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RESEARCH ARTICLE

Optimizing calf growth performance and nutrient digestibility with whey dangke-enriched green calf starter (fodder-based proteins) in Holstein Friesian

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ABSTRACT

Common calf starters often contain fish meal as a primary protein source; however, the strong odour of fish meal hinders calves from consuming the feed, necessitating alternative protein sources. Thus, the research aims to optimize calf growth performance and nutrient digestibility using whey dangke-enriched green calf starter (fodder-based proteins) in Holstein Friesian calves. Dangke is a local soft cheese from Enrekang District, South Sulawesi, Indonesia, prepared using fresh milk heated and mixed with the enzyme papain. The Holstein Friesian calves were distributed into a completely randomized design to assess the impact of two key factors: The type of concentrate feed (common feed, T0 *vs*. green calf starter, T1) and the level of enriched whey-dangke: 0% whey dangke + 5% water (W0); 2.5% whey dangke + 2.5% water (W1); and 5% whey dangke + 0% water from body weight (W2). The use of T1 significantly differed ($P < 0.01$) from T0 in crude protein (CP) digestibility, energy digestibility, and feed conversion ratio, and significantly differed ($P < 0.05$) in average daily weight gain (ADG). Additionally, whey dangke levels showed a highly significant effect ($P < 0.01$) on CP digestibility, energy digestibility, insulin-like growth factor 1 (IGF-1) hormone levels, and significantly differed ($P < 0.05$) in shoulder height and body length. The T1 promotes higher ADG (660 g d^{-1}) than the T0 (420 g d^{-1}). Whey dangke enrichment enhances CP digestibility and energy digestibility, increasing IGF-1 hormone levels from 56.06 ng mL^{-1} (W0) to 80.76 ng mL^{-1} (W2). Feeding calves, a combination of green and common calf starter with enriched whey dangke significantly boosts growth performance compared to a diet without enrichment.

Key words: Blood protein, green calf starter, Holstein Friesian dairy calves, insulin-like growth Factor-1, performance, whey dangke.

INTRODUCTION

Holstein Friesian dairy cows' management is closely tied to calves' feeding management, as they are kept to replenish the herd stock. Internal and external factors must be considered to ensure dairy calves' comfort and maximum productivity (Santoso et al., 2021). The performance of the calves directly impacts the milk production of lactating Holstein Friesian cows. High-performing calves produce superior replacement stock (Maharani et al., 2015). Improving the performance of calves will help boost the low milk production of dairy cows in Indonesia, ultimately reducing the need for imports (Asmarasari et al., 2023).

Genetic factors, environments, and their interaction influence the performance of Holstein Friesian dairy calves. The genetic factor is the breed, while environmental factors include feed, climate, altitude, and disease (Susanty et al., 2018). Nutrition from feed plays a crucial role in shaping the performance of dairy cow calves

(Hudaya et al., 2020), as it is vital for their growth. Good growth performance in calves indicates their future performance as adult dairy cows (National Academies of Sciences, Engineering, and Medicine, 2001). The nutrition of dairy calves has a lasting impact on their ability to produce milk once they reach maturity (Ockenden et al., 2023). The calving period is particularly critical, especially when the calf reaches 2 mo of age, as this stage is when the transition from maternal milk to concentrated feed takes place (Parsons et al., 2022).

A specific concentrate designed for calves at this stage is known as a calf starter. An ideal calf starter typically contains crude protein (CP) at 16%-20%, crude fat (CF) at 3%, total digestible nutrient (TDN) at 80%, Ca at 0.6%, and P at 0.4% (National Academies of Sciences, Engineering, and Medicine, 2001). Fishmeal is commonly used as a protein enrichment source in calf starters. However, calves do not prefer it due to its strong odour and limited availability for efficient use despite prices. Due to increased demand, local dairy farmers in Enrekang, South Sulawesi, Indonesia, face challenges in obtaining fishmeal, leading to a significant price surge. As a result, it is essential to explore alternative protein sources that can ensure availability (Hussain et al., 2024). Leguminous plants are a potential alternative due to their high quality and palatability as protein-source, self-sufficient materials for calf starters (Ako et al., 2024).

Legumes such as indigofera (*Indigofera zollingeriana* Miq.) and gliricidia (*Gliricidia sepium* (Jacq.) Kunth), can be utilized as green concentrates. Indigofera contains CP at 27.89%, CF at 3.70%, crude fibre (CFi) at 14.96%, and TDN at 79.00% (Wati et al., 2020). Conversely, gliricidia has a nutritional content of 25.7% CP and 13.3% CFi (Kefe et al., 2020). However, there is no report on using green concentrate containing indigofera and gliricidia as calf starters for dairy calves.

Furthermore, leguminous-based calf starter, also known as green calf starter, can be enriched with whey-dangke to improve its palatability and nutritional value. Whey dangke, a by-product of milk processing into dangke (Enrekang local soft cheese), is cost-effective, enhances palatability, and improves feed texture. It also helps to balance energy, protein, and mineral nutrients (Araújo et al., 2020). Considering the lack of information, further research is required to utilize fodder legumes as green concentrate enriched with whey-dangke on feed quality, dairy cows' performance, and blood protein profile, including 1GF-1 hormone, a crucial growth indicator. Hence, this research aims to explore the potential for optimizing the growth performance of Holstein Friesian dairy calves through a diet of green calf starter (leguminous-based calf starter) enriched with whey-dangke.

