Research Article

Influence of Protein-Energy Balance on Growth, Production, and Reproduction in *Pesisir* Heifers

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Abstract | The protein-energy ratio in *Pesisir* cattle diets plays a role in determining their productivity. We aimed to establish the most effective combination of crude protein (CP) and total digestible nutrients (TDN) in a ration to enhance the productivity of *Pesisir* heifers. We evaluated consumption, nutrient digestibility, production performance, and the percentage of first estrous in sixteen heifers at 12-15 m and averaging 100.09 kg in a randomized block design with a 2 x 2 x 4 factorial pattern, where factor A represented crude protein, and factor B represented the TDN level of the ration. The treatment combinations were A1B1 (CP 10% and TDN 60%), A1B2 (CP 10% and TDN 65%), A2B1 (CP 12% and TDN 60%), and A2B2 (CP 12% and TDN 65%). The results revealed a significant interaction between factors concerning consumption and nutrient digestibility. However, there was no significant interaction for production performance. Notably, the highest percentage of first estrous was found in heifers that received A1B2. A diet with 10% CP and 65% TDN could increase consumption, feed digestibility, production performance, and the estrous number of *Pesisir* heifers.

Keywords | Crude protein, Estrous, Performance, *Pesisir* Heifers, TDN

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INTRODUCTION

Cows are ruminant livestock that supply human protein needs and play a significant role in meeting both national and international demands for animal protein (Basyar, 2021). Pighin *et al.* (2016) stated that beef remains a popular choice due to its complete nutritional profile, including protein and fat, and its appealing taste. *Pesisir* cattle, a local Indonesian bread, exhibit high adaptability to low-quality feed and resistance to various diseases and parasites (Hartatik *et al.*, 2018). They demonstrate superior heat tolerance in hot environments compared to the simmental and ongole crossbreeds (Yetmaneli *et al.*, 2023). This adaptability in tropical regions is influenced by the presence of large sweat glands and their density, providing them with advantages in thermoregulation.

Recently, the development of *Pesisir* cattle has encountered challenges resulting in a decline in production. This decline is evident in both the population and productivity, attributed to limited environmental carrying capacity and competition from imported cattle. In 2020, the *Pesisir* cattle population recorded 85,031 individuals. This figure marked a significant decrease compared to the population in 2008, which reached 89,995 heads, with a decline rate of 5.84% (BPS, 2020). *Pesisir* cattle have a relatively high carcass percentage (50.6%) compared to buffalo, Ongole crossbreed, Madurese, and Ongole cattle. They rank second to Balinese cattle, which have a percentage of 56.9% (Hendri, 2013). One contributing factor to the suboptimal productivity of *Pesisir* cattle is the provision of feed with low nutritional content and inadequate community maintenance management. There is an urgent need for initiatives to boost the *Pesisir* cattle population through feed engineering, with a particular emphasis on *Pesisir* heifers, as they are prospective parent cattle.

The nutrients that livestock need are protein and energy (Pazla *et al*., 2023a). Protein plays a role in facilitating the growth or formation of body cells, particularly in calves and young cattle. Livestock experiencing nutritional deficiencies will encounter reduced reproductive efficiency and reproductive disorders, affecting both females and males. A deficiency in protein within the feed can lead to reproductive disorders in livestock, such as weak estrous, embryo death, and premature birth. The balance between protein and TDN in the ration significantly influences the efficiency of nutrient utilization, ultimately impacting livestock productivity (Pazla *et al*., 2021a). The balance of protein and TDN plays a crucial role in formulating cattle rations, if this balance is not maintained, it can result in an excess or deficiency of energy and protein intake in the animals. Nugroho *et al*. (2013) stated that there is a substantial need for a balance of protein and TDN, especially in young ruminants during their growth phase. We aimed to identify the optimal protein and TDN balance for *Pesisir* heifers concerning feed consumption, nutrient digestibility, body weight gain, production performance, and the percentage of first estrous.

MATERIALS AND METHODS

Experimental location

The trial was carried out at the Center for Breeding Superior Livestock and Forage for Animal Feed in Padang Mengatas, located at Jl. Raya Payakumbuh-Lintau, KM.9, Pekan Sabtu, Payakumbuh 26201, Indonesia, spanning from May to September 2023. The altitude of this area is 790-1014 m above sea level. The region experiences an average annual rainfall of 1800 mm, with temperatures between 18 and 28°C, and a relative humidity of around 70%. Watering source is from around the cage and *ad bilitum* availability. The cattle was feed with concentrate in the morning and forage was given twice in a day. The cage has good drainage and ventilation system. Lighting was provided in the night. The cage was cleaned everyday and also the cage and the person were sprayed with disinfectant. Anthelmintic procedure was given before experiment.

Research materials and methods

This research used a 2 x 2 factorial randomized block design method with four replications. Factor A represented protein content at 10 and 12%, while factor B represented TDN content at 60 and 65%. We used sixteen Pesisir heifers aged 12-15 months with an average body weight of 100.09± 14.85 kg. the animals were divided into four groups of four *Pesisir* heifers as follows: GI at 73.5-84.5 kg (20.03%), GII at 86-94 kg (16.07%), GIII at 106.5-112.5 kg (15.37%) and GIV at 115.5-119.5 (15.76%). The feed treatments were A1B1 (10% CP and 60% TDN), A1B2 (12% CP and 60% TDN), A2B1 (10% CP and 65% TDN), and A2B2 (12% CP and TDN 65%).

