Iranian Journal of Applied Animal Science

The Effect of Different Dietary Crude Protein Content on Growth Performance, Nutrient Digestibility, and Blood Metabolites of Cull Bali Cows Fed on Urea-Treated Rice Straw

Research Article

I.G.N. Jelantik[1*], G.E.M. Malelak[1], I. Benu[1] and C.L.O. Leo-Penu[2]

[1] Department of Animal Husbandry, Faculty of Animal Husbandry, Fishery and Marine Science, the University of Nusa Cendana, Kupang, Indonesia
[2] Department of Animal Production, Kupang State Agricultural Polytechnique, Kupang, Indonesia

Received on: 27 May 2023
Revised on: 21 Sep 2023
Accepted on: 6 Nov 2023
Online Published on: Dec 2023

*Correspondence E-mail: igustingurahjelantik@staf.undana.ac.id

Online version is available on: www.ijas.ir

ABSTRACT

Feeding cull cows with a balanced concentrate is a major strategy to improve meat production and quality. The optimal protein level of the concentrate is rarely determined specifically for indigenous cull Bali cows fed urea-treated rice straw. The present experiment was conducted to investigate the effects of feeding concentrate differing in crude protein (CP) content on the feed intake, nutrient digestibility, and growth performance of cull Bali cows fed urea-treated rice straw. We used 24 thin cull Bali cows that were randomly assigned into three groups of 8 animals to receive concentrates containing different CP levels as treatments, i.e. 12.6% (CP12), 14.2% (CP14), and 16.4% (CP16) at dry matter (DM) basis. The concentrate allowance was 2% live weight (LW) and urea-treated rice straw was offered at 1% LW. The experiment was conducted in a completely randomized design. The measured variables included the intake and digestibility of DM and nutrients, rumen ammonia and volatile fatty acids (VFA) concentration, blood plasma of glucose (GLUC), blood urea nitrogen (BUN), average daily gain (ADG), live weight gain (LWG) and feed conversion ratio (FCR). Dry matter intake (DMI) decreased linearly (P<0.05) with increasing CP content of the concentrate, but the digestibility of dry matter as well as other nutrients was not affected. There was a linear increase in rumen ammonia concentration (P<0.05) with increasing CP content in the concentrate. The ruminal total VFA concentration was not affected (P>0.05) by the treatments, but the concentrations of propionate, butyrate, and n-valerate were lowest (P<0.05) in CP16. The GLUC, BUN and blood profiles, including packed cell volume (PCV) and hemoglobin declined by feeding concentrate with increasing CP levels. The levels of ADG, LWG and FCR were significantly higher (P<0.05) in CP12 than in CP14 and CP16. In conclusion, feeding concentrate containing more than 12.6% CP reduces the intake, rumen parameters, blood metabolites and ADG of cull Bali cows when fed at 2% LW.

KEY WORDS concentrate, cull Bali cows, protein level, urea-treated rice straw, weight gain.

INTRODUCTION

A major proportion of beef supply in Indonesia, particularly in some cattle-producing areas where fattened bulls are mainly transported to major cities in the country, is contributed by slaughtering beef cows (Priyanti *et al.* 2017). In those areas, approximately 72 to 91% of the total cattle being slaughtered are cows, and approximately 81.7% (Suardana *et al.* 2013) to 90% (Priyanti *et al.* 2017) of those cows are considered productive. This has been considered the most important factor that contributes to the decline of the cattle population in Indonesia (Priyanti *et al.* 2017).

Hence, there is a strong need to reduce the number of productive cows being slaughtered and one obvious strategy is by fattening cull cows. Numerous studies have shown that thin cull cows could gain significant weight and have a higher dressing percentage when they were fed a high energy and protein diet (Gallo *et al*. 2017; Chainam *et al*. 2019; Santos *et al*. 2019). Moreover, beef quality from cull cows is also markedly improved when they are fed on concentrate-based diets (Couvreur *et al*. 2019).

Despite the numerous studies that have been undertaken, information on the optimal crude protein (CP) level in the concentrate for fattening cull cows is still lacking although studies on feeding concentrates containing different CP levels to dairy cows (Mantysaari *et al*. 2004; Teixeira *et al*. 2011; Katongole and Yan, 2020), crossed steers (Mendes *et al*. 2015), local Vietnamese cattle (Dung *et al*. 2013), or swamp buffaloes (Chanthakhoun *et al*. 2012) have been documented. It appears that the optimal CP level in the concentrates differs with the level of concentrate in the diets, cattle genotypes, the companion forages or roughages as well as other factors. Dung *et al*. (2013) reported that the optimal level of CP in the concentrates is 16% for male Vietnamese local cattle fed natural grass and rice straw. Meanwhile, for dairy cows or steers, a concentrate CP level of 17.6% is required for optimal milk production (Katongole and Yan, 2020). There is a possibility, however, that this optimal level may not simply apply to cull cows. It is commonly believed that the protein requirement of cull cows is somewhat lower than that of other classes of cattle since the composition of gain in finishing cull cows is high in fat content (Santos *et al*. 2017). In addition, Bali cows are an Indonesian indigenous cattle breed that have small mature size with a slow growth rate (Sampurna *et al*. 2014). Therefore, the animals may need a lower CP content in the ration.

The optimal level of protein content in the concentrate may also depend on the quality of the other feeds in the ration. Feeding urea-treated rice straw has been consistently demonstrated as a solution for combating ruminant feed scarcity in developing countries (Sheikh *et al*. 2018). Urea treatment generally improves the nutritive value and its utilization of rice straw for ruminant production (Aquino *et al*. 2020).

The utilization of urea-treated straw is variably affected by the supplementation of concentrate and protein feeds (Tekliye *et al*. 2018) which indicate different level of protein is required. The purpose of this experiment was to investigate the effect of increasing CP content in the concentrate mixtures on nutrient intake and digestibility, rumen fermentation products, the concentration of blood metabolites, and the performance of cull Bali cows fed urea-treated rice straw.

