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Nutritional Strategies for Healthy and Productive Pregnant Pesisir Cattle through Proper Protein and Energy Ratios in Rations

Roni Pazla [1], Mardiati Zain [1]*, Fauzia Agustin [1], Yetti Marlida [1], Jaswandi [2], Masrizal [2], Hendri [2], Windu Negara [3], Totti Tjiptosumirat [3], Ezi Masdia Putri [3], Taufik Rahman [4], Renny Romaito Harahap [4] and Firdaus [4]

[1]Department of Nutrition and Feed Technology, Faculty of Animal Science, Universitas Andalas, Padang, 25163, West Sumatera, Indonesia
[2]Department of Livestock Production Technology, Faculty of Animal Science, Universitas Andalas, Padang, 25163, West Sumatera, Indonesia
[3]Research Center for Animal Husbandry, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor Cibinong 16915, Indonesia
[4]Undergraduate Program, Animal Science study program, Faculty of Animal Science, Andalas University, Padang, West Sumatra, Indonesia
***Corresponding author:** mardiati@ansci.unand.ac.id

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ABSTRACT

This research evaluated the effect of protein and energy ratio in rations on consumption, digestibility, weight gain, blood mineral content, and blood serum parameters in pregnant Pesisir cows. The treatment factors included two levels of crude protein (10 and 12%) and two levels of TDN (60 and 65%). According to the results, protein and energy ratios did not interact significantly (P>0.05) with any measured parameters. However, the energy factor significantly affected nutrient digestibility and weight gain, with 65% TDN producing the highest values in both parameters. In addition, the protein ratio significantly affected blood glucose and manganese levels, where rations with 12% protein produced higher levels than those with 10% protein. The combination of rations with 12% protein and 65% TDN showed an increasing trend in almost all parameters, although not all were statistically significantly different. These outcomes recommend that growing the protein and energy ratio supports better metabolic performance in pregnant Pesisir cows, especially in improving feed digestibility, body weight gain, and blood metabolic status. Thus, rations with 12% protein and 65% TDN can be recommended as the best option to meet the nutritional needs of pregnant Pesisir cows during pregnancy.

Key words: Pesisir cattle, Protein, TDN, Digestibility, Body weight, Metabolism.

INTRODUCTION

Pesisir cattle are one of Indonesia's local cattle that have great potential to increase the productivity of smallholder farming. They can endure the tropical climatic conditions and use the poor-quality feeds found in the coastal regions (Yetmaneli et al. 2023). However, several factors affect the productivity of the Pesisir breeds of cattle, including the pregnant ones, which are rooted in proper nutritional management. During the gestation period, the cows' nutritional requirements shoot up, more so the requirement for protein and energy, for they have to cater for the growth of the fetus and maintain the health of the cow itself (Sguizzato et al. 2020).

Ration management is often an issue regarding animal husbandry, especially in pregnant Pesisir cows. Therefore, farmers must look at the ratio between protein and energy when feeding cows, more so the pregnant ones. The correct proportion between protein and energy is necessary to achieve the highest consumption, good digestibility, proper utilization of the nutrients for weight gain, stable mineral concentration and metabolic status (Pazla et al. 2021; Pazla et al. 2024a). This research assessed the effects of varying protein and energy concentrations in pregnant pesisir cattle rations on feed intake and digestibility, weight gain, and metabolic and blood mineral content.

In conclusion, it should be highlighted that food consumption is a critical variable in the performance of

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pregnant cows. This is important because with feed intake levels, the cattle can feed well throughout the day, and in pregnant cows, there is fetal development together with the healthy state of the cow. Zago et al. (2020) found that feed intake during pregnancy depends on the quality of the rations fed, specifically on the precise balance of the protein-to-energy ratio. Such an imbalance, for instance, energy deficit, can result in low feed intake, which is undesirable for fetal well-being and development. On the other hand, a ration that contains high energy density but low protein density results in high feed output without a relative increase in output. However, feed intake or feed intake variation equally determines the level or kind of protein and energy intake in a cow. Regarding the amino acids – the proteins in the diet that form new tissues: in growing a fetal cow, energy is required for the metabolic process and—the cow's activities throughout the day. It has been estimated that protein deficiency during pregnancy compromising vitamin and nitrogen depletion in pregnant cows may negatively impact health and reproduction (Lean et al. 2012).

