved 18 postpartum FH dairy cows aged 4 to 5 years with an average body weight of approximately 500 kg. The dairy cows were fed 3% of their body weight (BW) in dry matter (DM), consisting of 75% forage, specifically elephant grass (*Pennisetum purpureum*), and 25% concentrate. The study commenced with preparing the green concentrate and implementing concentrate feed testing on the postpartum FH dairy cows. The formulated green concentrate was administered to 18 postpartum FH dairy cows over a period of 50 days, including a 7-day adaptation phase. The cows were housed in treatment pens and were fed three times daily at 6:00 AM, 11:00 AM, and 4:00 PM. Weekly assessments of physiological parameters such as body temperature, respiratory rate, and heart rate—were recorded. Additionally, on day 50, hematological, liver function, and blood urea measurements were performed on the treated dairy cows.

Table 2: Nutritional content of commercial concentrate and green concentrate.

Nutritional Content (%)	CC	GCFI	GCF2
Crude protein	16.25	16.89	16.56
Crude fat	7.00	7.28	10.15
Fiber	8.50	8.05	9.30
TDN	75.00	72.42	75.30
Ash	7.00	7.63	5.92

CC: Commercial Concentrate; **GCF1:** Green Concentrate Formulation-1; **GCF2:** Green Concentrate Formulation-2; **TDN:** Total Digestible Nutrient.

Variables Measured

Microclimate conditions: The microclimate conditions examined in this study included ambient temperature, air humidity, and Temperature Humidity Index (THI). Temperature and humidity were recorded using a thermohygrometer (Thermohygrometer Clock HTC-2) during three key times of day: in the morning (from 07:00 to 08:00 AM), at mid-day (from 11:30 AM to 12:30 PM), and in the late-afternoon (at 5:00 PM). The THI was derived from these temperature and humidity measurements. This index indicates dairy cows comfort levels within their environment and is calculated based on the methodology outlined by Thompson and Dahl (2012).

$$THI=(1.8\times Ta+32)-[(0.55-0.0055\times RH)\times(1.8\times Ta-26)]$$

Ta: Ambient Temperature (°C).
RH: Relative Humidity (%).

Physiological status: The physiological values of dairy cows were measured at the following points:

Physiological status measurements were conducted weekly in the morning, mid-day, and late-afternoon, including body temperature, respiratory rate, and heart rate. Body temperature was calculated based on McLean *et al.* (1983) as follows:

$$Tb = 0.86\ Tr + 0.14\ Ts$$

Where Tb is body temperature, Tr is rectal temperature and Ts is skin temperature.

According to Suherman et al. (2017), skin surface temperature (Ts) was measured at four measurement locations: back (A), chest (B), upper limbs (C), and lower limbs (D) (Figure 1). Mean skin surface temperature was calculated based on the formula of McLean et al. (1983):

$$Ts = 0.25\ (A + B) + 0.32\ C + 0.18\ D$$

Rectal temperature (Tr) was measured by inserting a rectal thermometer approximately 10 cm into the cow's rectum until the thermometer beeps (point G). Respiratory rate was measured by observing the movements of the flank and rib cage (point E) during inspiration. Heart rate was measured using a stethoscope placed near the left axilla (left chest) (point F) (Figure 1).

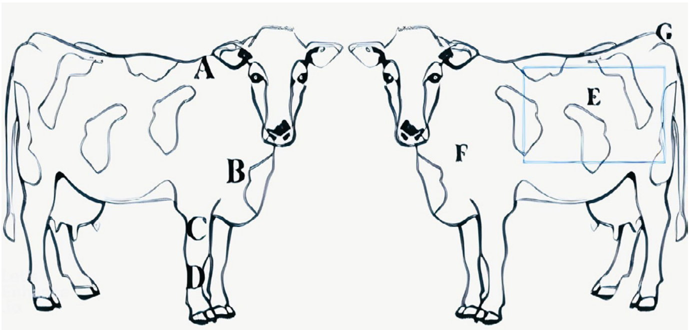

Figure 1: Measurement points for physiological values; **Source:** Utamy *et al*., 2024; Measurement points for physiological values: back (A), chest (B), upper leg (C), lower leg (D), respiratory frequency (E), heart rate (F), and rectal temperature.