MATERIALS AND METHODS

Animals, feeds and treatments

This experiment was conducted at the University of Nusa Cendana, Indonesia for 3 months with a 2-week adaptation period and 10 weeks of data collection from July to December 2021. A number of 24 thin Bali cows (BCS 1.5-2, i.e., based on a 1-5 scoring system; 1= extremely thin to 5= extremely fat) (Soares *et al*. 2011) were allocated in this experiment. The animals were purchased from the local market, moved to the barn and randomly placed into 1.5 m × 2 m individual concrete-floored barns. They were inspected for any tooth disorder and then dewormed with a 1 mL subcutaneous injection of Ivomec (Merck Sharp and Dohme B.V.). During the adaptation period, the animals were first fed with mainly fresh grass and gradually introduced to concentrate and urea-treated rice straw. This adaptation period lasted for 2 weeks, and the animals were then weighed in the morning before feeding.

The mean initial weight of the cows was 175 ± 18.1 kg, and they were randomly allotted into three groups of 8 cows. Each group was offered one of the concentrate mixtures containing different CP content, i.e., 12.6% (CP12), 14.2% (CP14), and 16.4% (CP16), respectively, and urea-treated rice straw. The experimental design was a completely randomized design with three treatments and 8 replications. The concentrates were offered at 2% LW divided into two meals given at 08.00 in the morning and 16.00 in the afternoon. The concentrates were composed of cornmeal, rice bran, and fishmeal (Table 1). Each cow was fed in a plastic bucket 1 h before urea-treated rice straw was introduced in the feed manger separated from the concentrate. Urea-treated rice straw was offered at 1% LW, but an additional amount of the straw was offered when it was completely consumed by the cows. Rice straw was treated with 4% urea (W/W) at approximately 65% moisture content and incubated for 4 weeks in a plastic container. The straw was removed from the container and spread on the floor for at least 6 hours before feeding. The amount of feeds on offer was adjusted every week for any changes in the cow's live weight. Water was available at all times in a plastic bucket for each cow. The ingredients and chemical composition of the concentrate mixtures and urea-treated rice straw are presented in Table 1.

Data collection and sampling

Approximately 200 g of sample was sampled when the concentrate was prepared, i.e. once in two weeks. Meanwhile, a similar amount of sample of urea-treated rice straw was taken at the time of container opening. About 50 g of those samples were directly determined for DM content.

Table 1 The ingredients and chemical composition of the concentrate mixtures and urea-treated rice straw

Item	Concentrate			Urea-treated rice straw
	CP_{12}	CP_{14}	CP_{16}	
Ingredient (% of DM)[1]				
Cornmeal	64	55	61	
Rice bran	30	35	25	
Fishmeal	5	9	13	
Mineral premix[1]	1	1	1	
Chemical composition (% of DM)[2]				
OM	94.1	91.6	91.2	77.5
CP	12.6	14.2	16.3	6.71
EE	4.54	5.51	5.98	0.22
CF	17.1	17.0	17.1	26.1
NFE	59.8	54.9	50.2	54.9
GE (MJ/kg DM)	17.91	17.8	17.9	13.9

[1] Ingredients (/kg): Calcium carbonate: 470 g; Phosphate flour: 310 g; Ferrum: 10 g; Cuprum: 0.5 g; Manganese sulfate: 0.3 g; Potassium iodide: 0.01 g; Sodium chloride: 270 g; Magnesium sulfate: 30 g and Zinc oxide: 10 g.
[2] OM: organic matter; CP: crude protein; EE: ether extract; CF: crude fiber; NFE: nitrogen free extract, calculated as OM-(CP+EE+CF); GE (MJ/kg DM): gross energy, calculated as CP (g/kg) × 24.237 + EE (g/kg) × 34.116 + (CF+NFE) (g/kg) × 17.300 (Hvelplund *et al*. 1995).

Meanwhile, the rest was kept after sundried and pooled at the end of the experiment for chemical analyses including organic matter (OM), CP, crude fibre (CF) and ether extract (EE). Feed refusals were collected daily before morning feeding. The daily refusals of concentrate and ammoniated rice straw were collected, weighed and sampled for DM determination. Dry matter intake (DMI) was estimated as the difference between dry matter feeds on offer and the refusals. Meanwhile, nutrient intake was estimated as dry matter intake multiplied by its nutrient content.

The digestibility of DM and nutrients, i.e. OM, CP, CF and EE were determined in the last week of the experiment. Daily fecal production was estimated by total fecal collection for five consecutive days. The daily collected feces were weighed every morning for every cow, mixed thoroughly, and sampled at approximately 5% for DM determination, and a composite sample of about 10% was formed by the cow and immediately frozen. At the end of the fecal collection period, the composited feces were then mixed, subsampled, and dried at 55 ˚C in a forced-air oven for 48 h. The dried fecal and feed samples were ground (1 mm screen using the Cyclotech Mill, Tecator, Sweden) before chemical determination (proximate analyses). Chemical analyses were performed according to AOAC (1990). The ash content was determined by ignition in a furnace at 600 ˚C for 4 hours (method 938.08). The total nitrogen content was analyzed using the Kjeldahl technique, and crude protein was calculated as % N × 6.25 (method no. 984.13) and method no. 920.85 for the determination of ether extract. The digestibility coefficient of DM, OM, EE, CP, and CF of the ration was calculated by the difference between intake and fecal matter.

To estimate the rumen pH, ammonia, and VFA concentration, rumen fluid was collected in one day on the last day of the experiment 2 h after morning feeding.

Samples were collected using a vacuum pump connected to a stomach tube, and the collected rumen fluid was filtered through three layers of cheesecloth. The ruminal pH was immediately measured using HANNA instrument (HI 8424 microcomputer, Singapore). Aliquots were thereafter centrifuged at 1643 g force for 15 minutes, and the supernatant was collected and acidified with concentrated sulfuric acid to pH <4 and frozen until analysis for ammonia (NH_3-N) and VFA concentrations. Individual VFAs were assayed using HPLC as described by Mathew *et al.* (1997), and the sum was assigned as the total VFAs. Briefly, 10 mL ruminal fluid was added with 5 mL of 20% stannous chloride solution and filtered after 10 mins. About 10 mL filtrate was then added with 2.5 mL H_3PO_4 and repeatedly filtered to produce a clear solution. The solution was then analyzed using HPLC with a 210 mm column and peaks were detected using a spectrophotometric detector at 210 nm. The colorimetric method as described by Chaney and Marbach (1962) was followed to determine the NH_3-N concentration.