Feed digestibility is an essential indicator of the quality of rations, which also explains the extent to which the cows utilize the given nutrients. The digestibility of feeds is affected by the formulation of the diet, especially the protein-to-energy ratio (Pazla et al. 2024b). If the protein content of the diet is higher than required, most of the protein will be poorly digested and excreted as nitrogen (Cole et al. 2005). On the contrary, a deficiency in dietary protein will reduce overall feed digestibility because the cows lack sufficient enzymes and amino acids to effectively utilize feeds (Lee et al. 2012). In their study, Han et al. (2019) indicated that adequate dietary protein levels can enhance microbial activities in the rumen, thus increasing crude fiber digestibility. Then, the energy and protein balance must maintain this microbial activity because the energy is a carbon source for the rumen microbes responsible for fiber digestion. Thus, a proper balance of protein with energy affects not only the digestibility of proteins themselves but even the total digestibility of feed, including fiber, considered to play a vital role in maintaining digestive function in pregnant cows.

Weight gain during pregnancy in cows is a crucial measure of energy and protein balance in the cow's body. The protein-to-energy ratio in the diet significantly affects the efficiency of energy deposition in the animal's body in the form of body tissue. Feeding cows a diet with an excess of protein but a shortfall of energy results in the catabolism of body protein to meet energy requirements, leading to loss of body weight or inadequate weight gain (Williams et al. 2003). Pregnant Pesisir cows must have enough energy levels to conserve their energy reserves and assist in the enlarging fetus. Insufficient energy can lead to loss of weight and may even be associated with instances of abortion; these situations also change with having too much energy but not enough protein, where too much fat gets deposited, resulting in excess fat and disorder within metabolism (Bohmanova et al. 2022). Pazla et al. (2024a) also discovered that the proper protein-to-energy ratio led to enhanced feed utilization and proper weight increases.

The level of minerals in the blood is one of the critical aspects to consider in the nutritional management of pregnant cows. Physiological processes like tissue-bone growth and fetus development depend on minerals, including calcium, phosphorus, and magnesium (Ciosek et al., 2021). The correct protein-energy ratio in the diet can help support blood mineral levels. Disintegration between protein and energy interferences may alter mineral absorption and metabolism. For instance, hypocalcemia may be provoked by a high protein feeding regime, which may promote calcium loss through urine, thus increasing the risk of reduced serum calcium levels a few days before calving (Yogeshpriya et al. 2024). Zimpel et al. (2021) assert that propionate produced in excess energy diets interferes with the metabolism of minerals, affecting the health of the cow and the fetus.

The metabolic status describes the energy and protein equilibrium of the body of pregnant dairy cows. As the pregnancy advances, the metabolic need of the pregnant dairy cows increases. The cow's energy metabolism may be compromised in a diet containing an abnormal protein-to-energy ratio. Ketosis is a metabolic imbalance that is likely to develop in pregnant cows. It occurs in cases where the cow's energy is deficient, and cows start catabolizing body tissues, especially fat, to release these harmful budgetary compounds known as ketones (Wu et al. 2020). Poor protein intake can cause metabolic disorders like lower blood albumin and urea (Merhan and others 2022). Results: Improper protein and energy ratio fed to pregnant cows indicates increased chances of reduced metabolic status, which has been linked with reproductive health and calf production (Glamočić et al. 2024). The present study aims to examine the effects of different ratios of protein energy in the diets of pregnant pesisir cattle on feed intake, digesta kinetics, body weight changes, blood electrolytes, and the metabolic status of the animals in order to make better suggestions for the nutrition of the pregnant pesisir cattle in the future.

MATERIALS AND METHODS

Ethical approval

This study has considered the ethics of science when using animals as per the government of the Republic of Indonesia law number 41/2014 regarding Animal husbandry veterinary.

Location and time of research

The study was undertaken at the Cattle Metabolic Pen of the Faculty of Animal Science of Universitas Andalas, Padang. Metabolic cages were used to facilitate more accurate data collection related to feed consumption, fecal disposal, and body weight measurement of pregnant Pesisir cows. The research was conducted in three stages: the adaptation stage, the implementation stage, and the evaluation stage. The study spanned a specific period of 33 days, including 14 days of the adaptation stage, 14 days of the preliminary phase, and five days of the collection phase.

Research design

This study utilized a randomized group design 2x2x4 factorial arrangement with two main factors: protein ratio (Factor A) and energy in the form of Total Digestible Nutrients (TDN) (Factor B) with four groups of cows that differ in gestation age. Factor A consisted of two levels of

protein, 10% and 12%, while Factor B consisted of two levels of energy in the form of TDN, 60% and 65%. The combination of these two factors resulted in four treatments applied to groups of pregnant cows.