MATERIALS AND METHODS

Experimental sites

This study was conducted in Lebang Village, Cendana District, Enrekang Regency, the center of Holstein Friesian dairy cows in South Sulawesi. Analysis of IGF-1 hormone concentration was conducted at Hasanuddin University Hospital, Makassar. The blood profile was tested at the Makassar Health Laboratory Center. Proximate feed characteristics and digestibility tests were conducted at the Dairy Animal Nutrition Laboratory, Bogor Agricultural University, Bogor, West Java, Indonesia.

Ethical approval

The Animal Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, has approved the ethical use of research animals. A certificate of ethics bearing the reference number B/104/UN14.2.9/PT.01.04/2024 has been issued for using research animals.

Treatments and experimental design

This study used a completely randomized design in a factorial pattern with two factors, i.e., the first factor is the type of concentrate consisting of common calf starter (T0) and green calf starter (T1). The second factor is the level of enriched whey-dangke, consisting of the following: 0% whey dangke + 5% water of body weight (W0); 2.5% whey dangke + 2.5% water of body weight (W1); and 5% whey dangke + 0% water of body weight (W2).

Methodology applied

The calves were reared in pens and fed a diet of green calf starter and forage at 1.25% of their body weight in DM. They had unrestricted access to water. The green calf starter was prepared according to the formulation in Table 1, meeting the nutritional requirements of Calf Starter-2 as per Indonesian National Standards, with nutrient content specified in Table 2. The green calf starter diet was enriched with whey dangke at the appropriate level. The feed's texture was liquid but not runny, making it easier for the calves to consume. Before starting the calf starter diet, the calves underwent a 7-d adaptation period. They were fed the green calf starter enriched with whey-dangke for 50 d. The research process can be seen in Figure 1.

Table 1. Formulation of common and green calf starters.

Feedstuff	Composition (DM %) of calf starter	
	Common	Green
Indigofera meal	0	10
Gliricidia meal	0	12
Rice brand	36	37
Coconut cake meal	29	20
Milled corn	27	20
Fish meal	7	0
Molasses	1	1
Total	100	100

Table 2. Nutritional contents of green calf starters enriched with whey dangke. $^{(a-e)}$Different superscripts on rows and columns within the same parameter significant differences were observed ($P < 0.01$). Different superscripts ($^{p-r}$) within the same row represent significant differences ($P < 0.01$), while different superscripts ($^{x, y}$) within the same column also indicate significant differences ($P < 0.01$). Additionally, different superscripts ($^{X, Y}$) in the same column indicate significant differences ($P < 0.05$). T0: Common calf starter; T1: green calf starter; W0: 0% whey dangke + 5% water from body weight; W1: 2.5% whey dangke + 2.5% water from body weight; W3: 5% whey dangke + 0% water from body weight; NFE: N-free extract. Data were presented as average ± standard deviation.

Parameter	Type of concentrate	Level enriched whey dangke (% of body weight)			Average
		W0 (0%)	W1 (2.5%)	W2 (5%)	
Crude protein, %DM	Common (T0)	15.01 ± 0.37	15.39 ± 0.43	15.17 ± 1.08	15.19 ± 0.63^{X}
	Green (T1)	16.22 ± 0.68	16.29 ± 1.17	16.85 ± 1.44	16.45 ± 1.03^{Y}
	Average	15.62 ± 0.82	15.84 ± 0.93	16.01 ± 1.46	
Crude fat, %	Common (T0)	7.36 ± 0.18	6.94 ± 0.21	6.14 ± 0.54	6.81 ± 0.61^{x}
	Green (T1)	9.55 ± 0.34	8.85 ± 0.58	7.70 ± 0.72	8.70 ± 0.94^{y}
	Average	8.45 ± 1.22^{q}	7.90 ± 1.11^{q}	6.92 ± 1.02^{p}	
Crude fibre, %DM	Common (T0)	5.38 ± 0.90^{c}	4.03 ± 0.10^{a}	4.75 ± 0.27^{b}	4.72 ± 0.60^{x}
	Green (T1)	6.94 ± 1.72^{e}	6.35 ± 0.29^{d}	6.01 ± 0.36^{d}	6.43 ± 0.47^{y}
	Average	6.16 ± 0.86^{q}	5.19 ± 1.28^{p}	5.38 ± 0.74^{p}	
Moisture, %FM	Common (T0)	12.57 ± 0.18	14.15 ± 0.71	15.47 ± 1.00	14.06 ± 1.40
	Green (T1)	12.62 ± 0.10	14.38 ± 0.66	15.67 ± 1.28	14.22 ± 1.51
	Average	12.59 ± 0.13^{p}	14.26 ± 0.62^{q}	15.57 ± 1.03^{r}	
Ash, %DM	Common (T0)	8.67 ± 0.23	10.29 ± 0.85	11.08 ± 1.00	10.01 ± 1.25^{y}
	Green (T1)	6.35 ± 0.57	7.89 ± 0.53	8.82 ± 0.98	7.68 ± 1.25^{x}
	Average	7.51 ± 1.33^{p}	9.09 ± 1.46^{q}	9.95 ± 1.52^{q}	
NFE, %DM	Common (T0)	64.47 ± 1.53	62.90 ± 1.72	63.48 ± 1.87	63.62 ± 1.63^{y}
	Green (T1)	60.29 ± 1.52	61.41 ± 1.69	61.40 ± 1.67	61.03 ± 1.52^{x}
	Average	62.38 ± 2.66	62.16 ± 1.73	62.44 ± 1.95	
Energy, cal g^{-1}	Common (T0)	4993.66 ± 111.46	5120.66 ± 211.89	5255.66 ± 220.58	5123.33 ± 198.42^{X}
	Green (T1)	5402.66 ± 337.46	5484.66 ± 399.83	5484.66 ± 421.76	5490.66 ± 345.16^{Y}
	Average	5198.16 ± 317.34	5302.66 ± 348.79	5420.16 ± 350.84	

