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Monensin Supplementation in the Feed for Lactating Murrah Buffaloes (*Bubalus bubalis*): Influence on Nutrient Utilization and Enteric Methane Emissions

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ABSTRACT

The present experiment was conducted to find out the effect of monensin supplementation on nutrients utilization and enteric methane emission in lactating Murrah buffaloes. Twelve lactating Murrah buffaloes (567.50 ± 44.3 kg body weight (BW); initial days in milk = 52.83 ± 10.24; milk yield = 6-8 kg/d) were divided in to two groups (n=6) based on the BW and days in milk. Both the groups were fed sugar graze and concentrate mixture (70:30 ratio) as a total mixed ration, without supplementation (control) or supplemented with monensin 24 mg/kg of dry matter intake (treatment) for sixty days. Nutrient utilization and enteric methane emissions were measured after 50 days of monensin supplementation. The daily intake (kg/d) and apparent digestibility (%) of nutrients were similar (P>0.05) in both the groups, However Methane emissions in terms of g/d, g/kg milk yield and g/kg dry matter intake (DMI) were found to be lower (P<0.05) by 8.55%, 13.20% and 9.02% respectively, in treatment group as compared to control. Methane energy loss as percent of Gross Energy (GE), Digestive Energy (DE) and Metabolizable Energy (ME) was reduced (P<0.05) in monensin supplemented group by 8.82, 11.11 and 11.45%, respectively compared to control. The results suggested that feeding 24 mg/kg DMI of monensin on high forage diets has the potential to reduce enteric methane emissions in lactating buffaloes without significant effect on nutrient utilization which will reduce the contribution of buffaloes to the global methane inventory and its negative impact on environment and increase environmental friendly milk production in the country.

Keywords: Enteric methane, Lactating Buffalo, Monensin, Nutrient Utilization

India is the world's largest milk producer; accounting for more than 18.5 % of the world's total milk production (GOI, 2016) and buffaloes contribute the highest (49.2%) share to milk production in India (Basic Animal Husbandry Statistics, 2017)**.** India has the world's largest number of livestock. Livestock production contributes 14.5% of anthropogenic greenhouse gas (GHG) emissions, which play a significant role to climate change (Gerber *et al*., 2013). Enteric fermentation in livestock is an important source of anthropogenic methane emission. Global attention received towards methane production through enteric fermentation from livestock because of its contribution to the accumulation of greenhouse gases in the atmosphere, as well as its waste of fed energy for the animal. Among livestock, methane production is greatest in ruminants, as methanogens are able to produce methane freely through the normal process of feed digestion. Buffaloes account for 42.8% of total enteric methane (CH_4) emissions of Indian livestock (Patra, 2014). Methane emission intensity for buffaloes (31 g CH_4/kg milk) is also higher compared to crossbred dairy cattle (25 g CH_4/kg milk) in India (Patra, 2012).

Dietary manipulation and inclusion of CH_4 abatement strategies like feed additives and management options like genetic selection of efficient feed utilizers or high producing animals can lower CH_4 emission; however studies showed that some of the available technologies like dietary supplementation with feed additive are highly cost effective in reducing enteric methane emissions. Sirohi *et al*. (2007) studied that the gross cost of CH_4

abatement (Per ton CO_2 equivalent) from use of feed additive (monensin premix) was lowered for Indian livestock than the dietary manipulation like increased concentrate feeding. Therefore, various rumen modifiers including monensin have been used in ruminants to increase feed utilization and production performance while reducing/maintaining environmental impact of milk production. Monensin is a carboxylic polyether ionophore obtained from *Streptomyces cinnamonensis*. Ionophores regulate the movement of monovalent cations across cell membranes of Gram-positive bacteria and protozoa, disrupting their normal function (Duffield and Bagg, 2000). Monensin has inhibitory effect on Gram-positive bacteria, thereby decreasing ammonia, and lactate production and increasing propionate production (McGuffey *et al*., 2001). The favorable effect of monensin feeding has been related to modification of feed intake, rumen microbial populations, volatile fatty acids (VFAs) proportions, feed digestibility, rumen fill and rate of passage (Akins *et al*., 2014; Ipharraguerre and Clark, 2003). The inhibition of methanogenesis is due to inhibitory effect on Gram-positive bacteria and protozoa which favours propionate production and decreases acetate, butyrate, and formate formation, resulting in reduced methane production (Kobayashi, 2010; Appuhamy *et al*., 2013). Feeding 300 mg/d monensin feed premix in dry Holstein cows reduced methane emission by 10.7% (Junior *et al*., 2017). Only a few studies have been carried out on efficacy of monensin supplementation on efficiency of feed utilization and methane emissions in buffaloes. Therefore, the objectives of the present study were to evaluate the effects of monensin supplementation on nutrient intake, total tract apparent digestibility and enteric methane emissions in lactating buffaloes.

