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The effect of dietary protein level on feed intake, digestibility and semen characteristics of Donggala Bulls

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Abstract

This experiment examined the effect of dietary crude protein (CP) level on dry matter (DM) intake, digestibility and semen characteristics of Donggala bulls. Four Donggala bull (weighing 440 ± 6.3 (SE), 36-40 months of age) were subjected to four dietary treatment containing different CP levels (11, 13, 15 and 17%) in 4x4 Latin square design. The experimental animals were fed with corn stover and mixed concentrate of 25 and 75%, respectively, from the total diet which was allocated at 3.0 % BW/day. For all dietary treatment metabolizable energy (ME) was fixed at 11.50-11.55 MJ/kg dry matter (DM). The experiment consisted of 4 runs, with one replicate (bull) per treatment level per run. After each run, bulls were randomly allocated to a different level of CP. In each run, the bulls were fed their diet in individual pens over a 14 d preliminary and 7 d collection period. Measurements of feed intake and digestibility were done during 7 day each collection period. Total DM intake was 2.81, 2.74, 2.75 and 2.78% BW/d, for Donggala bull given CP level of 11, 13, 15 and 17%, respectively. Increasing CP intake resulted in significant increase ($P<0.05$) in dry matter digestibility (DMD), semen volume, spermatozoa concentration and motility of Donggala bulls. However, increasing CP intake did not affect significantly ($P>0.05$) total DM intake, semen colour, consistency, pH, mass movement, sperm viability, mortality and abnormality. It was concluded that increased dietary CP intake increased feed digestibility and quality of semen and potentially to improve their reproductive performance of Donggala bulls.

Keywords: Donggala bull, protein level, semen quality

Introduction

Donggala (*Bos indicus*) cattle is one Indonesian native breed that has been acknowledged by Indonesian government based on the decree of Ministry of Agriculture of Republic Indonesia number 666/Kpts/SR.120/6/2014. These cattle are raised by famers in Central Sulawesi province and surroundings. Approximately, 40% of cattle population in Central Sulawesi is Donggala cattle. Recently, the government of Indonesia have identified and developed Donggala cattle in order

to conserve and to improve the genetic potential of the breed. In fact, Donggala cattle have some superiorities such as the adaptability to a hot climate and feed variation. Almost all Donggala cattle are raised by smallholder farmers with traditional management system. Under this system, Donggala cattle were given low quality forage such as corn stover as the main feed. Feeding cattle with low quality forages often associated with low feed intake and digestiblity that resulting low metabolisable energy intake. In addition, low quality forage provided a low crude protein content (less than 7%), which resulted in inadequate nutrient to support the growth and activity of microbes in the rumen.

One of the strategies to develop Donggala cattle is to select the good quality of bulls. Bulls play significant role of the beef herd since the extensive use of frozen semen in artificial insemination. Fertility of a bull is of paramount importance for the successful breeding program. It has been known that bull fertility is a low heritable trait and affected by many factors such as genetics, epigenetics and environment [1]. Other authors [2,3] noted that fertility of bull is governed by many factors including genetic, nutrition, management and environment (climate, stress, pollution and behaviour).

Protein is one of the important nutrients that influence the fertility of the bull. Several studies have documented the interrelationship between protein intake and fertility in ruminants [4,5]. Brown [6] noted that protein deficient feeding can reduce semen quality and sexual. Protein and and their deficiency may impair spermatogenesis and libido in males and fertility [4]

Nowadays, there is no information available with regard to the effect of dietary crude protein level on feed intake, digestibility and semen characteristics of Donggala bulls. This is due to new acknowledgment of Donggala cattle as Indonesia native breed. Therefore, this study was aimed to examine the effect of dietary crude protein level on feed intake, digestibility and semen characteristics of Donggala bulls.

Material and method

Sites, Animal and Experimental design

The experiment was done at Breeding Centre of Central Province located in Sidera, District of Sigi, Central Sulawesi province. Four Donggala bulls, approximately 36-40 months of age and weighing 440 ± 6.3 (SE) kg used in this experiment. They were selected and purchased from local market in Sigi and Palu districts. Bull were allocated to four dietary treatments. Before the experiment started, there was an acclimatisation phase of 14 d pre-adaptation period in which the bulls became accustomed to their supplements by gradually increasing the supplement intake and restricting the hay intake. The bulls were also injected with Ivomec (10 g/L Ivermectin, Merck and Co. Inc. White House Station, New Jersey USA) for the control of internal and external parasites at the beginning of this period.