About 10 µL rumen aliquots were poured into glass tubes. Thereafter, 1.5 mL of a phenol solution and 1.5 mL of sodium hypochlorite solution were added. The tubes were vortexed in a water bath at 39 ˚C for 15 minutes. The absorbance was then read at 630 nm in a spectrophotometer UV/Visible BEL Photonics 2000 UV.

Blood samples were taken on the same day as rumen fluid collection. Blood collection was performed by jugular puncture using a vacutainer containing disodium-EDTA as an anticoagulant approximately 2 h after feeding. Blood samples were centrifuged at 1643 g force for 15 minutes to collect blood plasma.

Blood plasma was then stored at -20 ˚C until the analyses for plasma concentrations of glucose (GLU) and blood urea nitrogen (BUN). GLU and BUN concentration was assessed using Roche Diagnostic GmbH, Jerman standard procedures with COBAS C111 (Freckmann *et al.* 2014).

Cows were weighed weekly before morning feeding. Live weight gain was calculated as the difference between the initial and final weight. The average daily weight gain (ADG) of individual cows was estimated from the slope of the regression line. In addition, ADG was also estimated for 0-30, 30-60, and 60-90 days after feeding. Feed conversion ratio (FCR) was calculated as the amount of DM required to achieve 1 kg of live weight gain.

Statistical analysis

Experimental data were analyzed by ANOVA with polynomial contrast for a completely randomized design using SPSS (2015). Data were analyzed using:

$$Y_{ij} = \mu + Ti + e_{ij}$$

Where:

Y_{ij}: observation from the animal j receiving treatment i.
μ: population mean.
Ti: treatment effect of the i^{th} treatment.
e_{ij}: random error.

Means ± SEM were reported. The effects of treatment were declared significant at $P < 0.05$. Probabilities at $0.05 < P < 0.10$ were considered a trend toward significance.

RESULTS AND DISCUSSION

As presented in Table 2, there was a linear decrease (P<0.01) in total dry matter intake (DMI) and the intake of CF and nitrogen free extract (NFE) in cull Bali cows fed on concentrate with increasing CP from 12.6% to 16.4% which correspondents to dietary CP content from 10.7% to 13.4%. However, the intake of CP and EE did not differ between treatments (P>0.05). In the present experiment, the decline in DMI resulted from the linear decrease in both concentrate and straw intake (P<0.05). The result of our experiment was contrary to other results that when the animals were offered concentrates with increasing CP content, DMI increased (Huhtanen *et al.* 2008; Kang *et al.* 2015) or maintained as shown by Puhakka *et al.* (2016) that DMI did not increase when CP in the concentrates was increased from 15.4 to 19%. These different results were apparently caused by various factors including the level of concentrate in the diet, as well as the ingredients of the concentrate and the quality of the companion roughage as suggested by Dung *et al.* (2013).

The result of our experiment showed that despite the decrease in DMI, CP intake in cull Bali cows did not differ between treatments (P>0.05).

It appears that the CP intake was maintained at a close range between 0.68 and 0.72 kg/d, and this level of intake could be the CP requirement of cull Bali cows weighing 187 to 204 kg and gaining 0.34 and 0.56 kg/d.

It is therefore understood that the animals fed higher CP in the concentrate had lower DMI to avoid excessive CP intake. The negative effect of excess CP on DMI is commonly mediated through the excess NH_3-N, which is the end product of protein degradation in the rumen (Forbes, 2007).

Rumen ammonia is absorbed from the rumen and should be converted into urea before being excreted in the urine. Urea synthesis in the liver requires energy, which is mainly derived from the citric acid cycle. In addition, the synthesis of urea also depletes α-ketoglutarate by increasing the formation of glutamate, thereby inhibiting ATP production in the Krebs cycle (Sampaio *et al.* 2010). This energy draining due to urea formation may reach a level that causes discomfort due to poor brain tissue functioning (Forbes, 2007). At this level, voluntary feed intake can be reduced (Detmann *et al.* 2009).

The reduced intake observed in the present experiment with increasing CP in the concentrates may be also due to an unbalanced protein:energy ratio of the absorbed nutrients (Forbes, 2007). The excess CP in relation to energy increases body heat production (Poppi and McLenann, 1995). The ratio of protein and energy can be estimated from CP:digestible organic matter (DOM) and DMI starts to fall when the ratio is above 210 g CP/kg DOM (Poppi and McLenann, 1995) or as high as 288 g CP/kg DOM (Detmann *et al.* 2014). In our study, the CP:DOM ratios were 171, 201, and 218 g CP/kg DOM for concentrates containing 12%, 14%, and 16% CP, which correspond to dietary CP contents of 10.6%, 11.7%, and 13.4%, respectively. In this case, it appears that CP was excessive in CP16, but they already reduced intake to a CP level of 14%. This lower trigger might be related to the lower CP requirement of Bali cows compared to exotic breeds. Forbes (2007) reported different levels of CP content-related energy that cause discomfort as the CP requirements differ.

The digestibility coefficients of DM and nutrients except CP were not affected (P<0.05) by the CP level of diet (Table 3). The results of our experiment were in agreement with the results of Amaral *et al.* (2016), who found no increase in DM and OM digestibility with increasing dietary CP.

However, the result of our experiment is contrary to other results from similar breeds. Mariani *et al.* (2016) reported that the DM digestibility of feeds was higher in diets containing higher CP. Increasing digestibility with increasing dietary CP levels was also reported in Bali cows (Jelantik, 2001).