MATERIALS AND METHODS

The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC/09/16 dated 05.11.2016) of the National Dairy Research Institute, Karnal, India. The study was conducted in the experimental animal shed at Livestock Research Center of NDRI, Karnal, India, located at an altitude of 250 meter above the mean sea level on 29.43°N latitude and 72.2°E longitude. The maximum ambient temperature goes up to 45°C during summer, minimum about 5°C during winter, relative humidity varies from 18 to 97 percent with an annual rain fall is approximately 760-960 mm most of which is received during the months of July to August (Central Soil Salinity Research Institute, Karnal, Haryana). The present experiment was conducted during mid-December to mid-February.

Animals, feeding and experimental design

Twelve lactating Murrah buffaloes having average body weight of (567.50 ± 44.3 kg of live weight; initial days-in milk = 52.83 ± 10.24; milk yield = 6-8 kg/d) were selected from the Institute Livestock Research Centre and identified by numbered ear tags, tethered with nylon rope individually in a well-ventilated stall (floor space = $4m^2$ per animal) provided with uniform management practices and having facilities for individual feeding. Animals were dewormed using Fenbendazole (Panacur®, Intervet, India) at 10mg/kg BW and treated against ectoparasites using Deltamethrin (Butox®) spray 10 d before the commencement of experimental feeding. After an adaptation period of 10 days, animals were divided into two groups of six animals in each on the basis of body weight and days in milk. Both groups were fed ration comprising of green sugar graze fodder chopped at 2–3 cm length and concentrate mixture (in g/kg as mixed: maize 330, groundnut cake 180, mustard oil cake 100, cotton seed cake 50, wheat bran 200, de-oiled rice bran 60, bajra 50, mineral mixture 20 and common salt 10) at a ratio 70: 30 without and with monensin supplementation (24 mg/kg of dry matter intake) in control and treatment group, respectively for sixty days. Monensin was top dressed on concentrate mixture in the form of Rumensin (Elanco, Division of Eli Lilly and company (NZ Limited), which contains monensin in a concentration of 20% Mill mix (Equivalent to 200g of monensin activity as monensin sodium per kg). All animals were provided clean and fresh drinking water twice daily in morning at 10.00 h and evening at 17:30 h.

Digestion trial

The metabolism study with 3 days adaptation period followed by 7 days collection period was conducted after 50 days of experimental feeding trial, during which daily intake of feeds and output of faeces were recorded. Gross energy of feed ingredients was calculated on the basis of reference values. Metabolizable energy and Digestible energy were estimated as per NRC (2001). Faeces excreted

were collected with into plastic containers, weighed, mixed and sampled once daily 1% of thoroughly mixed total faecal matter by fresh weight was used for Dry Matter (DM) determination by drying at 60°C for 48 h to constant weight. Then, faecal samples were dried immediately, composited and later used for chemical analysis. For N determination (Kjeldahl method), faeces samples (1/500 of daily voidance) were preserved in 30% sulphuric acid to make pooled samples of 7 d for individual animals. Oven-dried samples of the feeds offered, residues left and faeces voided during metabolism trial were pooled and ground in a Wiley mill to pass a 1-mm sieve and preserved for chemical analyses in an air tight container. The samples were analysed for proximate principles (AOAC, 2005) and cell wall constituents (Van Soest *et al*., 1991). For N determination (Kjeldahl method), faeces samples (1/500 of daily voidance) were preserved in 30% sulphuric acid to make pooled samples of 7 d for individual animals.