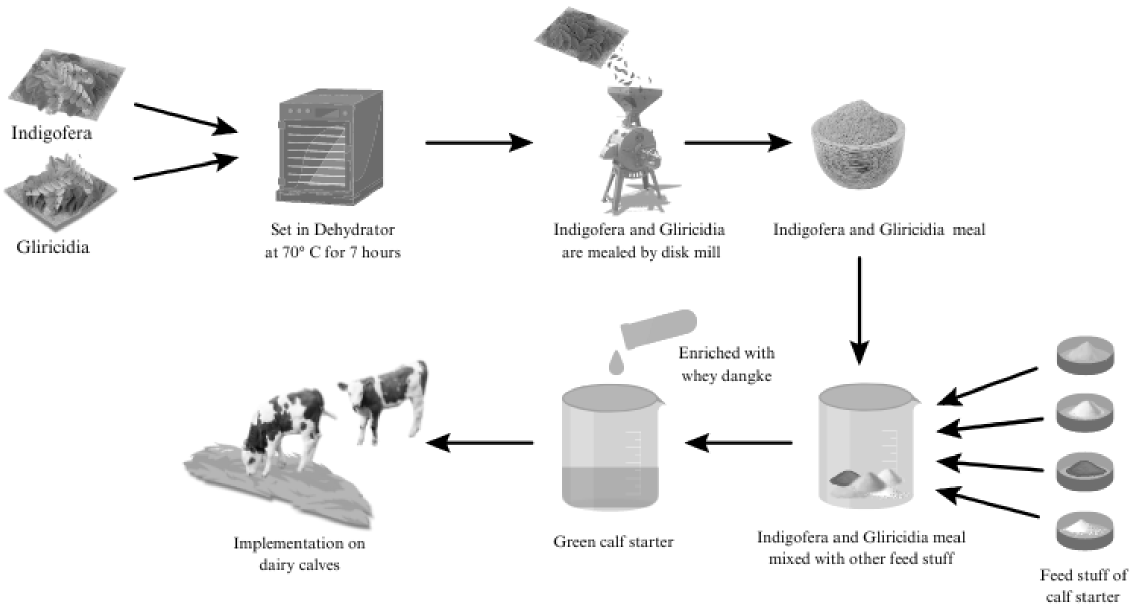

Figure 1. Research process.

Variables measured

This study evaluated the quality and nutrition of feed, including proximate analysis, gross energy test, and feed digestibility test, including DM digestibility, organic matter digestibility, CP digestibility, and energy digestibility.

Average daily gain (ADG) is obtained from the results of measurements accumulated in the following formula:

$$\text{ADG} = \frac{Final\ body\ weith\ (kg) - Initial\_body\ weith\ (kg)}{Experimental\ period\ (d)}$$

The DM intake (DMI) was calculated by subtracting the feed from the remaining feed. The feed conversion ratio (FCR) was determined by dividing the accumulated DMI by the daily gain. Body length was measured using a measuring tape to determine the distance from the protrusion of the shoulder bone to that of the pelvic bone or from the base of the pelvic bone to the base of the neck bone. These measurements were taken weekly throughout the study. Body length gain was calculated by dividing the average body length gain by the duration of the study. The chest circumference of the dairy cow was measured around the chest, specifically on the back of the front legs. Additionally, a stick measured the shoulder height from ground level to the shoulder just behind the front legs. The measurements of shoulder height were taken weekly, and the shoulder height gain was determined by dividing the average shoulder height gain by the duration of the study.

Blood samples were collected at the end of the study to measure the concentrations of insulin-like growth factor I (IGF-1) hormone and protein levels. Blood sampling was done at 08:00 h after the calves were fed the dietary. The samples were obtained from the jugular vein using a Flashback Vacutainer (Onemed, Jayamas Medica Industri, Sidoarjo, Indonesia) needle connected to a vacutainer tube without anticoagulant (Onemed). Approximately 3 mL blood was collected and centrifuged at 3000 rpm for 20 min. Following centrifugation, the blood cells and serum were separated, and the serum was subsequently frozen for analysis.