Heat tolerance: Heat tolerance was measured using HTC and BC. HTC measures the level of heat adaptation based on changes in body temperature from a comfortable environment (in the morning) to a heat-stressed environment (in the mid-day). BC measurement, in addition to body temperature, also uses respiratory rate to determine heat tolerance. HTC and BC were calculated using the formulas of Rhoad (1944) and Benezra (1954), modified by Soeharsono (1978), as follows:

Rhoad's formula, HTC:

$$HTC = 100\text{-}10\ (Tf\text{-}Ti)$$

Where;
HTC: Heat Tolerance Coefficient (Rhoad Coefficient).
Tf: Average body temperature at mid-day (°C).
Ti: Average body temperature in the morning (°C).
100: The number of perfect coefficients on Ti.
10: Constant.

Benezra's formula, BC:

$$BC = \frac{Tf}{Ti} + \frac{Rf}{Ri}$$

Where;
BC: Benezra coefficient.
Tf: Average body temperature at mid-day (°C).
Ti: Average body temperature in the morning (°C).
Rf: Average respiratory rate in mid-day (breaths/minute).
Ri: Average respiratory rate in the morning (breaths/minute).

Metabolite blood profile: Blood analysis was performed to assess the impact of green concentrate on blood profiles, focusing on hematology (including RBC, WBC, hematocrit value, and hemoglobin level), liver function tests (ALT, AST, and ALP), and blood urea levels. Hematological parameters were measured using a Prokan hematology analyzer model PE-6100, made in China. Liver function and blood urea levels were evaluated using the spectrophotometric method with a Thermo Scientific Indiko blood chemistry instrument, made in Finland.

Statistical Analysis

The data underwent analysis of variance (ANOVA) using the General Linear Model (GLM) procedure in SPSS for Windows version 16.0 (Chicago, IL, USA) with a 95% confidence level. Duncan's test was used if the experimental diet means showed significant effects (P<0.05).

RESULTS AND DISCUSSION

Microclimate Conditions

Alterations in microclimate conditions will impact the well-being of dairy cows. Microclimate conditions are important because conditions greatly affect physiological values, heat tolerance, and blood profile. Microclimate values are also carried out to ensure that the data from this study is influenced by the treatment and not by environmental conditions. Rising ambient temperatures and humidity levels will modify the physiological parameters of the dairy cows. Sukandi *et al.* (2023) revealed different hematologic status under different microclimate conditions. The interplay between ambient temperature and humidity is referred to as the THI, which indicates the comfort level of dairy cows within their environment. The microclimate conditions recorded during the study are presented in Table 3.

Table 3: Microclimate conditions.

No	Parameter	Measurement Time			p-Value
		Morning	Mid-Day	Late-Afternoon	
1.	Ta	26.77±1.02[a]	34.18±0.80[c]	29.68±0.64[b]	0.00
2.	RH	81.85±4.92[c]	52.72±2.47[a]	64.72±2.58[b]	0.00
3.	THI	78.14±0.94[a]	84.27±0.88[c]	80.03±0.76[b]	0.00

[a,b] Different superscripts in the same row indicate significant differences (p<0.05); **Ta:** Ambient Temperature; **RH:** Relative Humidity; **THI:** Temperature Humidity Index.