This research comprised four periods. The 1st is the Adaptation Period, which spanned 10 days to acquaint *Pesisir* heifers with new environmental conditions and enable them to adapt to the forthcoming treatment rations. The 2nd is the observation period (Preliminary), a 15-day designed to eliminate any influence from previous rations. The 3rd is the collection period of 5 days aimed to assess the digestibility of the ration via a collection method. During this period, daily measurements were taken for ration consumption, feces, and urine excretion. Approximately 10% of the daily feces and urine were collected and stored in designated containers. The feces were air-dried using Memmert Universal Oven Model UN55 from Germany, followed by an additional oven-drying in an oven at 60°C for 48 hours. Nutritional contents in food and feces samples were analyzed using proximate analysis based on the Association of Official Agricultural Chemists (2016), and fiber fractions were determined using the method developed by Van Soest *et al*. (1991). The 4th is the growth period for two months post-collection period, which involves measuring the initial and final body weights of the *Pesisir* heifers. Estrous was observed during the study by monitoring signs of estrous, where vaginal discharge indicated a normal cycle. The percentage of estrus cows was calculated as follows:

$$\text{Estrus cow (\%)} = \frac{\text{The number of estrus cows in each treatment group}}{\text{The number of cows in the group}} \times 100\%$$

The experimental diet was composed of a 60:40 ratio of forage to concentrate. Elephant grass was used as the forage, while the concentrate was comprised of bran, corn, palm kernel meal, and a blend of minerals for cattle. The specific composition of the feed ingredients in the diet can be found in Table 1, and the chemical composition of the diet is detailed in Table 2.

Table 1: Ration composition of treatment ration.

Composition (%)	A1B1	A1B2	A2B1	A2B2
Corn	7	25	2	17
Palm Kernel cake	6	2	15	14
Mineral	1	1	1	1
NaCl (table salt)	0.5	0.5	0.5	0.5
Rice bran	25.5	11.5	21.5	7.5
Elephant grass	60	60	60	60

A1B1 (CP 10% and TDN 60%), A1B2 (CP 10% and TDN 65%), A2B1 (CP 12% and TDN 60%), and A2B2 (CP 12% and TDN 65%).

Statistical analysis

The research data were analyzed using analysis of variance using Stastix 8 software. If significant differences in the results were obtained, the Duncan test, as outlined in Steel and Torrie (2002), was conducted.

RESULTS AND DISCUSSION

Feed consumption

The treatments showed a significant (P<0.05) interaction with dry matter (DM) consumption (Table 3). The A1B2 treatment exhibited the highest DM consumption (Figure 1), whereas the A1B1 treatment had the lowest. The difference in DM consumption was caused by differences in the protein and energy ratio between treatments. These two main nutrients play a crucial role in supporting the growth and activity of rumen microbes in degrading feed. Treatment A1B2 has a higher energy content compared to treatments A1B1 and A2B1 (Table 2). Energy is needed by rumen microbes to increase their population so it has an impact on the amount of enzyme secretion produced in the feed degradation process, which will have a direct impact on

Table 2: Nutritional composition of treatment ration.

Nutritional composition (%)	Treatments			
	A1B1	A1B2	A2B1	A2B2
Dry matter	89.59	90.99	91.71	90.87
Organic matter	88.32	84.33	91.48	91.46
Ash	11.67	15.67	9.51	8.53
Crude protein	10.32	10.03	12.26	12.38
Crude fiber	34.86	22.51	36.99	30.64
Crude fat	2.17	2.29	3.30	3.16
Nitrogen free extract	40.95	49.48	37.92	45.27
Total digestible nutrient	59.76	64.73	59.87	65.03
Neutral detergent fiber	69.76	57.52	60.86	65.71
Acid detergent fiber	51.39	38.81	43.61	51.94
Cellulose	31.01	34.99	28.69	16.72
Hemicellulose	18.36	18.70	17.25	13.77

A1B1 (CP 10% and TDN 60%), A1B2 (CP 10% and TDN 65%), A2B1 (CP 12% and TDN 60%), and A2B2 (CP 12% and TDN 65%).

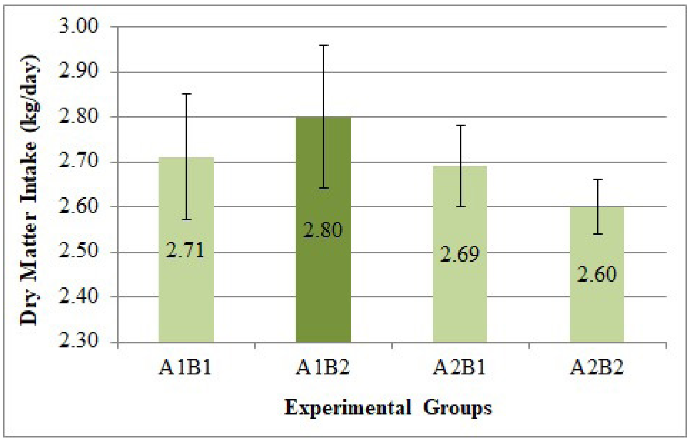

Figure 1: Dry matter intake of the treatments.

Table 3: *In vivo* daily consumption of treatment ration.

