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Metabolic Benefit of Bulls Being Fed Moringa Leaves Twigs and Branches as a Major Concentrate Ingredient

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The study was conducted to investigate nutrient metabolism and semen quality of bulls fed with moringa (*Moringa oleifera*) leaves, twigs, and branches as a major concentrate ingredient. Twenty-one Red Chittagong bulls of about 204 (±50) kg initial live weight (LW) were randomly divided into three equal LW groups. They were fed maize silage as a basal feedstuff for 65 days with the supplementation of concentrate mixtures at 1% of LW, consisting of either 0, 25, or 50% moringa mash on a fresh basis. Moringa mash was a sun-dried ground preparation of leaves, twigs, and branches of moringa. The results indicated that different levels of moringa in concentrate mixtures (0, 25, and 50%) did not change daily DM intake, digestibility, and LW gain of bulls ($p > 0.05$). However, increasing dietary moringa (up to 203 g/kg DM) significantly decreased production cost of methane (CH_4) (methane emission [kg/kg gain] = 1.6422−[0.0059 × moringa intake, g/kg DM], $n = 12$, $R^2 = 0.384$, $P = 0.032$) in a similar metabolizable energy intake level (0.21 ± 0.01 MJ/kg LW). Also, higher dietary moringa significantly reduced urinary nitrogen loss (urinary nitrogen [% digested nitrogen] = 43.0 – 0.069 × moringa intake [g/kg DM]; $R^2 = 0.3712$, $P = 0.034$). Thus, increasing moringa by 1 g/kg DM decreased CH_4 emission by 6 g/kg gain and absorbed nitrogen loss by 0.069 %. Also, progressive motility of sperm increased significantly (33.0, 51.0, and 60.1%, respectively; $p = 0.03$) in bulls fed with concentrate mixtures containing moringa at 0, 25, or 50%. It may be concluded that feeding moringa mash at 203 g/kg DM may decrease energy loss as methane and urinary nitrogen loss without impacting the production of beef cattle. Feeding moringa mash to beef cattle may abate dietary energy and nitrogen loss and consequently decrease the environmental pollution.

Keywords: bioenergetics, feed efficiency, methane conversion factor, moringa, nutrient metabolism

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INTRODUCTION

Dietary nutrient loss, particularly energy, and nitrogen, from beef cattle feeding may determine the level of the environmental impact of production. For example, anaerobic fermentation of feedstuff in the rumen of Zebu beef cattle in tropical developing countries fed with low-quality crop residues and byproducts incurs about 4.8–13.7% of dietary gross energy (GE) loss as methane (CH_4) production (Kaewpila and Sommart, 2016). The amount of volatile solids (VS) in manure, as determined by the manure energy content (fecal and urinary energy loss) and dietary organic matter (OM) level, may undergo anaerobic conditions and emit CH_4 at varying rates according to different manure management systems in different environmental temperatures (IPCC, 2019). Similarly, manure nitrogen may undergo microbial decomposition and emit N_2O by nitrification and denitrification processes. Also, organic manure nitrogen (urea in mammals) tends to be mineralized as ammonium nitrogen and converts to NH_3. Emissions of such greenhouse gases (CH_4 and N_2O) from farm animal production are a global concern for their substantial climate change impacts. Global livestock sector emission contributes to about 18% of anthropogenic greenhouse gas annually, leading to global warming (Gerber et al., 2013). In Bangladesh, livestock greenhouse gas emission was estimated to be about 70×10^3 Gg/year carbon dioxide equivalent (Das et al., 2020).

Mitigation of greenhouse gas emission from livestock entails increasing dietary efficiency. Increasing dietary nutrient utilization in animal production (particularly energy and protein) may minimize their unproductive wastes and subsequent CH_4 and N_2O emissions to the environment. There may be lower need for conversion of dietary GE to CH_4 when a diet with more digestible ingredients is fed to cattle (Kurihara et al., 1999; Liu et al., 2017). Therefore, dietary strategies were reported to be effective in reducing enteric CH_4 emissions in ruminants (Kebreab et al., 2010; Gastelen et al., 2019; Min et al., 2020). Regarding this, Benchaar et al. (2001) quantified that dietary manipulation may reduce up to 40% of enteric CH_4 emissions. Knapp et al. (2014) registered that improving feeds, feeding, and nutritional approaches may reduce up to 15% of enteric CH_4 emissions in dairy cattle production. An efficient diet may also produce less manure nitrogen and thus less anaerobic fermentation or aerobic decomposition of nitrogen to emit CH_4, N_2O, and NH_3 into the air.