The experimental design was 4x4 Latin Squares, with four levels of dietary crude protein namely 11, 13, 15 and 17%. In addition, the energy levels of the diets were kept equal (11.50-11.55 MJ/kg DM). The study involved 4 runs, with one replicate (bull) per treatment level per run. Thus, there were 4 replications of each level of feeding overall. After each run, bulls were randomly allocated to a different CP level. In each run, the bulls were fed their diet in individual pens over a 14 d preliminary period and 7 d collection period. The bulls were weighed at the beginning of the preliminary and collection period to adjust feed allocation. The dietary treatment was feed formulation with crude protein (CP) content of 11, 13, 15, and 17 of dry matter (DM). The feed formula was made up as a combination of several types of ingredients including corn stover (*Zea mays*), and mixed concentrate. The freshly chopped corn stover and mixed concentrate were given to each bull at 25% and 75% of the total feed on a DM base (3.0% of liveweight), respectively. The concentrate was made up of ground maize, rice bran, gliricidia leaves, copra meal, limestone, mineral mix. In addition, the proportion of mineral mixtures include calcium 165 g, phosphorus 52 g, sodium 157 g, iron 2.5 mg, copper 2.5 mg, manganese 2 g, iodine 0.125 g, cobalt 0.05 g, zinc 5 g, and selenium 0.01 g per kg. Except for

limestone and mineral, the proportion of each feed ingredient was different to create 4 treatments with varying level of CP and approximately similar ME content (11.50-11.55 MJ/kg DM). In addition, both corn stover and mixed concentrate were given twice a day in separate portions. Corn stover was given at 0700 h and 1200h while the mixed concentrate was offered at 0800h and 1300 h. Fresh drinking water was freely available at all times. The proportion of feed ingredients of concentrate in each dietary treatment and chemical composition feed ingredients given to bulls are presented in Table 1 and 2, respectively.

Table 1. The proportion of feed ingredients of concentrate in each dietary treatment

Feed ingredients (%)	Dietary crude protein level (%)			
	11	13	15	17
Ground maize	41.50	31.50	20.50	14.50
Rice bran	49.00	41.00	30.00	14.00
Gliricidia leaves	3.00	12.00	24.00	29.00
Copra meal	4.00	13.00	23.00	40.00
Limestone	0.50	0.50	0.50	0.50
Mineral mix	2.00	2.00	2.00	2.00
Total	**100**	**100**	**100**	**100**

Table 2. Chemical composition feed ingredients given to bulls during experimental period

Feed ingredient	DM (%)	OM (% DM)	CP (% DM)	NDF (%DM)	ADF (% DM)	ME (MJ/kg DM)
Corn stover	26.33	86.37	6.86	65.90	43.89	7.20[6]
Ground maize	85.03	92.88	11.22	10.65	5.18	13.50[7]
Gliricidia leaves	25.29	90.20	22.85	42.72	35.63	11.50[8]
Rice bran	87.34	91.68	14.27	44.37	28.89	13.10[9]
Copra meal	89.01	90.73	22.90	54.64	32.64	12.80[10]

DM = dry matter, OM = organic matter, CP = crude protein, NDF =neutral detergent fibre, ADF =acid detergent fibre, ME = metabolizable energy

Sampling and Measurements

Feed intake was recorded every day during each collection period, by measuring the amount of feed consumed and refused by the individual bull. Feed digestibility was measured 7 days during collection period, by calculated the data of feed intake and faecal output, which was collected from concrete floor using a plastic bucket placed behind each bulls. Daily fecal samples (5% of output)

from individual bulls and were collected and stored (-20^{o}C), then thawed and mixed at the end of fecal samples collection. A subsample from each bull was taken, dried and ground before chemical analysis. Faecal pH was measured on day 7 on each collection period.