In vivo consumption (Kg/day)	Factor A (Crude protein)	Factor B (TDN)		Average	SEM
		B1 (60%)	B2 (65%)		
Dry matter (kg/day)	A1 (10%)	2.71aA±0.14	2.80aB±0.08	2.76	0.04
	A2 (12%)	2.69bA±0.08	2.60bB±0.06	2.65	
	Average	2.70	2.71		
Organic matter (kg/day)	A1 (10%)	2.39aA±0.13	2.37aB±0.06	2.38	0.04
	A2 (12%)	2.43bA±0.07	2.38bB±0.05	2.41	
	Average	2.41	2.37		
Crude protein (kg/day)	A1 (10%)	0.27aA±0.01	0.28aA±0.01	0.28a	0.01
	A2 (12%)	0.33bA±0.01	0.32bA±0.01	0.33b	
	Average	0.30	0.30		

Different lowercase letters in the row and different uppercase letters in the same column have a significantly different effect (P<0.05).

feed digestibility. Another factor that determines the high consumption of DM is the palatability and composition of the feed ingredients that make up the ration (Arief *et al*., 2023a, b; Jamarun *et al*., 2023; Pazla *et al*., 2023b). The A1B2 ration is more palatable because the main energy source comes from corn, whereas in the A1B1 and A2B1 treatments, the main energy source comes from rice bran. The A2B2 treatment is lower than A1B2 because the percentage of corn used is lower than A1B1.

The A1B2 treatment contained a lower ration CP compared to A2B1 and A2B2. Diet protein is needed to provide a nitrogen source for microbes (Pazla *et al*., 2021b; Zain *et al*., 2023). However, there needs to be a balance with energy availability because balanced protein and energy will increase the rate of microbial growth. Nitrogen from protein will be used as a nitrogen source for the formation of microbial protein, whereas apart from nitrogen, the process of forming microbial protein also requires ATP, which comes from available energy. This condition will increase the rate of feed leaving the rumen (rate of passage) so that the rumen empties more quickly and stimulates livestock to consume more ration. DM consumption in the A1B2 treatment was higher than in Usman *et al*. (2013), namely 2.72 kg with a balance of CP 13.67% and TDN 60%.

Organic material is the largest component of dry matter rations, consisting of crude protein, crude fat, crude fiber, and extracts without nitrogen. Consumption of organic material is an indicator of energy adequacy for livestock. The treatments showed a significant ($P<0.05$) interaction with organic material consumption (Table 3). Treatment A1B1 showed higher OM consumption than A1B2 and A2B2, but the highest consumption was found in A2B1. The high OM consumption in A2B1 was due to the high organic material content of the ration in A2B1. The organic material content of the ration plays a very important role in determining the amount of organic material consumed (Febrina *et al*., 2017).

The treatments showed a significant ($P<0.05$) interaction with crude protein consumption (Table 3). The highest crude protein consumption was in treatment A2B1, which was not significantly different from A2B2. The high consumption of CP in this treatment was due to the high protein content of the diet. CP consumption is highly dependent on the protein content of the diet consumed by livestock (Arief and Pazla, 2023).

Digestibility of dry matter, organic matter, and crude protein

The impact of varying the ratio of CP and TDN in the treated feed had a significant ($P<0.05$) interaction on the OMD, DM, and CP in *Pesisir* cattle, as shown in Table 4. Numerically, the highest levels of DM and OM digestibility were achieved in treatment A1B2, followed by A1B1, A2B1, and A2B2. The high digestibility of DM and OM in treatment A2B1 can be attributed to the balanced values of CP and TDN in the ration. Rumen microbes require a balance of protein and energy for their activity and growth. The greater the rumen microbial population, the more food substances that can be digested (Jamarun *et al*., 2017a, b). Apart from that, the CP and TDN ratios of A1B2 were lower than the others. Several studies show that reducing the CP and TDN ratio results in better DM and OM digestibility. The current CP and TDN ratio of 0.25 in the feed led to decreased digestibility in male *Pesisir* cattle, in contrast to the ratio of 0.23 as reported by Pazla *et al*. (2021a). These findings align with Purbowati *et al*. (2008) and Nugroho *et al*. (2013), both of which indicated that an increase in the CP: TDN ratio harmed feed digestibility values.

The pattern of OMD is linear with DMD because it constitutes the largest component of DM. This linear pattern has been previously observed by Antonius *et al*. (2023); Elihasridas *et al*. (2023a). The organic material components in the A1B2 treatment are more easily degraded by rumen microbes, resulting in higher OMD than other treatments. Montcho *et al*. (2017) stated that easily soluble and degraded organic matter helps increase rumen microbial activity, enabling effective feed degradation.

Providing feed containing a balanced ration of protein and energy activates rumen microbes, which can increase the population of proteolytic and deamination bacteria, thereby enhancing feed digestibility. Efficiency in the synthesis of microbial protein is dependent on the sequential availability of ammonia, followed by the availability of energy and carbon skeletons (Febrina *et al*., 2016; Pazla *et al*., 2018a; Elihasridas *et al*., 2023b). In cases where ammonia becomes available more rapidly than carbohydrate fermentation, the utilization of ammonia for microbial protein formation can become inefficient. Optimal conditions for microbial protein synthesis are achieved when the availability of fermented carbohydrates aligns with the availability of the protein source (Christiyanto *et al*., 2005). Table 4 shows that the A1B2 showed better CP digestibility values compared to other treatments.

CP digestibility is the percentage of crude protein consumed in the diet that does not appear in feces. This digestibility is affected by the crude protein content in the feed. An increase in the crude protein content in the diet leads to higher microbial reproduction rates and a larger rumen population, which, in turn, enhances digestibility (Haryanto, 2014; Tedeschi *et al*., 2015). Nevertheless, an increase in protein levels in rations, when not adequately balanced with sufficient non-protein nitrogen, fails to

Table 4: *In vivo* digestibility of treatment ration.

