The effect of supplementation with different protein source on feed intake, digestibility and semen characteristics of Donggala Bulls given corn stover and rice bran

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Abstract—**The effects of supplementation with different protein sources on intake, digestibility and semen characteristics of Donggala bulls fed corn stover and rice bran were assessed. Four Donggala bulls were used, with initial average weight 425±5.9 kg. The experiment consisted of 4 experimental periods, according to a 4x4 Latin square design. There were 4 dietary treatments include (1) corn stover (CS) ad libitum plus rice bran (RB) (0.5% body weight (W)/day (CSRB) (2) CSRB plus gliricidia (0.5% W/day) (CSRBG) (3) CSRB plus palm kernel meal (PKM, 0.5% W/day) (CSRBPKM) (4) CSRB plus copra meal (CM, 0.5%W/day) (CSRBCM). Bulls were randomly allocated to a different treatment after each period. In each period consisted of 14 and 7 days for adaptation and measurement period, respectively. Dry matter intake (DMI), dry matter digestibility (DMD) and semen characteristics were measured during 7 day each collection period. TDMI, DMD and some semen characteristics were significantly affected (P<0.05) by different protein supplementation. CM supplementation resulted in the highest increase (P<0.05) in TDMI, DMD, semen volume, spermatozoa concentration and motility of Donggala bulls. However, supplementation with different supplement source did not affect significantly (P>0.05) semen colour, consistency, pH, mass movement, sperm viability, mortality and abnormality. It was concluded that inclusion CM as source of protein in addition with energy source can increase feed intake, digestibility and semen quality of Donggala bull fed low quality forage.**

Key words : Donggala bull, protein source, semen quality

I. INTRODUCTION

Donggala cattle (*Bos indicus*) are one of Indonesian native breed which are spread out in Central Sulawesi province and its surroundings. This breed has been acknowledged by Ministry of Agriculture of Republic Indonesia since 2014 with decree number 666/Kpts/SR.120/6/2014. As a new native breed, its existence needs to be preserved and developed. Most of the Donggala cattle are raised by smallholder farms with traditional management system or reared extensively. In general, the cattle only get feed based on native grass or agricultural byproducts. Under these conditions, the growth and production performance of Donggala cattle are often below their genetic potential.

In rearing cattle, feed plays an important role because about 60–70% of production costs come from feed. Single feeding in the form of grass or agricultural byproduct alone, has not been able to meet the nutritional needs of both the rumen microbes and the livestock themselves, so other feed ingredients are still needed as a complement [1]. Cattle given low quality forages is often associated with low feed intake and digestiblity that resulting in inadequate nutrients to support the growth and activity of microbes in the rumen, other production and reproduction performance. For this reason, it is necessary to provide feed supplement to meet animal nutrient requirements and production targets.

One of the key strategies to preserve and develop Donggala cattle is to select the good quality of bulls. This is because the good quality bulls can produce good offspring through the extensive use of frozen semen in artificial insemination. In order to be success in

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breeding program of Donggala cattle, the fertility of a bull need to be maintained. In fact, many factors affect bull fertility including genetics, epigenetics and environment [2]. In addition, some authors [3-4] suggested that nutrition and management also play important role in affecting fertility of bull.

One of the nutritional needs of cattle that must be provided to maintain the fertility of bull is protein. Previous studies [5-7] reported the effect of protein intake on fertility in ruminants. They documented that ruminants with protein deficiency had low semen quality. Smith and Akinbamijo [6] noted that ruminants with protein deficiency had low libido and fertility. The appropriate feed source containing protein in animal feed will be able to increase microbial protein synthesis in the rumen so that feed absorption becomes better and more efficient [8] However, the price of feed containing high protein is relatively expensive, especially at farmer level. The use of locally available feed resources containing high in protein such as gliricidia, palm kernel meal and copra meal could help farmers in maintaining the fertility Donggala bulls at village level.

Nowadays, there is limited information available regarding to the effect of different protein source on feed intake, digestibility and semen characteristics of Donggala bulls. This is because Donggala cattle are relatively new acknowledged from Indonesian government as native breed. Hence, this study was done to examine the effect of different protein source on feed intake, digestibility and semen characteristics of Donggala bulls.