This study observed significant differences (P=0.00) in ambient temperature and humidity throughout the morning, mid-day, and late-afternoon. The relationship between ambient temperature and humidity was inversely correlated; in the morning, lower temperatures coincided with higher humidity levels, whereas at mid-day, temperatures increased while humidity levels declined. This phenomenon was primarily attributed to sunlight intensity. During the daytime, stronger sunlight increases temperatures and decreases humidity, as it causes water molecules to evaporate (Sukandi *et al*., 2023). Livestock, particularly dairy cows, are highly sensitive to stress caused by variations in temperature and humidity. FH dairy cows in tropical countries like Indonesia are especially vulnerable to heat stress due to these fluctuations. Originally from subtropical regions, FH cows face challenges in the tropical climate of Indonesia (Asmasari *et al*., 2023). At our study location, the temperature and humidity ranged from 26–34ºC and 52–81%, respectively. These conditions differ significantly from the optimal temperature and humidity for FH dairy cows to achieve peak production performance, which is ideally around 18.3ºC and 55% (Utamy *et al*., 2024).

High humidity can impede heat dissipation from dairy cows' bodies, making them vulnerable to heat stress (Hernández-Castellano *et al*., 2019). The stress or comfort level of dairy cows in their environment can be assessed using the THI, which reflects the interaction between temperature and humidity. Elevated THI values signify that dairy cows suffer heat stress, which can adversely impact feed intake, milk production, milk fat content, and the number of somatic cells in milk (Nam *et al*., 2024). When THI values exceed 80, milk production can diminish by as much as 19% (Kim *et al*., 2014). A THI value below 72 represents a no-stress or comfort zone, while a value between 72 and 79 indicates mild stress. Values ranging from 80 to 89 signal moderate stress, 90 to 98 denote severe stress, and values at or above 98 suggest a critical risk of dairy cow mortality (Moore *et al*., 2024).

Physiological Values of *Friesian holstein* Dairy Cows Suffering Negative Energy Balance Fed Green Concentrate

An NEB in dairy cows can lead to alterations in physiological values, which serve as early indicators of the dairy cows' health status. The results of the study indicated that the physiological values of FH dairy cows suffering NEB and fed a green concentrate significantly affected body temperature (P<0.05) and heart rate (P<0.01), as presented in Table 4.

Measurement time and concentrate type significantly influenced dairy cows' body temperature. Specifically, the measurement time had a notable impact (p<0.01), with body temperatures peaking at mid-day and dipping in the morning. This pattern is attributed to rises in ambient temperature during the day, which subsequently elevates the cows' body temperature. Elevated body temperature can be a key indicator of heat stress in dairy cows, linked to their microenvironment (Yameogo *et al*., 2021). Moreover, the type of feed also plays a critical role in body temperature regulation. Different concentrates significantly affected body temperature (p<0.05). In particular, FH dairy cows receiving green concentrates (GCF1 and GCF2) exhibited body temperatures that differed (p<0.05) from those consuming commercial concentrates (CC). Generally, feeds with higher calorie content lead to increased body temperatures in dairy cows compared to those with lower calorie counts. Such temperature increments can benefit metabolism and growth but must be carefully managed to prevent heat stress. The calorigenic effect associated with feed consumption contributes to temperature increases. High-protein and high-energy feeds elevate body temperature as cows metabolize these nutrients, generating heat as a metabolic byproduct (Utamy *et al*., 2024). Both energy and amino acid metabolism are implicated in the potential for heat stress in dairy cows (Zhao *et al*., 2022). Consequently, it is vital to choose feeds with suitable calorie content to ensure the health and productivity of dairy cows. For example, high-calorigenic feeds can be advantageous for body temperature maintenance in cold weather, while low-calorigenic feeds are preferable to mitigate heat stress in hot or dry conditions. In our study, the average body temperature recorded was 38.64°C, which aligns with the normal range identified by Asmasari *et al*. (2023), where the typical body temperature of dairy cows falls between 38.2°C and 39.10°C.

