Effect of Graded Levels of Fishmeal or Urea Supplementation on Rumen Environment and Ruminal Feed Degradation in Bali Cows

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Abstract. An experiment to study the effects of supplementation of graded levels of urea or fishmeal on rumen environment and ruminal feed degradation in Bali cows fed low quality tropical grass hay (crude protein, CP = 3.53%) was conducted according to a 5 x 5 Latin square experimental design with five animals and five periods. In each period lasting for 3 weeks, five non-pregnant Bali cows were given ad libitum access to grass hay (G) or supplemented daily with two levels of urea, i.e. 38 g (U₃₈) and 74 g (U₇₄), or two levels of fishmeal, i.e. 156 g (FM₃₁₂) and 312 g (FM₆₂₄). Supplementation of both urea and fishmeal reduced significantly (P<0.01) the average rumen pH from 6.89 in G to 6.74 in FM₃₁₂. Rumen ammonia concentration increased linearly (P<0.01) with increasing levels of supplementation and the increase was more pronounced with urea than with fishmeal supplementation. Rumen ammonia concentration was significantly higher (P<0.01) in urea than fishmeal supplemented cows at both levels of supplementation. Ruminal total as well as individual VFAs (Acetate, Butyrate and Propionate) concentrations were not affected by the increasing level of supplementation of both urea and fishmeal. Supplementation improved significantly (P<0.05) in sacco rumen degradation of DM but not protein. Ruminal DM effective degradability was increased significantly with increasing level of fishmeal supplementation but not with increasing level of urea supplementation. Supplementation of increasing level of both urea and fishmeal improved rumen environment and feed degradability in Bali cows maintained on low quality tropical grass hay with fishmeal was proven to be the better supplement over urea.

1. Introduction
Rumen degradation of tropical low quality grass is generally low due to a shortage of rumen degradable nitrogen. Mature tropical grass contains very low level of degradable crude protein, i.e. commonly lower than 6% [1]. When cows are fed mainly with the kind of grass, the rumen ammonia concentration may fall to between 20 and 40 mg/l [2]. In contrast, a ruminal ammonia concentration in the range from 50 to 80 mg/l is required for sufficient microbial growth and activity particularly those microbes responsible for fibre degradation [2-3]. For low quality roughages, the rumen ammonia requirement is even higher which may reach as high as 200 mg/l [4]. It seems advisable, therefore, that when the diets are composed mostly of mature tropical grass, a source of rumen degradable nitrogen should be supplemented [5].
Urea is the most widely used nitrogen supplement in ruminant animals because it is an extremely concentrated nitrogenous compound and most importantly it is relatively inexpensive. However, improvement in intake and nutrients supply to the animals is often not sufficiently large to support the desired level of animal production [5, 7, 8]. It has been commonly shown that supplementation of true protein to low quality roughage improves rumen degradation to a larger extent compared to supplementation with urea. This larger effect is argued due to the fact that in addition to nitrogen, the breakdown of true protein also produces branch-chained fatty acids, peptides, pre-form amino acids, and minerals which are required for maximum rumen microbial growth [9, 10]. In addition, the rate of protein degradation and the release of nitrogen during protein degradation in the rumen is also much slower than in urea. Hence, the supply of ammonia for rumen microbes is also maintained more evenly [11] and avoid temporary deficiency as in urea supplementation [12]. However, few studies were conducted to directly compare urea supplementation to fishmeal (FM) supplementation. FM contains less RDN compared to common true protein sources [13] hence the relative supply of those nutrients is much less. It is also particularly interesting is that those studies, however, were mostly conducted with European breeds and very few studies were done in indigenous tropical cattle breeds such as Bali cattle. Bali cattle are known to thrive well with low quality forages because they have a seemingly high rate of urea recycling [14]. With this characteristic, it is possible that Bali cows are able to utilize urea as supplemental nitrogen equal to FM when fed low quality grass hay basal diet. Therefore, this experiment was designed to assess the effect of supplementation of different levels of urea and fishmeal on rumen environment and rumen degradation in Bali cows maintained on low quality tropical grass hay.