Efforts to reduce dietary energy loss as CH_4 include supplementation of diet with fats (<5%) (Johnson and Johnson, 1995), organic acids (Castillo et al., 2004), plant secondary metabolites (Beauchemin et al., 2008), essential oils (Tamminga et al., 2007), and probiotics, ionophores, antibiotics, and so on (Su and Chen, 2020). In this context, leaves, foliage, and pods of moringa (*Moringa oleifera*), which are rich in secondary metabolites (Premi and Sharma, 2017; Su and Chen, 2020) and have the potential to reduce rumen CH_4 production and gain in nutrient metabolism and animal production, were found promising. Dong et al. (2019) found that supplementing dairy cattle diet with 6% moringa (rachises and twigs) changed the composition and diversity of fecal methanogens (lower count of *Methanobrevibacter ruminantium*, a methanogenic bacteria), indicating modification of rumen microbiomes and producing less enteric CH_4. Soliva et al. (2005) found 17% reduction of enteric CH_4 emission *in vitro* and reported moringa leaves to be an inhibitor of methanogens and as an alternative to antibiotic feed additives of cattle. Another *in vitro* study registered up to 50% reduction of rumen CH_4 by replacing soybean meal with moringa leaf meal (Elghandour et al., 2017). A linear reduction of rumen CH_4 was registered in cattle when supplementation of moringa seeds in the concentrate mixture was increased up to 40% (Lins et al., 2019).

In addition to CH_4 emission reduction, improvement in digestion and utilization of nutrients of concentrate mixtures (*in vitro*) was found when conventional ingredients were replaced with moringa leaves at 25–50% level (Nouala et al., 2006). Greater utilization of dietary nutrients was reported when 75% of berseem clover diet of Nubian goats was replaced with moringa leaves (Kholif et al., 2018). Consequently, supplementing moringa leaves and their extracts in diets increased the quality of goat meat (Qwele et al., 2013), presumably because of its abundant secondary metabolites, vitamins, flavonoids, phenols, and carotenoids (Su and Chen, 2020). Considering biomass yield, CH_4 emission reduction efficiency, animal production efficiency, and cost-benefit ratio, moringa was ranked on top of common forages in Bangladesh (Huque et al., 2017).

Along with growth performances, carotenoids, and vitamin E in moringa leaf (Qwele et al., 2013; Su and Chen, 2020) may help promote the reproduction of animals and maintain various physiological functions of bones, epithelial tissues, visceral, and mucosal epithelial secretions, and cellular immunity by protecting cells from harmful free radicals. Greater litter size, birth weight, and survival in mice were registered when a normal diet was supplemented with 4% moringa leaf (Zeng et al., 2019). When rice straw was replaced with 3% moringa leaves from the diet of Bali bulls (0.15% LW), the libido and progressive sperm motility was found to increase significantly (Syarifuddin et al., 2017). Therefore, the objectives of the study were to investigate the efficiency of dietary nutrient utilization and semen quality of bulls fed with moringa leaves, twigs, and branches as a major ingredient in the concentrate.

MATERIALS AND METHODS

Study Location and Ethical Statement

The study was conducted at Cattle Research Farm of Bangladesh Livestock Research Institute (BLRI), Savar Dhaka, Bangladesh (latitude 23.89°N, longitude 90.27°E). During the feeding trial in bulls (September-November, 2019), the average air temperature and humidity were 28(±3)°C and 73(±6)%, respectively. The care and management of experimental bulls were in accordance with the procedures of Curtis and Nimz (1988) and approved by the Annual Research Evaluation Committee of BLRI (2019).

Production of Moringa Mash

Moringa mash was produced by collecting leaves, twigs, and branches (2–3 cm) from a previously established plot at BLRI Cattle Research Farm, Savar, Dhaka in May–June 2019. The collected biomass was mechanically chopped into pieces (3–5 cm) and dried in sun for about 24–32 h (3–4 days). The dried biomass was ground in an electric grinder by passing through a 3-mm sieve, and the moringa mash thus produced was stored in plastic drums until feeding to animals. The stored moringa mash was used to produce different concentrate mixtures according to their fresh ingredient composition (**Table 2**), similar to conventional ones.

Ensiling of Maize

The maize (*Zea mays*) was harvested at 85 days of cultivation in April 2019, chopped into 2–3 cm pieces, and ensiled in a pit at the BLRI Cattle Research Farm. No additives and fermenters were

TABLE 1 | Composition of diets.

Concentrate ingredient (% fresh)	CM$_0$	CM$_{25}$	CM$_{50}$	Moringa mash	Maize silage
Wheat bran	37	23	20	–	–
Ground maize	30	24	7	–	–
Soybean meal	30	25	20	–	–
Moringa	0	25	50	–	–
Common salt	1	1	1	–	–
Dicalcium phosphate	2	2	2	–	–
Total	100	100	100	–	–
Chemical composition (% DM)					
DM (% fresh)	90.4	91.2	90.1	87.6	22.3
OM	91.0	91.2	91.2	89.9	90.5
CP	19.6	19.5	19.4	13.4	8.0
NDF	34.4	34.5	35.7	44.6	50.3
ADF	19.5	20.2	21.1	32.7	29.1
Hemicellulose	14.7	14.4	14.9	11.9	21.2
GE	20.1	17.2	16.2	16.1	18.7

CM$_0$, concentrate mixture containing 0% moringa mash; CM$_{25}$, concentrate mixture containing 25% moringa mash; CM$_{50}$, concentrate mixture containing 50% moringa mash; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; GE, gross energy.

TABLE 2 | Nutrient intake and live weight changes in bulls.