An increase in respiratory rate is a key response of dairy cows to fluctuations in ambient temperature. Research findings indicate a significant difference (p<0.01) in respiratory rates based on the measurement time, with dairy cows exhibiting a notable rise in respiratory rate during mid-day compared to the morning or late-afternoon. In warmer conditions, or when ambient temperatures rise, dairy cows often show an elevated respiratory rate as a mechanism for thermoregulation to prevent overheating. This physiological response aids in expelling body heat through the respiratory tract (Asmasari *et al*., 2023). The respiratory rate indicates heat stress, which is closely associated with reduced CO_2 levels in body tissues and increased O_2 intake (Utamy *et al*., 2024). Typically, the respiratory rate of FH dairy cows ranges from 39 to 49 breaths per minute; however, at mid-day, this rate can elevate to a significant 66.43 breaths per minute. According to Asmarasari et al. (2023), the respiratory rate for FH dairy cows spans from 25 to 65 breaths per minute. This increase in respiratory rate is part of the cows' efforts to maintain thermal equilibrium when confronted with heat stress caused by elevated ambient temperatures and the internal heat produced during digestion (Sulistyowati *et al*., 2019). Therefore, offering a balanced diet can help mitigate internal heat generation during the digestive process.

Table 4: Physiological values of friesian holstein dairy cows experiencing negative energy balance given green concentrate.

Parameter	Type of Concentrate	Measurement Time			Average
		Morning	Mid-Day	Late-Afternoon	
Body temperature (°C)	CC	37.77 ±0.16	38.88 ±0.14	38.64 ±0.12	38.43 ±0.51^{P}
	GCF1	38.01 ±0.26	39.36 ±0.28	38.76 ±0.46	38.71 ±0.65^{Q}
	GCF2	38.27 ±0.48	39.19 ±0.08	38.91 ±0.41	38.79 ±0.52^{Q}
	Average	38.02 ±0.37^{a}	39.15 ±0.27^{c}	38.77 ±0.35^{b}	
Respiratory rate (breaths/min)	CC	38.66 ±5.32	68.55 ±14.64	44.08 ±2.98	50.43 ±15.90
	GCF1	38.33 ±6.42	62.91 ±12.40	46.00 ±11.31	49.08 ±14.25
	GCF2	40.00 ±2.10	67.83 ±11.91	57.50 ±12.83	55.11 ±15.12
	Average	39.00 ±4.55^{a}	66.43 ±12.08^{c}	49.19 ±10.98^{b}	
Heart rate (beats/min)	CC	70.77 ±13.39	79.44 ±4.30	76.16 ±8.02	75.46 ±9.24^{p}
	GCF1	80.33 ±3.13	90.77 ±2.83	80.88 ±2.04	84.00 ±5.57^{q}
	GCF2	78.66 ±4.25	82.55 ±5.34	77.77 ±3.13	79.66 ±4.48^{pq}
	Average	76.59 ±8.68^{A}	84.25 ±6.32^{B}	78.27 ±5.05^{A}	

^{a,b,c} Different superscripts in the same row indicate significant differences (p<0.01). ^{ABC} Different superscripts in the same row indicate significant differences (p<0.05); ^{pqr} Different superscripts in the same column indicate significant differences (p<0.01); ^{PQR} Different superscripts in the same column indicate significant differences (p<0.05); **CC:** Commercial Concentrate; **GCF1:** Green Concentrate Formulation-1; **GCF2:** Green Concentrate Formulation-2.

In addition to body temperature and respiratory rate, heart rate can also be used as an indicator to measure the level of stress or comfort of dairy cows in their environment. The study results demonstrated that measurement time significantly affected heart rate (p<0.05). Mirroring the trends of body temperature and respiratory rate, the highest heart rate was recorded at mid-day, reaching 84.25 beats per minute. Generally, heart rate increases with rising body and respiratory rates, facilitating faster blood circulation to distribute heat and cool the dairy cow's body effectively. Higher ambient temperatures lead to elevated heart rates due to sympathetic nervous system activity. The autonomic nervous system, particularly its sympathetic component, plays a crucial role in heart rate regulation (Kasahara *et al*., 2021). Furthermore, internal heat from feed metabolism can also elevate heart rates. For instance, FH dairy cows suffered NEB that received green concentrate (GCF1 and GCF 2) and exhibited higher heart rates than those fed commercial concentrate (CC). This difference is linked to the protein and energy content in the concentrates. Green concentrate, comprised of indigofera and gliricidia, has a high protein and amino acid content, generating more heat during metabolism. Protein digestion requires more energy, producing heat and raising the heart rate. Utomo *et al*. (2010) indicated that FH cows consuming a diet with 12% protein had elevated heart rates compared to those on a 10% protein diet. Notably, the heart rate observed in this study was greater than the heart rate range of 62–69 beats per minute reported by Utamy *et al*. (2024). Energy imbalance can make dairy cows more susceptible to stress, and the presence of NEB exacerbates their physiological challenges. This imbalance accelerates feed metabolism and increases heat production (Ghiardien *et al*., 2016).