2. Method

2.1. Animal and Experimental Design

Five dry non-pregnant Bali cows weighing on average 189 kg and fitted with permanent rumen cannulae were used in this experiment. The experiment was arranged in a 5 x 5 Latin square design and each period consisted of 2 weeks adjustment period followed by a two-week collection period. In each period, the animals were randomly allotted to five tested diets, i.e. native grass hay alone (G) or supplemented with two levels of urea, i.e. 38 and 74 gd⁻¹ (U₃₈ and U₇₄) and two levels of fishmeal, i.e. 156 and 312 gd⁻¹ (FM₁₅₆ and FM₃₁₂). The hay was obtained from native pasture grass dominated by Botryochloa spp, that was cut about one month after flowering and contained 3.53% CP. Fishmeal supplementation was made iso-nitrogenous to the level of urea supplementation. For the animal receiving the supplemental urea, the diets were supplemented with Na₂SO₄ in the amount that gave a 1:13 ratio to urea. Grass hay was offered twice a day at 08:00 and 16:00 in an amount that gave about 10 to 15% refusal. The supplemental feeds were fed at the same time. Urea was diluted into 200 to 300 ml of water and sprayed onto about one-third of the grass hay on offer. Additional hay was further offered after the urea-added hay was about to be completely consumed, i.e. about one hour. Water was available on a rubber bucket and offered frequently during day and night.

Within each period which lasted for four weeks, the first 2 weeks were allocated for the adjustment period and the last two weeks were for the collection period. Of the two-week collection period, one week (third week) was devoted to rumen incubation of the prepared samples. At the end of the fourth week, a 24-hour collection of rumen fluid was conducted.
Table 1. The composition of the experimental diets

| Ingredient            | (kg DM) | G   | U_{38} | U_{74} | FM_{156} | FM_{312} |
|-----------------------|---------|-----|--------|--------|----------|----------|
| Grass hay             | Ad libitum* | Ad libitum | Ad libitum | Ad libitum | Ad libitum |
| Fishmeal              | -       | -   | -      | -      | 0.156    | 0.312    |
| Urea                  | -       | 0.038 | 0.074  | -      | -        | -        |
| Sodium sulfate        | -       | 0.0028 | 0.0057  | -      | -        | -        |

* The hay allowance is about 20% excess of *ad libitum* intake which is obtained during two weeks preliminary period when all animals were given a similar diet i.e. FM_{156}

Table 2. Chemical compositions of feeds used in the experiment

| Chemical composition (%) | Grass hay | Fishmeal | Urea |
|--------------------------|-----------|----------|------|
| CP                       | 3.53      | 63.7     | 288.0|
| EE                       | 1.39      | 5.97     | -    |
| CF                       | 26.8      | 0.1      | -    |
| Ash                      | 7.8       | 19.5     | -    |
| NFE                      | 60.5      | 10.7     | -    |
| OM                       | 92.2      | 80.5     | 100  |
| NDF                      | 72.6      | 0        | -    |

2.2. Parameters, measurement, and calculations

To estimate the rumen pH, ammonia, and VFA concentrations, rumen fluid was collected in one day on the last day of the third week for every period. Rumen fluid was collected using a vacuum pump 12 times a day at the two-hour interval. The collected rumen fluid was directly measured for pH and thereafter centrifuged at 2500 rpm for 15 minutes and the supernatant was acidified with concentrated sulphuric acid to pH <4 and frozen until analysis for ammonia and VFA. VFA concentration was determined on a pooled sample per cow and period.

In *Sacco* nylon bag technique was used in this study to estimate ruminal degradation of dry matter and protein of hay and fishmeal. Hay was incubated in the rumen of all animals, whereas fishmeal was limited in animals receiving fishmeal supplements. Feeds were ground to pass a 1.5 mm screen and about 1 g of the sample was then weighed into a 7.5 x 10 cm bag made of nylon cloth with a pore size of 37 x 37 m^2. The bags were thereafter incubated in the rumen for 0, 4, 8, 16, 24, 48, and 96 hours.
At the time of removal, the bags were then directly frozen. After all bags have been removed from the rumen, they were washed under running tap water for 1 hour. The residues were transferred from the bag into a nitrogen-free filter paper and dried at 105°C for 20 hours. The degradation data were then fitted to the exponential equation using a simultaneous model as described by Dhanoa [15]:

\[ Y(t) = a \text{ for } t < t_0 \]  
\[ Y(t) = a + b(1 - e^{-c(t-t_0)}) \text{ for } t > t_0 \]

Where \( Y(t) \) is the degraded part at time \( t \), \( a \) is the water-soluble fraction, \( b \) is the insoluble but potentially degradable fraction, \( c \) is the degradation rate constant (in h\(^{-1}\)), \( t \) is the incubation time (in h), and \( t_0 \) is the lag time (in h). Whereas the effective degradability (ED) was calculated as \( \text{ED} = a + \frac{bc}{c+k} \cdot e^{-kt_0} \), where \( a, b, \) and \( c \) values are from the previous model and \( k \) is the fractional rate of passage.

