Effect of the inclusion of different sources of carbohydrate in the fattening ration based on Leucaena leaves on the intake, digestibility, and weight gain of Culled Bali Cows

G E M Malelak, I G N Jelantik, and I Benu

Department of Animal Husbandry, The University of Nusa Cendana, Indonesia, 35111

Corresponding author e-mail: fapetuberales@gmail.com / geminimalelak@staf.undana.ac.id

Abstract. An experiment with the objective of improving the utilization of leucaena leaves by culled Bali cows by the inclusion of different sources of carbohydrate was conducted following a completely randomized design using 16 thin cull Bali cows. Four diets were allotted to each animal at any period including La: the animal was offered a diet consisting of 80% dried leucaena leaves and 20% rice straw as a control, LaLb: added with pumpkin, LaJg: added with cornmeal, and LaLbJg: added with pumpkin and cornmeal. All supplemented diets were set to have similar energy and protein level. Variables measured included intake and digestibility of nutrients, rumen environments, and daily weight gain. Results showed that the inclusion of pumpkin and especially cornmeal significantly increased (P<0,05) dry matter and nutrient intake. DM and nutrient digestibility did not differ among treatments except for crude fiber. Rumen concentration of volatile fatty acids was significantly increased but rumen ammonia concentration was reduced with the addition of carbohydrate. Daily weight gain was significantly (P<0,05) improved and the inclusion of cornmeal produced the highest daily weight gain. It can be concluded that the intake and the utilization of leucaena leaves for weight gain by culled Bali cows was improved by the inclusion of carbohydrates with cornmeal exert a better effect over pumpkin.

1. Introduction

Most of the cattle delivered to Java, especially to the island of Java to meet the demand for meat in several big cities in Java, especially Jakarta, are Bali bulls which are fattened with the Amarasi system. This fattening system is the most popular and most successful in the Province of Nusa Tenggara Timur due to largely the use of leucaena leaves as the basal feed. As a legume plant, leucaena (Leucaena leucocephala) has a very high crude protein content, up to 24.4% [1]. The most important characteristic that causes the high quality of leucaena forage as feed for ruminants is the high proportion of protein that escapes degradation in the rumen (bypass protein or protein escape) [2]. This occurs because, in addition to high protein content, the leaves also have considerable tannin content which protects protein from rumen degradation [3]. Hence, leucaena potentially supplies a very large amino acid, and feeding cattle can produce high body weight gain [4].

Although the weight gain achievement in the Amarasi system fattening is classified as the highest in NTT, [5] reported that the fattening ration consisting of 100% can be increased body weight gain from 0.4 to 0.6 kg per day with the addition of grass. This shows that even though the forage has a very high quality (eg with its ability to supply amino acids), its utilization by Bali cattle has not been optimal. This is due to the presence of an excess protein that is degraded in the rumen relative to the energy availability...
in the rumen. Even though it has low protein degradability (i.e. 36.7%), there is still an excess of 337 grams of degraded protein in the rumen if the cattle consume 6 kg dry matter of leucaena per day. This is due to the low energy content of the forage. Each kg of dry matter forage only contains 0.2 kg of digested carbohydrates (DCHO) in the rumen [6] so that only 1.2 kg of DCHO is available. On the other hand, the efficiency of microbial protein synthesis was highest when there was 1 kg of DCHO for 179 grams of degraded protein in the rumen. In other words, it takes only 214.8 grams of RDP to compensate for the 1.2 kg of DCHO available in the rumen. Therefore, there is an excess of 534 grams of RDP in cattle that consume 6 kg DM of Leucaena leaves.

Thus, a clear strategy to increase the efficiency of using leucaena for the growth of cattle is to add carbohydrate feed sources that are easily digested in the rumen. Pumpkin is a local feed that is known to have a very high degradability of carbohydrates in the rumen. [7] reported that pumpkin contained 90.6% carbohydrates and 73.5% was potentially digested in the rumen. Thus, the addition of pumpkin in the cattle ration consisting of leucaena leaves is expected to significantly increase the efficiency of the use of leucaena RDP for the development of microbial population and activity in the rumen. This in turn will have an impact on increasing the production of VFA and microbial protein so that the supply of energy and amino acids will increase significantly. Meanwhile, corn is a feed that is known to have a high proportion of by-pass carbohydrates. The provision of these two supplementary feeds will have a different effect on the utilization of nutrients and the efficiency of meat production in fattened cull Bali cows. The purpose of this study was to examine the effect of supplementing cornmeal and pumpkin on the utilisation of leucaena for fattening cull Bali cows.