The measurement of IGF-1 hormone is performed using the enzyme-linked immunosorbent assay (ELISA) method (IGF-I Bovine kit Elisa, Wuhan Fine, Biotech, Wuhan, China). This test utilizes the competitive inhibition enzyme immunoassay ELISA technique, which involves a microplate coated with goat anti-rabbit antibodies. Both standards and samples are added to the microplate with an antibody specific for IGF-1 and IGF-1 conjugated with horseradish peroxidase (HRP, conjugate). A competitive inhibition occurs between the HRP enzyme-labelled IGF-1 and non-HRP-labelled IGF-1 with the antibody. After incubation, a substrate solution is added, causing a colour change to blue. The intensity of the colour is inversely related to the concentration of

IGF-1. A stop solution is introduced to stop the enzymatic reaction, changing the colour to yellow. The intensity of the resulting colour is then measured using an ELISA reader (NS-100 Nanoscan, Hercuvan, London, UK) at a wavelength of 450 nm. Finally, the concentration of IGF-1 was calculated using the Microplate Manager-6 (MPM 6) program (Bio-Rad, Hercules, California, USA).

The total blood protein was analysed using the biuret method, while albumin levels were determined using electrophoresis (Thermo Fisher Scientific, Indiko Clinical Chemistry Blood Equipment, Vantaa, Finland). Globulin levels were calculated by subtracting the albumin from the total protein value.

Statistical analysis

The research data were analysed using ANOVA using the General Linear Model-Univariate with a significance level of 5%. If it shows a significant effect, it will be continued with the Duncan multiple range test for the difference between any pair of means with significant levels of 1% and 5%. Further data were examined by Pearson correlation and linear regression regarding the relationship between feed quality and growth performances.

RESULTS

Nutritive value of green calf starter enriched with whey dangke

The proximate analysis and gross energy test, as presented in Table 2, reveal notable differences ($P < 0.01$) in the nutritional composition between common calf starter and green calf starter, particularly in terms of CF, CFi, ash content, N-free extract (NFE), and energy; a significant disparity ($P < 0.05$) in CP; with no substantial impact ($P > 0.05$) on DM content. Furthermore, incorporating enriched whey dangke in green calf starters showed a highly significant impact ($P < 0.01$) on fat, CFi, DM, and ash content while exhibiting nonsignificant influence on the protein, NFE, and energy content. Notably, a highly significant interaction ($P < 0.01$) was observed between the calf starter type and the enriched whey-dangke level, particularly regarding CFi content.

Digestibility of green calf starter enriched with whey-dangke

Based on the feed digestibility study data presented in Table 3, it is evident that there is a significant difference ($P < 0.01$) in the digestibility of protein and energy between common and green calf starters. The enriched whey dangke level also significantly impacted protein and energy digestibility ($P < 0.01$). Furthermore, a notable interaction ($P < 0.01$) was observed between the calf starter type and the enriched whey dangke level regarding DM digestibility, organic matter, CP, and energy.

Performance of calves fed green calf starter enriched with whey dangke

According to Table 4, the research data on dairy calves' performance reveals a significant difference ($P < 0.01$) in FCR between the common and the green calf starter. The ADG showed nonsignificant difference ($P > 0.05$) between the calf starters. Moreover, the green calf starter enriched with whey dangke had a highly significant effect ($P < 0.01$) on the concentration of IGF-1 hormone while showing a significant effect ($P > 0.05$) on shoulder height and body length. It was observed that there was no interaction between the type of calf starter and the level of enriched whey dangke on the performance of dairy cows.

Protein profile of calf blood fed green calf starter enriched with whey dangke

Calf starter type and whey dangke enriched level had nonsignificant effect ($P > 0.05$) on the profile of dairy calves, and there was no interaction between calf starter type and whey dangke enriched level.

Table 3. Digestibility of green calf starter enriched with whey dangke. (a-f)Different superscripts on rows and columns within the same parameter indicate significant differences (*P* < 0.01); (p-r)different superscripts on the same row indicate significant differences (*P* < 0.01); (x, y)different superscripts on the same column indicate significant differences (*P* < 0.01). T0: Common calf starter; T1: green calf starter; W0: 0% whey dangke + 5% water from body weight; W1: 2.5% whey dangke + 2.5% water from body weight; W3: 5% whey dangke + 0% water from body weight. Data were presented as average ± standard deviation.