- Factor A (Protein Level):
  - A1: 10% Protein
  - A2: 12% Protein
- Factor B (Energy/TDN Level):
  - B1: TDN 60%
  - B2: TDN 65%
- Groups of cows based on gestation age:
  - Group 1: Pregnant cows 1-3 months
  - Group 2: Pregnant cows >3 to 5 months old
  - Group 3: Pregnant cows >5 to 7 months old
  - Group 4: Pregnant cows >7 to 9 months old

Sixteen pregnant Pesisir cows were used as research subjects, and each gestational age group consisted of four cows. Each group was treated based on a predetermined combination of protein and energy ratios.

Research period

This study consisted of three periods:

1. Adaptation Period (14 days): Cattle were fed the treatment rations for the first 14 days to familiarize them with the feed. During this period, cattle were monitored to ensure they were accustomed to the new environment and ration.
2. Introduction Period (14 days): During this period, cattle received the complete treatment ration. During this period, preliminary data on ration consumption and the physical condition of the cattle were observed.
3. Collection Period (5 days): In this period, the emphasis was on intensive data collection, which consisted of feces collection, ration intake measurement, body weight weighing and blood sampling to analyze minerals and metabolic state. Feces were collected daily, and body weights were taken at the start and the end of the collection period.

Feed and ration composition

The feed used in this study was formulated based on protein and energy content according to the treatment. The ration consisted of forage (grass) and concentrate mixed according to the level of protein (10 and 12%) and energy in the form of TDN (60 and 65%).

a. Forage: Corn stover silage was used in all treatments as the primary feed in fixed amounts.

b. Concentrates: Concentrates were adjusted to achieve the appropriate protein and TDN levels. The ingredients of the concentrates were analyzed in the laboratory to ascertain their nutrient content.

The nutrient composition of the ration and nutrients in the feed were evaluated by following the standard methods of the Association of Official Analytical Chemists (2016) in the Ruminant Nutrition Laboratory. Such analysis involves measuring dry matter, organic matter, and potentially fermentative compounds, including crude protein, fiber, fat, and energy content. The composition of the dietary components is presented in Table 1 above, and the treatment ratio is shown in Table 2 below.

Parameters observed

It is a fact that the other parameters were still explored in an attempt to estimate the impact of protein and energy balance in the diet on the performance of pregnant Pesisir cows. The parameters observed were:

Table 1: Research ration formulation

Ingredient	A1B1	A1B2	A2B1	A2B2
Corn Cob Silage	40	40	40	40
Field Grass	10	10	10	10
Corn	5	1	3	6
Rice Bran	34	14	24.5	3.5
Cassava	3	29.5	1.5	28
Coconut Meal	1	0.5	5.5	
Molasses	2.5	2.5	2.5	2.5
Palm Kernel Cake	3	1	11.5	3.5
Minerals	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Probiotics	0.5	0.5	0.5	0.5
Total	100	100	100	100

Table 2: Nutrient content of the research ration

Nutrient content (%)	Ration			
	A1B1	A1B2	A2B1	A2B2
Dry matter	57.80	58.11	57.43	57.86
Organic matter	89.49	91.94	89.71	92.13
Ash	12.21	8.32	11.17	8.24
Crude protein	10.16	10,42	12.09	12.17
Crude Fiber	23.32	21.74	24.91	21.47
Crude fat	2.88	2.95	2.97	2.76
Nitrogen free extract	51.43	56.58	48.86	55.37
TDN	60.04	65.09	59.86	65.91
NDF	61.56	59.17	60.69	58.48
ADF	41.58	40.11	40.79	40.13
Cellulose	31.03	32.05	31.14	33.23
Hemicellulose	19.98	18.06	19.91	18.35
Lignin	7.00	6.41	6.58	5.59
Silica	3.57	1.65	3.07	1.32

1. Ration Consumption: It also comprises dry matter (DM), organic matter (OM), and crude protein (CP), which is determined by the amount of the feed given and then subtracting what is left. Data on feed intake was collected on the feed consumed per group per day during the feed collection period.
2. Nutrient Digestibility: dry matter, Organic matter, and crude protein digestibility were determined by dividing the hay and grain consumed by fecal output. All the feces produced during the collection period were accumulated and tested to ascertain the digested and excreted food material.
3. Body Weight Gain (BWG): All the animals lost body weight during the collection period, and therefore, body weight at the beginning and end of the collection period was used to estimate the daily body weight gain. BWG equals the final body weight minus the initial body weight divided by the number of days of measurements.
4. Blood Minerals: Values recorded for blood mineral content were Ca, P, Mn, Zn, Se, and Cu. Blood samples were collected at the end of the study, and collecting these minerals was determined by the spectrophotometric method in the laboratory.
5. Blood Serum or Plasma (Metabolic Status): Another category of the metabolic status parameters assayed was total protein, cholesterol, and glucose. Blood samples were collected from the jugular vein in the last five minutes of the collection period. The samples collected were subjected to laboratory analysis of total proteins, cholesterol, and glucose, indicating the cattle's metabolic state.

