Reformulation of dairy cow diets based on rumen degradable protein and total digestible nutrient with varying levels on \textit{in vitro} fermentability and digestibility

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Abstract. Rumen degradable protein (RDP) needs to be balanced with the adequacy of rumen undegradable protein (RUP) and energy for optimal microbial growth. Therefore, this study aimed to determine the optimal level of the RDP:RUP ratio and the energy level of dairy cattle rations using the \textit{in vitro} method. The rumen inoculum used to carry out this research, was obtained from two bull rumen fistulated of Friesian Holstein. The treatments consisted of 3 levels of RDP:RUP ratio, namely 50:50, 55:45 and 60:40, while the energy levels consisted of total digestible nutrient (TDN) levels of 65.6% and 68.6%. The experimental design was a factorial randomized block, while data were analyzed using ANOVA and Duncan multi range’s test. The result showed the ratio of RDP:RUP had an effect on DMD, OMD, NH$_3$, and rumen microbe. Furthermore, the higher TDN content increased significantly DMD, OMD, total VFA, and partial VFA. The increase in the microbial population was associated with a rise in total VFA and NH$_3$ concentrations. This research concluded, the rations with RDP:RUP (60:40) ratio increased the population of bacteria and protozoa, while the availability of ammonia in the rumen, and the high level of TDN provided a higher supply of VFA, DMD, and OMD.

Keywords: rumen degradable protein, rumen fermentation, digestibility, in vitro

1. Introduction
The increased demand for domestic milk production in Indonesia has led to the insufficiency of feed supply and nutrient requirements in dairy cows. This demand greatly affected productivity, both in terms of quality and quantity. Dairy cow productivity in the country amounted to relatively 13.5 liter/head/day which are equivalent to 4100 liter/head/year [1]. According to BPS, 560,061 heads of cattle population, produce 996,442 tons of dairy milk, which is relatively 16.5 liter/capita/year (4,405,500 tons). This data further reported that 22.6% and 77.4% of dairy milk were locally supplied and imported, respectively. According to the Center for Agricultural Data and Information System domestic fresh milk only contributes 20% in meeting Indonesia’s diary needs, while those imported constitutes 80%. The increase in milk production must be realized by providing high feed quality in order to meet the availability of milk.

Feed is extremely relevant in livestock development. However, when its availability fluctuates, it results in varying qualities thereby affecting production. Feed contains widely nutritional elements depending on species, types, and conditions of the ingredients in terms of texture and structure. Its
nutritional elements are generally composed of water, minerals, protein, fat, carbohydrates and vitamins. The administration of protein sources such as concentrates and legumes used for feed ingredients is based on the National Research Council (NRC) standard \[2\]. This standard was adopted by Indonesia’s cooperate firms for the manufacture rations offered to farmers which yielded less optimum result since Indonesia is a tropical country that needs accurate nutrient requirements. Therefore, feed formulation based on RDP and RUP is needed to enhance the productivity of dairy cows.

In Indonesia, it is solely dependent on total digestible nutrient (TDN), crude protein, fat, and fiber including minerals. However, the formulation adopted in this study is based on the RDP and RUP proportion in accordance with the feed database on the online website. Fulfilling the needs of dairy cows triggers protein efficiency and saves costs.

The importance of RDP and RUP needs to be considered. The recommended proportion is 60:40 \[2\]. Dairy cows given RDP below the expected requirement, undergoes a depression in milk productivity and quality \[3\]. Bahrami-yekdagi et al. \[4\] reported that the utilization of N-protein in early lactating dairy cows is increased by giving them 9.3% RDP (%DM) to produce milk and its excretion in urine is minimized with adequate RUP.

Rumen energy supply is an important factor for microbial protein synthesis \[5\]. According to Anggraeny et al. \[6\], high digestibility ration indicates the effective supply of rumen degradable protein (RDP) and energy, in the form of readily available carbohydrate (RAC) which is sufficient for microbial growth. The rations’ high protein content depicts the amount of N-protein available for degradation and digestion. This increases digestibility, improves growth and supplies enormous amino acids \[7\]. Imran et al. \[8\] stated that highly digestible and absorbed protein in post-rumen organs increases milk production in dairy cows.