Table 2 Effects of dietary protein level on dry matter and nutrient intake

Intake (DM bases)	Treatment			P-value	
	CP_{12}	CP_{14}	CP_{16}	Linear	Quadratic
Concentrate (kg/d)	4.12±0.27[a]	3.44±0.51[b]	3.32±0.80[b]	0.010	0.253
Rice straw (kg/d)	2.41±0.23[a]	2.15±0.26[ab]	1.85±0.66[b]	0.018	0.899
Total (kg/d)	6.54±0.41[a]	5.59±0.69[b]	5.17±1.29[b]	0.005	0.489
Total (g/kg $LW^{0.75}$)	129±10.2[a]	118±16.40[ab]	107±28.1[b]	0.040	0.970
OM	5.75±0.36[a]	4.82±0.61[b]	4.46±1.10[b]	0.003	0.380
CP	0.68±0.04	0.63±0.08	0.72±0.17	0.548	0.170
EE	0.19±0.01	0.19±0.0.03	0.20±0.05	0.570	0.788
CF	1.58±0.11[a]	1.36±0.16[ab]	1.23±0.33[b]	0.006	0.653
CHO	4.88±0.31[a]	3.99±0.50[b]	3.54±0.88[b]	<0.001	0.418
NFE	3.78±0.23[a]	3.06±0.38[b]	2.67±0.67[b]	<0.001	0.408
GE (MJ/d)	107.49±6.67[a]	91.0±11.53[b]	85.6±20.90[b]	0.006	0.377
CP in the DM intake (%)	10.4±0.13[a]	11.3±0.23[b]	14.0±0.92[c]	<0.001	0.001

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; CF: crude fibre; NFE: nitrogen free extract; CHO: total carbohydrate estimated as CF + NFE and GE: gross energy

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Effects of dietary protein level on nutrient digestibility and digestible nutrient intake

Parameters	Treatment			P-value	
	CP_{12}	CP_{14}	CP_{16}	Linear	Quadratic
Digestibility (%)					
DM	71.9±4.56	74.6±6.12	76.5±5.35	0.109	0.868
OM	76.7±3.16	76.4±3.32	78.3±1.67	0.288	0.394
CP	72.3±3.41[a]	74.7±6.04[a]	80.9±2.70[b]	0.001	0.337
EE	81.7±10.0[ab]	76.2±6.90[a]	85.4±5.36[b]	0.411	0.043
CF	80.6±3.11	81.0±1.84	82.6±1.98	0.138	0.584
GE	76.6±3.16	76.3±3.47	78.7±1.42	0.189	0.292
Digestible nutrient intakes					
DDMI (kg/d)	4.710±0.51[a]	4.20±0.79[a]	3.48±0.80[b]	0.067	0.712
DOMI (kg/d)	4.42±0.40[a]	3.69±0.53[b]	3.48±0.80[b]	0.006	0.319
DCPI (kg/d)	0.49±0.03[a]	0.47±0.08[a]	0.58±0.14[b]	0.089	0.136
DEI (MJ/d)	82.5±7.42[a]	69.5±10.16[b]	67.2±15.50[b]	0.015	0.299

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; GE: gross energy; DDMI: digestible dry matter intake; DOMI: digestible organic matter intake; DCPI: digestible protein intake and DEI: digestible energy intake.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The positive results of increasing dietary CP on digestibility were commonly observed when protein (or nitrogen) is the limiting factor for optimal rumen fermentation. In the present experiment, however, rumen fermentation might not be limited by nitrogen availability in the rumen since about 60% of the intake was coming from concentrate, which should have been easily fermented in the rumen. Moreover, all diets contained sufficient rumen degradable protein (RDP) for every kg of organic matter intake. In addition to that, the CP difference between treatments in the present experiment may not be sufficiently large to affect DM digestibility. Promkot and Wanapat (2005) reported that to affect DM digestibility, the difference in dietary CP level should be at least 4%. In our research, the difference in CP content of the dietary CP level was only 3.4%.

In the present experiment, there was a significant effect of treatments (P<0.05) on the digestible organic matter intake (DOMI) and digestible energy intake (DEI). In this case, DOMI and DEI increased in CP14 but no further increase was observed in CP16.

Meanwhile, DCP intake (DCPI) was slightly higher in CP16 compared to other treatments. DOMI has been known to have a close relationship with weight gain (Salah *et al.* 2014).

It is therefore clear from the study that the digestible energy intake has been optimized when the concentrate contains 14.2% CP.

In many circumstances, intake and digestibility in ruminant animals are predominantly determined by the rate and extent of rumen degradation by ruminal microorganisms through physical rumen fill and their metabolic effect on the end product of rumen degradation (Allen *et al.* 2019). Hence, the determination of several fermentation products, such as rumen ammonia and VFA concentrations, as well as rumen pH would help to indicate the rate and extent of rumen fermentation (Mapato *et al.* 2010; Daneshvar *et al.* 2015). Rumen ammonia, VFA concentration, and pH in culled Bali cows fattened with a diet containing different levels of crude protein in the concentrate are presented in Table 4.

In general, rumen pH was slightly acidic, but it was under the range of 6.0 to 6.8, which is considered by Ososanya *et al.* (2013) to be required to maintain the stability of the rumen ecosystem and function.

The result of the present experiment showed that there was no difference among treatments (P>0.05) in the rumen pH. In contrast with our result, Amaral *et al.* (2016) reported increasing rumen pH with an increasing dietary CP from 10 to 14%. Rumen pH is affected by the degradation rate of dietary carbohydrates, particularly grain (Metzler-Zebeli *et al.* 2013), and hence by the ratio between concentrate and roughage (Phesatcha *et al.* 2020). In the present experiment, the concentrate:straw ratios of the consumed feed were 63:37% in CP12 and 64:36% in CP16. In all treatments, the concentrate portion was higher than the straw. According to Faniyi *et al.* (2019), rumen pH is commonly acidic in cows fed concentrate-based diets.

Rumen ammonia concentration increased linearly (P<0.05) with increasing CP in the concentrate as shown in Table 4. A similar result was reported by Amaral *et al.* (2016), who recorded a linear increase in rumen ammonia concentration when dietary CP was increased gradually from 10 to 14%. Previously, Chanthakhoun *et al.* (2012) and Chen *et al.* (2010) also reported a similar trend, i.e., increasing NH_3-N with increasing dietary CP. Ammonia is the product of protein degradation in the rumen therefore an increase in NH_3-N is commonly observed. Nevertheless, the rumen ammonia concentration in the present experiment, even at the lowest level of CP (NH_3-N=126 mg/L), was above the minimum level required for optimal growth and activities of rumen microbes, which was reported by Satter dan Slyter (1974) to be 50 mg/L. The level of NH_3-N in the present experiment is also higher than 120 mg/L which is the required level to optimize intake and digestibility in Bali cattle consuming low-quality roughages (Jelantik and Belli, 2010). This indicates that nitrogen is probably not the limiting factor for optimal rumen fermentation even at the lowest CP content of the concentrate mixture in this study.