In vivo digestibility (%)	Factor A (Crude protein)	Factor B (TDN)		Average	SEM
		B1 (60%)	B2 (65%)		
Dry matter	A1 (10%)	64.92bA±3.70	66.99bA±1.96	65.95b	1.12
	A2 (12%)	61.34aA±2.10	56.15aA±1.18	58.75a	
	Average	63.13A	61.57A		
Organic matter	A1 (10%)	66.44bA±3.81	68.60bA±1.77	67.52b	1.27
	A2 (12%)	62.92aA±3.46	58.35aA±1.09	60.64a	
	Average	64.68A	63.47A		
Crude protein	A1 (10%)	80.08bB±3.02	80.83bA±1.56	80.46b	1.04
	A2 (12%)	79.45aB±2.50	72.18aA±1.21	75.82a	
	Average	79.76B	76.51A		
Crude Fat	A1 (10%)	65.88bA±2.80	69.72bA±1.68	67.80b	1.15
	A2 (12%)	58.45aA±2.32	51.33aA±2.38	54.89a	
	Average	62.16A	60.52A		
Crude Fiber	A1 (10%)	58.92bA±3.12	59.83bA±3.22	59.87b	1.34
	A2 (12%)	53.28aA±2.88	49.33aA±1.51	51.30a	
	Average	56.10A	55.08A		
Neutral detergent fiber	A1 (10%)	57.09bA±3.99	61.02bA±1.56	59.05b	1.32
	A2 (12%)	51.26aA±3.69	45.26aA±1.33	48.26a	
	AVERAGE	54.18A	53.14A		
Acid detergent fiber	A1 (10%)	55.43bA±4.30	57.98bA±3.05	56.70b	1.58
	A2 (12%)	49.43aA±4.55	43.97aA±0.75	46.70a	
	Average	52.43A	50.97A		
Cellulose	A1 (10%)	58.99bA±4.60	63.52bA±2.54	61.25b	1.52
	A2 (12%)	52.78aA±4.09	47.23aA±0.96	50.01a	
	Average	55.88A	55.38A		
Hemicellulose	A1 (10%)	60.55bA±4.34	63.85bA±0.50	62.20b	1.12
	A2 (12%)	54.90aA±2.99	50.13aA±1.11	52.52a	
	Average	57.73A	56.99A		
Extract material without nitrogen	A1 (10%)	68.16bA±3.38	71.45bA±2.19	69.81b	1.03
	A2 (12%)	63.99aA±1.72	57.72aA±0.94	60.86a	
	Average	66.08A	64.59A		
Total digestible nutrient	A1 (10%)	65.44bA±1.05	67.39bA±1.69	66.42b	0.74
	A2 (12%)	61.64aA±2.45	56.07aA±0.76	58.85a	
	Average	63.54A	61.73A		

Different lowercase letters in the row and different uppercase letters in the same column have a significantly different effect (P<0.05)

promote microbial growth in the rumen. Similarly, Teti *et al.* (2018) noted that achieving a balance between protein and total digestible nutrients (TDN) is crucial for optimal fermentation efficiency. In line with this, Rosmalia *et al.* (2022a) emphasized that the addition of protein sources alone may not stimulate rumen microbial growth without the supplementation of soluble carbohydrates. Rosmalia *et al.* (2022b) pointed out that rations with higher TDN content typically undergo more extensive fermentation compared to those with lower TDN content. Moradi and Zadeh (2013) indicated that achieving synchronization or balance between the availability of energy and protein in the rumen can boost microbial activity and microbial protein synthesis in the rumen. In the current study, the A1B2 treatment produced better digestibility compared to the A1B1, A2B1, and A2B2 treatments.

Extract ether digestibility (EED)

The treatments revealed a significant (P<0.05) interaction regarding crude fat digestibility, with the highest value found in treatment A1B2 and the lowest in A2B2. In Table 4, it is evident that fat digestibility tends to decrease with increasing protein with the same TDN content. However, fat digestibility tends to increase with raising TDN content with the same protein level. A high crude fat concentration in the A2B2 treatment diet led to decreased fat digestibility, whereas a low crude fat concentration in the A1B1 treatment resulted in increased crude fat digestibility. The crude fat content in the treatment influences the level of crude fat digestibility. As shown in Table 1, treatment A2B2 has the highest fat content among the other treatments, resulting in the lowest fat digestibility. The substantial use of palm kernel cake in A2B1 and A2B2 impacts the level of crude fat digestibility. This aligns with Polii *et al.* (2020) and Makmur *et al.* (2019) who stated that high crude fat content in rations reduces nutritional digestibility. Alhadas *et al.* (2023) also reported that an increase in protein levels and a decrease in NFC (Non-fiber Carbohydrate) levels in low-fat DDGS-based rations and high-protein concentrates influenced a decrease in crude fat digestibility due to the higher crude fat composition in the treatment rations.