Parameter (%)	Type of concentrate	Level enriched whey dangke (% of body weight)			Average
		W0 (0%)	W1 (2.5%)	W2 (5%)	
Dry matter digestibility	Common (T0)	73.00 ± 0.94^{a}	77.48 ± 1.32^{b}	77.60 ± 0.52^{b}	76.02 ± 2.42
	Green (T1)	79.09 ± 0.42^{c}	73.30 ± 0.56^{a}	73.96 ± 0.35^{a}	75.45 ± 2.77
	Average	76.04 ± 3.40	75.39 ± 2.46	75.78 ± 2.03	
Organic matter digestibility	Common (T0)	70.61 ± 0.98^{a}	75.31 ± 1.36^{c}	75.16 ± 0.69^{c}	73.69 ± 2.48
	Green (T1)	78.58 ± 0.47^{d}	71.89 ± 0.46^{ab}	72.40 ± 0.29^{b}	74.29 ± 3.24
	Average	74.59 ± 4.41	73.60 ± 2.08	73.78 ± 1.58	
Crude protein digestibility	Common (T0)	46.84 ± 0.44^{a}	54.61 ± 0.87^{c}	57.46 ± 0.55^{d}	52.97 ± 4.79^{x}
	Green (T1)	62.00 ± 0.51^{f}	52.80 ± 0.31^{b}	60.36 ± 0.64^{e}	58.39 ± 4.27^{y}
	Average	54.42 ± 8.31^{p}	53.70 ± 1.14^{p}	58.91 ± 1.67^{q}	
Energy digestibility	Common (T0)	58.66 ± 0.86^{a}	63.96 ± 0.69^{b}	64.94 ± 0.68^{b}	62.52 ± 2.99^{y}
	Green (T1)	67.79 ± 0.66^{c}	58.08 ± 0.87^{a}	58.35 ± 0.69^{a}	61.41 ± 4.83^{x}
	Average	63.22 ± 5.04^{q}	61.02 ± 3.29^{p}	61.65 ± 3.66^{p}	

Table 4. Performance of calves fed green calf starter enriched with whey dangke. (a, b)Different superscripts in the same row indicate significant differences (*P* < 0.01); (A, B)Different superscripts in the same row indicate significant differences (*P* < 0.05); (x, y)Different superscripts in the same column indicate significant differences (*P* < 0.01); (X, Y)different superscripts in the same column indicate significant differences (*P* < 0.01). T0: Common calf starter; T1: green calf starter; W0: 0% whey dangke + 5% water from body weight; W1: 2.5% whey dangke + 2.5% water from body weight; W3: 5% whey dangke + 0% water from body weight. ADG: Average daily gain; DMI: DM intake; FCR: feed conversion ratio; IGF-1: insulin-like growth factor-1. Data were presented as average ± standard deviation.

Parameter	Type of concentrate	Level enriched whey dangke (% of body weight)			Average
		W0 (0%)	W1 (2.5%)	W2 (5%)	
ADG, g d^{-1}	Common (T0)	360 ± 150	420 ± 90	490 ± 108	420 ± 140^{X}
	Green (T1)	560 ± 300	700 ± 140	710 ± 80	660 ± 180^{Y}
	Average	460 ± 240	560 ± 180	600 ± 170	
DMI, kg d^{-1}	Common (T0)	0.76 ± 022	0.93 ± 0.21	1.07 ± 0.39	0.92 ± 0.28
	Green (T1)	1.03 ± 0.69	1.24 ± 0.39	1.20 ± 0.24	1.16 ± 0.43
	Average	0.89 ± 0.48	1.08 ± 0.33	1.14 ± 0.30	
FCR	Common (T0)	2.22 ± 0.41	2.20 ± 0.09	2.19 ± 0.45	2.20 ± 0.21^{y}
	Green (T1)	1.78 ± 0.34	1.75 ± 0.21	1.68 ± 0.15	1.73 ± 0.22^{x}
	Average	2.00 ± 0.41	1.97 ± 0.28	1.93 ± 0.29	
Shoulder height, cm d^{-1}	Common (T0)	0.06 ± 0.03	0.10 ± 0.07	0.16 ± 0.07	0.10 ± 0.67
	Green (T1)	0.08 ± 0.02	0.13 ± 0.07	0.15 ± 0.01	0.12 ± 0.04
	Average	0.07 ± 0.02^{A}	0.11 ± 0.06^{AB}	0.15 ± 0.04^{B}	
Body length, cm d^{-1}	Common (T0)	0.10 ± 0.01	0.13 ± 0.06	0.18 ± 0.05	0.14 ± 0.05
	Green (T1)	0.14 ± 0.05	0.17 ± 0.06	0.22 ± 0.02	0.18 ± 0.05
	Average	0.12 ± 0.04^{A}	0.15 ± 0.05^{AB}	0.20 ± 0.04^{B}	
Chest circumference, cm d^{-1}	Common (T0)	0.07 ± 0.03	0.14 ± 0.03	0.14 ± 0.02	0.11 ± 0.04
	Green (T1)	0.13 ± 0.06	0.14 ± 0.03	0.20± 0.17	0.15 ± 0.10
	Average	0.10 ± 0.05	0.14 ± 0.02	0.17 ± 0.11	
Hormone IGF-1, ng mL^{-1}	Common (T0)	50.35 ± 7.23	73.28 ± 8.80	74.96 ± 19.15	66.19 ± 16.52
	Green (T1)	61.77 ± 8.24	74.25 ± 11.30	86.56 ± 20.79	74.19 ± 16.82
	Average	56.06 ± 9.42^{a}	73.77 ± 9.39^{b}	80.76 ± 19.51^{b}	

DISCUSSION

The calf starter type significantly impacts crude protein (CP), crude fat (CF), crude fibre (CFi), N-free extract (NFE), and energy in feed nutrition (Table 2). Green calf starters demonstrate superior nutritional quality compared to common calf starters as they contain sufficient protein content that meets the CP requirements of Starter Calves-2, with a minimum CP level of 16%. However, the CF content of the green calf starter exceeds the normal threshold (Gunun et al., 2023). On the other hand, the common calf starter fails to meet the minimum CP value, and the ash content in the calf starter feed is within the normal limit. The higher quality of green calf starter is attributed to its composition of indigofera and gliricidia, which are legumes containing essential nutrients such as CP, minerals, and high digestibility, enhancing feed quality (Ako et al., 2024).