Parameters	Dietary groups			SEM	*P*-values
	CM$_0$	CM$_{25}$	CM$_{50}$		
DM intake, kg/d	5.0	5.4	5.0	0.20	0.598
DM intake, % LW	2.3	2.5	2.3	0.05	0.245
DM intake from maize silage, kg/d	2.8	3.2	2.9	0.12	0.417
Concentrate intake, % DM intake	43.0	41.5	42.8	0.54	0.477
Moringa intake, % LW of bulls	0.0^{c}	0.3^{b}	0.5^{a}	0.05	<0.01
Moringa intake, g/kg DM intake	0.0^{c}	104.0^{b}	214.0^{a}	17.57	<0.01
OM intake, g/kg DM intake	908	908	908	0.10	0.177
CP intake, g/kg DM intake	130	128	129	0.62	0.366
NDF intake, g/kg DM intake	440	438	435	0.94	0.073
ADF intake, g/kg DM intake	257^{a}	254^{b}	250^{c}	0.75	<0.01
Hemicellulose intake, g/kg DM intake	184	183	185	0.41	0.251
GE intake, MJ/kg DM	18.5^{a}	18.1^{a}	17.6^{b}	0.08	<0.01
Initial LW, kg	204	208	200	10.81	0.962
Final LW, kg	239	244	237	11.15	0.974
Gain, g/d	541	550	572	0.02	0.792

CM$_0$, concentrate mixture containing 0% moringa mash; CM$_{25}$, concentrate mixture containing 25% moringa mash; CM$_{50}$, concentrate mixture containing 50% moringa mash; LW, live weight; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; GE, gross energy.

SEM, standard error of mean; $P < 0.05$, significant.

^{abc}means with different superscripts within same raw are significantly different.

used in the ensiling process. The silage was fed to trial bulls as a basal feedstuff to appetite in September–November 2019.

Selection and Management of Bulls

Twenty-one bulls of about 25–32 months of age were selected from a large Red Chittagong Cattle (RCC) herd at BLRI, Savar, Dhaka, and weighed at 700 h before morning feeding. They were housed individually in concrete stalls (1.0 × 2.5 m^2) where there was 24 h supply of adequate clean drinking water. They were fed maize silage with a supplementation of a conventional concentrate mixture (CM$_0$, **Table 1**), representing 1% live weight (LW) for a 15-day adjustment period. Maize silage was weighed and supplied in two equal parts at 900 and 1,600 h daily. About 20% extra maize silage was supplied than the intake of the previous day to ensure *ad libitum* intake. The concentrate mixture was fed about 30 min before feeding silage, and no refusals of concentrate mixtures were found. The daily supply and refusal of maize silage and intake of concentrate mixture were recorded. During adjustment, they were dewormed by drenching with an anthelmintic drug (Trilev-Vet® Bolus, Square Pharmaceuticals Limited, Bangladesh) according to prescribed doses. After adjustment, they were weighed (initial LW 204 ± 50 kg) at 700 h before morning feeding, divided into three equal LW groups, and fed experimental diets for 65 days.

Feeding Management of Bulls

After the adjustment period, bulls were weighed fortnightly before morning feeding (700 h) to adjust their daily concentrate mixture allowances (1% LW). During the whole feeding trial (after adjustment), CM_0 concentrate mixture was supplemented to bulls of the control group, whereas concentrate mixtures containing either 25 or 50% of moringa mash (CM_{25} and CM_{50}, respectively; **Table 1**) were supplemented to bulls of other groups. All the concentrate mixtures were iso-nitrogenous (**Table 1**). The concentrate mixtures were produced weekly according to their ingredient composition, and a representative portion of samples (about 250 g) were kept in air-tight sample bags and stored in a deep freeze (−20°C) until analysis. The ingredient composition of concentrate mixtures and chemical composition of maize silage and concentrate mixtures are presented in **Table 1**.

Metabolism Trial

On the 51st day of the feeding trial, four bulls from each group were weighed and transferred to metabolic crats individually to study digestibility and metabolism of nutrients. Before the collection period (7 days), bulls were given a 7-day adjustment period to feeding and management in metabolic crats. The supply of feeds and refusals were recorded as described earlier. The overnight fasted LW of bulls were taken before and at the end of the collection period. The feces and urine samples were weighed and recorded at 700 h daily. Feces were collected in a plastic bin with a lid. Feces collected every 24 h were weighed, mixed thoroughly, and about 10% of samples were kept in properly labeled air-tight sample bottles. A portion of the fresh sample (about 20 gm) was used in determining DM, whereas the remaining was kept frozen (−20°C) for further laboratory analysis. The urine was collected into a plastic bucket containing 200 ml of 20% H_2SO_4 (v/v), weighed, diluted to 20 L by adding fresh clean tap water, kept in properly labeled sample bottles (100 ml), and stored in a deep freeze (−20°C) until analysis. In the end, samples of feedst offered, refusals, and feces were thawed to room temperature; aliquots for each bull were pooled and mixed thoroughly. The subsamples (about 2.5 kg) were dried in a forced air oven at 60°C for 72 h and ground by passing through a 1-mm sieve to prepare them for further laboratory analysis. The urine samples of each bull were mixed in a bucket and composited, and a subsample (about 100 ml) was taken in a properly labeled sample bottle and sent to the laboratory for analysis.