Heat Tolerance of *Friesian holstein* Dairy Cows Suffering Negative Energy Balance Fed Green Concentrate

Heat tolerance refers to the capacity of dairy cows to adapt to environmental changes, especially in the face of heat stress. This tolerance is assessed by comparing physiological values observed in a comfortable environment (morning) to those recorded during heat-stressed conditions (midday). A significant increase in physiological values suggests a reduced ability to adapt. According to the study's findings, no change in the heat adaptation of FH dairy cows suffering NEB was observed when fed a green concentrate diet (Table 5).

Table 5: Heat tolerance of *Friesian holstein* dairy cows suffering negative energy balance fed green concentrate.

Parameter	Type of Feed			p-Value
	CC	GCF1	GCF2	
HTC	89.92±1.69	87.54±1.87	91.17±4.84	0.30^{NS}
BC	2.81±0.33	2.67±0.27	2.71±0.29	0.80^{NS}

^{NS} Non significant effect (p>0.05); **CC:** Commercial Concentrate; **GCF1:** Green Concentrate Formulation-1; **GCF2:** Green Concentrate Formulation-2; **HTC:** Heat tolerance coefisien; **BC:** Benezra coefisien.

The HTC obtained in this study ranged from 87.54 to 91.17, suggesting that FH dairy cows exhibit a good heat tolerance capacity, even under mild heat stress conditions. A higher HTC value indicates a greater capacity for dairy cows to adapt to hot environments, with optimal heat tolerance approaching an HTC value of 100 (Nurhidayat *et al*., 2024). Another important measure of heat tolerance is the BC, where a lower BC value signifies better adaptation to heat. In this study, BC values ranged from 2.67 to 2.81; values above 2 indicate the presence of heat stress, which requires significant energy for maintaining homeostasis (Omniski *et al*., 2002). Factors that can elevate HTC values include air temperature, humidity, and the THI. Increased air temperatures can lead to higher HTC values, suggesting that cows have heat stress, as those in warmer environments typically show elevated body temperatures.

Additionally, high humidity can exacerbate the effects of elevated air temperatures, hindering the cows' ability to dissipate heat through evaporation. The THI, which combines air temperature and humidity to assess the level of heat stress experienced by cows, generally correlates with higher HTC values, indicating a more pronounced heat stress response. Consequently, environments characterized by high temperature and humidity can increase HTC values, signifying substantial heat stress among FH dairy cows. Implementing effective feed management strategies can improve the heat tolerance of FH dairy cows, thereby promoting optimal productivity in tropical regions. HTC and BC values serve as valuable indicators for selecting dairy cows and can assist farmers and stakeholders in determining the appropriate actions for their herds. This strategy maximizes milk production, as heat stress can significantly reduce yield (Utamy *et al*., 2024). The HTC and BC are derived from the differences in physiological measurements between a comfortable state and under heat stress. It's important to highlight that dairy cows' responses to heat stress can vary significantly and are influenced by factors such as diet, age, and breed. The calorigenic effect of feed can worsen stress levels, resulting in a decreased capacity for heat tolerance in dairy cows. In this study, the feed treatment did not significant effect, as the amount of concentrate provided was lower during the day compared to the morning and evening. This approach aimed to mitigate the risk of heat stress in dairy cows by minimizing the additional heat generated from their food intake.