2. Materials and methods

2.1. Animals and experimental design

This experiment was carried out following a completely randomized design using 16 cull Bali cows weighing 184.6±12.3 kg. The animals were randomly grouped into four groups to receive the four experimental diets i.e. La: cows were given 80% fresh leucaena leaves and 20% rice straw, LaLb: La + pumpkin, LaJg: La + cornmeal, and LaLbJg: La + a combination of pumpkin and cornmeal. The level of addition of the pumpkin and cornmeal was as much as to produce a balance of 179 grams of RDP per kg of DCHO which is proven by Hvelplund and Madsen (1985) as the optimal RDP: DCHO balance to produce optimal population and activity, microbial protein synthesis.

2.2. Parameters and measurements

Parameters measured included intake and digestibility of dry matter and nutrients (CP, OM, CHO, energy, and the intake of digestible organic matter), as well as the rumen ammonia and VFA concentration.

Feed intake was determined by calculating the difference between given and residual in dry matter. Feed residues were collected every morning and weighed before the next feed was introduced. The daily residues were then sampled for DM determination. The dry matter content of the residues was determined by placing the residues sample in an oven at 105°C for a minimum of 20 hours (AOAC, 1990). Another feed sample and residues were taken daily and sun-dried. They were composited for each animal and at the end of the study, they were sampled for further chemical analyses i.e organic matter, protein, and fat. The nutrient intake (OM, protein, fat, and carbohydrate) was calculated as dry matter intake multiplied by the content of each of these nutrients.

Digestibility trial was conducted by total fecal collection for 5 days at 10 weeks of the experiment. Fecal matter was collected daily before morning feeding. Feces were weighed and sampled for DM which was conducted daily. Fecal samples for determination of nutrient content were taken daily and sun-dried. At the end of the collection period, they were constituted for every cow and sampled for nutrient content.

To determine the concentration of ammonia and VFA in the rumen fluid, on the last day of the data collection period, the rumen fluid was collected using oro-pharyngeal tubes connected to a vacuum
pump. The collected rumen liquid was strained with four layers of cheese. Samples were taken and directly acidified with a few drops of H2SO4 to reduce the pH to about less than 3 so that rumen microbial activity was stopped. This experiment was carried out following a completely randomized design using 16 cull Bali cows weighing 184.6±12.3 kg. The animals were randomly grouped into four groups to receive the four experimental diets i.e. La: cows were given 80% fresh leucaena leaves and 20% rice straw, LaLb: La + pumpkin, LaJg: La + cornmeal, and LaLbJg: La + a combination of pumpkin and cornmeal. The level of addition of the pumpkin and cornmeal was as much as to produce a balance of 179 grams of RDP per kg of DCHO which is proven by Hvelplund and Madsen (1985) as the optimal RDP: DCHO balance to produce optimal population and activity, microbial protein synthesis.

2.3. Statistical analysis
All data collected were analyzed using Proc. Anova by employing SPSS 23.

3. Results and discussion
3.1. Intake and digestibility
Table 1 presents the effect of supplementation of pumpkin or cornmeal as carbohydrate sources on the dry matter (DM) and nutrient intake of cull Bali cows fed leucaena leaf. Supplementation of both carbohydrate sources and their combination significantly improved (P<0.05) the intake of dry matter and nutrients but crude protein (CP). A similar result was reported by [8] when their supplement cross-bred beef cows fed a diet of the mixture of leucaena leaf and elephant grass with two sources of carbohydrates contrasting in rumen degradability (rice polishing vs cane molasses. In their study, intake was improved from 12.4 kg/d in control cows to 15-18 kg/d in cows fed supplemental carbohydrates. The increase in intake in the present study might not due to the improvement of rumen fermentation since it is believed that the control diet already provides sufficient substrate especially nitrogen for optimal microbial growth and activity. Rather, the elimination of the intake barrier due to excessive ammonia concentration in Bali cows may the reason for the increased intake in the supplemented groups. Excessive ammonia is absorbed through the rumen wall to circulating ammonia which needs to be converted to urea and excreted via urine. This high blood ammonia triggers a discomfort condition due to poor brain tissue functioning and this condition stimulates voluntary feed intake to decline [9]. Hence when energy is available in the supplemented groups, the intake can be increased.

| Parameter                      | Treatment    | P-value |
|--------------------------------|--------------|---------|
| Dry matter (kg/d)              | La           | 4.765a  |
|                                | LaLb         | 5.442c  |
|                                | LaJg         | 5.574c  |
|                                | LaLbJg       | 5.108b  |
| Organic matter (kg/d)          |              | <0.001  |
| Crude Protein intake (kg/d)    |              | <0.001  |
| Crude fat intake (kg/d)        |              | 0.203a  |
| Crude Fibre (kg/d)             |              | 0.814b  |
| Carbohydrate (kg/d)            |              | 3.07a   |
| Nitrogen Free Extract (NFE) (kg/d) |          | 2.795a  |
| Gross Energy (MJ/d)            |              | 84.017a |
| DE intake (MJ/d)               |              | 45.883a |

Values followed by different superscript on the same row indicate significant differences (P<0.05).