Chemical Analysis of Samples

The dry matter (DM), ash, and crude protein (CP) or nitrogen of feeds, concentrate mixtures, feed refusals, and feces were determined according to methods described by the AOAC (2006). Briefly, the methods of determining DM, ash, and CP (or nitrogen) were methods 934.01, 934.05, and 981.10, respectively. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following the methods of Van Soest et al. (1991). The GE contents of feeds, refusal, and feces were determined in a Shimadzu auto-calculating bomb calorimeter (Shimadzu CA-4PJ, Shimadzu Corporation, Japan).

Calculations

The GE intake was calculated as the difference between GE supplied and refused in orts. The digestible energy (DE) intake was calculated as the difference between GE intake and fecal energy (FE) outgo. Urinary energy (UE) excretion was calculated according to Ramin and Huhtanen (2013) by the following equation:

$$\begin{aligned}\text{UE (MJ/d)} &= -2.71 + 0.028 \times \text{CP (g/kg DM)} \\ &\quad +0.589 \times \text{DMI(kg/d)}.\end{aligned}$$

The enteric methane conversion factor (Y_m, % GE intake) was calculated according to the following equation (Jaurena et al., 2015):

$\text{Y}_\text{m} = 2 - 0.243 \times \text{DMI} + 0.0059 \times \text{NDF} + 0.0057 \times \text{DDM}$, where Y_m, is the methane conversion factor (%GE intake); DMI, DM intake (kg/d); NDF, NDF of the diet (g/kg DM); and DMD, DM digestibility (g/kg). From the Y_m value and GE intake, GE loss as methane emission was calculated. The methane emission factor was calculated by the following equation (IPCC, 2019), using the Y_m values of the present study:

$\text{EF} = \frac{\text{GEI} \times \left(\frac{\text{Y}_\text{m}}{100}\right) \times 365}{55.65}$, kg methane/animal/year, where EF, enteric methane emission factor (kg/animal/year); GEI, GE intake (MJ/d); 55.65, energy content of methane (MJ/kg methane). The UE and energy loss as methane was subtracted from DE intake to estimate metabolizable energy (ME) intake. The amount of VS in manure was calculated by calculating manure energy loss (FE and UE outgo) and ash fraction of dietary DM intake of bulls (ASH) according to IPCC (2019) (Equation 10.24). The fasting heat production was estimated according to the following equation (Blaxter, 1962):

$\text{Fasting Heat} = 1.15 \times 0.53 \times \left(\frac{\text{LW}}{1.08}\right)^{0.67}$ MJ/d. The retained energy (RE) was calculated by subtracting heat production energy from ME. The ME for maintenance (ME_m) and partial efficiency of utilization of ME for gain (k_g) was calculated by constructing a linear regression of RE ($\text{kJ/kgW}^{0.75}$) as a function of ME intake ($\text{kJ/kgW}^{0.07}$) according to a model $\text{RE} = \beta_0 + (\beta_1 \times \text{ME})$; where β_0 is intercept and β_1 is the slope which represents the efficiency of gain (k_g). When retained energy is zero, ME intake represents the maintenance level of ME (ME_m). Metabolizable energy for gain was the difference between retained energy and ME_m.

The nitrogen balance (NB) was calculated by the following equation: NB = [(nitrogen supply – nitrogen refused in orts) – (fecal nitrogen + urinary nitrogen)]. The total nitrogen outgo (fecal and urinary) was converted to nitrogen excretion rate (nitrogen, kg/1,000 kg LW/d).

Semen Quality of Bulls

After completion of a 65-day feeding trial, four bulls of similar age (28–32 months) from each group were managed in the

TABLE 3 | Digestibility of nutrients.

Digestibility (%)	Dietary groups			SEM	*P*-values
	CM_0	CM_{25}	CM_{50}		
DM	61.5	62.5	64.8	0.73	0.353
OM	63.3	64.5	66.5	0.76	0.217
CP	67.0	70.0	70.3	0.70	0.277
NDF	40.3	42.5	49.5	1.59	0.274
ADF	36.3	34.3	39.3	1.40	0.270
Hemicellulose	46.0	54.0	62.5	2.92	0.120
DE (% GE)	67.6	65.3	68.0	0.63	0.051

CM_0, concentrate mixture containing 0% moringa mash; CM_{25}, concentrate mixture containing 25% moringa mash; CM_{50}, concentrate mixture containing 50% moringa mash; LW, live weight; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; GE, gross energy.
SEM, standard error of mean; $p < 0.05$, significant.

previous feeding and management regime for the next 15 days. They were given the training to jump on dummy bull, ejaculate semen, and subsequent collection using artificial vagina three times in a 2-day interval. Then, semen volume and evaluation were done by collecting semen every 2 days with a 2-day interval at the end. Handling of semen samples was done according to Susilawati (2017) by collecting semen using artificial vaginas. Initially, semen volume and color were recorded, and finally, semen quality was evaluated by using Computer Assisted Semen Analyzer (CASA) with Sperm VisionTM software (version 3.7.5).