Semen was collected from each bull using an artificial vagina for 1 ejaculation on day 7 of collection period. Immediately after collection, semen was assessed macroscopically and microscopically from each individual bull. Macroscopic assessment includes: volume, color, consistency (viscosity), and the degree of acidity (pH). Semen volume was recorded by reading from graduated tubes and sperm concentration was measured a using Neubauer haemocytometer counting chamber according to the method of the [11]. pH of semen was measured immediately after complete liquefaction of semen using digital pH meter. Microscopic assessment includes: mass movement, motility and sperm concentration, sperm viability, mortality and abnormality. The concentration of spermatozoa was measured using Hemocytometer by using fixing solution (3% sodium chloride) according to the method of [12] Sperm motility, was determined using method of [13] by examination of a drop of diluted semen under cover slip at magnification of 20X. The sperm number moving straight in forward direction in the field of microscope was recoded. Sperm viability, mortality and abnormality were measured using the method of the [11]

Chemical Analysis

Prior to chemical analysis, samples of feeds, refusal and faeces were grinded using a blender (1 mm screen). Samples were analysed for DM contents by drying representative samples to a constant weight at 70^{o}C. Ash content was determined by drying at 600^{o}C for 6 hours [14] Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis were performed according to the methods of [15] while Kjeldahl procedures were used to analyse total CP content [14]. Ether extract (EE) was determined with petroleum ether as solvent [16]

Statistical Analysis

The data obtained from DMI, DMD, faecal pH and semen characteristic of Donggala bulls were subjected to statistical analysis. This was performed with analysis of variance using Minitab 16 statistical package, and the mean differences were determined by Duncan test

Results and Discussion

The effects of dietary crude protein level on total DM intake, DMD and faecal pH are presented in Table 3. None of the bull consumed 100% of corn stover or concentrate allocation. Total DM intake ranged from 91 to 94% of total feed allocation. The hard stem of the corn stover was rejected by the bull therefore corn stover intake could not reach 100% of allocation. With the current total DM intake, the actual percentage CP intake were 10.65, 12.13, 14.03, and 15.59 (Table 3).

The dietary CP level significantly increased ($P<0.05$) the DMD of the experimental diets as presented in Table 3. The lowest DMD was found on diet with 11% of CP. Increased dietary CP level up to 15%, resulted in the significant ($P<0.05$) increase of DMD. However, further increase in dietary protein level up to 17% was not followed by significant increase ($P>0.05$) the DMD. The dietary CP level of 15% can provide a sufficient nitrogen in the rumen, therefore further increase in CP level was not followed by increase in DMD . This finding is in agreement with earlier studies [17,18]. In their study with [18] found that nitrogen digestibility increased from 71.48% with dietary CP level of 10% to 77.56% with dietary CP level 16%. These authors suggest that the addition of CP level more than 16% did not increase nitrogen digestibility.

Increasing protein intake did not affect significantly ($P>0.05$) faecal pH which ranged 6.38-6.64. High intake of grinded maize is potentially to reduced rumen pH that may cause the depression in feed digestion [19]. However, the addition of limestones in each dietary formulation may lead to stabilitation of rumen pH. In addition, the feed was offered twice a day to slow the rate of starch consumption. The faecal pH was in the normal range which indicated low

escapes of starch from rumen, and small intestine to hind gut.

Table 3. Effect of dietary crude protein (CP) level on total dry matter (DM) intake, dry matter digestibility (DMD), faecal pH, crude protein intake of Donggala bulls

Parameters	Dietary crude protein level (%)			
	11	13	15	17
Corn stover DM intake (% BW/d)	0.56±0.01	0.55±0.02	0.58±0.01	0.57±0.02
Concentrate DM intake (% BW/d)	2.25±0.05	2.19±0.05	2.17±0.04	2.21±0.05
Total DM intake (% BW/day)	2.81±0.05	2.74±0.06	2.75±0.05	2.78±0.06
% CP in diet	10.65±0.13	12.13±0.11	14.03±0.09	15.59±0.13
CP intake (%BW/day)	0.34±0.01	0.38±0.02	0.43±0.01	0.48±0.01
ME intake (MJ/kg DM)	11.55±	11.50±	11.55±	11.50±
DMD (%)	62.27±1.59^{a}	67.76±1.26^{b}	71.04±2.47^{c}	70.96±4.45^{c}
Faecal pH	6.35±0.11	6.37±0.12	6.78±0.07	6.67±0.14

Means with different superscripts in same raw are significantly different ($P < 0.05$)

Table 4 show the effect of dietary crude protein level on semen characteristics. The characteristics of colour, consistency, pH, mass movement, sperm viability, mortality and abnormality were not influenced significantly ($P>0.05$) by increasing CP intake. However, semen volume, sperm concentration, motility in experimental Donggala bulls were significantly affected ($P<0.05$) by dietary CP intake.