Enteric methane estimation by SF_6 tracer technique

Enteric methane production by the animals was measured by sulphur hexafluoride (SF_6) tracer technique (Johnson *et al*., 1994). After collection of a sample, the canisters were pressurized with nitrogen and the concentration of SF_6 in the canisters was analyzed by gas chromatography (Nucon 5700, Nucon Engineers, New Delhi), fitted with an electron capture detector (250 ºC) to determine SF_6 and 3.3 m molecular sieve column with an i.d of 0.32 mm. Another gas chromatograph instrument was fitted with a flame-ionization detector (100 ºC) and stainless steel column packed with Porapak-Q (length 1.5; o.d. 3.2 mm; i.d. 2 mm; mesh range 80-100) to determine CH_4 concentration. The column and injector temperatures were 50 and 40 ºC in both the instruments. All samples were analyzed in duplicate except standards, which were analyzed in triplicate. Nitrogen was used as the carrier gas at a pressure of 1kg/cm^2. The standards were run in the beginning and end of each day with the methane standard run every 10 samples throughout the day. Gas concentrations (SF_6 and CH_4) were determined from peak areas and identified from their different retention times relative to the known standards. The methane output calculated using following formula:

$$CH_4\ (g/d) = \left(\frac{S_{CH4} - B_{CH4}}{S_{SF6} - B_{SF6}}\right) \times \left(\frac{M_{CH4}}{M_{SF6}}\right) \times Q_{SF6} \times 1000$$

Where, S_{CH4} and B_{CH4} are methane concentrations in sample and background's canisters (ppm), S_{SF6} and B_{SF6} represent the concentrations of SF_6 in sample and background's canister's (ppt), M_{CH4} and M_{SF6} are molecular weight of methane and SF_6 (g), respectively and Q_{SF6} represents release rate of SF_6 (mg/d).

Statistical Analysis

All the data collected during study were subjected to the statistical analysis as per Snedecor and Cochran, (1994). Independent sample t-test was done to find out the significant difference between groups using software package IBM SPSS statistics version 16.0, 2010 (SPSS, 2010).

RESULTS AND DISCUSSION

Chemical composition and energy contents

Chemical composition of ingredients of basal diet has been presented in Table 1. The chemical composition of all the ingredients were within normal range reported previously (Das *et al*., 2014, Prusty, 2015, and Sharma, 2017).

Table 1: Chemical composition and energy contents of offered feedstuffs

Parameter (%DM)	Concentrate Mixture	Sugar graze Green fodder
DM	90.39	25.21
OM	94.54	90.41
CP	21.78	10.75
EE	3.9	1.73
TA	5.45	9.59
NFC	44.77	17.91
NDF	24.1	60.02
ADF	11.49	36.16
Hemicellulose	12.6	23.86
Cellulose	6.87	30.98
ADL	3.96	5.18
TDN	76.33	56.30
DE (MJ/kg DM)	14.08	10.39
ME (MJ/kg DM)	12.34	8.61

Nutrients intake

Nutrients intake during metabolic trial are presented in Table 2. There was no difference (P>0.05) observed in the daily DM, organic matter (OM), crude protein (CP), either extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicelluloses (HC), non fibrous carbohydrate (NFC) carbohydrate (CHO) and energy intake (kg/d) between the groups. Comparable to the present study, Ali Haımoud *et al*., (1995) found no change (P>0.05) in dry matter intake (DMI) of lactating cows were fed ration supplemented with monensin (33 mg/kg of DM). Martineau *et al*., (2007) also reported that DMI was unaffected (P>0.05) by monensin supplementation (24 mg/kg DM) to mid lactating Holstein cows. Similar to these findings Lamba *et al*., (2013) also observed no difference (P>0.05) in nutrients intake by monensin (300 mg/d) supplementation in lactating crossbred cows fed on seasonal green fodder and concentrate mixture. On other hand Cant *et al*., (1997) found decreased DMI of lactating cows fed ration supplemented with monensin (14.5 mg/kg of DM). Different response of monensin supplementation on nutrient intake could be attributed to variation in experimental conditions such as rate of inclusion of monensin, type of offered feedstuffs and different physiological stages and species of animals.