This study aimed to determine the characteristics of feed protein with different TDN levels and varying proportion balances of RDP and RUP in accordance with local ingredients. It also evaluates the reformulation of dairy cow ration on RDP and RUP using in vitro method.

2. Material and methods

2.1. Rumen Degradable Protein (RDP) and Rumen Undegradable Protein (RUP) analysis
This study was carried out from December 2020 to February 2021 at the Laboratory of Dairy Nutrition, Faculty of Animal Science, IPB University. The observed research variables include fermentation characteristics (pH, ammonia concentration (NH3), partial, and total VFA production, and microbe’s activity) and nutrient digestibility (dry and organic matter). The RUP and RDP content in animal feed was analyzed using a literature study method. The Indonesian local feed protein content was compared with existing databases, including the similarities in percentages. The database was obtained through online websites such as https://www.feedtables.com/ and https://www.feedipedia.org/. The rations used were a combination of forage and concentrate at a ratio of 40:60 in accordance with 2 levels of concentrate containing low (65.6%) and high (68.6%) TDN. However, that feed to the Dairy cattle were formulated according to the adequacy and balance ratio of the rumen undegradable protein (RUP) and rumen degradable protein (RDP). These were prepared according to the NRC \[2\]. The feed composition and nutrient content of the research ration are shown in Table 1.

2.2. In vitro analysis
The feed used comprises of forage and concentrate at a ratio of 40:60, in accordance with 2 levels of concentrate containing low (65.6%) and high (68.6%) TDN. The concentration of ammonia, partial, and total VFA production, dry and organic matter digestibility, and rumen microbe’s activities were analyzed.

In vitro analysis was carried out by adopting Tilley and Terry’s \[9\] method. The rumen fluid was drawn from 2 fistulated dairy cows, based on standard laboratory procedures. Furthermore, 0.5 gr of the ration and 40 ML of McDougall solution was poured into fermenter tubes. 10 mL of rumen fluid was added followed by CO2 (anaerobic condition). Fermenter tubes were incubated in the shaker water bath
for 48 h at 39°C. The pH, ammonia (NH3), partial and total VFA concentration, and rumen microbe activities were observed within 4 h after the incubation. Nutrient digestibility (DMD and OMD) measurements were carried out by incubating the fermenter tubes filled with the ration at 39°C for 48 h in the shaker water bath.

### Table 1. Feed composition and nutrient content of the dairy cow ration.

| Ingredients            | P1   | P2   | R1   | R2   | R3   | R1   | R2   | R3   |
|------------------------|------|------|------|------|------|------|------|------|
| Elephant grass         | 40.0 | 40.0 | 40.0 | 40.0 | 40.0 | 40.0 |      |      |
| Corn                   | 7.0  | 5.0  | 3.0  | 20.5 | 11.1 | 11.1 |      |      |
| Rice bran              | 23.0 | 17.2 | 9.9  | 12.3 | 3.4  | 0.9  |      |      |
| Cassava waste          | 5.4  | 9.0  | 13.4 | 5.0  | 16.8 | 20.0 |      |      |
| Wheat pollard          | 7.0  | 3.0  | 2.0  | 2.0  | 2.0  | 2.0  |      |      |
| Coconut meal           | 3.0  | 3.0  | 1.0  | 3.0  | 3.0  | 1.0  |      |      |
| Palm kernel meal       | 6.8  | 15.0 | 22.4 | 7.5  | 12.5 | 14.7 |      |      |
| Soybean meal           | 5.4  | 3.4  | 1.0  | 6.6  | 5.0  | 2.0  |      |      |
| Corn gluten meal       | 0.0  | 2.1  | 1.9  | 2.2  | 1.4  | 1.2  |      |      |
| CaCO3                  | 2.2  | 2.1  | 1.9  | 2.2  | 1.4  | 1.2  |      |      |
| DCP                    | 0.2  | 0.3  | 0.6  | 0.4  | 1.2  | 1.5  |      |      |