Statistical Analysis

The study was conducted in a completely randomized design with three dietary treatments having sevenbulls as experimental units in each. Data were analyzed according to the general linear model procedures using IBM SPSS statistical software (version 20 for windows, SPSS Inc., Chicago, IL, USA). The mathematical model of the procedure is: $Y_{ij} = \mu + T_i + \varepsilon_{ij}$; where Y_{ij} = observed data, μ = overall mean, T_i = effect of dietary treatment, and ε_{ij} = error. Means were separated by conducting Duncan's multiple range test and presented by calculating SEM. Significant differences between means were declared at $p < 0.05$, and a tendency of difference was declared at $p < 0.10$.

RESULTS

Nutrient Intake and LW Changes in Bulls

The dietary DM intake of bulls was not different (**Table 2**; $P > 0.05$) when moringa mash was added to up to 214 g/kg DM, replacing convectional concentrates. The DM intake represented about 2.3–2.5% of LW of bulls, wherein the concentrate represented about 41–43%. Intake of OM, CP, NDF, and hemicellulose of bulls was also similar ($P > 0.05$). However, intake of ADF and GE from diets decreased with the increase of moringa mash in the concentrates ($P < 0.05$). As a consequence of feeding moringa at 104–214 g/kg DM (**Table 2**), final LW and daily gain of bulls were not affected ($P > 0.05$).

Digestibility of Nutrients

Addition of moringa up to 203 g/kg DM as a concentrate ingredient did not affect the digestibility of DM and nutrients in diets significantly (**Table 3**; $p > 0.05$). A tendency of greater DE (% GE) was found with the increase of moringa in diet ($p = 0.051$).

Metabolism of Nutrients

Dietary supplementation of moringa up to 203 g/kg DM did not exert any significant effect ($P > 0.05$) on the metabolism of energy and nitrogen in bulls (**Table 4**). However, nitrogen balance showed a tendency to increase ($P = 0.082$) with the addition of moringa in diets (up to 203 g/kg DM). All bulls were in positive energy and nitrogen balance during the study.

A relationship between dietary moringa level, ME intake, and calculated methane emission (**Figure 1**) during the metabolic study period showed that, with the increase of moringa in diet (0–203 g/kg DM), the CH_4 cost of beef cattle production decreases significantly (from 2,962 to 427 CH_4 g/kg gain; $n = 12$, $R^2 = 0.384$, $P = 0.032$) in a similar ME intake level (0.21 ± 0.01 MJ/kg LW), suggesting that moringa may help increase the efficiency of utilization of retained energy for growth and reduce the environmental cost of beef cattle production in a similar dietary plane of energy. The regression equation (methane emission, kg/kg gain = 1.6422 – [0.0059 × moringa intake, g/kg DM]) suggests that, if moringa intake is increased by 1 g/kg DM in diet, methane emission may be decreased by about 6 g/kg gain, presumably because of better retained energy utilization for growth.

Also, a significant power relationship between LW gain (g/d) and CH_4 emission (g/kg gain) shows that, with the increase of daily gain, methane cost of beef cattle production decreases significantly ($n = 12$, $R^2 = 0.9687$, $P < 0.01$) (**Figure 2**). The relationship explains that, by increasing 1 g/kg of LW gain of bulls (from 35 to 246 g/d), methane cost of gain reduces by about 13.6 g/kg gain (from 3,325 to 457).

A linear regression of retained energy on ME intake of bulls (kJ/kgW$^{0.75}$; **Figure 3**) is significant (n =12, $R^2 = 0.9253$, $P < 0.01$). The relationship illustrates that ME requirement for

TABLE 4 | Metabolism of nutrients.

Parameters	Dietary groups			SEM	*P*-values
	CM_0	CM_{25}	CM_{50}		
GE intake, MJ/d	97	109	100	3.77	0.463
FE loss, MJ/d	32	38	32	1.63	0.288
DE intake, MJ/d	65	71	68	2.27	0.581
UE, MJ/d	3.8	4.4	4.0	0.13	0.259
Volatile solids, kg/d	1.9	2.3	2.0	0.10	0.314
Volatile solids, kg/1,000 kg LW/d	7.5	8.0	7.4	0.25	0.630
Methane energy loss, MJ/d	6.1	6.8	6.3	0.20	0.390
Methane conversion factor (Y_m)	6.3	6.2	6.3	0.05	0.619
Methane emission, kg/kg gain	1.8	0.9	0.7	0.24	0.138
Methane conversion factor (D_m)	9.4	9.5	9.3	0.09	0.711
Metabolizable energy intake, MJ/d	55	60	57	2.01	0.634
Metabolizability (Q)	0.6	0.6	0.6	0.01	0.100
Energy efficiency, ME/DE	0.85	0.84	0.85	0.001	0.274
Metabolizable energy for maintenance (ME_m), MJ/d	22.8	24.8	23.3	0.85	0.650
Metabolizable energy for gain (ME_g), MJ/d	32.5	35.5	34.5	1.12	0.584
Feeding level	2.5	2.5	2.5	0.01	0.767
Heat energy, MJ/day	24	26	24	0.85	0.650
Retained energy (NE), MJ/d	31	34	34	1.10	0.570
Retained energy, % GE intake	32	31	33	0.45	0.234
N intake, g/d	102.0	121.3	110.3	4.75	0.280
N in feces, g/d	34.3	36.0	33.3	1.82	0.857
N in urine, g/d	30.0	27.5	24.0	1.41	0.231
N balance, g/d	38.0	57.8	53.0	3.88	0.082
N excretion rate in manure, kg/1,000 kg LW/d	0.25	0.23	0.22	0.01	0.470