Indigofera is a valuable source of protein and has the potential to be utilized as a raw material for feed, replacing commercial protein sources and reducing feed costs. Additionally, this fodder offers antibacterial, antioxidant, anti-inflammatory, and immunomodulatory properties, which can enhance the immune system of dairy cows and contribute to their overall health. As a result, the optimal growth of dairy cows can be achieved (Kim et al., 2022a). Gliricidia plants are also a promising alternative feed to address nutritional deficiencies in calves, particularly during their growth stage. Feeds derived from greenery provide greater energy obtained from the breakdown of forage cellulose. The level of enriched whey dangke significantly impacted CF, CFi, water content, and ash content (Table 2). The CF and CFi decreased as the levels of whey dangke increased. This is attributed to the presence of nutrients in whey dangke that bacteria can utilize as a source of nutritional growth (Faridah, 2019). Lactic acid bacteria (LAB) are commonly found in whey dangke and can break down feed components into simpler compounds (Liu et al., 2022). As a result, the activity of LAB in whey dangke leads to a reduction in CF and CFi content in calf starters.

Moreover, increasing the level of whey dangke contributes to higher water and ash content in the calf starter. This is due to the composition of whey-dangke, which contains approximately 92%-94% water, 5.09%-8.18% solids, and 0.32%-0.58% ash (Amalfitano et al., 2024). Higher water content in the feed can compromise its quality, leading to quicker deterioration caused by microorganism activity. On the other hand, ash content denotes the mineral content in calf starters. An increase in ash content indicates a higher mineral content in the feed (Liu, 2019), particularly essential minerals like Ca and P, which are crucial for bone and muscle formation in dairy calves during their growth period. The calf starter type and enriched whey dangke level impacted feed CFi content (Table 2). The addition of whey dangke decreased CFi, attributed to the presence of LAB. Calves should consume solid feed with low CFi to promote their reticulo-rumen development (Maharani et al., 2015). Feeding high CFi feed to calves may lead to digestive organ abnormalities and long-term effects on their growth, health, and milk production as cows. Enriched whey dangke is suitable for feeding green calf starter to calves, especially as forages generally contain high CFi (Khan et al., 2011). Additionally, whey dangke offers the added benefits of increasing palatability, improving feed texture, and balancing energy, protein, and mineral nutrients.

Feed digestibility is an important indicator that can be used to determine the amount of nutrients and feed the digestive tract can absorb. Feed quality is not only determined by its nutritional content but also its digestibility. Feeding ingredients that contain high nutrients but low digestibility will result in dairy cows experiencing nutritional deficiencies (Mayulu et al., 2018). The results showed that protein and energy digestibility differed between common and green calf starters (Table 3). The protein digestibility of green calf starter is higher than that of commercial calf starter. This is because indigofera and gliricidia have a high protein content of 25%-30% with a digestibility of 72%-78%, thus potentially increasing digestibility in the ratio (Ako et al., 2024).

Conversely, energy digestibility is higher in calf starters than in green ones. The common calf starter feed comprises higher raw coconut meal and ground corn materials. Both raw materials contain high energy, showing high energy digestibility (Zewdie, 2019). As the level of enriched whey dangke increases, the digestibility of CP increases while energy digestibility decreases (Table 3). This is due to the ability of the protein from whey dangke to enhance protein digestibility, as noted by Zewdie (2019), the presence of lactose in whey dangke, a fermentable sugar, and leads to a decrease in energy digestibility. If lactose binds with other energy sources, it forms more complex compounds, reducing energy digestibility.

An interaction is observed between the calf starter type and the level of enriched whey dangke (Table 3). In typical calf starters, there was an increase in digestibility with the addition of enriched whey dangke. Conversely, supplementing whey dangke can reduce the digestibility of green calf starters. This is attributed to lactose binding with lysine, forming complex compounds that reduce feed digestibility. Indigofera and gliricidia contain high levels of lysine at 1.60% and 1.87% respectively (Tirajoh, 2022). Weaned dairy cows will experience a reduced ability to produce lactase, resulting in a decreased ability to digest lactose (Zhao et al., 2021). Enriching 5% whey into the calf starter diet resulted in improved protein digestibility. Rumen microbes rapidly convert the N in whey into protein. Studies have shown that whey boasts a high protein digestibility of around 70%, and even in pre-ruminant calves, it can reach up to 91% (Anderson, 1975).