Hematological Status of *Friesian holstein* Dairy Cows Suffering Negative Energy Balance Fed Green Concentrate

The hematological status is a vital indicator for evaluating feed's impact on dairy cows' health. If the hematological values of dairy cows deviate from the established normal range, it can be inferred that the feed harms their health. The findings of the study demonstrated a significant impact (p<0.05) of providing green concentrate to FH dairy cows undergoing NEB on RBC count, hemoglobin levels, and hematocrit (Table 6).

Table 6: Hematological status of *Friesian holstein* dairy cows suffering negative energy balance fed green concentrate.

Parameter	Type of Feed			p-Value
	CC	GCF1	GCF 2	
RBC (1×10^6/µl)	4.99±0.18^a	5.23±0.37^{ab}	5.81±0.50^b	0.04
WBC(1×10^3/µl)	7.16±1.23	8.26±1.65	7.93±0.12	0.44^{NS}
Hemoglobin (mg/dl)	8.10±0.35a	9.86±0.75^b	8.98±0.74^{ab}	0.01
Hematocrit (%)	24.10±1.11^a	28.46±2.67^b	26.72±2.14^{ab}	0.04

^{a,b} Different superscripts in the same row indicate significant differences (p<0.05); ^{NS} Non significant effect (p>0.05); **CC:** Commercial Concentrate; **GCF1:** Green Concentrate Formulation-1; **GCF2:** Green Concentrate Formulation-2; **RBC:** Red Blood Cell; **WBC:** White Blood Cell.

The study's findings indicated that the administration of green concentrate to FH dairy cows suffering from NEB significantly influenced RBC, hemoglobin, and hematocrit levels (p<0.05). These hematological serve as indicators of the nutritional adequacy and overall health of dairy cows (Fahreza *et al*., 2020). Notably, the RBC levels in the treatment group GCF2 were significantly higher (p<0.05) than those in the NEB group and exceeded those in group CC. Adequate nutrition plays a pivotal role in maintaining normal and elevated RBC levels in the blood of dairy cows. An increase in RBC count suggests enhanced oxygen transport in the blood, positively influencing metabolic activity within the body (Utamy et al., 2021a; 2021b). Furthermore, the diet's balance of protein and energy is critical for maintaining healthy hematological parameters. Properly formulated feeds with appropriate protein and energy levels can support cows' blood health and physiological functions. Mohsin and Setiowati (2021) discovered that high-protein substances like whey can accelerate recovery and increase the number of RBCs in the body, thereby enhancing nutrient utilization in mice.

In addition to RBC, WBC are essential for the immune system of livestock; their levels can serve as indicators of feed utilization. This study revealed that WBC values stayed within the normal range and exhibited no significant differences between the treatment. An elevation in WBC values may signal an infection in dairy cows or potential toxicity related to the provided feed. However, the introduction of green concentrate did not lead to any signs of toxicity or infection among the livestock (Mahgoub *et al*., 2008). Thus, in this study, the WBC values remained within normal ranges, suggesting that the feed did not induce inflammation (Utamy et al., 2021b). The normal reference range for RBC is between 4.8 and 7.6 × 10^6 / µL, while for WBC, it is between 4.4 and 10.8 × 10^3 /µL (Brooks *et al*., 2022).