**Chemical Composition (%)**

| Ingredient   | P1   | P2   | R1   | R2   | R3   | R1   | R2   | R3   |
|--------------|------|------|------|------|------|------|------|------|
| Dry mater    | 60.9 | 61.3 | 61.4 | 60.7 | 61.0 | 61.0 |      |      |
| Crude protein| 14.08| 14.08| 14.08| 14.08| 14.08| 14.08|      |      |
| RDP          | 8.45 | 7.74 | 7.04 | 8.45 | 7.7  | 7.0  |      |      |
| RUP          | 5.6  | 6.3  | 7.0  | 5.6  | 6.3  | 7.0  |      |      |
| TDN          | 65.6 | 65.6 | 65.6 | 68.6 | 68.6 | 68.6 |      |      |
| Ca           | 1.2  | 1.2  | 1.2  | 1.2  | 1.2  | 1.2  |      |      |
| P            | 0.7  | 0.7  | 0.6  | 0.6  | 0.6  | 0.6  |      |      |

**2.3. Collection and measurement samples**

The observed rumen fermentation characteristics include ammonia (NH3) concentration determined with the Conway micro diffusion method [10], pH (analyzed by pH meter), total, and partial VFA analyzed using steam distillation and gas chromatography respectively. Nutrient digestibility consisting of dry and organic matter, were measured after 48 h of incubation. In addition, 2 drops of HgCl2 were added to the substrate after 48 h incubation period, to stop microbe’s activity, and centrifuged at 3000 rpm in 15 minutes to separate the supernatant from the solid component. In addition, the supernatants were eliminating and 50 mL of pepsin solution added to the solid component in each tube. The solutions were incubated in the shaker water bath at 39°C for 48 h after which pepsin-HCl was added to the tubes. Then, the supernatants were eliminated and the solids were washed with hot water and filtered using a vacuum pump and Whatman filter paper. For dry matter measurements, the substrate was placed in the porcelain cup and dried in an oven (at 105°C for 24 h). Conversely, they were incinerated in the furnace for 6 h (600°C) to obtain the organic matter measurements.

**2.4. Microbe’s activity calculation**

The total protozoa population was measured using the Ogimoto and Imai [11] method. Furthermore, 1 ml of rumen fluid was mixed with 1 ml of Trypan Blue Formal Saline (TBFS) solution, which was dropped into the counting chamber and observed under a microscope. The bacterial population was determined using the living bacterial colony counting method [11]. The calculation is based on the principle that the rumen fluid is diluted serially and further cultured in a
Hungate tube after which it was added to the glycerol medium for 4 h in vitro incubation. It was also calculated using the Brain Heart Infusion (BHI), a type of growth medium.

2.5. Experimental design
The experimental diets of in vitro using Factorial Randomized Block Design with 2x3 treatments and 4 replications, factors A is RDP levels with symbol (R), as follows R1 = RDP 60%, R2 = RDP 55% and R3 = 50%. Factors B is TDN levels with symbol (P), as follows P1 = TDN level 65.6 % and P2 = TDN 68.6%.

2.6. Data analysis
The data were analyzed using ANOVA and the significantly different treatments were determined with the Duncan multiple range test in accordance with the SAS University edition. The differences were considered significant at \( p < 0.01 \) and \( p < 0.05 \).

3. Results and discussion

3.1. RDP and TDN levels on in vitro fermentation
Reformulated dairy cow ration based on RDP and TDN level had an effect \( (p <0.05) \) on total protozoa, and rumen bacteria, including ammonia (NH3) concentration. However, pH value and volatile fatty acid (VFA) concentration were not affected by the treatments. The effects of RDP and TDN level on ruminal fermentation are shown in Table 2.

The in vitro fermentation process tends to be optimal supposing the rumen fluid and microbial environment has a suitable pH. The ration given to dairy cows needs to provide adequate proportion of nitrogen for the rumen microbes (RDP), as well as the undegradable protein (RUP) as a direct source for the animal. In this study, the rumen pH ranges from 6.78 to 6.85 which proves that under normal conditions, these values support fermentation activities [12].

The optimal balance ratio of RDP:RUP are needed to efficiently optimize the dairy cow’s production. Putri et al. [13] reported that ration with higher RUP resulted in the depression of microbial protein production, lowered the VFA production, and decreased in the ability of ruminal microbes to perform fermentation. Reformulated RDP:RUP ratio at different TDN level causes a \( (p <0.05) \) decrease in the total bacteria population (from 10.82 to 10.31 log cell ml\(^{-1}\)). This lowered the RDP ratio added to the ration, according to McDonald et al. [12], under normal population, it ranges between 9 and10 log CFU ml\(^{-1}\). This condition predicts that degradable protein level in R1 has higher RDP ratio which fulfils the requirements of the host animals [13].