CM_0, concentrate mixture containing 0% moringa mash; CM_{25}, concentrate mixture containing 25% moringa mash; CM_{50}, concentrate mixture containing 50% moringa mash; N, nitrogen; GE, gross energy; DE, digestible energy; ME, metabolizable energy; UE, urinary energy.

SEM, standard error of mean; $p < 0.05$, significant.

maintenance of RCC bulls was 350 kJ/kgW$^{0.75}$ at a feeding level of 2.5 (±0.05) (**Table 4**).

The linear regression analysis shows a significant reduction of urinary nitrogen loss with increasing dietary moringa level, even when digested nitrogen intake was increasing (**Figure 4**), implying greater efficiency of absorbed nitrogen utilization. According to the relation, even increasing one unit of dietary moringa (g/kg DM) reduced urinary loss of absorbed nitrogen by 0.069% (urinary nitrogen [% digested nitrogen] $= 43.004 - 0.0688 \times$ moringa intake [g/kg DM]; $R^2 = 0.3712$, $P = 0.034$).

Semen Quality of Bulls

Supplementing moringa to diets increased progressive motility of bull sperm significantly ($P = 0.026$; **Table 5**). It also exerted a tendency to decrease coil-tailed abnormal sperm ($P = 0.111$).

DISCUSSION

Nutrient Intake and LW Changes in Bulls

The present study showed that the addition of moringa of up to 214 g/kg DM of diet did not affect the dietary intake of bulls. This might be due to similar NDF intake of bulls fed from different diets (440, 438, and 435 g/kg DM, respectively in CM_0, CM_{25}, and CM_{50} groups; $p = 0.073$, **Table 2**). The DM intake of RC bulls (2.3–2.5%; **Table 2**) was similar to the findings of Roy et al. (2016) who fed sole maize silage to BLRI Cattle Breed-1 (BCB 1) bulls for 75 days and reported about 2.5% of LW. Even, replacing a maize silage diet of cows with moringa leaf postulated similar DM intake (Zeng et al., 2018). The CP intake of bulls (about 650–691 g/d; calculated from **Table 2**) of 273 (±49) kg LW experiencing 541–572 g/d gain was consistent with the recommended requirements of BSTI (2008). The CP requirements of a 250–300 kg bull with 500 g/d gain is about 623–678 g/d (BSTI, 2008).

Digestibility of Nutrients

Intake of similar NDF (435 to 440 g/kg DM) and ADF (250 to 257 g/kg DM) from different diets (**Table 2**) might result in their similar digestibility. Digestibility of diet DM (62–65%; **Table 3**) was higher than the values reported for sole maize silage (60%) by Roy et al. (2016). Supplementation of concentrate at 43% (**Table 2**) might be responsible for higher digestibility. The higher trend of digestibility of DM and other nutrients (**Table 3**) in moringa-supplemented diets (104 and 214 g/kg DM; **Table 2**) agrees with the findings of Nouala et al. (2006), who reported higher *in vitro* true digestibility of concentrate mixtures

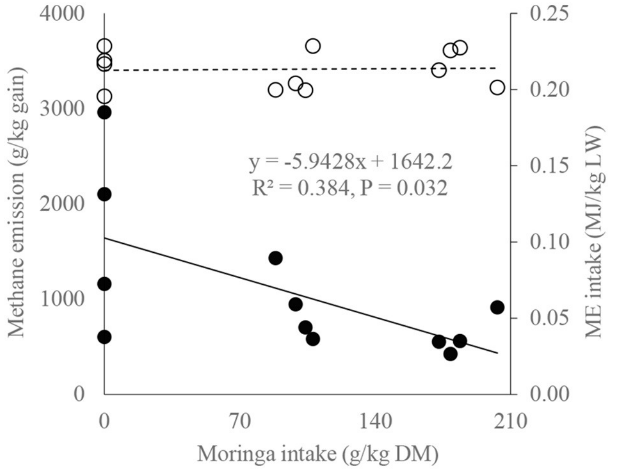

FIGURE 1 | Methane emission and metabolizable energy (ME) intake of bulls as a function of moringa intake [solid line and bold markers represent methane emission (g/kg gain), and dotted line with circle markers represent ME intake (MJ/kg LW)].