II. MATERIAL AND METHOD

2.1. Sites, Animal and Experimental design

The experiment was undertaken at Animal Breeding Centre of Central Province located in Sidera village, District of Sigi, Central Sulawesi province.

Four Donggala bulls, with the mean initial weight 425 ± 5.9 kg (mean and SE) kg used in this experiment. The bulls were randomly allocated on a stratified unfasted weight basis to treatment. Before the experiment started, the bulls were dewormed against internal and external parasites with Ivomec (10 g/L Ivermectin, Merck and Co. Inc. White House Station, New Jersey USA). The experimental bulls were penned individually during all experimental period and introduced feed supplements during 14 d pre-adaptation period.

The experimental design was 4x4 Latin Squares, with four dietary treatment namely (1) corn stover (CS) ad libitum plus rice bran (RB) (0.5% body weight (W)/day (CSRB) (2) CSRB plus gliricidia (*Gliricidia sepium*) (G, 0.5% W/day) (CSRBG) (3) CSRB plus palm kernel meal (PKM, 0.5% W/day) (CSRBPKM) (4) CSRB plus copra meal (CM, 0.5%W/day) (CSRBCM). The experiment involved 4 periods, so there were one replicate (bull) per treatment per period. Thus, overall, there were 4 replications of each dietary treatment. After the completion each period, bulls were randomly allocated to a different dietary treatment. Each run lasted 21 days, consisting of a 14 days adaptation period followed by 7 days of collection. The liveweight of the bulls were recorded at the commencement of the preliminary period and then every seven days including the beginning and completion of the collection period. Feed supplement (RB and different protein sources) allocation was determined by the liveweight at the beginning of the preliminary period and then adjusted just prior to the collection period. Corn stover was chopped into length of 5-10 cm, and offered individually ad libitum twice a day at 08.30h and 14.30h with offered amount adjusted to give 10-15% refusal. Rice bran and protein source were given separately to the CS in plastic container placed in the feed bin and offered individually twice daily at 0700 h and 1200h. Water for drinking was freely available at all times. Composition feed used foe experiment is presented in Table 1.

Table 1. Chemical composition of experimental feed ingredients given to Donggala bulls during experimental period

Feed ingredient	Nutrient content					
	DM (%)	OM (% DM)	CP (% DM)	NDF (%DM)	ADF (% BK)	EM (MJ/kg DM)
Corn stover	28.63	90.17	8.21	65.46	46.12	7.90 [9]
Rice bran	89.17	92.83	12.73	43.19	26.71	13.10 [10]
Gliricidia	28.24	91.23	20.41	44.06	36.12	11.50 [11]
Palm kernel meal	90.06	92.64	17.19	63.72	36.84	11.60 [12]
Copra meal	90.12	91.47	21.76	60.16	42.17	12.80 [13]

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

2.2. Sampling and Measurements

Feed intake was measured daily during experimental period by recording the amount of feed offered and refused. Daily sub-samples of CS, RB, Gliricidia, PKM, CM were collected and bulked for DM, OM, CP, NDF, ADF and EE analysis. Feed digestibility was calculated from feed intake and faecal data. The daily faecal collection for each bull was mixed, and a 5% aliquot was taken, frozen and stored (-20^{o}C). At the end of the collection period, these samples were thawed, bulked, mixed and a sub-samples were taken for each bull for DM analysis.

Semen from each bull was collected on day 7 of collection period using an artificial vagina. Immediately after collection, semen from each bull was tested macroscopically and microscopically. The parameters measured from semen macroscopic include: volume, color, consistency (viscosity), and the degree of acidity (pH). Semen volume was recorded by reading from graduated tubes. The measurement of sperm concentration was done using Neubauer haemocytometer counting chamber based on the method of the [14]. The measurement of semen pH was carried out immediately after complete liquefaction of semen using digital pH meter. The parameters recorded from semen microscopic include: mass movement, motility and sperm concentration, sperm viability, mortality and abnormality. The detemination of spermatozoa concentration was conducted using Hemocytometer using the method of [15] Sperm motility, was measured using method of [16] by examination of a drop of diluted semen under cover slip at magnification of 20X. Sperm viability, mortality and abnormality were determined according to the method of [14]