Data collection

a. Fecal Collection: While the collection phase lasted, feces came from each cow daily. The weight of feces each cow produces was evaluated along with dry matter, organic matter and undigested crude protein to calculate the content.

b. Body Weight Measurement: At the outset and conclusion of the collection period, measurements of cow body weight were performed to determine daily body weight gain.

c. Blood Sampling: On the last day of blood collection, we withdrew blood from the jugular vein of cows. Afterward, blood samples were examined to measure total protein, cholesterol, glucose, and the content of blood minerals.

Data analysis

The data analyzed utilized the analysis of variance (ANOVA) method to identify the effect of treatment on ration consumption, nutrient digestibility, body weight gain, blood mineral content, and metabolic status. Should significant differences emerge, additional tests were performed using Duncan's Multiple Range Test (DMRT) to assess which treatment gave the superior result. All data analyses were conducted using SPSS v25.0 software (IBM Corp, New York, USA).

RESULTS & DISCUSSION

Ration consumption

The findings indicated that there was no significant change in the consumption of dry matter, organic matter, and crude protein among the different treatments concerning the interaction between Factor A - the ratio of protein and Factor B - the ratio of energy (P>0.05) (Table 3). This suggests that the combination of the two factors has no feeding effect. However, there was a marked difference in the amount of crude protein consumed among the groups, especially in the protein ratio that was supplied between the groups containing 10% crude protein (CP) and 12% CP. With the ration containing 12% CP, the protein consumption increased to 0.60 kg/head/day.

The increase in protein consumption in rations with 12% CP is due to pregnant cows' more significant body requirements for protein intake to support fetal development and maintenance of maternal body tissues. Lopes et al. (2020) claim that cows need enhanced protein intake as they prepare for labor to ensure proper fetal development in the final months of their pregnancy. The rise in demand explains why protein consumption surges as the protein percentage of the ration grows. This system affords a substantial protein supply, which acts as a beautiful nitrogen substrate for metabolism and tissue growth, thus allowing cows to meet their protein requirements while keeping their body reserves whole.

Such results are based on findings by Du et al. (2013) that raising protein levels in the diet of pregnant cows considerably increases protein consumption and nutrient use efficiency, which is essential for the growth of the fetus. Conversely, the 10% CP group's low protein intake may produce nitrogen deficiency, influencing growth and cow reproduction (Edouard et al. 2016). As a result, the protein ratio in the ration is of utmost importance for ideal reproductive health and performance in expectant cows.

Digestibility of dry matter, organic matter and crude Protein

The results revealed that variations in protein amount (Factor A) compared to energy as TDN (Factor B) did not produce any statistically significant interactions concerning the digestibility of dry matter, organic matter, and crude protein (P>0.05) (Table 4). Regardless, digestibility parameters reacted strongly to energy levels, especially in the case of the difference between 60% TDN (B1) and 65% TDN (B2). The digestibility level was most significant for that group receiving 65% TDN, as the results showed that figures under 60% TDN emerged from another group. With 65% TDN and 12% protein, the mixing illustrated, if not statistically validated, improved digestibility of total feedstuffs.

The significant effect of TDN on digestibility, especially in the 65% TDN group, reflects that increasing the energy content of the ration plays an essential role in increasing the efficiency of feed utilization by cows. TDN is a parameter that measures the energy content of feed that can be digested and utilized by livestock. As the energy content of feed increases with TDN, the rumen microbes receive more energy, which is a prerequisite for the fermentation and breakdown of feed ingredients, especially the fibers. Moreover, Zain et al. (2024) study illustrated that the energy supply parameter is among the basics that determine the proper work of rumen microbes, which stimulates the digestibility of fibers and other organic materials. Thus, the rations with a higher TDN did it this way by increasing the fermentation activity in the rumen, which was followed by higher dry matter, organic matter, and crude protein digestibility.