Diets, ingredients, residues, feces, and rumen digesta were analyzed for nutrient contents. The samples were dried in a forced-air oven at 40°C for 48 hours, ground using a Wiley mill (1 mm screen), and analyzed for crude protein, EE, ash, CF, NDF, and ADF. Ash content was determined by ignition in a furnace at 600°C for 4 hours. Total nitrogen content was analyzed using the Kjeldahl technique and crude protein was calculated as \( \% N \times 6.25 \). Those chemical analyses were according to AOAC [16]. NDF and ADF contents were determined using detergent analysis as Van Soest et al. [17] excluding the use of alpha-amylase enzyme. Individual VFA was assayed using HPLC and the sum was assigned for total VFA. Ammonia-nitrogen was determined using the steam distillation technique.

2.3. Animal and Experimental Design

All data were statistically analyzed using Proc. GLM (SAS Institute). The model used was consistent with Latin square design: \( Y = \mu + C + P + T + E \) or \( Y = \mu + C + P + \text{Urea} + \text{FM} + E \), where \( \mu \): overall mean, \( P \): systematic effect of period, \( C \): systematic effect of cow, \( T \): fixed effect of treatment, \( \text{Urea} \): linear effect of urea, \( \text{FM} \): linear effect of fishmeal, and \( E \): residual error.

3. Results and Discussion

3.1. Rumen environment

Data on the effect of graded level of fishmeal (FM) or urea supplementation on rumen pH and ammonia as well as VFA concentration in Bali cows fed low quality grass hay is presented in Table 3. Rumen pH was significantly affected by supplementation of both levels of FM supplementation but not by urea supplementation. Rumen pH was significantly lower (\( P<0.05 \)) in FM supplemented cows than in cows fed the control diet and urea supplemented diet. Since rumen pH is controlled by the balance between acid production, buffering capacity of saliva and the production of ammonia as a weak base [18], the lower pH in FM supplemented cows can be due to one of those factors.

Higher rumen VFA concentration was observed in this study for urea supplemented cows, indicating a higher fermentation rate in those cows and it can potentially reduce rumen pH. At the same time, however, ammonia concentration was also significantly higher (\( P<0.01 \)) in urea supplemented cows. This may cancel any pH reduction due to increased VFA concentration in urea supplemented Bali cows. A similar observation was reported by Bento et al. [19] who found that no reduction in rumen pH was detected despite the increase of VFA concentration as a result of protein supplementation in steers consuming tropical grasses.
As expected, rumen ammonia concentration increased (P<0.05) with both urea and fishmeal supplementation. In this case, rumen ammonia concentration was higher (P<0.01) in urea supplemented cows than in fishmeal supplemented cows, but both urea and fishmeal improved significantly the rumen ammonia concentration from a sub-optimal level in G to more than 50 mg/l all level of supplementation of both urea and fishmeal. It means that the level of ammonia concentration in the supplemented cows was above the minimal level required for optimal rumen fermentation as stated by Satter and Slyter [20]. It seems that in this study, Bali cows require a low level of supplementation to achieve the threshold level of rumen ammonia concentration.

The higher concentration of rumen ammonia in urea compared to FM supplemented cows in this study is possibly due to a higher rate of rumen degradation of urea than that of fishmeal. Ammonia concentration in the rumen fluid is highly correlated to the rate of deamination during protein degradation [19], hence its level is highly dependent upon the rate of protein degradability of protein supplement in the rumen. Urea is known to be quickly and completely degraded in the rumen, while fishmeal is only partly degraded [21].