2.3. Chemical Analysis

Prior to the chemical analysis, samples of feeds, refusal and faeces were grinded using a blender (1 mm screen). Samples of feed offer, refusal, faeces were dried for constant weight in an oven at 70^{o}C for 3-4 days to determine DM content. Ash content was determined by combusting approximately 1 g of oven dried ground sample in a furnace at 600^{o}C for 6 hours. OM content was determined as 100-ash (%) [17] Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis were done according to the methods developed by [18] The kjeldahl procedure was used to analyse total CP content [17] Ether extract (EE) was determined with petroleum ether as solvent [19]

2.4. Statistical Analysis

The experimental (DMI, DMD, and semen characteristic of Donggala bulls) were subjected to analyses of variance using Minitab 16 statistical package and the mean differences were determined by Duncan test

III. RESULTS AND DISCUSSION

The effect of supplementation with different protein sources on feed intake and digestibility

The effects of supplementation with different protein sources on total DM intake, DMD of Donggala bull fed CS and RB and are presented in Table 2. More than 90% of supplements (rice bran and protein sources) allocation were eaten by bulls. Total DMI increased significantly (P<0.05) in association with addition of protein. Bull supplemented with CM had the highest (P<0.01) total DMI, whereas bull without protein supplementation had the lowest (P<0.05) total DMI. Previous studies [20-21] suggested that increased total DMI caused in associated with increased protein supplementation were due to increased digestibility, and reducing the gut fill effect on reticulorumen.

Protein supplementation significantly increased (P<0.05) the DMD of the experimental diets as presented in Table 2. The lowest DMD was found on diet without protein supplementation. Addition of CM to bull fed CS and RB, resulted in the highest increase (P<0.05) in DMD. This finding is in agreement with earlier studies [22-23] In their study with Kivircik lambs, Keser and Bilal [22] recorded that addition of CP in the diet from 10% to 16% increased nitrogen digestibility from 71.48% to 77.56%. Yasser et al. [23] found that addition of protein in the diet increased the digestibility of CP, EE and CF for lambs. The highest DMD of CM supplemented bull was presumably a result of a higher digestible substrate supply, such as carbohydrate and protein in supplemented bull compared to other treatments.

Table 2. Effect of different protein source on total dry matter intake (DMI), dry matter digestibility (DMD), of Donggala bulls fed corn stover and rice bran

Parameters	Dietary treatments			
	CSRB	CSRBG	CSRBPKM	CSRBCM
Corn stover DMI (% W/day)	2.37 ± 0.05	2.24 ± 0.01	2.30 ± 0.02	2.61 ± 0.05
Rice bran DMI (% W/day)	0.45 ± 0.16	0.49 ± 0.22	0.48 ± 0.17	0.48 ± 0.33
Gliricidia DMI (% W/day)	0.00±0.00	0.48±0.10	0.00±0.00	0.00±0.00
PKM DMI (% W/day)	0.00 ± 0.00	0.00 ± 0.00	0.48 ± 0.11	0.00 ± 0.00
CM DMI (% W/day)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.47 ± 0.03
Total DMI (% W/day)	2.82 ± 0.05[a]	3.21 ± 0.18[b]	3.26 ± 0.21b	3.56 ± 0.12[c]
DMD (%)	56.88 ±3.98[a]	62.24 ± 0.31[b]	63.04 ±1.72b	66.34 ±2.85[c]

CSRB = Corn stover *ad libitum* + rice bran (0.5% W/day)
CSRBG = corn stover *ad libitum* + rice bran (0.5% W/day) + Gliricidia(0.% W/day))
CSRBPKM = corn stover *ad libitum* + rice bran (0.5% W/day)+ palm kernel meal (0.5% W/day)
CSRBCM= corn stover *ad libitum* + rice bran (0.5% W/day) + copra meal (0.5% W/day)
Means with different superscripts in same raw are significantly different (P < 0.05)

The effect of supplementation with different protein sources on semen characteristic of Donggala bulls fed corn stover and rice bran

Table 3 show the effect of supplementation with different protein sources on semen characteristics of Donggala bulls fed CS and RB. Semen characteristics such as colour, consistency, pH, mass movement, sperm viability, mortality and abnormality were not affected significantly (P>0.05) by protein supplementation. However, other characteristics such as semen volume, sperm concentration, motility of Donggala bulls were significantly influenced (P<0.05) by the addition protein in the diet with CM supplemented bull the highest value.