The protozoa population ranges from 6.53 to 6.68 log cell ml\(^{-1}\) as observed in Table 2. The average value was within the normal range proposed by McDonald et al. [12], i.e. 5 to 6 log cells ml\(^{-1}\). The normal value of total protozoa was 10\(^3\) ml\(^{-1}\) within the high crude fiber feed and this tends to increase to approximately 10\(^5\) per ml\(^{-1}\) in the rumen which has adapted to a source that contained a lot of dissolved sugars (16%). Protozoa comprises of approximately 50% of the microbial mass and contributes less (11%) compared to those that enter the duodenum, due to their mobility and ability to resist the rumen wall [14].

| Item                      | RDP          | TDN          | Average ± SD |
|---------------------------|--------------|--------------|--------------|
| pH                        | R1           | 6.78         | 6.84         | 6.81 ± 0.08 |
|                           | R2           | 6.79         | 6.81         | 6.80 ± 0.09 |
|                           | R3           | 6.83         | 6.85         | 6.84 ± 0.08 |
|                           | Average ± SD | 6.80 ± 0.06  | 6.83 ± 0.10  |
| Protozoa (log cfu ml\(^{-1}\)) | R1           | 6.68         | 6.65         | 6.67 ± 0.06a |
|                           | R2           | 6.65         | 6.59         | 6.62 ± 0.07b |
|                           | R3           | 6.53         | 6.57         | 6.55 ± 0.06c |
|                           | Average ± SD | 6.62 ± 0.10  | 6.61 ± 0.05  |
al factors in the rumen depends on 3 catabolic processes, namely proteolysis, peptidolysis, and deamination. Enzymes found in the bacteria include endo and exo-proteases that leads to a rise in ammonia concentration. Feed protein in the rumen of ruminants tends to be partly broken down by microbial proteolytic enzymes and converted to ammonia [1]. This affects the ammonia concentrations are shown in Table 2. The degradation of protein in the rumen depends on 3 catabolic processes, namely proteolysis, peptidolysis, and deamination. Enzymes found in the bacteria include endo and exo-peptidase which binds to the cell surface thereby allowing interaction with the substrate. However, several factors lead to metabolism in microbes which results in proteolytic activities [17]. This affects the ammonia concentration. Feed protein in the rumen of ruminants tends to be partly broken down by microbial proteolytic enzymes and converted to ammonia [17].

[18] reported that increase in dry matter digestibility is in line with microbial activity in the rumen and this triggers the RDP level. This does not only increase the concentration of ammonia however, it also helps the proliferation of ruminal bacteria population [19]. An increase in the RDP level in the feed, leads to a rise in NH3 concentration and high blood urea nitrogen in accordance with amino acids and might be used for energy [20].

VFA is a source of energy and carbon derived from livestock and microbial protein synthesis [21]. It is converted from biodegraded fat to fatty acids and glycerol [22]. The average value of total VFA concentration with RDP level was within the range of 100.24 to 127.37 mM. [23] reported that it ranges from 80 to 160 mM, which is regarded as the normal value in ruminants. Pilachai et al. [24] stated that the rumen fermentation was increased after the feeding of RDP proportion. However, this affects the VFA, ammonia (NH3) and histamine concentration.

3.2. RDP and TDN levels on Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD)

Dry matter digestibility describes the feed protein, carbohydrate, fat, and mineral compounds that tends to be digested by livestocks. Organic matter digestibility is based on the ability to digest such substances

| Bacteria total (log cfu ml⁻¹) | R1  | 10.82 | 10.88 | 10.85 ± 0.58a |
|-------------------------------|-----|-------|-------|---------------|
| R2                            | 10.48 | 10.52 | 10.50 ± 0.11ab |
| R3                            | 10.24 | 10.31 | 10.28 ± 0.16b |
| Average ± SD                  | 10.51 ± 0.41 | 10.57 ± 0.43 |

| NH3 (mM)                      | R1  | 13.95 | 13.94 | 13.94 ± 1.66a |
|-------------------------------|-----|-------|-------|---------------|
| R2                            | 11.44 | 12.23 | 11.84 ± 1.95b |
| R3                            | 9.89  | 9.91  | 9.90 ± 2.33c  |
| Average ± SD                  | 11.76 ± 2.87 | 12.03 ± 2.31 |

| VFA (mM)                      | R1  | 108.91 | 123.37 | 116.14 ± 11.76 |
|-------------------------------|-----|-------|-------|---------------|
| R2                            | 102.48 | 113.77 | 108.13 ± 11.10 |
| R3                            | 100.24 | 117.63 | 108.93 ± 11.47 |
| Average ± SD                  | 103.88 ± 8.37b | 118.25 ± 9.78a |