Table 2: Effect of Monensin supplementation on nutrients intake in lactating buffaloes

Parameter	Control	Treatment	P value
Milk yield (kg/d)	8.92±0.18	9.02±0.30	0.84
DMI (kg/d)	14.53± 0.27	14.04±0.18	0.17
CPI (kg/d)	1.94±0.06	1.86±0.03	0.24
TDNI (kg/d)	8.85±0.12	8.70±0.17	0.59
DE (MJ/d)	163.16±2.22	160.37±3.21	0.59
ME (MJ/d)	137.44±1.74	135.53±2.91	0.68
OMI (kg/d)	13.30±0.25	12.86±0.17	0.17
EEI(kg/d)	0.35±0.00	0.34±0.00	0.18
NDFI (kg/d)	7.10±0.18	6.79±0.11	0.17
ADFI(kg/d)	4.19±0.10	4.00±0.07	0.15
HCI (kg/d)	2.92±0.08	2.79±0.05	0.20
NFCI (kg/d)	3.90±0.02	3.86±0.02	0.24
CHOI(kg/d)	11.01±0.19	10.65±0.13	0.17

Nutrients digestibility

Nutrients digestibility (%) during metabolic trial in lactating buffaloes are depicted in Table 3. There was no variation (P>0.05) in the nutrient digestibility coefficient between the groups. Similar results observed by Lamba *et al*. (2013) and Martineau *et al*. (2007) who reported no effect of monensin supplementation on nutrient digestibility in lactating crossbred cows and Holstein cows, respectively. Reed and Whisnant, (2001); Osborne *et al*. (2004); Oliveira *et al*. (2007) and Benchaar, (2016) also found no significant effect of monensin supplementation on apparent nutrient digestibility. Monensin supplementation had no significant effect on crude protein digestibility reported by Benchaar *et al*. (2006) and Khorrami *et al*. (2015) support recent findings. Funk *et al*. (1986) reported that ionophore feedings changes the site of digestion of dietary carbohydrate fractions and digestion of starch in rumen may be decreased, but increased post ruminally to the extent that total tract digestibility is unchanged. Allen and Harrison, (1979) observed that fiber digestibility is largely unaffected by ionophores feeding due to Increased numbers of ionophore-resistant fibrolytic bacteria and reduced numbers of ionophore-sensitive ruminococci along with longer rumen retention time caused by ionophores may contribute to normal fiber digestion (Lemenager *et al.*, 1978).

Table 3: Effect of Monensin supplementation on nutrient digestibility (%) in lactating buffaloes

Parameter	Control	Treatment	P value
DM	61.54±0.45	62.30±0.67	0.48
OM	63.93±0.44	64.96±0.55	0.26
CP	60.55± 1.05	61.23±0.62	0.55
EE	80.19±0.53	81.62±0.38	0.68
NDF	52.89±0.70	51.88±0.45	0.25
ADF	43.12±1.65	42.78±0.58	0.81
HC	66.92± 3.44	64.92±1.13	0.49
NFC	84.24±0.99	88.12±1.82	0.16

Enteric methane emissions

The energy intake and loss of energy in the form of CH_4 from the lactating buffaloes fed on ration with and without monensin supplementation is provided in Table 4. The energy (MJ/day) intake in terms of gross energy

(GE), digestible energy (DE) and metabolizable energy (ME) was similar between groups. Methane emissions in terms of g/d and g/kg milk yield were found to be lower (P<0.05) by 8.55% and 13.20%, respectively in monensin supplemented group as compared to control, respectively. Similar to findings of present study Odongo *et al*., (2007) and Kobayashi, (2010) found that long term feeding of 24 mg of Rumensin Premix/kg DM in diet (forage to concentrate ratio of 60:40) to lactating Holstein lactating cattle (620±5.9 kg of BW; 92.5±2.62 d in milk) reduced production of CH_4 (g/d) by about 7 percent. Enteric methane emissions found to be lower (P<0.05) around 9.02% for g/kg DM, 8.95 % for g/kg OM and 9.13 % for g/kg NDF in monensin supplemented group in comparison to control.