The reduction in fat digestibility was correlated with a decrease in the digestibility of DM and OM, as demonstrated in Table 4. There was a decrease in DDM and DOM in A2B2. The decrease in fat digestibility is caused by an imbalance in the concentration of protein and energy in the ration, resulting in microbes experiencing deficiencies in their body's protein synthesis, which impairs feed digestion activities. The recent study explained that a ration with a 10% CP and a 60-65% TDN demonstrates an optimal balance of protein and energy for increasing fat digestibility. In line with Han *et al.* (2022), who reported that the balance of protein and energy impacts crude fat digestibility. A balance of protein and energy capable of optimizing the protein formation process in rumen microbial bodies will enhance the productivity of rumen microbes in digesting feed (Putri *et al.*, 2019, 2021; Sari *et al.*, 2022; Zain *et al.*, 2020).

There was a significant ($P<0.05$)interaction between protein and energy levels on extract ether digestibility (EED). The highest EED was observed in the A1B2 treatment, while the lowest was in the A2B2 treatment. As shown in Table 4, an increase in protein levels at the same TDN level tended to decrease EED. Conversely, an increase in TDN levels at the same protein level tended to increase EED. It can be explained that a high concentration of extract ether in A2B2 depressed its digestibility, while the lowest concentration of extract ether in A1B1 improved its digestibility. The composition of extract ether in the experimental ration affected EED. Table 2 showed that A2B2 consisted of 3.16% extract ether, which is higher than in other treatments, resulting in the lowest EED value. The higher composition of palm kernel cake in the ration, especially in A2B1 and A2B2 was associated with a decrease in EED. This aligns with Polii *et al.* (2020), who noted that an elevated content of ether extract in the diet contributes to a reduction in nutrient digestibility. Alhadas *et al.* (2023) also reported that an increase in protein levels and a decrease in NFC (Non-fiber Carbohydrate) levels in low-fat dried distillers grains without soluble (DDGS) and high-concentrate rations affected the decrease in extract ether digestibility due to an increase in ether extract composition in the ration.

In addition to being influenced by extract ether composition, EED is associated with a decrease in DMD and OMD. As seen in Table 4, the decrease in DMD and OMD corresponds with the decrease in EED. This correlation can be explained by the fact that dry matter consists of all nutrients, while OM consists of all nutrients except ash. Furthermore, the decrease in EED was attributed to the lack of synchronization between protein and energy in the rumen, leading to a deficiency in microbe protein synthesis. This, in turn, results in a reduction in digestibility activities in the rumen. The recent study explains that a ration consisting of 10% protein and 60-65% TDN demonstrated the optimal protein and energy ratio that can increase EED. This is in line with Han *et al.* (2022), who reported that the balance of protein and energy influences the increase in ether extract digestibility. The protein and energy ratio can optimize microbe protein synthesis. The efficiency of nutrient digestibility is influenced by a high population of rumen microbes, as indicated by Putri *et al.* (2019, 2021), Sari *et al.* (2022), and Zain *et al.* (2020).

Table 5: Body weight gain of treatment ration.

Parameter	Factor A (Crude protein)	Factor B (TDN) B1 (60%)	B2 (65%)	Average	SEM
Body weight gain (Kg/day)	A1 (10%)	0.49±0.14	0.52±0.16	0.50	00.06
	A2 (12%)	0.47±0.09	0.44±0.06	0.46	
	Average	0.48	0.48		

Nitrogen free extract digestibility (NFED)

The levels of protein and energy in the diet significantly ($P<0.05$) influenced the digestibility of NFE, with the highest digestibility value found in the A1B2 treatment and the lowest in the A2B2 treatment. It showed that the A1B2 is more synchronized than the A2B2. In Table 6, it can be seen that increasing the TDN level at the same protein level tends to increase nitrogen-free extract (NFE) digestibility, although not significantly. Meanwhile, increasing the protein level at the same TDN level results in the NFE digestibility value tending to decrease significantly ($P<0.05$). It indicates that an increase in protein levels without a TDN balance results in protein and energy asymmetry in the protein formation process of the microbial body, reducing the ability of the microbes to digest food. Increasing protein levels will not stimulate microbial growth without a balanced energy source (Sahroni *et al.*, 2021). However, adding the TDN level to a protein level of 10% showed the highest NFE digestibility (10% and TDN of 60-65%). This showed that 65% of TDN has met the energy needs of livestock and rumen microbes. The most dominant components of NFE are non-structural carbohydrates, such as starch and monosaccharides. This component is found in many cereal plants, including corn. The corn composition of 25% in A1B2 contributed to the high digestibility of NFE compared to other treatments. In line with Wiryawan *et al.* (2017), which states that NFE with high starch content will contribute to a high energy source. This energy will be used by rumen microbes in the body's protein synthesis process. Increasing the efficiency of microbial protein formation will increase nutrient digestibility (Pazla *et al.*, 2018a; Putri *et al.*, 2021).

Table 6: Ration efficiency of treatment ration.

Parameter	Factor A (Crude protein)	Factor B (TDN) B1 (60%)	B2 (65%)	Average	SEM
Ration efficiency (%)	A1 (10%)	18.33±0.73	18.59±2.10	18.46	0.98
	A2 (12%)	17.61±3.05	17.02±2.00	17.32	
	Average	17.96	17.81		

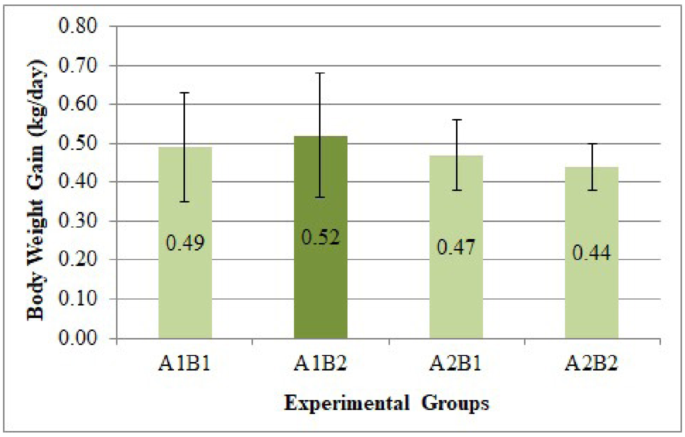

Figure 2: Body weigh gain of the treatments.