The performance of dairy calves greatly influences the performance of production during lactation. High performance during the calf period produces superior replacement stock (Maharani et al., 2015). Values for average daily gain (ADG) and feed conversion ratio (FCR) are crucial for enhancing calf production efficiency (Gunun et al., 2023). A significant difference in ADG and FCR values was observed between dairy calves fed with green and common calf starters (Table 4). Cows fed with green calf starter exhibited a higher ADG value than those fed with common calf starters, as the former contains higher protein content to optimize calf growth (Beretta et al., 2020). This study found a positive correlation between feed protein and ADG (r = 0.44; medium category), DM intake (DMI) and ADG (r = 0.93, very strong category), and a negative correlation between FCR and ADG (-0.54, moderate category) (Table 5). This suggests that the high quality of feed protein and digestibility positively impacted the growth performance of dairy cow calves. The study found a positive correlation between weight gain and enriched whey dangke (0.46 in common and green calf starter, moderate category) (Figure 2). The higher the whey level, the higher the daily weight gain, which is linked to the availability and digestibility of feed intake, particularly protein. Protein is a crucial precursor for the growth process. This aligns with Kim et al. (2022b) that feed protein plays a strategic role in dairy cows' growth performance.

Moreover, the study indicated that dairy calves fed with green calf starters exhibited a lower FCR than those fed with common calf starters. This can be attributed to the higher CP content in green calf starters, which optimizes calf growth (Table 2). The FCR is influenced by feed nutrition, indicating that feed quality impacts the FCR value (Davison et al., 2023).

Table 5. Pearsons's correlations between feed quality attributes and calf performance. ADG: Average daily gain; SH: shoulder height; CC: Chest circumference; BL: body length; DMI: DM intake; FCR: feed conversion ratio; IGF-1: insulin-like growth factor-1. Feed quality: CP: crude protein; GE: gross energy; *P-value ($P < 0.05$); **P-value ($P < 0.01$).

Pearsons's correlations	ADG	SH	CC	BL	DMI	FCR	IGF-1	CP	GE
ADG	1.00								
SH	0.38	1.00							
CC	0.47*	0.50*	1.00						
BL	0.74**	0.63**	0.35	1.00					
DMI	0.93**	0.44*	0.46*	0.46*	1.00				
FCR	-0.54**	-0.16	-0.25	-0.25	-0.27	1.00			
IGF-1	0.76**	0.47*	0.45*	0.76**	0.80**	-0.19	1.00		
CP	0.44*	0.18	0.21	0.21	0.44*	-0.36	0.35	1.00	
GE	0.33	0.03	0.02	0.02	0.26	-0.47*	0.40	0.69**	1.00

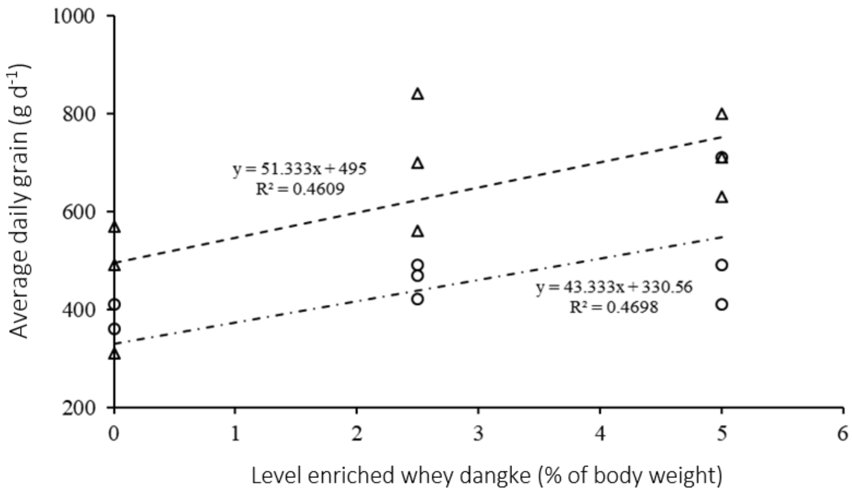

Figure 2. Correlation between whey dangke enriched level and daily body weight gain.

The whey dangke enriched levels can increase shoulder height, body length, and the insulin-like growth factor I (IGF-1) hormone concentration of dairy calves (Table 4). This increase aligns with the increase of whey dangke enriched levels in calf starters. Not only was shoulder height positively correlated with IGF-1 hormone (r = 0.47, medium category), but also body length (r = 0.76, strong category). The IGF-1 concentration influences growth performance as a hormone that regulates growth and development (Wang et al., 2021). There was a correlation between enriched whey dangke levels and IGF-1 concentration (0.80 in common calf starter; 0.64 in green calf starter, strong category) (Figure 3). There was a correlation between enriched whey dangke levels and IGF-1 concentration (0.80 in common calf starter; 0.64 in green calf starter, strong category) (Figure 3). The IGF-1 is a single-chain peptide hormone composed of 70 amino acids (Bailes and Soloviev, 2021). Whey dangke, as a by-product of milk processing, contains various amino acids similar to cheese whey, including histidine (17.68 mg g^{-1} protein), isoleucine (45.65 mg g^{-1} protein), lysine (83.98 mg g^{-1} protein), cysteine (15.08 mg g^{-1} protein) and so on. One of the main constituent amino acids of the IGF-1 hormone is cysteine. Calves need these amino acids to optimize their growth and performance. A notable outcome of this dietary intervention was the significant elevation in IGF-1 hormone concentration in the blood of the calves fed with the enriched green calf starter. The IGF-1 is a crucial indicator of growth and development in calves, suggesting that the enriched green calf starter positively impacts metabolic and physiological processes associated with calves' growth.