Semen volume of Donggala bull increased from 5.42 ml at dietary CP level of 11%, to 7.64 ml at dietary CP level of 15%, and no further increase in semen volume at dietary CP level of 17%. There is no study concerning the direct relationship between dietary CP level and semen volume in cattle. However, the current study showed that increasing the dietary CP level up 15% increased significantly ($P<0.05$) the semen volume of Donggala bull. Semen volume is one of the important parameters in semen evaluation and reproduction performance in the males [20]. Previous studies have demonstrated that increasing protein intake led to the increase of the spermatogenesis. This effect has been associated with the increase in testicular size which is mainly due to an increase in the volume of seminiferous epithelium and in the diameter of seminiferous tubules [21,22] The results presented in this work confirm that sperm volume per ejaculate, was affected by increased CP level in the diet. This result is consistent with the study of [5]. The semen volume of Donggala bull is categorised as the normal volume recommended by [23] which ranged 5-8 ml.

Table 4. The effect of dietary crude protein level on macroscopic and microscopic characteristics of Donggala bulls

Parameters	Dietary crude protein level (%)			
	11	13	15	17
Macroscopic				
Volume (ml)	5.42±0.52[a]	6.83±0.14[b]	7.64±0.41[c]	6.98±0.24[c]
Colour	White	White	White	White
Consistency	Viscous/thick	Viscous/thick	Viscous/thick	Viscous/thick
pH	6.47±0.06	6.52±0.10	6.56±0.12	6.64±0.17
Microscopic				
Concentration (10^6/ml)	606.75±0.01[a]	636.25±0.01[b]	678.52±0.01c	703.53±0.02[c]
Motility (%)	72.75±0.48[a]	75.25±0.48[a]	77.50±1.71[b]	82.00±1.68[b]
Mass movement*	++	++	++	+++
Viability (%)	73.75±1.03	74.75±0.85	74.50±1.04	73.50±1.19
Mortality (%)	19.25±0.85	18.75±0.75	19.50±1.19	18.75±0.48
Abnormality (%)	2.50±0.29	2.50±0.25	2.50±0.29	2.25±0.48

*(-) poor, (+) medium, (++) good, (+++) very good

Means with different superscripts in same raw are significantly different ($P < 0.05$)

The dietary CP level did not affect significantly ($P<0.05$) semen colour, pH and mass activity, sperm viability, mortality and abnormality (Table 4). In the present study the color of all semen samples was white, creamy with normal appearance. This is consistent with the finding of Rehman et al [24] who reported that normal color of bull semen is white to creamy white.

The pH of the semen of Donggala bulls given feed with different CP level in this study ranged from 6.47 to 6.64. This data indicates that the seminal pH range was in a normal condition [25]. Mass activity found (2.25) in the current study was slightly lower than the value reported in the previous studies (2.65, in Nili Ravi bulls [26] and 2.54 in Indian bulls [2]. This lower value may be attributed to the effect of warm climatic conditions, in which sperm might be less active due to high temperature.

Increased CP intake was significantly increased sperm motility (Table 4). This is consistent with the finding of the previous study [28]. Sperm concentration increased significantly ($P<0.05$) due to increased CP level in the diet (Table 4). Singh et al., [29] reported that increasing CP level in the diet was associated with the increase of the motility of spermatozoa then followed with the increase in sperm concentration.

Sperm viability, mortality and abnormality of Donggala bull in the current experiment were not significantly affected ($P>0.05$) by the dietary CP level with mean value 74.13, 19.06 and 2.44%, respectively. These values indicated that the semen quality met the standard of good quality semen. Hafez [30] noted that when sperm mortality less than 20% is categorised as a good quality semen The value of semen abnormality was low compared with other studies of [31] and [32] with value of 14.1 and 15.9%, respectively.

Generally, the sperm abnormality is not related the diet. Santos et al. [28] suggested that other factors such as the environment, semen processing and human error often influence the sperm abnormality.

Conclusions

It was concluded that increasing dietary protein level from 11% to 15% of Donggala bulls increased significantly feed digestibility, CP intake, semen volume, sperm concentration, sperm motility. No further increase was detected at dietary CP level above 15%. However, the dietary CP level did not affect faecal pH, semen colour, consistency, pH and sperm viability, mortality and abnormality of Donggala bulls. It is strongly recommended that dietary CP level of Donggala bull was provided up to 15% in order to improve the bull reproductive performance.

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