Table 3: In vivo consumption (kg/head/day) of pregnant Pesisir cattle rations treated with crude protein content and total digestible nutrients in the ration

In Vivo Consumption	Factor A (CP%)	Factor B (TDN%)		Average	SEM
		B1 (60%)	B2 (65%)		
Dry Matter	A1 (10%)	4.42±0.99	5.34±0.88	4.88	0.30
	A2 (12%)	4.95±1.77	5.09±0.91	5.02	
	Average	4.68	5.22		
Organic Matter	A1 (10%)	3.88±0.88	5.07±0.83	4.48	0.27
	A2 (12%)	4.45±1.59	4.84±0.86	4.65	
	Average	4.17	4.96		
Crude Protein	A1 (10%)	0.44±0.09	0.53±0.08	0.48a	0.03
	A2 (12%)	0.59±0.21	0.61±0.11	0.60b	
	Average	0.52	0.57		

Values bearing different alphabets under same paramter in the same column differ significantly (P<0.05).

Table 4: In vivo digestibility (%) of pregnant pesisir cattle rations treated with crude protein content and total digestible nutrients

In Vivo Digestibility	Factor A (CP% Ration)	Factor B (TDN% Ration)		Average	SEM
		B1 (60%)	B2 (65%)		
Dry Matter	A1 (10%)	61.11±1.50	76.43±2.98	68.77	1.32
	A2 (12%)	64.73±4.07	77.52±1.28	71.13	
	Average	62.92a	76.98b		
Organic Matter	A1 (10%)	78.61±3.93	84.12±2.51	81.36	0.97
	A2 (12%)	80.19±3.33	85.44±1.16	82.81	
	Average	79.40a	84.78b		
Crude Protein	A1 (10%)	73.16±3.75	80.57±2.46	76.86	1.02
	A2 (12%)	78.89±3.07	84.08±4.26	81.49	
	Average	76.02a	82.33b		

Values bearing different alphabets under same paramter in the same row differ significantly (P<0.05).

Table 5: Body weight gain (Kg/head/day) of pregnant Pesisir cattle rations treated with crude protein content and total digestible nutrients

Parameter	Factor A (CP % Ration)	Factor B (TDN % Ration)		Average	SEM
		B1 (60%)	B2 (65%)		
Body weightgain	A1 (10%)	0.42±0.30	0.76±0.16	0.59	0.10
	A2 (12%)	0.69±0.22	0.89±0.27	0.79	
	Average	0.56a	0.83b		

Values bearing different alphabets under same paramter in the same row differ significantly (P<0.05).

In a different study done by Wang et al. (2024), it was reported that a total dietary energy-dense diet has a better capacity in aid of intra-rumen fermentation. This is due to the observation that energy loves to enhance the effectiveness of the microbial breakdown of fibrous elements coupled with the use of proteins to repackage amino acids inside the rumen. Therefore, increased energy availability in the 65% TDN group allowed cows to utilize feed more efficiently, thereby increasing the digestibility values of dry matter and organic matter.

Although there was no significant interaction among protein and energy factors on crude protein digestibility, the group with 12% protein showed a trend towards increased protein digestibility, especially when combined with 65% TDN. Those above may be reasoned by the diet incorporating protein with a more significant nitrogen fraction, allowing the rumen microbes to utilize nitrogen more efficiently to enhance their microbial population. Protein nitrogen is essential in the growth and reproduction of rumen microbes, which helps in fiber digestion among other substrates (Zain et al. 2023).

As reported by Wei et al. (2022), the incorporation of higher protein levels in the dietary regimes of cattle translates into improved nitrogen usage efficiency by the rumen microbes; this is especially true where energy in the form of TDN is available in sufficient quantities. The findings of this study support, to a certain extent, those of Wei et al. (2022); in the 12% protein and 65% TDN groups, digestibility was generally higher, although the difference was not significant statistically. However, it is observed that this increased digestibility is not in use due to the feed protein sources alone but due to the energy efficiency, which is also available and allows for microbial digestion of the available protein.

The findings of this research are consistent with those of Pazla et al. (2024a), who demonstrated that as the energy level of cattle feeds increased, the digestibility of organic matter and crude protein improved. Higher energy content allows rumen microbes to utilize protein more efficiently, increasing feed digestibility. On the other hand, this study also supports the views of Mustafa et al. (2017), who stated that a balanced combination of protein and energy ratios can improve digestibility and feed utilization efficiency in pregnant cows.

However, some other studies found slightly different results. For example, Yu et al. (2024) reported that increasing protein above the optimal requirement only sometimes increases crude protein digestibility, especially if the feed has insufficient energy. This suggests that an increase in protein should always be accompanied by an increase in energy to achieve a synergistic effect on digestibility.

Although there was no significant interaction between protein and energy ratios in this study, the combination of 12% protein with 65% TDN showed an increasing trend in digestibility. This suggests that cows may be better able to utilize protein when energy in the form of TDN is available in sufficient amounts. This combination supports more effective fermentation and breakdown of feed in the rumen, especially in pregnant cows requiring greater nutrient intake to support fetal growth and maintain body condition.