The total VFA concentration (TVFA) in the rumen did not differ (P>0.05) among cull Bali cows receiving concentrate differing in the CP level. The result of the present experiment was similar to the results reported by Amaral *et al.* (2016) that there was no increase in TVFA or the proportion of individual VFAs with increasing dietary CP levels. Chanthakhoun *et al.* (2012) also did not find an increase in total VFA when the concentrate differing in CP level (12.4 to 18.1%) was supplemented to buffaloes consuming rice straw basal diets. Ruminal VFA concentration is highly related to the proportion of concentrate in the diet and its ratio to roughage (Phesatcha *et al.* 2020). As the ratio between concentrate and urea-treated rice straw did not markedly vary in the present experiment, it would be expected that TVFA did not differ among treatments.

The concentrations of propionate, butyrate, and n-valerate differed significantly between treatments (P<0.05) and they were lowest in CP16 (Table 4). This finding is in contrast with the report of Chanthakhoun *et al.* (2012), who found increasing propionate concentration with increasing CP in the concentrate mixture. Commonly, the positive effect of dietary CP on propionate concentration is related to the ratio between concentrate and straw; the propionate concentration and molar proportion in the rumen increase with increasing concentrate:forage ratio (Wang *et al.* 2020). Although concentrate intake was indeed reduced in the present experiment, the ratio between concentrate and straw did not change since straw intake was also reduced. In the present experiment, the ratio of concentrate:straw in the consumed diet was relatively similar, i.e., 64%:36% in CP16 compared to 63:37 in CP12. Hence, the reduction of the concentration and molar proportion of propionate, butyrate, and n-valerate may be due to the partial substitution of starchy feeds (maize and rice bran) with fishmeal to increase the protein content of the concentrate in CP16. This small level of substitution may be sufficient to cause a reduction in propionate concentration in the rumen. Propionate production is primarily from the fermentation of starch in the rumen and varies from different sources of starch (Allen *et al.* 2019); hence, the decline in propionate in CP16 can be attributed to the fact that CP16 has the lowest proportion of cornmeal and rice bran, which have a high starch content.

Means of the concentrations of blood metabolites and blood constituents in cull Bali cows fed urea-treated rice straw and concentrate containing different levels of crude protein are presented in Table 5. The glucose concentration declined linearly (P<0.05) with increasing CP in the diet. This finding is different from previous results that plasma glucose concentration increased with increasing dietary CP (Tahuk *et al.* 2018). This decline in glucose concentration found in the present experiment might be related to the decline in propionate concentration in the rumen as previously discussed. Propionate is known as one of the glucose precursors in ruminant animals (Bannink *et al.* 2006). Beef cows mostly rely on the gluconeogenesis mechanism from either amino acids or propionate to suffice the glucose requirement because glucose absorption from the small intestine is commonly low (Allen *et al.* 2019).

The concentration of BUN in the present experiment tended to be linearly reduced (P=0.075) in cull Bali cows fed a higher CP diet. This finding is unexpected since in the present experiment there was a significant increase in rumen NH_3-N with increasing dietary CP.

Table 4 Effects of protein level in the concentrate mixture on rumen environment of cull Bali cows

Variable[1]	Treatment			P-value	
	CP_{12}	CP_{14}	CP_{16}	Linear	Quadratic
pH	6.56±0.34	6.70±0.21	6.58±0.37	0.891	0.385
Ammonia nitrogen (mg/L)	126±44.3^{a}	148±72.7^{a}	216±68.3^{b}	0.010	0.470
Acetate (mM)	71.0±17.00	64.4±17.07	68. 6±20.92	0.796	0.511
Propionate (mM)	25.9±6.78^{b}	28.0±5.72^{b}	18.9±7.35^{a}	0.046	0.065
Butyrate (mM)	12. 9±3.54^{b}	12.4±4.25^{b}	8.91±3.35^{a}	0.045	0.375
Iso-Valerate (mM)	2.24±0.49	2.32±0.88	1.73±0.65	0.151	0.276
n-Valerate (mM)	1.25±0.34^{a}	1.26±0.51^{a}	0.75±0.33^{b}	0.024	0.159
Total VFA (TVFA)	113.3±25.32	108.4±24.60	98.8±30.28	0.294	0.843
Acetate (% of TVFA)	62.57±3.08^{a}	59.3±5.69^{a}	69.5±3.61^{b}	0.004	0.002
Propionate (% of TVFA)	22.8±3.48^{ab}	26.1±4.02^{a}	19.0±3.99^{b}	0.060	0.005
Butyrate (% of TVFA)	11.4±1.78^{a}	11.2±2.28^{a}	8.94±2.01^{b}	0.025	0.239
Ratio C3:(C2+C4)	0.311±0.058^{a}	0.375±0.086^{a}	0.246±0.065^{b}	0.078	0.005

VFA : volatile fatty acids; C2: acetate; C3: propionate and C4: butyrate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 5 The effect of CP levels in the concentrate mixture on blood metabolites in cull Bali cows fed urea-treated rice straw

Parameter[1]	Treatment			P-value	
	CP_{12}	CP_{14}	CP_{16}	Linear	Quadratic
Glucose (mg/dL)	55.3±6.07^{a}	51.7±6.64^{ab}	46.2±5.84^{b}	0.010	0.731
BUN (mg/dL)	15.7±2.46	14.9±1.67	13.5±2.82	0.075	0.780
TPP (g/dL)	8.45±0.74^{a}	8.00±0.28^{ab}	7.65±0.58^{b}	0.011	0.828
PCV (%)	36.2±4.07^{a}	32.6±5.34^{ab}	30.0±4.75^{b}	0.022	0.811
Hb (g/dL)	12.0±1.36^{a}	10.9±1.78^{ab}	10.0±1.58^{b}	0.022	0.811
Erytrocite ($\times10^{6}$ cells/mm^{3})	16±1.96	14.6±2.71	13.7±2.48	0.077	0.827
Leucocyte ($\times10^{3}$ cells/mm^{3})	13.4±1.53	14.9±2.77	15.1±3.88	0.279	0.596