The levels of protein and energy in the experimental ration significantly (P<0.05) influenced the digestibility of NFED, with the highest value found in the A1B2 treatment and the lowest in the A2B2 treatment. This demonstrates that a ration consisting of 10% protein and 65% TDN is more synchronous than a ration with 12% protein and 65% TDN. In Table 6, it can be observed that increasing TDN levels at the same protein level tends to increase NFED, although not significantly. On the other hand, increasing protein levels at the same TDN level results in a significant (P<0.05) decrease in NFED. This indicates that an increase in protein levels without a balanced TDN source results in unsynchronized protein and energy levels. This imbalance interferes with microbial protein synthesis, reducing the ability of microbes to digest feed. An increase in protein levels alone will not be sufficient to stimulate microbial growth without a balanced energy source (Sahroni *et al.*, 2021). However, increasing TDN levels to 60-65% at a protein level of 10% showed the highest NFED. This suggested that a TDN level of 65% meets the needs of cattle and rumen microbes. The most dominant component of NFE is non-structural carbohydrates, such as starch and monosaccharides, which are found in many cereal plants, including corn. The corn composition in A1B2 contributed to the high NFED compared to other treatments. This aligns with Wiryawan *et al.* (2017) who stated that NFE with a high starch content provides a significant energy source. This energy is utilized by rumen microbes in the process of protein synthesis, ultimately enhancing nutrient digestibility (Pazla *et al.*, 2018a; Putri *et al.*, 2021).

Total digestible nutrient (TDN)

Total digestible nutrient is the energy that can be digested and utilized by livestock, obtained from the sum of the digestibility of protein, crude fiber, NFE, and crude fat. The research results showed that the levels of protein and TDN in the ration influence the amount of TDN that can be utilized by livestock. We demonstrated that increasing protein and TDN levels significantly (P<0.05) affected TDN digestibility. Increasing TDN levels at the same protein level tended to increase TDN digestibility, although not significantly. The increase in digestibility in A1B2 was due to the higher TDN content. In line with Teti *et al.* (2018), who stated that a high TDN content in the ration is more easily degraded by rumen microbes. In contrast, increasing the protein level at the same TDN level resulted in a significant (P<0.05) decrease in TDN digestibility. This explains that the increase in protein levels is not balanced with the TDN content, resulting in suboptimal nutrient absorption. Consistency in the synchronization between protein and energy levels will increase microbial protein synthesis. Energy that degrades easily must be balanced with protein that degrades easily, ensuring that no nutrients go to waste (Syamsi *et al.*, 2022).

Total digestible nutrient represents the energy that livestock can digest and utilize, derived from the summation of protein, crude fiber, NFE (Nitrogen-Free Extract), and crude fat digestibility. The current research has demonstrated a significant (P<0.05) influence of protein and TDN levels on TDN utilization in livestock. An increase in TDN levels at the same protein level tended to enhance TDN digestibility, although not statistically significant. The observed improvement in digestibility in A1B2 can be attributed to the increased TDN content. This finding aligns with the study conducted by Teti *et al.* (2018), which emphasized that rations with higher TDN content are more readily degraded by rumen microbes. Conversely, raising the protein level at a consistent TDN level resulted in a significant (P<0.05) decrease in TDN digestibility. This discrepancy suggests that the elevated protein levels lack balance with the TDN content, leading to suboptimal nutrient absorption. Achieving a harmonious synchronization between protein and energy levels is crucial for enhancing microbial protein synthesis. This ensures that easily degradable energy complements readily degradable protein, minimizing nutrient wastage (Syamsi *et al*., 2022).

Crude fiber digestibility (CFD)

Crude fiber plays a pivotal role as an energy source for rumen microbes and serves as a rumen filler for ruminants. We demonstrated that variations in the combination of protein and energy levels in the ration lead to significant (P<0.05)differences in crude fiber digestibility levels. As depicted in Table 4, there is a trend towards increased crude fiber digestibility with higher TDN levels at consistent protein levels, even though this difference is not statistically significant. Conversely, when keeping the protein level at 12%, there is a tendency toward decreased crude fiber digestibility, although not statistically significant. However, significantly (P<0.05), varying the protein level in diets with the same TDN level results in a notable decrease in crude fiber digestibility.

This observation aligns with previous studies that report increased TDN levels lead to enhanced crude fiber digestibility, while higher protein levels tend to decrease it (Teti *et al*., 2018). Zhou *et al*. (2015) reported that elevating energy levels at consistent protein levels positively impacts crude fiber digestibility. This phenomenon can be explained by the fact that 65% of TDN meets the energy requirements of cattle, with further enhancement achievable through balanced protein levels. The level of feed digestibility, including crude fiber, is significantly influenced by the composition of the feed ration. As the crude fiber content in the ration increases, digestibility tends to decrease. The observed reduction in crude fiber digestibility in A2B1 and A2B2 can be attributed to the high crude fiber content in these rations.