Body weight gain

The outcomes showed that there was no significant interaction (P>0.05) between protein ratio (Factor A) and TDN (Factor B) on body weight gain of pregnant Pesisir cows (Table 5). However, the energy factor (TDN) had a significant effect (P<0.05) on body weight gain. The group receiving 65% TDN (B2) showed higher weight gain than the 60% TDN group (B1). Although not statistically significant (P>0.05), the combination of 12% protein with 65% TDN showed a tendency to increase body weight gain, indicating that higher energy tends to support the achievement of more optimal weight gain.

The energy content in terms of TDN significantly influences the weight gain of food animals, particularly cattle. Primarily, TDN indicates the amount of energy contained in the feed that the cow can assimilate for her various needs, including but not limited to maintaining the body and growth and development of the calf. Energy availability is particularly crucial for pregnant cows as the energy needed increases during gestation. When enough energy is supplied from feed, the cows will not only grow

fetal cows but also take care of their body, enabling them to gain weight appropriately.

As reported by Zhu et al. (2023), it has been observed that beef cattle fed on energy-dense diets were able to partition their nutrients more effectively for tissue deposition, resulting in enhanced weight gain. This was in line with the present observation, in which the group with 65% TDN recorded a higher gain in body weight than the group with a TDN of 60%. The provision of appropriate energy in the form of 65% TDN makes sure that there is enough energy in the cows to allow for growth of both body tissues and fetal tissues with no use of any body fat energy reserves, and this is vital in preserving a good body condition during pregnancy.

Despite no significant interaction between energy and protein on overall weight gain, a provision of 12% protein and 65% TDN appeared to promote weight gain. This can be attributed to adequate protein nutrition for tissue development, such as fetal tissues, and positive nitrogen balance in the cow's body.

Protein is a crucial element within the diet, enhancing muscle as well as body tissues. The work of Lopes et al. (2020) is focused on the effect of two types of diets on the weight gain of pregnant cows, which are distinguished by the high and low protein contents in the diets. Protein serves to build up new tissues and, along with that, for growth enhancement in the fetus. Protein levels increase the body condition score in cows more effectively, enabling tissues to be deposited on the body and the fetus more efficiently.

Nonetheless, protein is essential for proper growth; however, the maximum weight increase would still depend on balancing the available protein with the energy put into the system. In the case of an energy-deficient diet, cows will start using protein for energy purposes, thus causing inefficiency in using protein for weight gain purposes. Research by Sguizzato et al. (2020) showed that increasing protein in the ration must be accompanied by sufficient energy availability to be used efficiently by the cow's body. Therefore, although the combination of 12% protein with 65% TDN showed an increase in body weight gain, this result was not statistically significant, which may indicate that cows require a more appropriate balance between protein and energy to achieve maximum results.

This research supports the results obtained by Pazla et al. (2024a) that suggested improvement in cattle feed utilization efficiency with better weight gain achieved by increasing the energy content of the feed. In this trial, it was reported that the 65% TDN group had a higher body weight gain than the 60% TDN group, which further substantiates that a ration with more energy supports better utilization of nutrients; it is more so in pregnant cows that need more energy for the growing fetus.

Research by VandeHaar et al. (1999) and Kang et al. (2022) also showed that cows fed rations with higher energy content showed better body weight gain because adequate energy allows cows to use the protein more efficiently for body and fetal tissue growth. In this study, the combination of 12% protein and 65% TDN also showed a tendency to increase body weight gain, although not statistically significant, indicating the importance of the balance between protein and energy to achieve optimal results.

Blood mineral content

This study showed that different ratios of protein (Factor A) and TDN (Factor B) did not produce significant interactions ($P>0.05$) on blood mineral content, namely calcium, manganese, phosphorus, zinc, selenium, and copper. However, the significant difference ($P<0.05$) observed between the fed groups at 10 and 12% protein ratios showed that the former had higher manganese (Table 6). The increase in the blood manganese content of the group fed 12% protein underlines the critical role of protein in the metabolism and absorption of some minerals.

Manganese (Mn) is a crucial trace element required for several bodily functions, such as bone development, reproductive health, and energy and nutrient metabolism (Erikson and Aschner 2019; Pazla et al. 2020). The functional role of manganese is also as a cofactor for certain enzymes involved in the processes of polypeptide biosynthesis, glycometabolism, and bone hypertrophy (Freeland-Graves et al. 2014). The increase in manganese levels in the group given rations with 12% protein can be explained by the body's increased need for manganese to support protein synthesis and optimal enzymatic function.