NH3= ammonia; VFA= volatile fatty acid; P1R1= RDP: RUP (60: 40) with TDN level 65.6%. P1R2= RDP: RUP (55: 45) with TDN level 65.6%. P1R3= RDP: RUP (50: 50) with TDN level 65.6%. P2R1= RDP: RUP (60: 40) with TDN level 68.6%. P2R2= RDP: RUP (55: 45) with TDN level 68.6%. P2R3= RDP: RUP (50: 50) with TDN level 68.6%.

a, b, c Different superscripts in the same row with various letters show significant differences (p <0.05).
in food ingredients except minerals. The DMD value ranges from 60.92% to 67.09% while the OMD is from 61.81% to 65.93%. However, both DMD and OMD are shown in Table 3.

Energy supply in the rumen is an important factor in the utilization of N-protein for microbial protein synthesis [5]. According to [6], high digestibility of dry matter indicates the provision of rumen degradable protein (RDP) and energy in the form of readily available carbohydrate (RAC) which is sufficient for rumen microbial growth. The high protein content of the ration determines the amount of N-protein available for degradation and digestion, thereby increases digestibility, supplies high amino acid and improves growth [7].

Microorganisms in the rumen aid in the digestion process in ruminant feed. Its growth is influenced by the availability of protein and energy in the feed. Meanwhile, protein and energy deficiency in livestock inhibits the microorganisms’ activities and reduces feed digestibility [25]. Dry matter digestibility was affected by the protein and energy content in ration. Therefore, an increase in the protein level is not followed by the enhancement of dry matter digestibility [26].

[27] reported that an increase in dietary RUP had no effect on DMD and DMO. However, the rumen ecosystem activity is unable to be obtained, assuming the optimal ratio between RDP and RUP are imbalanced.

### Table 3. Effect of RDP level on in vitro.

| Item  | RDP  | P1  | P2  | Average ± SD |
|-------|------|-----|-----|--------------|
| DMD   | R1   | 60.92 | 64.84 | 62.88 ± 2.92 |
|       | R2   | 60.68 | 66.35 | 63.52 ± 3.91 |
|       | R3   | 59.40 | 67.09 | 63.24 ± 4.44 |
|       | Average ± SD | 60.33 ± 2.39 | 66.09 ± 2.01 | |
| OMD   | R1   | 61.81 | 63.13 | 62.47 ± 2.21 |
|       | R2   | 58.37 | 65.35 | 61.86 ± 4.40 |
|       | R3   | 58.46 | 65.35 | 62.19 ± 4.36 |
|       | Average ± SD | 59.55 ± 2.78 | 64.80 ± 2.29 |

DMD = dry matter digestibility; OMD = organic matter digestibility. P1R1= RDP: RUP (60: 40) with TDN level 65.6%, P1R2= RDP: RUP (55: 45) with TDN level 65.6%, P1R3= RDP: RUP (50: 50) with TDN level 65.6%, P2R1= RDP: RUP (60: 40) with TDN level 68.6%, P2R2= RDP: RUP (55: 45) with TDN level 68.6%, P2R3= RDP: RUP (50: 50) with TDN level 68.6%.

### 3.3. RDP and TDN levels on partial VFA

The results of the dairy cow ration based on RDP and TDN level had an effect (p < 0.05) on propionic (C3) and n butyric (nC4). However, acetic (C2), iso butyric (iC4), iso valeric (iC5) and n valeric acids (nC5) are not affected by the treatments. The effects of RDP and TDN level on partial VFA are shown in Table 4.

The 3 volatile fatty acids (VFA) majors produced are acetic, propionic and butyric acids. The various VFA ratios depends on the feed types digested in the rumen [28]. Acetate is an end product of the fiber fermentation in the rumen. The result showed that the RDP:RUP ratio had no effect on acetic acid concentration. Conversely, the TDN level had an effect on the concentration of acetic acid. It was predicted that the ration is highly fibrous although low in energy that led to microbial populations which produces high ratio of acetate to propionate [28]. The result also showed that P1 had a higher concentration of acetic acid than P2. It is reflected that P1 had a higher percentage of feed in crude fiber.