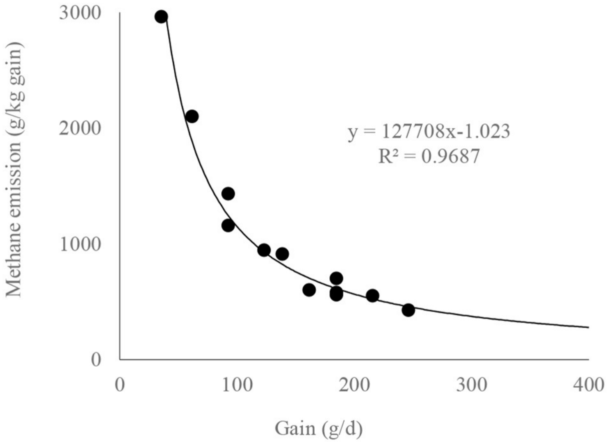

FIGURE 2 | Methane emission (g/kg gain) as a function of live weight gain of bulls (g/d).

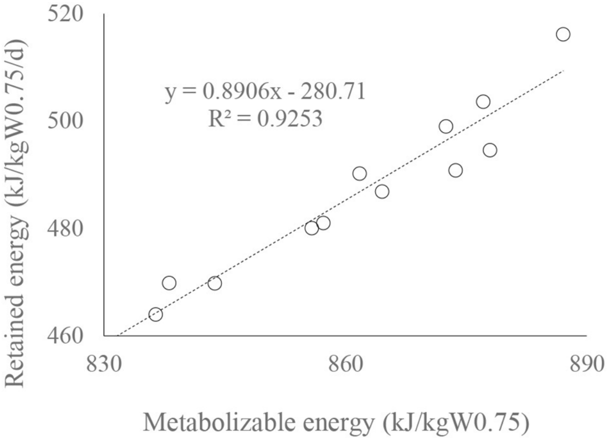

FIGURE 3 | Fit plots for retained energy (kJ/kgW$^{0.75}$) as a function of ME (kJ/kgW$^{0.75}$) intake of Red Chittagong bulls.

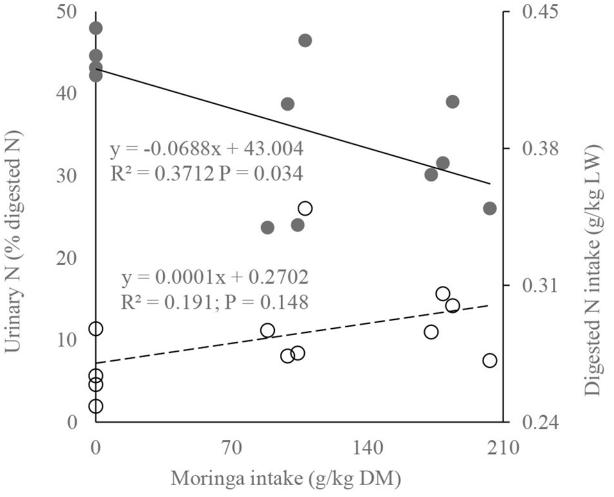

FIGURE 4 | Fit plots of digested nitrogen and urinary nitrogen as a function of dietary moringa level [solid line and bold markers represent urinary nitrogen (% digested nitrogen), whereas dotted line and circle markers represent digested nitrogen intake (g/kg LW)].

containing 25% or 50% moringa leaves. Also, the addition of moringa at different levels might be responsible for changes in the digestibility of other nutrients. For example, feeding of moringa foliage to cows at 264 g/kg DM of the diet reported significantly greater digestibility of DM, OM, and other cell wall contents (Sánchez et al., 2006). The level of moringa in the diet of bulls represented up to 203 g/kg DM during the digestibility study.

Metabolism of Nutrients

The study represents fecal energy loss of 32–35% GE intake (**Table 4**; calculated), which corroborates with the findings of da Fonseca et al. (2019), who reported about 33.6% fecal GE loss in bulls fed with tropical forages. The estimated urinary energy loss represents 3.9–4.0% of GE intake (**Table 4**; calculated), which is similar to the default values (4% in forage-based diet) reported by IPCC (2019). The amount of VS in manure (7.4–8.0 kg/1,000 kg LW) of this study is about 38% less than recommended default values of IPCC (2019) (12.0–13.5 kg/1,000 kg LW; Table 10.13A [new]) for beef cattle in Indian subcontinent. Methane conversion factor (Y_{m}, % GE intake) (6.2–6.3; **Table 4**) was similar to the recommended values of IPCC (2019). The average ME intake of bulls (237 ± 49 kg; 55–60 MJ/d; **Table 4**) with LW gain of 143 (±64) g/d during the metabolic study was higher than the recommended level (39 MJ/d; BSTI, 2008). The energy efficiency (ME/DE) of the study is higher than the value reported by NRC (2000) (0.80) but within the range (0.84–0.88) reported by Chaokaur et al. (2015). About 1% higher energy retention (% GE intake) was found in bulls fed with a diet containing 214 g/kg DM compared with control ($P = 0.234$; **Table 4**). Nitrogen

TABLE 5 | Reproductive performances of bulls.