Crude fiber fraction digestibility

Fiber is found in many cell walls and comprises the main components of hemicellulose, cellulose, and lignin. Cell walls that are insoluble in neutral detergents are referred to as NDF, consisting of lignin, cellulose, hemicellulose, and small amounts of protein, nitrogen bonds, minerals, and cuticle. NDF plays a crucial role for ruminants as it is closely linked to dry matter intake. This study reports a significant (P<0.05) influence of protein and energy balance on NDF digestibility. The highest NDF value was observed in treatment A1B2, while the lowest was in A2B2. Furthermore, the highest ADF value was found in A1B2, and the lowest was in A2B2. In line with these findings, the highest digestibility values for cellulose and hemicellulose were recorded in A1B2, and the lowest values were in A2B2 (Table 4). These results indicated that A1B2 offers the most optimal protein and energy ratio for enhancing digestibility. Increasing the TDN level at the same protein level tended to enhance the digestibility of the fiber fraction, although this difference was not statistically significant. Conversely, increasing the protein level at the same TDN level resulted in a significant (P<0.05) decrease in the digestibility of the fiber fraction. This suggests that TDN levels of up to 65% at 10% protein levels provide an optimal balance of protein and energy. The availability of nitrogen and protein degradation, in conjunction with VFA production from energy degradation, fosters favorable conditions for microbial protein synthesis. Microbes become more active in degrading the fiber fraction, in agreement with Emmanuel *et al*. (2015), who emphasized that a balanced availability of nitrogen and soluble energy sources enhances feed fermentation in the rumen.

Body weight gain, efficiency, and ration conversion

The findings from the study regarding the average daily body weight gain, feed efficiency, and feed conversion ratio of the experimental diets given to *Pesisir* heifers are displayed in Tables 5, 6, and 7. It is evident from Table 5 and Figure 2 that the A1B2 treatment achieved the highest average daily body weight gain, although no significant interaction was observed compared to the other treatments. This weight gain is accompanied by high DM consumption, also observed in A1B2. The lowest body weight gain is found in treatment A2B2. Animal body weight is consistently directly proportional to the level of feed consumption. Increased feed consumption leads to greater body weight, and this also affects population growth, as indicated by Ningrat *et al*. (2020) and Malindo *et al*. (2023). Weight gain occurs when the quantity of food consumed exceeds the animal's requirements, and the surplus nutrients are converted into meat and fat tissue increasing body weight. Ningrat *et al*. (2018) demonstrated that when the amount of feed consumed is insufficient to meet the livestock's needs, it results in a decrease in body weight.

Table 7: Feed conversion ratio of treatment ration.

Parameter	Factor A (Crude protein)	Factor B (TDN) B1 (60%)	Factor B (TDN) B2 (65%)	Average	SEM
Feed conversion ratio	A1 (10%)	5.53±0.73	5.38±2.10	5.46	0.98
	A2 (12%)	5.72±3.05	5.91±2.00	5.32	
	Average	5.96	5.81		

The body weight gain was higher than the body weight gain of male *Pesisir* cattle weaned until the age of 2 and 3-4 years. The body weight gain was less than what was reported by Afdal and Khasrad (2006), who recorded a PBB range of 0.58-0.80 kg/head/day for *Pesisir* cattle. The higher PBB value in their study was attributed to the ration provided, which had a TDN value of 68%, and the experimental livestock experienced a compensatory growth effect.

In A2B2, the weight gain was at its minimum. The conversion rate for the A2B2 diet indicated that to achieve an increase of 1 kg in body weight per day, 5.91 kg of dry ration material is necessary (Table 7). It can be concluded that providing the A2B2 treatment does not result in efficient feed conversion. Therefore, the feed conversion in the A2B2 treatment is suboptimal due to a significant amount of feed being consumed with less efficient meat production. However, this combination did not have a significant impact on rumen microbes. Rumen microbes require balanced CP and TDN values for their growth. In alignment with this, Pazla *et al.* (2018b) have also illustrated that the quality of the feed, particularly its protein, energy, and crude fiber content, significantly influences livestock's capacity to convert feed into meat.

The treatment with the lowest feed conversion rate was A1B2. An effective feed conversion rate is achieved when modest feed consumption leads to a substantial increase in body weight. This is likely because the feed consumption in treatment A1B2 meets the physiological requirements of the livestock with an optimal balance of protein and energy content. It has been noted by Pazla *et al.* (2021a) that a more favorable conversion ratio is achieved when there is a higher nutritional value in the ration.

The feed conversion values in treatments A1B1 and A2B1 were suboptimal. The results indicated that ration conversion in treatment A1B2 was lower than in the other treatments. This implies that the presence of energy sources in treatment A1B2 is more efficient, enabling rumen microorganisms to operate with greater efficiency.

The best feed conversion was achieved in A1B2. This situation demonstrates that the A1B2 ration is of excellent quality for livestock, as it exhibits high palatability. As noted by Suyitman *et al.* (2020), feed quality plays a significant role in determining feed conversion. Additionally, high-quality feed leads to higher Average Daily Gain (ADG). Feed conversion is influenced by factors such as the type of animal, genetics, the cow's condition, feed palatability, seasonal conditions, age, management, the animal's ability to digest feed, types of feed ingredients, nutrient availability in the ration, and ADG (Pazla *et al*., 2018b; Suyitman *et al*., 2020; Jusman *et al*., 2020). According to Sugiharto *et al.* (2004), cattle fed with local feed, following the practices of dryland breeders, attain a feed conversion ratio of 13.6. This is in contrast to the ideal feed conversion for beef cattle, which is 9, as suggested by Tillman *et al.* (2005).