The digestibility of DM, OM, and CP did not differ (P>0.05) between treatments. Meanwhile, crude fiber was slightly reduced (P<0.05) by the supplementation of both carbohydrate sources. This decline may be due to lowered rumen pH as a result of the higher fermentation rate of the supplement. The
decrease in pH will have an impact on the suppression of the population and the activity of microbes that digest fiber which can cause a decrease in the rate of degradation and ultimately the total tract fiber digestibility.

### Table 2. Effect of adding a carbohydrate source on digestibility.

| Digestibility coefficient (%) | Treatment | P-value |
|-------------------------------|-----------|---------|
| Dry Matter                    | La        | LaLb    | LaJg    | LaLbJg   |         |
| Organic Matter                | 64.47     | 61.97   | 65.16   | 62.93    | 0.106   |
| Crude Protein                 | 68.29     | 61.30   | 63.15   | 61.25    | 0.123   |
| Crude Fat                     | 81.04     | 81.28   | 79.81   | 79.03    | 0.522   |
| Crude Fibre                   | 73.58<sup>a</sup> | 57.37<sup>b</sup> | 58.23<sup>b</sup> | 52.23<sup>b</sup> | 0.006   |
| Total Carbohydrates           | 67.64<sup>a</sup> | 55.57<sup>b</sup> | 53.91<sup>b</sup> | 55.03<sup>b</sup> | 0.020   |
| Nitrogen Free Extract         | 63.27     | 54.97   | 61.66   | 58.10    | 0.104   |
| Energy                        | 74.21     | 69.61   | 74.56   | 71.81    | 0.128   |

Values followed by different letters on the same line indicate significant differences (P < 0.05).

Unlike the case with the fiber fraction, the addition of carbohydrate sources generally does not affect protein digestibility. This is due to the ability of protein-digesting microbes (i.e. proteolytic microbes) to withstand changes in rumen pH. Protein digesting microbes are quite tolerant of decreasing rumen pH. In conditions of rumen pH approaching 5. Proteolytic bacteria in general can still survive and carry out temporary activities of cellulolytic bacteria [10].

#### 3.2. Ruminal VFA and ammonia concentration

Various rumen parameters such as VFA and ammonia concentrations, in general, can be used as main indicators of metabolic processes that occur in the rumen which include the rate of degradation of carbohydrates and proteins and absorption by microbes and the walls of the rumen. The results of this study indicate that the VFA concentration increased significantly with the addition of carbohydrate sources, especially the addition of pumpkin. This is indeed expected because the degradability of the pumpkin is higher than the leucaena so that it increases at a higher extent compared to the supplementation of cornmeal. Increasing the VFA concentration will have an impact on increasing the energy supply for the animal. VFA is the main energy source in ruminants and at the cellular level will be used as an energy source (ATP) and a carbon skeleton for the formation of meat tissue.

Meanwhile, the addition of cornmeal did not have a significant effect on increasing the VFA concentration in the rumen fluid. This may be due to two main factors. First, the rate of degradation of corn carbohydrates is slow so that if it is accompanied by an increase in VFA absorption rate along with an increase in the pH of the rumen fluid. The concentration of VFA in the rumen tended to be relatively low even though the animals get a higher supply of VFA. The second factor is the VFA precursor (hexose) in the fermentation of cornmeal carbohydrates which is used by microbes as an energy source and carbon skeleton [11]. Thus, the VFA production rate was not too high and the rumen VFA concentration did not increase. However, the availability of energy sources and a carbon skeleton will increase the use of ammonia for the production of microbial cells so that the microbial population and activity increases. Another important effect is that the increase in MPS will increase the post-rumen supply of amino acids that are absorbed in the small intestine.