According to research by Johnson and Korynta (1990), higher protein intake can increase mineral absorption, including manganese. This is because of the heightened activity of manganese-dependent enzymes in the body, especially in pregnant cows, which require the nutrients for fetal development. In this study, when the protein level was raised to 12%, it enhanced the enzymatic activity and manganese absorption compared to the 10% protein level. Furthermore, in line with Ivan and Veira (1981), manganese that is included in the diet amino acids have a higher bioavailability because high protein diets increase the solubility of manganese in the gut. Therefore, more manganese can be absorbed and used by the body.

Furthermore, this rise in blood manganese levels is noteworthy because it is known that the demand for manganese by pregnant cows is high for fetal bone and tissue development. Bone structures, reproduction, and the unborn child may be affected due to deficiencies in Manganese minerals (Carvalho et al. 2010). Thus, the increase in manganese levels in the group with 12% protein indicates that higher protein rations can support the needs of this mineral during gestation.

The results showed no significant differences ($P>0.05$) in the content of other minerals, such as calcium, phosphorus, zinc, selenium, and copper, due to protein ratio differences or TDN. Such could happen as these minerals may have been provided in the diets in adequate quantities; hence, an increase in the protein or energy ratio did not significantly improve the levels of these minerals in the blood of the cows.

These results align with previous research by Sathler et al. (2017), which showed that protein intake can affect the absorption and metabolism of certain minerals, especially manganese, but does not necessarily significantly impact other minerals if their basic needs are met. Moreover, Sathler et al. (2017) also demonstrated that small protein or energy ratio adjustments do not entail a risk in calcium and phosphorus levels if such ratios are already sufficient to provide these minerals.

Table 6: Blood mineral content of pregnant Pesisir treatments

Parameter	Factor A (CP%)	Factor B (TDN%)		Average	SEM
		B1 (60%)	B2 (65%)		
Calcium (mg/dL)	A1 (10%)	0.15±0.01	0.14±0.01	0.15	0.01
	A2 (12%)	0.17±0.02	0.15±0.01	0.16	
	Average	0.16	0.14		
Copper (mg/dL)	A1 (10%)	0.11±0.01	0.11±0.002	0.11	0.005
	A2 (12%)	0.12±0.01	0.10±0.003	0.11	
	Average	0.12	0.11		
Mangan (mg/dL)	A1 (10%)	0.96±0.02	0.95±0.02	0.96a	0.01
	A2 (12%)	1.06±0.04	1.09±0.02	1.08b	
	Average	1.01	1.02		
Phosphorus (mg/dL)	A1 (10%)	1.62±0.29	1.38±0.05	1.50	0.14
	A2 (12%)	1.57±0.29	1.69±0.37	1.64	
	Average	1.59	1.54		
Selenium (mg/dL)	A1 (10%)	0.03±0.006	0.02±0.005	0.03	0.003
	A2 (12%)	0.03±0.005	0.02±0.006	0.03	
	Average	0.03	0.02		
Zink (mg/dL)	A1 (10%)	0.11±0.01	0.11±0.01	0.11	0.004
	A2 (12%)	0.12±0.01	0.10±0.005	0.11	
	Average	0.11	0.11		

Values bearing different alphabets under same paramter in the same column differ significantly (P<0.05).

The finding that clay adjusted in higher protein rations contains more manganese correlates with the studies conducted by Spears (2003), who noted that pregnant cows requiring excessive protein had observed increased uptake of manganese needed for reproduction and growth of the fetus.

Serum/Blood plasma content: total protein, cholesterol, and glucose

This study shows that distinctions in the ratio of protein (Factor A) and TDN (Factor B) do not produce significant interactions (P>0.05) on the total protein, cholesterol, and glucose content in the blood (Table 7). Nonetheless, referring to the parameter of glucose, differences in protein levels (Factor A) are profound enough to have some influence, whereby a group provided with a diet containing 12% protein fraction (A2) exhibited much-elevated glucose levels when compared to another group which was provided with 10% protein (A1) diet. This seems to show that protein consumption has an influence on the glucose metabolism of pregnant coastal cows, where the total protein and cholesterol means did not vary significantly between treatments.

Glucose is the primary energy source for cows, especially during pregnancy, when the energy needs for fetal development increase significantly. The glucose levels in the group with a protein ratio of 12% increased, suggesting that higher protein intake enhances the energy metabolism essential in sustaining pregnancy.

Physiologically, increased protein intake can affect glucose metabolism through several mechanisms. First, higher protein in the diet can increase the production of insulin, a hormone that plays a role in regulating blood glucose levels. Research by Sletmoen-Olson et al. (2000) showed that cows with higher protein intake tend to have a better insulin response, which can facilitate glucose uptake by body tissues. This may explain why the 12% protein group showed higher glucose levels. Protein in the diet serves as a substrate for tissue synthesis and affects metabolic hormones such as insulin, which affect glucose regulation.