BUN: blood urea nitrogen; TPP: total protein plasma and Hb: hemoglobin.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 6 Live weight gain of cull Bali cows fed different CP levels in the concentrate mixture

Variables[1]	Treatment			P-value	
	CP_{12}	CP_{14}	CP_{16}	Linear	Quadratic
Initial LW (kg)	178±20.13	168±12.83	178±20.63	0.978	0.222
Final LW (kg)	236±21.96^{a}	212±8.90^{ab}	218±25.21^{b}	0.082	0.096
Total LWG (kg)	57.9±6.53^{a}	43.8±11.42^{b}	39.9±18.72^{b}	0.013	0.381
ADG (kg/d)	0.630±0.079^{a}	0.462±0.136^{b}	0.420±0.223^{b}	0.013	0.381
FCR (kg DM/kg LWG)	9.596±1.267^{a}	10.6±2.838^{ab}	12.6±4.768^{b}	0.082	0.697

LW: live weight; LWG: live weight gain; ADG: average daily gain and FCR: feed conversion ratio estimated as kg DM feed required by the cows to gain 1 kg daily.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

As stated by Kang *et al.* (2015), there is a particularly strong positive relationship between ammonia concentration in the rumen and BUN. Similarly, the decrease of BUN in this experiment cannot be explained by its positive relationship with energy intake as stated by Garcia *et al.* (2017).

Blood profiles, including PCV and hemoglobin, were significantly reduced (P<0.05) with increasing CP levels in the concentrate. The erythrocyte concentration tended to decline (P=0.08) with increasing CP levels. However, leucocytes did not differ between treatments (P>0.05). The decline in erythrocytes and hemoglobin may be attributed to the decline in DOMI, which may indicate a lower metabolism rate in Bali cows fed concentrate with a CP of more than 12%.

Merdana *et al.* (2020) reported that erythrocyte synthesis is stimulated by increasing oxygen demands, such as during the later stages of gestation. It is possible that when DOMI decreased the requirement for oxygen transport also declined. Hematocrit and hemoglobin values are directly proportional to the number of erythrocytes (Adam *et al.* 2015).

The results of our experiment showed that ADG and LWG were significantly reduced (P<0.05) in Bali cows fed the concentrate mixture with increasing CP content. LWG in CP12 was about 36 to 50% higher than that in CP14 and CP16 respectively. This finding was different from several results of other experiments with Bali cattle in which LWG was improved when the animals were fed diets with increasing CP content. Quigley *et al.* (2009) reported that LWG was significantly improved when the CP level of the supplemental concentrate was increased from 9% to 24% in young male Bali cattle. Similarly, Tahuk *et al*. (2020) reported that ADG increased when dietary CP was increased from 11 to 13%. However, our results were in line with some reports. Lee *et al*. (2011) presented data that multiporous Haenwoo cows fed high-energy diets containing 14.28% CP found in one farm had lower ADG compared to a diet containing 12.39% CP. The decrease of ADG in the present experiment was best explained by the decline of DOMI in cows fed increasing CP in the concentrate mixture. DOMI is commonly used to estimate metabolizable energy intake (Ülger *et al*. 2018) and Salah *et al*. (2015) concluded that at a similar intake of digestible crude protein, ADG is reduced with declining ME intake.

Nevertheless, this experiment demonstrates that feeding concentrates containing low CP to cull Bali cows provides a considerable practical alternative to reduce the number of productive cattle being slaughtered. With LWG of 57.9 kg obtained in 70 days of the feeding period, it is expected that the number of productive female cattle that need to be slaughtered could be reduced by about one-third assuming the average LW of the slaughtered Bali cows in Indonesia is between 180-200 kg. With this reduction, cattle productivity can be significantly increased.

CONCLUSION

Feeding concentrate containing CP of more than 12.6% to cull Bali cows maintained on urea-treated rice straw reduces live weight gain and feed conversion ratio. The decline is mainly due to the reduced digestible organic matter intake as well as the concentration in the rumen propionate and blood metabolites.

ACKNOWLEDGEMENT

The authors would like to thank Mr. Illi, Mr. Ly and Ms. Sadipun for their technical support in this study as well as The Indonesian Ministry of Higher Education for its financial support.

REFERENCES

Adam M., Lubis T.M., Abdyad B., Asmilia N., Muttaqien M. and Fakhrurrazi F. (2015). Total erythrocytes count and haematocrit value of Aceh and Bali cattle in Leumbah Seulawah, Aceh Besar. *J. Med. Vet.* **9(2),** 115-118.

Allen M.S., Sousa D.O. and VandeHaar M.J. (2019). Equation to predict feed intake response by lactating cows to factors related to the filling effect of rations. *J. Dairy Sci.* **102,** 7961-7969.

Amaral P.D.M., Mariz L.D.S., Benedeti P.D.B., Silva L.G.D., Paula E.M.D., Monteiro H.F. and Faciola A.P. (2016). Effects of static or oscillating dietary crude protein levels on fermentation dynamics of beef cattle diets using a dual-flow continuous culture system. *PLoS One*. **11(12),** e0169170.

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Aquino D., Del Barrio A., Trach N.X., Hai N.T., Khang D.N., Toan N.T. and Hung N.V. (2020). Rice straw-based fodder for ruminants. Pp. 111-130 in Sustainable Rice Straw Management. M. Gummert, N.V. Hung, P. Chivenge and B. Douthwaite, Eds., Springer Nature, Switzerland.

Bannink A., Kogut J., Dijkstra J., France J., Kebreab E., Van Vuuren A.M. and Tamminga S. (2006). Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. *J. Theor. Biol.* **238(1),** 36-51.

Chainam J., Opatpatanakit Y., Kiatbenjakul P., Lin R.S. and Kiratikarnkul S. (2019). Carcass characteristics, meat quality and eating quality of culled dairy cows. *Sci. Technol. Asia*. **26,** 47-58.