Ammonia concentration in the rumen fluid is an important indicator of the adequacy of nitrogen availability for microbes for cell formation. In this study, the concentration of ammonia varied from 4.31 mmol/l or 73 mg/l in cattle that received additional pumpkin and 5.44 mmol/l or 93 mg/l of rumen fluid in cattle that consumed leucaena basal feed. This concentration is generally a level sufficient for ammonia needs in the rumen. The optimal level required for microbial growth and activity in the rumen is 50-70 mg/l [11]. Below this level, in general, the growth and activity of rumen microbes are disturbed.
In cattle that consume the concentrate, lower ammonia levels still result in adequate livestock production. Meanwhile, for livestock consuming low-quality feed, even higher concentrations of up to 200 mg/l are required. [1] reported that the optimal level for degradability and intake of low-quality feed by Bali cows is around 100 mg/l.

### Table 3. Effect of carbohydrate sources on rumen parameters.

| Parameter | Treatment | P-value |
|-----------|-----------|---------|
| VFA (mmol/l) | La | 151.14<sup>a</sup> | 173.45<sup>c</sup> | 155.09<sup>ab</sup> | 161.71<sup>b</sup> |
| NH3 (mmol/l) | La | 5.44<sup>b</sup> | 4.31<sup>a</sup> | 4.56<sup>ab</sup> | 5.1<sup>ab</sup> |

Values bearing different superscript on the similar rows indicate significant differences (P <0.05).

Apart from this debate and the adequacy of ammonia concentration in this study, the main reason for adding carbohydrate sources in this study is to reduce the production and concentration of ammonia in the rumen. In this study, it is assumed that although leucaena is classified as one of the feeds that have low protein degradability there is still an excess of the protein which is converted in the rumen into ammonia. The excess ammonia is then absorbed and excreted in the urine in the form of urea. Thus, feed protein is used inefficiently by the animals. In addition, energy is needed to break down ammonia into urea so that it will reduce the efficiency of feed energy utilization by Bali cows for production purposes (weight gain). The low concentration of ammonia in the animals receiving pumpkin and its combination with cornmeal was partly due to the lower protein content of the ration of the animals that received pumpkin compared to the control (La) and the addition of cornmeal (LaJg). Another factor is that giving the carbohydrate sources provides a higher energy source so that microbes use peptides to form cell proteins [12] so that ammonia is not formed in the rumen. In this condition, there will be an increase in microbial protein production due to the higher efficiency of feed protein utilization, although it cannot be ascertained yet due to the increased energy requirements used to transport peptides across the microbial cell walls.

#### 3.3. Nutrient supply and weight gain

Increasing body weight is the main goal of adding different sources of carbohydrates in cull Bali cows that consume leucaena leaf basal diet. In this study as shown in Table 4, the weight gain of cows for cows increased significantly with carbohydrate supplementation. The positive effect of supplementing a source carbohydrate to Leucaena fed Bali cattle is also reported by [13]. In the study, the author offered additional cassava tuber (0.5% LW) to Bali steer consuming 80% Leucaena leaf 20% grass or banana stem improved daily gain to almost double from 0.28 kg/d to 0.47 kg/d.

### Table 4. Effect of carbohydrate supplementation on the intake of digested nutrients and weight gain.

| Parameter | Treatment | P-value |
|-----------|-----------|---------|
| DDMI (kg /d) | La | 2.954<sup>a</sup> | 3.779<sup>b</sup> | 3.631<sup>b</sup> | 3.216<sup>a</sup> |
| DOMI (kg /d) | La | 2.615<sup>a</sup> | 3.265<sup>c</sup> | 3.028<sup>bc</sup> | 2.744<sup>ab</sup> |
| DCPI (kg / d) | La | 0.949<sup>c</sup> | 0.779<sup>b</sup> | 0.711<sup>a</sup> | 0.728<sup>a</sup> |
| DE intake (kg / h) | La | 52.799<sup>a</sup> | 65.944<sup>b</sup> | 64.167<sup>b</sup> | 66.746<sup>a</sup> |
| ADG (kg /d) | La | 0.512<sup>a</sup> | 0.577<sup>ab</sup> | 0.708<sup>bc</sup> | 0.804<sup>c</sup> |

DDMI: digestible dry matter intake
DOMI: digestible organic matter intake
DCPI: digestible crude protein intake
ADG: average daily gain

Means followed by different letters on the same line indicate significant differences (P <0.05).
The highest increase in body weight in this study was achieved in cull Bali cows that received additional cornmeal combined with pumpkin. Pumpkin that is easily digested in the rumen will provide VFA and energy (ATP) for microbes thereby increasing the production of microbial protein and thus the supply of amino acids for cows. The combination of increased amino acids from microbes, amino acids from the leaves of leucaena which escape degradation in the rumen and is digested in the small intestine, and VFA and glucose from the fermented pumpkin in the rumen and digestion of corn in the small intestine generally provide the best supply and balance of nutrients from a combination of corn and pumpkin.