Semen volume of Donggala bull increased from 5.39 ml at control diet (no protein supplementation), to 5.98; 6.04 and 6.80 ml for bull supplemented with gliricidia, PKM and CM respectively. The current study showed highest increase in semen volume was found in Donggala bull supplemented with CM. Ax et al. [24] noted that semen volume is one of the important parameter in the evaluation of reproduction performance of bull. Earlier works have demonstrated that protein supplementation led to the increase of the spermatogenesis. This is in association with the increase in testicular size as a result of the increase in the volume of seminiferous epithelium and in the diameter of seminiferous tubules [25-26] The results in the present study confirm that sperm volume per ejaculate, was affected protein supplementation [7] The semen volume of Donggala bull in the current study was within the normal range 5-8 ml as recommended by [27]

The protein supplementation did not affect significantly semen colour, pH and mass activity, sperm viability, mortality and abnormality (Tabel 3). The semen color collected from all bulls were white, creamy with normal appearance. This semen colour is consistent with data found in the previous study by [6] These author documented 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 [28] 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 [29] and 2.54 in Indian bulls [30]. This lower value may be attributed to the effect of warm climatic conditions, in which sperm might be less active due to high temperature.

Protein supplementation was significantly increased sperm motility (Table 4). This is consistent with the finding of the previous study [31]. Sperm concentration increased significantly ($P<0.05$) due to increased CP level in the diet (Tabel 4). Singh et al. [32] reported that increasing CP 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 protein supplementation. Mean value of semen viability, mortality and abnormality for all treatments were 72.65, 18.23 and 2.59%, respectively. The current values indicated that the semen quality of experimental bull is good quality semen. Previous worker [33] suggested that when sperm mortality is less than 20%, it is categorised as a good quality semen The abnormality level of semen of the current bull was lower than other studies reported by [34] and [35] who noted an abnormality value of 14.1 and 15.9%, respectively. Infacr, the sperm abnormality is not related to the ration. Santos et al., [33] recommended such factors such as the environment, semen processing and human error are often associated with the sperm abnormality.

Table 3. The effect of supplementation with different protein source on semen characteristics of Donggala bulls fed corn stover and rice bran

Parameters	Dietary treatments			
	CSRB	CSRBG	CSRBPKM	CSRBCM
Macroscopic				
Volume (ml)	5.39 ± 0.12^{a}	5.98 ± 0.23^{b}	6.04 ± 0.33^{b}	6.80 ± 0.31^{c}
Colour	white	white	white	white
Consistency	Viscous/thick	Viscous/thick	Viscous/thick	Viscous/thick
pH	6.44±0.02	6.92±0.15	7.11±0.09	6.82±0.02
Microskopic				
Concentration (10^6/ml)	590.23 ± 0.02^{a}	602.31 ± 0.02^{b}	623.50 ± 0.02^{b}	742.86 ± 0.01^{c}
Motility (%)	71.29 ± 0.23^{a}	74.33 ± 0.29^{b}	75.30 ± 1.26^{b}	79.06 ± 0.28^{c}
Mass movement*	++	++	++	+++

Viability (%)	72.37±2.23	71.39±1.22	72.38±1.04	74.49±0.23
Mortality (%)	18.30,±1.72	19.87±0.83	18.46±1.31	16.32±1.47
Abnormality (%)	2.35±0.33	2.84±0.76	2.84±0.29	2.33±1.22

CSRB = Corn stover *ad libitum* + rice bran (0.5% W/day)
CSRBG = corn stover *ad libitum* + rice bran (0.5% W/day) + Gliricidia(0.% W/day))
CSRBPKM = corn stover *ad libitum* + rice bran (0.5% W/day)+ palm kernel meal (0.5% W/day)
CSRBCM= corn stover *ad libitum* + rice bran (0.5% W/day) + copra meal (0.5% W/day)
*(-) poor, (+) medium, (++) good, (+++) very good
Means with different superscripts in same raw are significantly different ($P < 0.05$)

IV. CONCLUSIONS

It can be concluded that supplementation with copra meal led to improve total intake, digestibility, semen volume, sperm concentration, sperm motility of Donggala bull fed corn stover and rice bran. Copra meal is better protein source to feed Donggala bull as compared to other sources of protein such as gliricidia and palm kernel meal.

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