Chaney A.L. and Marbach E.P. (1962). Modified reagents for determination of urea and ammonia. *Clin. Chem*. **8,** 130-132.

Chanthakhoun V., Wanapat M. and Berg J. (2012). Level of crude protein in concentrate supplements influenced rumen characteristics, microbial protein synthesis and digestibility in swamp buffaloes (*Bubalus bubalis*). *Livest. Sci*. **144(3),** 197-204.

Chen S.C., Paengkoum P., Xia X.L. and Na-Lumpang P. (2010). Effects of dietary protein on ruminal fermentation, nitrogen utilization and crude protein maintenance in growing Thai-indigenous beef cattle fed rice straw as roughage. *J. Anim. Vet. Adv*. **9(18),** 2396-2400.

Couvreur S., Le Bec G., Micol D. and Picard B. (2019). Relationships between cull beef cow characteristics, finishing practices and meat quality traits of longissimus thoracis and rectus abdominis. *Food.* **8(4),** 141-149.

Daneshvar D., Khorvash M., Ghasemi E., Mahdavi A.H., Moshiri B., Mirzaei M. and Ghaffari M.H. (2015). The effect of restricted milk feeding through conventional or step-down methods with or without forage provision in starter feed on performance of Holstein bull calves. *Anim. Sci. J.* **93(8),** 3979-3989.

Detmann E., Paulino M.F., Mantovani H.C., Valadares Filho S.D.C., Sampaio C.B., de Souza M.A. and Detmann K.S. (2009). Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis–Menten kinetics. *Livest. Sci*. **126(1),** 136-146.

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. *Livest. Sci*. **162,** 141-153.

Dung D., Ba N.X., Van N.H., Phung L.D., Ngoan L.D., Cuong V.C. and Yao W. (2013). Practice on improving fattening local cattle production in Vietnam by increasing crude protein level in concentrate and concentrate level. *Trop. Anim. Health Prod.* **45,** 1619-1626.

Faniyi T.O., Adegbeye M.J., Elghandour M.M.M.Y., Pilego A.B., Salem A.Z.M., Olaniyi T.A., Adediran O. and Adewumi M.K. (2019). Role of diverse fermentative factors towards microbial community shift in ruminants. *J. Appl. Microbiol.* **127,** 2-11.

Forbes J.M. (2007). A personal view of how ruminant animals control their intake and choice of food: minimal total discomfort. *Nutr. Res. Rev*. **20,** 132-146.

Freckmann G., Baumstark A., Schmid C., Pleus S., Link M. and Haug C. (2014). Evaluation of 12 blood glucose monitoring systems for self-testing: system accuracy and measurement reproducibility. *Diab. Technol. Ther*. **16(2),** 113-122.

Gallo L., Sturaro E. and Bittante G. (2017). Body traits, carcass characteristics and price of cull cows as affected by farm type, breed, age and calving to culling interval. *Animal*. **11(4),** 696-704.

García C.A.C., Prado F.M.G., Galicia L.L. and Borderas T.F. (2017). Reference values for biochemical analytes in Mexican dairy farms: interactions and adjustments between production groups. *Arq. Bras. Med. Vet. Zootec.* **69,** 445-456.

Huhtanen P., Rinne M. and Nousiainen J. (2008). Evaluation of concentrate factors affecting silage intake of dairy cows: A development of the relative total diet intake index. *Animal.* **2(6),** 942-953.

Hvelplund T., Andrieu J., Weisbjerg M.R. and Vermorel M. (1995). Prediction of the energy and protein value of forages for ruminants. Pp. 205-227 in Proc. 4th Int. Symp. Nutr. Herb. INRA, Paris.

Jelantik I.G.N. (2001). Improving Bali Cattle (*Bibos banteng* Wagner) Production through Protein Supplementation. Ph D. Thesis. The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Jelantik I.G.N. and Belli H.L.L. (2010). Effect of urea or coconut cake supplementation on nutrient intake and digestion of Bali cows maintained on tropical grass hay. *J. Trop. Anim. Vet. Sci*. **15(3),** 196-204.

Kang S., Wanapat M., Phesatcha K. and Norrapoke T. (2015). Effect of protein level and urea in concentrate mixture on feed intake and rumen fermentation in swamp buffaloes fed rice straw-based diet. *Trop. Anim. Health Prod*. **47,** 671-679.

Katongole C.B. and Yan T. (2020). Effect of varying dietary crude protein level on feed intake, nutrient digestibility, milk production, and nitrogen use efficiency by lactating Holstein-Friesian cows. *Animals*. **10(12),** 2439-2447.

Lee C.D.H., Yoon W.J., Choi N., Ryu K.S., Oh Y.K., Jang S.S., Choi C.W., Joo J.W., Cho S.B. and Kim E.J. (2011). Effects of high protein diet on meat productivity and quality in multiparous Hanwoo cull cows. *J. Life Sci*. **21(9),** 1251-1258.

Mäntysaari P., Huhtanen P., Nousiainen J. and Virkki M. (2004). The effect of concentrate crude protein content and feeding strategy of total mixed ration on performance of primiparous dairy cows. *Livest. Prod. Sci.* **85(2),** 223-233.

Mapato C., Wanapat M. and Cherdthong A. (2010). Effects of urea treatment of straw and dietary level of vegetable oil on lactating dairy cows. *Trop. Anim. Health Prod*. **42,** 1635-1642.

Mariani N.P., Mahardika I.G., Putra S. and Partama I.B.G. (2016). Protein and energy ration that optimize performance of male Bali cattle. *J. Vet.* **17(4),** 634-640.

Mathew S., Sagathevan S., Thomas J. and Mathen G. (1997). An HPLC method for estimation of volatile fatty acids in ruminal fluid. *Indian J. Anim. Sci*. **67,** 805-807.

Mendes F.B.L., Silva R.R., de Carvalho G.G.P., da Silva F.F., Lins T.O.J.D., da Silva A.L.N. and Guimarães J.O. (2015). Ingestive behavior of grazing steers fed increasing levels of concentrate supplementation with different crude protein contents. *Trop. Anim. Health Prod.* **47,** 423-428.