In addition, this increase in glucose levels may also be related to increased rumen microbial activity in breaking down feed with higher protein content, which can produce more amino acids for energy metabolism. A physiological process termed gluconeogenesis, which is present in cattle, is capable of producing glucose from non-carbohydrate sources such as amino acids when converting deaminated amino acids into glucose. Qin et al. (2022) revealed that ruminant cattle depend on gluconeogenesis synthesis to prevent blood glucose levels from dropping too low due to energy expansion, for example, in pregnant cows.

Total protein in blood

The outcomes of this research presented that different protein and energy ratios did not significantly affect the total protein in the blood. This may be because rations with 10 and 12% protein content still meet the minimum protein requirements for pregnant coastal cows, so there was no significant difference in total blood protein among the two treatments.

The total quantity of protein in the bloodstream portrays the protein status of the cow's body, including albumin and globulin, which have functions in transporting nutrients, defense mechanisms, and regulating fluid balance. Pregnant cows need to increase their protein ingestion, according to Lopes et al. (2020), particularly during the last third of gestation, since it ensures proper fetus growth and maintenance of the mother's body condition. However, if the essential protein intake is sufficient, further increases in the protein ratio only sometimes lead to an increase in total blood protein. This may explain why differences in protein ratios in this study did not result in significant differences in total blood protein.

Blood cholesterol

Cholesterol is essential in fat metabolism, especially for synthesizing steroid hormones, such as the reproductive

Table 7: Serum or plasma content of pregnant Pesisir cattle given a crude protein control ration and different total digestible nutrients in concentrate

Parameter	Factor A (CP%)	Factor B (TDN%)		Average	SEM
		B1 (60%)	B2 (65%)		
Total Protein (g/dL)	A1 (10%)	7.34±0.35	7.21±0.54	7.28	0.26
	A2 (12%)	8.02±0.49	7.32±0.56	7.67	
	Average	7.68	7.27		
Cholesterol (mg/dL)	A1 (10%)	170.70±38.45	130.57±37.14	150.64	18.28
	A2 (12%)	157.60±33.10	155.15±34.28	156.37	
	Average	164.15	142.86		
Glucose (mg/dL)	A1 (10%)	53.67±13.22	44.00±4.77	48.84a	4.95
	A2 (12%)	60.75±13.24	66.87±0.79	63.81b	
	Average	57.21	55.44		

Values bearing different alphabets under same paramter in the same column differ significantly (P<0.05).

ones, critical during pregnancy. This study did not observe any significant blood cholesterol level changes between treatment groups subjected to varying protein and TDN ratios. This implies that the level of protein and energy in the rations provided is adequate to keep the lipid metabolism balanced; thus, the cholesterol levels do not differ markedly.

Cholesterol is also influenced by fat intake and fat metabolism activity in the body. According to research by Humer et al. (2019), cholesterol levels in cows tend to be stable if energy and fat intake are sufficient, so increasing protein intake does not directly affect cholesterol levels unless there is an energy deficiency or imbalance in lipid metabolism. In this case, rations with TDN content of 60 and 65% seem sufficient to support fat metabolism and maintain cholesterol levels within normal limits.

The results of this study confirm the prior research of Chelikani et al. (2009), in which it was mentioned that increased protein intake in cattle raises glucose levels mainly because of elevated energy metabolism and applicable hormones. Likewise, Nichols et al. (2019) showed that higher protein intake might influence glucose levels in cattle. However, the impact might vary depending on the animal's health status and the dietary proportions of nutrients.

However, Hudaya et al. (2021) conducted a study that indicated that surpassing the optimal protein levels in the diet does not necessarily translate into elevated total or serum cholesterol levels in the blood, especially after fulfilling the primary protein requirements. The findings in this study agree with this, as a higher protein ratio did not cause significant variation in total protein and cholesterol levels in the blood.

Conclusion

Variations in the levels of protein and energy provided for the pregnant coastal cows did not result in marked interactions on feed intake, digestibility, weight gain, blood mineral levels, and total blood serum conditions. In contrast, among the energy measures incorporated as TDN, the digestibility of dry matter and weight gain were significantly improved, with the highest TDN being 65%. In addition, the protein factor significantly affected blood glucose and manganese levels, with 12% protein increasing both parameters. The best treatment to support metabolic performance and growth in pregnant pesisir cattle is a combination of 12% protein and 65% TDN.

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