Hemoglobin is the primary component of erythrocytes, serving as a carrier for oxygen and carbon dioxide. Meanwhile, hematocrit, often called packed cell volume (PCV), indicates the proportion of erythrocytes in 100 mL of blood (Sukandi *et al*., 2023). The main purpose of hematocrit testing is to screen for conditions such as anemia, as hematocrit levels reflect the percentage of RBCs within the total blood volume. Variations in hemoglobin levels across different treatments can result from nutritional adequacy, particularly the role of protein in hemoglobin synthesis (Azzahra *et al*., 2023). An increase in hematocrit levels, indicating hemoconcentration, occurs due to elevated RBC levels, often attributed to sufficient protein in the diet. The RBC membrane consists of proteins bound to lipids, with hemoglobin itself being a crucial protein found within the RBCs. Ornay *et al*. (2021) stated that laying hens fed with high-protein and high-iron diets would have increased hemoglobin and hematocrit values. In a study of dairy cows suffering from NEB, those fed green concentrate formulation-1 (GCF1) exhibited higher hemoglobin and hematocrit levels than those receiving commercial concentrate (CC). This suggests that the green concentrate provided greater nutritional protein adequacy. According to Astuti *et al*. (2021), this study's hemoglobin and hematocrit levels fell within the normal range, with hemoglobin levels ranging from 8 to 15 g/dL and hematocrit levels from 24 to 46%. Overall, incorporating green concentrate in the feed did not adversely affect the hematological status of FH dairy cows. Adequate protein intake will increase hemoglobin and hematocrit levels in the blood, thereby optimizing the process of nutrient and oxygen transport within the body, which in turn will accelerate the recovery of dairy cows from NEB incidents.

Liver Function and Blood Urea of *Friesian holstein* Dairy Cows Suffering Negative Energy Balance Fed Green Concentrate

Liver function serves as a key indicator of liver health, with values such as AST, ALT, and ALP reflecting the severity of NEB. When NEB occurs, there is an increased mobilization of energy reserves into beta-hydroxybutyrate (BHB). Although the liver plays a crucial role in this process, prolonged NEB can lead to fat accumulation within the liver, resulting in ketosis and affecting the AST, ALT, and ALP values associated with liver performance. Blood urea levels indicate the effectiveness of feed protein utilization in livestock. Research findings indicate that liver function and blood urea levels in FH dairy cows suffering NEB, when supplemented with green concentrate, significantly influenced AST and ALP (p<0.05) and had a very significant effect on blood urea levels (p<0.01), as detailed in Table 7.

Liver function values, including AST, ALT, and ALP, are crucial indicators for evaluating liver performance. Dairy cows suffering from NEB often show increased fat mobilization by the liver, which can lead to various metabolic disorders such as ketosis, ruminal acidosis, and fatty liver syndrome. Liver dysfunction can result in abnormal AST, ALT, and ALP values (Zhang *et al*., 2020). Findings indicate that dairy cows suffering NEB when provided with green concentrate formulation-2 (GCF2), exhibited significantly higher AST and ALP values than those receiving a commercial concentrate (CC). Lower AST, ALT, and ALP levels suggest severe liver damage, while elevated levels indicate milder damage. The liver produces these enzymes in response to abnormalities; however, during instances of severe liver damage, it may fail to produce them altogether (Setiawati *et al*., 2021). Consequently, AST, ALT, and ALP values are more stable in livestock provided with green concentrates (GCF1 and GCF2). According to Yu *et al*. (2019), AST values in dairy cows range from 16.0 to 77.0 U/L, while ALT values range from 11 to 40 U/L. Also, FH dairy cows' ALP values range between 46 and 200 U/L (Cozzi *et al*., 2011). The provision of green concentrate has been shown to mitigate the incidence of NEB in dairy cows, as evidenced by the normalization of liver function values associated with its use. Adequate feed intake can be assessed through AST, ALT, and ALP values, which may also serve as indicators of feed toxicity (Azizah *et al*., 2020). The non-significant effect on ALT values can be attributed to the fact that ALT is produced not only in the liver but also in substantial amounts in other organs, particularly the heart. When abnormalities arise, especially within liver cells and cardiac muscle, this enzyme is released into the bloodstream. ALT serves primarily as a biomarker indicating disturbances in the liver and heart (Lomanorek *et al*., 2016). Consequently, the non-significant values likely suggest that the liver and heart of the livestock were in good condition.

Table 7: Liver function and blood urea of *Friesian holstein* dairy cows suffering negative energy balance fed green concentrate.