In general, the effect of the addition of carbohydrates on the weight gain of rejected cows in this study was due to an increase in the efficiency of nutrient utilization compared to an increase in the supply of nutrients (energy). This is evident from the absence of a relationship between several parameters which are indicators of energy supply. such as intake of digested organic matter and intake of digestible energy (DE) with PBB. Previously [1] had a very close relationship between energy supply (DOMI) and weight gain in cattle that consume low-quality feed supplemented with protein source feed. This close relationship was achieved in the protein-deficient animals in the study. In this study, cows were having excess protein. Increased nutrient utilization occurs through two mechanisms. First, the addition of carbohydrate sources in cows that consume leucaena leaves can increase energy utilization by reducing the energy requirements needed to convert ammonia into urea before it is excreted in the urine. This will provide more energy for meat deposition. This is evident from the fairly close relationship between ingested protein intake and ADG (r² = 0.541). Secondly, as previously explained, the increase in energy utilization is caused by an increase in the quality of the absorbed nutrients. [11] argued that increasing the P/E ratio could increase the efficiency of energy use in ruminants.

4. Conclusion

The utilization of leucaena forage for the fattening of cull Bali cows can be increased by the addition of both pumpkin and milled corn alone or in combination. This increase is achieved through the increased intake, rumen metabolism, and increased efficiency in the utilization of digested nutrients. Cornmeal is a better supplement in increasing the weight gain of cull Bali cows compared to pumpkin.

References
[1] Jelantik I G N 2001 Improving Bali Cattle (Bibos banteng Wagner) Production through Protein Supplementation. PhD Thesis. The Royal Veterinary and Agricultural University, Copenhagen, Denmark
[2] Pal K, Patraa A K, Sahoo A and Kumawat PK 2015 Evaluation of several tropical tree leaves for methane production potential, degradability and rumen fermentation in vitro Livestock Science 180 98–105
[3] Mahanani M M P, Kurniawati A, Hanim C, Anas M A, and Yusiati L M 2020 Effect of (Leucaena leucocephala) Leaves as Tannin Source on Rumen Microbial Enzyme Activities and In Vitro Gas Production Kinetics IOP Conf. Series: Earth and Environmental Science 478 012088 IOP Publishing doi:10.1088/1755-1315/478/1/012088
[4] Siaw D, Osuji P, andNsahlai I 1993 Evaluation of multipurpose tree germplasm: The use of gas production and rumen degradation characteristics The Journal of Agricultural Science 120 (3) 319-330. doi:10.1017/S0021859600076486
[5] Bamualim A and Wirdahayati R B 2003 Nutrition and Management Strategies to Improve Bali Cattle Productivity in Nusa Tenggara. ACIAR Proceedings No. 110 pp. 17-22.
[6] Mlay P S, Pereka A, Phiri E C, Balthazary S, Jelantik I G N, Hvelplund T, Weisbjerg M R, and Madsen J 2006 Feed value of selected tropical grasses, legumes and concentrates. Vet. Arhiv 76 (1) 53-63
[7] Jelantik I G N 2006 Chemical composition and energy value of some feedstuffs for ruminants from East Nusa Tenggara. Nutrition Bulletin 9 no. 3 115-120.
[8] Flores-Cocas J M, Aguilar-Pérez C F, Ramírez-Avilés L 2021 Use of rice polishing and sugar
cane molasses as supplements in dual-purpose cows fed Leucaena leucocephala and Pennisetum purpureum. *Agroforest Syst.* 95 43–53 https://doi.org/10.1007/s10457-019-00434-z

[9] Detmann E, Valente E E L, Batista E D, and Huhtanen P 2014 An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. *Livestock Science* 162 141-153

[10] Hobson P N and Stewart C S 1997 *The Rumen Microbial Ecosystem*. 2nd ed. Chapman & Hall. UK

[11] Leng R A 1990 Factors affecting the utilisation of poor quality forages by ruminants particularly under tropical condition. *Nut. Res. Rev.* 3 277-303.

[12] Nolan J V 1993 Nitrogen kinetics. In: Forbes J M and France J (eds) *Quantitative aspects of Ruminant Digestion and Metabolism* pp.123-143

[13] Nulik J 2014 Studies of Leucaena Based Feeding on the Growth Path of Bali Cattle and its Adoption in East Nusa Tenggara. *Proceedings of the 16th AAAP Animal Science Congress* Vol. II, Gadjah Mada University, Yogyakarta, Indonesia. Pp 316-319