The type of calf starter significantly impacts the calf's blood protein profile. Similarly, as the level of enriched whey dangke increases, the data indicates a corresponding increase in the blood protein profile (Table 6). Whey dangke serves as an additional source of protein in the feed, leading to elevated protein levels in the blood. Insufficient protein in the blood can result in poor growth and health issues for livestock. This study's total blood protein levels ranged from 4.60 to 5.20 g mL^{-1}. This aligns with the findings of Immler et al. (2022), which reported a range of 3.6-9.4 g mL^{-1}.

The feed consumed by dairy cows, which is high in protein, plays a crucial role in influencing their blood protein levels. Digestible feed protein is converted into amino acids in the digestive tract and then transported through the blood, ultimately affecting blood protein levels. The protein content in the feed directly impacts the level of blood protein. Increased feed protein consumption results in elevated blood protein levels. As Permana et al. (2020), using indigofera as a protein source instead of concentrate, calves help maintain blood protein levels in dairy cows. Furthermore, a high-protein diet can optimize growth and weight gain.

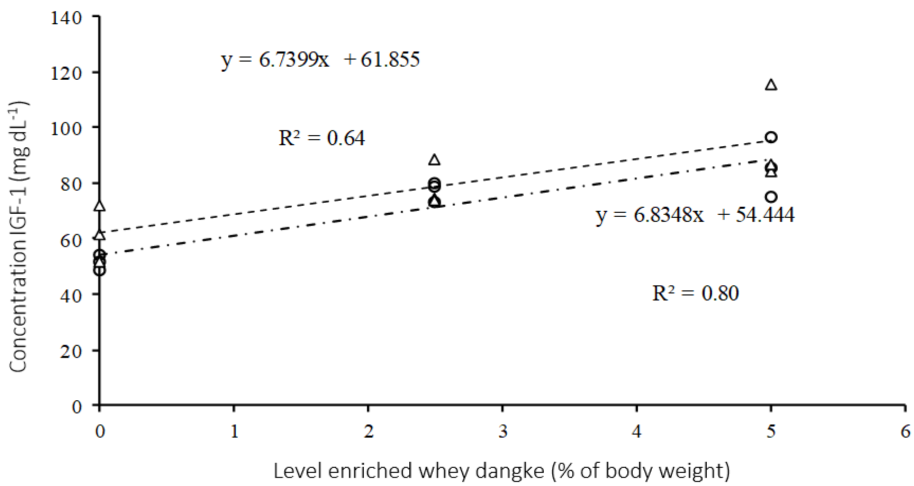

Figure 3. Correlation between whey dangke enriched level and insulin-like growth factor I (IGF-1) concentration.

Table 6. Protein profile of blood-fed green calf starter enriched with whey dangke. T0: Common calf starter; T1: green calf starter; W0: 0% whey dangke + 5% water from body weight; W1: 2.5% whey dangke + 2.5% water from body weight; W3: 5% whey dangke + 0% water from body weight; NFE: N-free extract. Data were presented as average ± standard deviation.

	Type of	Level enriched whey-dangke (% of body weight)			
Parameter (g mL^{-1})	concentrate	W0 (0%)	W1 (2.5%)	W2 (5%)	Average
Protein blood	Common (T0)	4.60 ± 0.87	4.86 ± 0.66	5.13 ± 0.55	4.86 ± 0.65
	Green (T1)	4.93 ± 1.22	4.70 ± 0.00	5.20 ± 0.10	4.94 ± 0.65
	Average	4.76 ± 0.96	4.78 ± 0.47	5.16 ± 0.35	
Albumin	Common (T0)	2.55 ± 0.05	2.70 ± 0.36	2.80 ± 0.00	2.68 ± 0.21
	Green (T1)	2.63 ± 0.30	2.50 ± 0.20	2.63 ± 0.05	2.58 ± 0.19
	Average	2.59 ± 0.19	2.60 ± 0.28	2.71 ± 0.09	
Globulin	Common (T0)	2.05 ± 0.86	2.16 ± 0.50	2.76 ± 0.61	2.32 ± 0.57
	Green (T1)	2.23 ± 1.15	2.20 ± 0.20	2.56 ± 0.15	2.33 ± 0.61
	Average	2.14 ± 0.91	2.18 ± 0.34	2.66 ± 0.41	

CONCLUSIONS

Utilization of green calf starters results in a greater daily weight gain compared to common calf starters. Additionally, the enrichment of whey enhances both crude protein digestibility and energy digestibility, as well as increasing levels of the IGF-1 hormone. Feeding calves with either the green calf starter or the common calf starter supplemented with whey leads to improved growth performance compared to a diet without this enrichment.

Author contribution

Conceptualization: R.F.U., A.A., H.H. Methodology: R.F.U., A.A., H.H., Z.R. Validation: R.F.U., Y.I., S.G. Data analysis: R.F.U., A.A., H.H., Z.R., M.M.R. Resources: R.F.U., Z.R., M.I.D. Data curation: N.A., K.U. Writing-original draft: R.F.U-P. All co-authors reviewed the final version and approved the manuscript before submission.

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