Parameters	Dietary groups			SEM	*P*-values
	CM_0	**CM_{25}**	**CM_{50}**		
Semen volume (ml)	3.5	3.8	3.8	0.19	0.840
Total count (million/ml)	245.0	384.0	210.0	42.18	0.289
Morphology					
Normal sperm (% total count)	83.0	82.0	84.0	0.59	0.534
Band tail (% total count)	9.9	9.3	4.9	1.51	0.364
Coil tail (% total count)	4.2	2.3	1.6	0.57	0.111
Motility					
Static (% total count)	42.0	38.0	23.0	4.87	0.234
Motile (% total count)	58.0	62.0	77.0	4.87	0.234
(a) Progressive motile (% total count)	33.0^{b}	51.0^{ab}	60.1^{a}	5.19	0.026
(b) Slow motile (% total count)	1.3	1.1	0.5	0.46	0.838

CM_0, concentrate mixture containing 0% moringa mash; CM_{25}, concentrate mixture containing 25% moringa mash; CM_{50}, concentrate mixture containing 50% moringa mash.
SEM, standard error of mean.
$p < 0.05$, significant.
^{ab}means with different superscripts within same raw are significantly different.

excretion rate of bulls (0.22–0.25 kg/1,000 kg LW/d) were lower than the default IPCC values for the beef cattle in Indian subcontinent [0.40–0.63 kg/1,000 kg LW/d; (IPCC, 2019)].

Lower energy cost of LW gain by increasing moringa in the diet in a similar plane of dietary energy level (**Figure 1**) might be due to the manipulation of ruminal methanogenic communities caused by different antioxidants and secondary metabolites in moringa, as registered in previous studies (Soliva et al., 2005; Dong et al., 2019). The regression between LW gain and CH_4 emission (g/kg gain **Figure 2**) corroborates the findings of Kurihara et al. (1999), who reported that CH_4 production (CH_4, g/kg LW gain) decreases curvilinearly with the increase of daily LW gain of bulls. Such relationships might also be consisted in increasing the digestibility tendency of energy (**Table 3**), as Hristov et al. (2013) postulated decreased enteric CH_4 with increasing digestibility. The ME_m requirement of RC bulls (ME_m =350 kJ/kg$W^{0.75}$; **Figure 3**) at 2.5 feeding level is well below the value recommended by Kearl (1982; 493 kJ/kg$W^{0.75}$) but close to the value of Liang and Young (1995; 335 kJ/kg$W^{0.75}$ for growing Kedah Kelantan bulls) and Subepang et al. (2019; 388 kJ/kg$W^{0.75}$ for Thai native cattle), at 1.1–2.0 feeding level. Unproductive dietary nitrogen loss in urine increases linearly with increasing dietary nitrogen intake (Kebreab et al., 2010), and such nitrogen losses are associated with emitting N_2O and NH_3 as a byproduct of aerobic or anaerobic microbial metabolism (Liu et al., 2017), causing environmental pollution. In this study (**Figure 4**), it is evident that, without increasing dietary nitrogen intake, reduction of urinary nitrogen loss may be possible by supplementing the diet with moringa at 203 g/kg DM. The impact of feeding moringa to increase LW gain in bulls is also evident in this study (**Table 2**) where the daily gain is 31 g/d higher in the moringa diet (214 g/kg DM) compared with control.

Semen Quality of Bulls

Higher progressive motility of sperms from bulls fed with moringa mash (25% of 50% of concentrate mixtures) might be due to certain nutritional constituents of moringa mash. Eghbali et al. (2010) and Princewill et al. (2015) reported that higher plasma content of Ca and P results in greater bovine sperm motility. Other nutrients that contribute to increased total sperm motility and progressive motility include arginine, carnitine, Zn, vitamin B12, vitamin C, vitamin E, glutathione, selenium, and Coenzyme Q-10 Begum et al. (2009). Moringa was reported to be a great source of all these nutrients (Su and Chen, 2020). Feeding moringa mash might increase the plasma level of these nutrients, which might cause better progressive sperm motility. Similar findings were also reported in Bali bulls and buffalo (Syarifuddin et al., 2017 and Wafa et al., 2017, respectively).

CONCLUSION

The results of the study indicate that replacement of conventional concentrate ingredient (particularly ground maize, soybean meal, and wheat bran) with up to 50% of mixtures with moringa mash may not affect DM intake and LW gain of bulls. However, when the inclusion of moringa represented 203 g/kg DM of the diet of a bull, it may significantly increase ME utilization and reduce urinary nitrogen loss, even when the dietary level of energy and nitrogen is similar. When dietary moringa addition is increased by 1 g/kg DM, calculated methane emission is decreased by 6 g/kg gain, with simultaneous absorbed nitrogen loss reduction by 0.069 %. Thus, increasing the efficiency of dietary energy and nitrogen utilization in bulls may be achieved by adding moringa mash to concentrate mixtures at 50% and fed at 1% LW. Reduction of dietary energy and nitrogen loss of bulls may also help reduce subsequent environmental pollution.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by Annual Research Evaluation Committee of Bangladesh Livestock Research Institute, 2019.

AUTHOR CONTRIBUTIONS

ND and NS conceived and designed the study, conducted trial and laboratory works, interpreted the data, and drafted and finalized the manuscript. MK and GD were involved in semen evaluation, data analysis, and drafting of manuscripts. MI was involved in the preparation and analysis of feeding trial samples.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.