Table 4: Effect of Monensin supplementation on energy loss as enteric methane emissions in lactating buffaloes

Parameter	Control	Treatment	P value
GEI (MJ/d)	334.16±3.87	335.60±5.56	0.84
DE (MJ/d)	164.36±3.73	159.77±2.61	0.35
ME (MJ/d)	139.23±3.19	134.75±2.24	0.29
CH_4 (g/d)	244.43^{a}±1.72	223.54^{b}±1.67	0.00
CH_4 (g/kg milk yield)	27.50^{a}±0.44	23.87^{b}±0.26	0.003
Methane g/kg Nutrient intake			
DMI	17.30^{a}±0.31	15.74^{b}±0.35	0.02
OMI	18.89^{a}±0.33	17.20^{b}±0.39	0.02
CPI	132.82±3.60	120.58±4.40	0.08
NDFI	36.05^{a}±0.83	32.76^{b}±1.00	0.04
DDMI	28.11^{a}±0.50	25.27^{b}±0.57	0.01
DOMI	29.54^{a}±0.52	26.48^{b}±0.60	0.01
DCPI	219.36±5.94	196.94±7.18	0.05
DNDFI	68.16±1.56	63.14±1.91	0.09
Methane energy loss as %			
GE	4.08^{a}±0.05	3.72^{b}±0.06	0.005
DE	8.55^{a} ±0.15	7.60^{b} ±0.16	0.006
ME	10.13^{a} ±0.18	8.97^{b} ±0.20	0.005

Means bearing different superscripts $^{a, b}$ in same row differ significantly (P<0.05).

Values for CH_4 emissions g/kg of DMI were 17.30±0.31 and 15.74±0.35; g/kg of OMI 18.89±0.33 and 17.20±0.39 and g/kg of NDFI 36.05±0.83 and 32.76±1.00 for control and monensin supplemented group, respectively. Similar results reported by Van vugt *et al*., (2005) who conducted a series of feeding experiments to measure the effect of monensin on enteric methane production by dairy cows fed indoors ryegrass-dominant pastures alone or with white clover, or maize silage. They found that methane production was reduced (P<0.01) by 9% from 16.9 to 15.3 g/kg DM after 72 days the monensin capsule was given. Different response for enteric methane emission could be attributed to variation in type and composition of offered feedstuffs, dose of monensin, and production level of lactating animals and stages of lactation. The CH_4 emissions g/kg of digestible dry matter (DDM) and digestible organic matter (DOM) intake were also lower (P<0.05) around 10.10 and 10.36%, respectively in monensin supplemented group in comparison to control. Methane energy loss as percent of GE, DE and ME was reduced (P<0.05) in monensin supplemented group by 8.82, 11.11 and 11.45%, respectively compared to control. Beauchemin and McGinn, (2006) reported higher CH_4 reduction in term of GE intake (about 9%) by monensin supplementation in growing beef cattle. This might be due to higher OM digestibility of the feeds in that study in comparison to the present study leading to higher CH_4 emissions. The monensin supplementation reduced CH_4 production support Russell and Houlihan, (2003) who stated that monensin inhibits growth of H_2 producing rumen bacteria which supply H_2 to methanogens and indirectly decrease CH_4 production and Orskov *et al*., (1991) who reported that an increased level of propionic acid could decrease the CH_4 production.

CONCLUSION

It was concluded that monensin supplementation at 24 mg/kg DMI to buffaloes had no effect on feed intake, feed digestibility and milk production but reduced daily methane emission (-8.55%) and methane emission intensity (-13.2%) which will reduce the contribution of lactating buffaloes to green house gases emissions and their impact on the environment. The number of animals per treatment was very small, and more long term studies are needed for evaluating efficacy of monensin on methane mitigation.

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