Table 3. Effect of levels and sources of nitrogen supplementation on pH, Ammonia and VFA concentrations of rumen liquid of Bali cows maintained on low quality tropical grass hay

| Parameters | Treatment | SEM | P |
|------------|-----------|-----|---|
|            | G | U₃₈ | U₇₄ | FM₁₅₆ | FM₃₁₂ | Treat | Urea | FM |
| pH         |   |     |     |       |       | 0.03  | <0.01 | 0.5  | 0.02 |
| Ammonia-N (mg/l) | 34.6 | 104 | 146 | 64    | 96    | 8.80  | <0.01 | <0.01 | <0.01 |
| VFA (mmol/l): |         |     |     |       |       |      |       |       |
| Acetate    | 47.4 | 58.1 | 63.8 | 42.4  | 58.5  | 5.4   | 0.08  | 0.02  | 0.2  |
| Propionate | 15.4 | 17.3 | 15.9 | 13.4  | 14.4  | 3.4   | 0.9   | 0.8   | 0.7  |
| Butyrate   | 3.5  | 3.0  | 4.1  | 3.2   | 4.2   | 0.5   | 0.4   | 0.3   | 0.2  |
| Total      | 66.3 | 78.3 | 83.8 | 59.0  | 77.1  | 6.8   | 0.1   | 0.03  | 0.4  |
| VFA (mol/100 mol): |         |     |     |       |       |      |       |       |
| Acetate    | 73.2 | 72.7 | 76.1 | 71.4  | 76.5  | 4.0   | 0.9   | 0.5   | 0.5  |
| Propionate | 21.3 | 23.3 | 18.9 | 23.1  | 18.4  | 3.9   | 0.9   | 0.5   | 0.5  |
| Butyrate   | 5.4  | 4.1  | 4.9  | 5.6   | 5.1   | 0.7   | 0.6   | 0.5   | 0.9  |

In contrast to rumen ammonia, ruminal VFA concentration which is another indicator for rumen fermentation was improved (P=0.03) with urea supplementation but not with both levels of FM supplementation. In this case, the increase was due to the significant increase (P=0.02) of Acetate concentration. This finding is quite surprising and in contrast to previous results. Stritzler et al.[12]
reported that total VFA concentration in rumen liquid was higher in fishmeal than in urea supplemented cows.

3.2. Rumen degradation

Table 4 shows the effect of urea and fishmeal supplementation on rumen degradation of dry matter, protein, and nitrogen-free dry matter of grass hay in Bali cows fed low quality tropical grass hay. As expected soluble (a) and potentially digestible (b) fractions of DM, crude protein and protein-free DM of grass hay did not differ (P>0.05) with both graded levels of urea and FM supplementation. Those fractions are considered as feed characteristics that are not affected by rumen fermentation processes. Meanwhile, the rate of degradation (c) is the degradation parameter that is highly affected by ruminal fermentative activities. In this study, the DM and protein-free DM degradation rate were improved significantly (P<0.05) with FM supplementation but not with urea supplementation. Consequently, DM and protein-free DM effective degradability (ED₁ and ED₂) were following this trend. In urea supplemented cows, rumen degradation increased after the cows were given supplemental urea at 38 gd⁻¹ and a declined rumen degradation was observed when the animals were given urea at 74 gd⁻¹. Meanwhile, with FM supplementation, DM and protein-free DM degradation continued to increase with increasing levels of supplementation. This finding shows a different and inconsistent trend with the effect of urea and FM supplementation on rumen ammonia concentration. As previously discussed, rumen ammonia concentration was higher when Bali cows were supplemented with urea than with FM. Therefore, it seems that a different rumen ammonia level is required when different nitrogen sources are supplemented to cows fed low quality grass hay. Previously, Neto et al. [22] reported that the optimum level of rumen ammonia concentration for optimal NDF rumen degradation was 177.6 mg/l when urea was used to supplement low quality buffelgrass.

Another interesting finding in the present experiment is that rumen DM degradation was increased by a small amount of fishmeal supplementation to a level even higher than the by the second level of urea supplementation. This strongly indicates that fishmeal is superior to urea in stimulating rumen degradation. which is previously also shown from several reports. Stritzler et al. [12] reported that in cows fed low quality roughage rumen degradation was improved with nitrogen or protein supplementation with fishmeal exerted higher effect compared to urea or other true protein. The common argument to explain the superiority of fishmeal over urea in improving rumen degradation is that in addition to nitrogen, the breakdown of fishmeal, as well as other true protein, also produce branch-chained fatty acids, peptide, pre-form amino acids and minerals which are required for maximum rumen microbial growth [8]. The rate of protein degradation and the release of nitrogen from fishmeal degradation is also much slower than from urea breakdown. Hence, the supply of ammonia for rumen microbes is also maintained more evenly [23] and avoids temporary deficiency as in urea supplementation [12].