The RDP:RUP ratio at different TDN level showed a significant effect on propionic acid. This was caused by the fermentation of starch and sugars in the rumen due to the high TDN level given to ruminant. This result also predicted that the ration had a different TDN level with both high and low percentage which affects the propionate proportion. Moran [28] reported that feed high in rapidly fermentable carbohydrates enhances bacteria populations thereby producing increased propionate and butyrate compared to acetate. Propionate is considered a more efficient energy source due to its fermentation. Total digestible nutrient (TDN) known as an energy source for ruminants tend to enhance propionic acid concentrations.
Propionic, acetic and butyric acids play a role in rumen fermentation. In glucogenesis, propionic acid is the substrate. Acetic acid is an important substrate for lipogenesis and when combined with butyric, it is mainly used in the citric acid cycle for energy sources by host animals [29].

**Table 4. Effect of reformulated RDP level on partial VFA.**

| Item                | RDP     | TDN | Average ± SD |
|---------------------|---------|-----|--------------|
| Acetic (C2)         |         |     |              |
| R1                  | 61.93   | 64.76 | 63.35 ± 3.09 |
| R2                  | 62.94   | 64.14 | 63.54 ± 3.02 |
| R3                  | 63.02   | 64.93 | 63.97 ± 3.27 |
| Average ± SD        | 62.63 ± 3.45 | 64.61 ± 2.31 |
| Propionic (C3)      |         |     |              |
| R1                  | 17.57   | 18.92 | 18.24 ± 2.02 |
| R2                  | 18.30   | 18.17 | 18.23 ± 2.69 |
| R3                  | 17.49   | 19.00 | 18.24 ± 1.69 |
| Average ± SD        | 17.79 ± 1.12 | 18.69 ± 2.75 |
| Iso butyric (iC4)   |         |     |              |
| R1                  | 2.84    | 3.04  | 2.94 ± 1.09  |
| R2                  | 2.52    | 3.07  | 2.80 ± 1.49  |
| R3                  | 2.91    | 2.62  | 2.76 ± 1.37  |
| Average ± SD        | 2.75 ± 1.53 | 2.91 ± 1.06 |
| N butyric (nC4)     |         |     |              |
| R1                  | 10.80   | 12.05 | 11.43 ± 1.08 |
| R2                  | 11.40   | 11.89 | 11.64 ± 1.42 |
| R3                  | 11.12   | 11.64 | 11.38 ± 1.14 |
| Average ± SD        | 11.10 ± 0.89 | 11.86 ± 1.36 |
| Iso valeric (iC5)   |         |     |              |
| R1                  | 2.76    | 2.57  | 2.66 ± 0.67  |
| R2                  | 2.34    | 2.47  | 2.40 ± 0.81  |
| R3                  | 2.34    | 2.42  | 2.38 ± 0.82  |
| Average ± SD        | 2.48 ± 0.67 | 2.48 ± 0.87 |
| N valeric (nC5)     |         |     |              |
| R1                  | 1.27    | 1.49  | 1.38 ± 0.29  |
| R2                  | 1.30    | 1.46  | 1.38 ± 0.41  |
| R3                  | 1.21    | 1.30  | 1.21 ± 0.39  |
| Average ± SD        | 1.26 ± 0.31 | 1.42 ± 0.47 |

P1R1= RDP: RUP (60: 40) with TDN level 65.6%, P1R2= RDP: RUP (55: 45) with TDN level 65.6%, P1R3= RDP: RUP (50: 50) with TDN level 65.6%, P2R1= RDP: RUP (60: 40) with TDN level 68.6%, P2R2= RDP: RUP (55: 45) with TDN level 68.6%, P2R3= RDP: RUP (50: 50) with TDN level 68.6%.

Different superscripts in the same row with various letters show significant differences (p <0.05).

4. Conclusion
The optimum ratio of RDP:RUP was 60:40, with an increase in bacteria population, protozoa and the availability of ammonia (NH3) in the rumen. High TDN level provides a higher supply of VFA, as well as DMD and OMD.

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