Treatment A1B2 demonstrated the best feed efficiency, resulting in higher body weight gain in this treatment compared to treatments A1B1, A2B1, and A2B2 (Table 6). The optimal balance of protein and energy in treatment A1B2 had a positive influence on the performance of rumen microbes in breaking down feed, ultimately leading to increased feed efficiency, as reported by Pazla *et al.* (2021a). The lower feed efficiency in treatments A1B1, A2B1, and A2B2 was attributed to an imbalance in crude protein and TDN content in the ration. Following Ariesafera (2019), who emphasized the close relationship between the CP and TDN ratio in rations and feeding efficiency. Efficient feed consumption in Pesisir cattle is achieved when there is a well-balanced ratio of CP and TDN in their diet. The feed efficiency observed exceeded that reported by Jusman *et al.* (2020), which was 7.78%, and that of Suyitman *et al.* (2020), which was 12.22%. This variation can be attributed to factors such as the cattle type, the composition of feed ingredients in the diet, and the quality of the feed used.

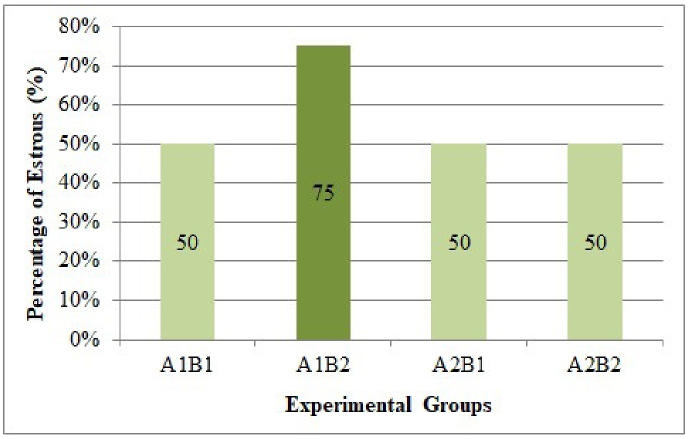

Figure 3: Percentage estrous of the treatments.

Estrous percentage

Figure 3 shows that treatment A1B2 achieved the highest estrous percentage. The speed of estrous in cows is greatly influenced by both the quantity and the nutritional quality of the ration provided. Treatment A2B1 had the highest dry matter consumption (Table 3), along with the highest level of digestibility (Table 4). Optimal nutrients consumed and utilized by the livestock's body stimulate the production of reproductive hormones, leading to quicker estrous. Conversely, when ration consumption and nutrient digestibility are low, it slows down the process of entering estrous due to insufficient nutrients to stimulate the formation of reproductive hormones. Inadequate feed consumption directly affects protein intake, which plays a vital role in hormone production, including reproductive hormones. Insufficient protein leads to a shortage of reproductive hormones, resulting in reproductive disorders such as silent heat and ovarian issues. Yendraliza (2013) noted that reduced food levels generally delay the onset of puberty, while high food levels can accelerate it and increase body weight. Wiltbank *et al.* (2006) added that rapid weight gain between birth and weaning, as well as between weaning and 396 days of age, speeds up the onset of puberty in heifers. Delayed puberty due to insufficient food may result from low levels of gonadotropins produced by the adenohypophyseal gland, a lack of ovarian response, or possibly the ovaries' failure to produce sufficient amounts of estrogen (Yendraliza, 2013). Dairy cows demonstrate that this combination can delay sexual maturity and suppress the signs of heat (Salisbury and Vandemark, 1985). In the ovaries, low feed intake delays puberty, accompanied by a decrease in the development of ovarian follicles, so low feed intake in female cows can cause the dominant follicle to remain smaller (Bergfeld *et al.*, 1994).

CONCLUSION

Providing a ration containing 10% CP and 65% TDN increased consumption, feed digestibility, production performance, and the estrous percentage in Pesisir heifers.

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NOVELTY STATEMENT

We identified the ideal protein to energy ratio for enhancing nutrient intake, production performance, nutrient digestibility, and the percentage of first estrous in *Pesisir* cattle. This research represents a novel contribution, as no previous studies have investigated this aspect.

AUTHOR'S CONTRIBUTION

Conceptualization: MZ, ZU, TT, WN, J and FA. Data curation: RP, MZ, FA, and EMP. Formal analysis: FA, RP, and EMP. Funding acquisition: MZ and FA. Methodology: FA, YM, H, and M. Project administration: MM and EMP. Supervision: MZ and ZU. Validation: MZ, FA, and RP. Writing-original draft: RP, FA, and EMP. Writing-review and editing: RP.

Ethical approval

All research procedures carried out have been approved and monitored by the Research Ethics Committee of the Faculty of Medicine, Andalas University, Padang, West Sumatra with the following numbers 297/UN.16.2/KEP-FK/2023. The conducted *in-vivo* trial also followed research ethics related to livestock as outlined in Section 66 of the Republic of Indonesia Government Law Number 18 of 2009 concerning the management, breeding, euthanasia, and appropriate treatment and welfare of animals. We followed the guidelines provided in the 'Guide for the Care and Use of Agricultural Animals in Research and Teaching' outlined by the Federation of Animal Science Societies (American Dairy Science Association, 2020).

Conflict of interest

The authors have declared no conflict of interest.

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