| Parameter | Treatment | SEM | P-value |
|-----------|-----------|-----|---------|
| DM        | U₃₈U₇₄FM₁₅₆FM₃₁₂ |     |         |

Dry matter:
|       | a     | b     | c     | Lag   | Fill  | ED1   | ED2   |
|-------|-------|-------|-------|-------|-------|-------|-------|
| a     | 9.2   | 9.7   | 9.2   | 9.9   | 10.5  | 0.3   | 0.02  | 0.08  | 0.18  |
| b     | 49.1  | 49.7  | 52.3  | 50.1  | 49.9  | 2.3   | 0.90  | 0.62  | 0.91  |
| c     | 0.018 | 0.020 | 0.016 | 0.028 | 0.025 | 0.004 | 0.24  | 0.04  | 0.16  |
| Lag   | 3.5   | 4.0   | 3.5   | 4.0   | 5.2   | 0.8   | 0.63  | 0.66  | 0.69  |
| Fill  | 1.46  | 1.41  | 1.44  | 1.371 | 1.343 | 0.03  | 0.06  | 0.03  | 0.02  |
| ED1   | 38.6  | 41.0  | 40.1  | 42.5  | 44.4  | 1.1   | 0.03  | 0.04  | 0.03  |
| ED2   | 30.1  | 32.2  | 30.9  | 34.2  | 35.6  | 1.3   | 0.06  | 0.02  | 0.02  |

Crude Protein:
|       | a     | b     | c     | Lag   | Fill  | ED1   | ED2   |
|-------|-------|-------|-------|-------|-------|-------|-------|
| a     | 10.8  | 8.5   | 7.8   | 11.3  | 9.8   | 1.1   | 0.2   | 0.3   | 0.9   |
| b     | 46.8  | 41.5  | 49.6  | 49.7  | 45.5  | 4.0   | 0.6   | 0.6   | 0.5   |
| c     | 0.024 | 0.033 | 0.049 | 0.031 | 0.048 | 0.01  | 0.2   | 0.3   | 0.9   |
| Lag   | 0.35  | 1.11  | 2.02  | 0.06  | 0.00  | 0.4   | 0.03  | 0.07  | 0.7   |
| Fill  | 41.3  | 39.8  | 46.6  | 47.5  | 47.0  | 3.0   | 0.3   | 0.9   | 0.9   |
| ED1   | 34.0  | 33.6  | 40.0  | 40.1  | 41.4  | 3.0   | 0.3   | 0.9   | 0.9   |
| ED2   | 38.3  | 40.8  | 39.8  | 42.2  | 44.1  | 1.1   | 0.03  | 0.05  | 0.03  |
| * linear effect

Protein-free dry matter:
|       | a     | b     | c     | Lag   | Fill  | ED1   | ED2   |
|-------|-------|-------|-------|-------|-------|-------|-------|
| a     | 9.0   | 10.5  | 9.5   | 9.6   | 10.1  | 0.5   | 0.3   | 0.3   | 0.1   |
| b     | 49.1  | 47.5  | 52.8  | 50.2  | 50.2  | 2.6   | 0.7   | 0.4   | 0.6   |
| c     | 0.018 | 0.022 | 0.016 | 0.028 | 0.027 | 0.004 | 0.1   | 0.02  | 0.05  |
| Lag   | 4.35  | 7.60  | 3.65  | 4.16  | 5.88  | 0.9   | 0.05  | 0.2   | 0.1   |
| Fill  | 1.46  | 1.42  | 1.45  | 1.38  | 1.35  | 0.03  | 0.06  | 0.02  | 0.02  |
| ED1   | 38.3  | 40.8  | 39.8  | 42.2  | 44.1  | 1.1   | 0.03  | 0.05  | 0.03  |
| ED2   | 29.7  | 32.0  | 30.6  | 33.9  | 35.2  | 1.3   | 0.06  | 0.02  | 0.02  |
4. Conclusion

Supplementation of increasing level of both urea and fishmeal improved rumen ammonia supply to a sufficient level for microbial growth and activities. As nitrogen requirement was fulfilled, rumen environment was improved, and DM degradability in Bali cows maintained on low quality tropical grass hay with fishmeal was proven to be the better supplement over urea.

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