Parameter	Type of Feed			p-Value
	CC	GCF1	GCF 2	
AST (U/L)	59.00±5.94^a	67.00±2.94^{ab}	71.32±3.39^b	0.03
ALT (U/L)	27.75±3.59	28.50±0.57	28.00±1.41	0.89^{NS}
ALP (U/L)	49.25±0.95^a	58.25±11.08^{ab}	65.00±4.00^b	0.02
Urea (mg/dl)	15.25±1.25^A	15.75±1.25^A	19.00±0.81^B	0.00

^{a,b} Different superscripts in the same row indicate significant differences (p<0.05); ^{A,B} Different superscripts in the same row indicate significant differences (p<0.01). ^{NS} Non significant effect (p>0.05); **CC:** Commercial Concentrate; **GCF1:** Green Concentrate Formulation-1; **GCF2:** Green Concentrate Formulation-2; **AST:** Aspartat Aminotransferas; **ALT:** Alanin Aminotransferase; **ALP:** Alkalin Fosfatase.

Blood urea levels serve as indicators of protein metabolism efficiency in livestock feed. In this study, the blood urea levels of dairy cows suffering NEB and fed green concentrate ranged from 15.25 to 19.00 mg/dl. These values fall within the normal range of Andjelić *et al.* (2022), which is between 10.84 and 20.20 mg/dl. Notably, cows receiving the green concentrate formulation-2 (GCF2) exhibited the highest urea levels, indicating effective protein adequacy for metabolism in these dairy cows. Blood urea levels are notably influenced by the protein content in the feed, as most urea originates from the breakdown of dietary protein (Luan *et al.*, 2020). Additionally, these levels are affected not only by feed intake but also by the rate of protein catabolism occurring in the liver. Measuring blood urea levels in dairy cows can provide farmers with valuable insights into their dairy cows' nutritional status and health (Rizal *et al.*, 2020).

Research indicates that feeding green concentrate can effectively reduce the occurrence of NEB in dairy cows. Additionally, this concentrate has demonstrated no adverse effects on the health of the dairy cows. As a result, farmers may want to consider adopting green concentrate as an alternative feed to help decrease the incidence of NEB. Composed of indigofera and gliricidia—both readily available in tropical regions—this green concentrate enables farmers to significantly lower their costs. These plants are abundant in nature and can be easily cultivated, making them an excellent resource for feed production.

CONCLUSIONS AND RECOMMENDATIONS

The incorporation of green concentrate has demonstrated a reduction in the occurrence of Negative Energy Balance (NEB) in cows during the early lactation period. Evaluation indicators reveal that cows consuming green concentrate exhibit improved nutritional intake, which is evident in their physiological condition and metabolic blood profiles. Farmers can use green concentrate as an effective strategy to reduce NEB and as a cost-effective alternative to commercial concentrates, ultimately improving dairy farmer welfare.

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NOVELTY STATEMENTS

This study explored the potential of green concentrate as a dietary solution to alleviate negative energy balance in early lactation dairy cows. Currently, there has been no specific research investigating the effects of green concentrate on the negative energy balance in these dairy cows. The use of green concentrate is viewed as a promising strategy, given its rich nutrient profile that may help mitigate negative energy balance. Additionally, the formulation of green concentrate includes indigenous product-sourced raw materials that are easily accessible to farmers, providing a viable alternative to commercial concentrates.

AUTHOR'S CONTRIBUTIONS

Renny Fatmyah Utamy: Conceived and designed the experiments, performed the field experiments, analyzed data, and wrote the paper; Ambo Ako and Zyahrul Ramadan: Conceived and designed the experiments, performed the field experiments, performed, and analyzed data, and wrote the paper; Muhammad Ihsan Andi Dagong, Herry Sonjaya, Fatma Maruddin, : Performed the data tabulation, analyzed data, and wrote the paper; and Azisah Nurfadilah, Nur Fajri Muhlis, Andi Akmal Haerul, Putri Intani: Performed the field experiments and data tabulation.

Conflict of Interest

The authors have declared no conflict of interest.

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