Merdana I.M., Sulabda I.N., Tiasnitha N.M.W.A., Gunawan I.W.N.F. and Sudira I.W. (2020). Erythrocyte, hemoglobin and hematocrit profile of Bali cattle during the various periods of parturition. *J. Anim. Health Prod*. **8(2),** 75-79.

Metzler-Zebeli B.U., Schmitz-Esser S., Klevenhusen F., Podstatzky-Lichtenstein L., Wagner M. and Zebeli Q. (2013). Grain-rich diets differently alter ruminal and colonic abundance of microbial populations and lipopolysaccharide in goats. *Anaerobe*. **20,** 65-73.

Ososanya T.O., Odubola O.T. and Shuaib-Rahim A. (2013). Intake, nutrient digestibility and rumen ecology of WestAfrican Dwarf sheep fed palm kernel oil and wheat offal supplemented diets*. Int. J. Agric. Sci*. **3,** 380-386.

Phesatcha K., Phesatcha B., Wanapat M. and Cherdthong A. (2020). Roughage to concentrate ratio and saccharomycescerevisiaeinclusion could modulate feed digestion and *in vitro* ruminal fermentation. *Vet. Sci.* **7(4),** 151-161.

Poppi D.P. and McLennan S.R. (1995). Protein and energy utilization by ruminants at pasture. *Anim. Sci. J.* **73(1),** 278-290.

Priyanti A., Inounu I. and Ilham N. (2017). Prevention of productive cows slaughter through management of local state enterprises. *Indonesian Bull. Anim. Vet. Sci*. **27(2),** 53-66.

Promkot C. and Wanapat M. (2005). Effect of level of crude protein and use of cottonseed meal in diets containing cassava chips and rice straw for lactating dairy cows. *Asian-Australasian J. Anim. Sci*. **18(4),** 502-511.

Puhakka L., Jaakkola S., Simpura I., Kokkonen T. and Vanhatalo A. (2016). Effects of replacing rapeseed meal with fava bean at 2 concentrate crude protein levels on feed intake, nutrient digestion, and milk production in cows fed grass silage–based diets. *J. Dairy Sci*. **99(10)**, 7993-8006.

Quigley S.P., Poppi D.P. and McLennan S.R. (2009). Strategies to increase growth of weaned Bali calves. Research report of ACIAR Project LPS/2004/023, ACIAR , Australia.

Salah N., Sauvant D. and Archimède H. (2014). Nutritional requirements of sheep, goats and cattle in warm climates: A meta-analysis. *Animal.* **8(9),** 1439-1447.

Sampaio C.B., Detmann E., Paulino M.F., Valadares Filho S.C., de Souza M.A., Lazzarini I. and de Queiroz A.C. (2010). Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Trop. Anim. Health Prod*. **42,** 1471-1479.

Sampurna I.P., Saka I.K., Oka G.L. and Putra S. (2014). Patterns of growth of Bali cattle body dimensions. *ARPN J. Sci. Technol.* **4(1),** 20-30.

Santos P.B., Júnior H.A.S., Araújo M.J., Oliveira A.P., Freitas T.B. and Viana P.T. (2017). Production and economic viability of feedlot beef cattle categories. *Acta Sci. Anim. Sci*. **39(2),** 195-199.

Santos L.V., Silva R.R., Silva F.F., Silva J.W.D., Barroso D.S., Silva A.P.G. and Santos M.C. (2019). Increasing levels of palm kernel cake (*Elaeis guineensis* Jacq.) in diets for feedlot cull cows. *Chilean. J. Agric. Res.* **79(4),** 628-635.

Satter L.D. and Slyter L.L. (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro*. *British J. Nutr.* **32(2),** 199-208.

Sheikh G.G., Ganai A.M., Reshi P.A., Bilal S., Mir S. and Masood D. (2018). Improved paddy straw as ruminant feed: A review. *Agric. Rev*. **39(2),** 137-143.

Soares F.S. and Dryden G.M. (2011). A body condition scoring system for Bali cattle. *Asian-Australasian J. Anim. Sci.* **24(11),** 1587-1594.

SPSS Inc. (2015). Statistical Package for Social Sciences Study. SPSS for Windows, Version 23.0. Chicago SPSS Inc., USA.

Suardana I.W., Sukada I.M., Suada, I.K. and Widiasih D.A. (2013). The analysis of amount and various age of productive female Bali cattle slaughtered at abbatoirs, Bali province. *J. Sain Vet*. **31(1),** 43-48.

Tahuk P.K., Budhi S.P.S., Panjono P. and Baliarti E. (2018). Nitrogen balance, microbial protein synthesis and blood metabolites in fattening of male Bali cattle fed ration with different protein levels in smallholder farms. *J. Indonesian Trop. Anim. Agric*. **43(1),** 43-53.

Tahuk P.K., Nahak O.R. and Bira G.F. (2020). The effect of complete feed to carcass characteristics and meat quality of male Bali cattle fattened in West Timor, Indonesia. *Vet. World*. **13(11),** 2515-2527.

Teixeira R.M.A., Lana R.D.P., Fernandes L.D.O., Oliveira A.S.D., Campos J.M.D.S. and Pimentel J.J.D.O. (2011). Concentrate and crude protein levels in diets for dairy Gyr lineage cows grazing elephant-grass during the rainy season. *Rev. Bras. Zootec.* **40,** 1347-1355.

Tekliye L., Mekuriaw Y. and Asmare B. (2018). Nutrient intake, digestibility, growth performance and carcass characteristics of Farta sheep fed urea-treated rice straw supplemented with graded levels of dried *Sesbania sesban* leaves. *Agric. Food Secur.* **7,** 77-85.

Ülger I., Küçük O., Kaliber M. and Ayaşan T. (2018). Chemical composition, organic matter digestibility and energy content of apple pomace silage and its combination with corn plant, sugar beet pulp and pumpkin pulp. *South African J. Anim. Sci.* **48(3),** 497-503.

Wang L., Zhang G., Li Y. and Zhang Y. (2020). Effects of high forage/concentrate diet on volatile fatty acid production and the microorganisms involved in VFA production in cow rumen. *Animals*. **10(2),** 223-231.