



Effect of Dietary Monensin Supplementation on Faecal Nitrogen Excretion and Blood Metabolites in Non Pregnant Non Lactating Murrah Buffaloes

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ABSTRACT

The present study was conducted to evaluate the efficacy of monensin supplementation in reducing faecal nitrogen excretion in non pregnant non lactating Murrah buffaloes and its effect on blood metabolites. The nitrogen released by the livestock in faecal matter contributes to the nitrous oxide (N₂O) content of environment, which is a potent green house gas. Fourteen dry Murrah buffaloes were randomly divided into two groups of seven animals each on the basis of body weight (597.62 kg). Both groups were fed as per ICAR, (2013) feeding standard without and with monensin supplementation (350 mg/head/day) in control and treatment group, respectively for sixty days. The daily nitrogen intake, urinary and total nitrogen out go and nitrogen balance (g/d) were not ($P > 0.05$) affected by monensin supplementation. However, faecal nitrogen excretion (g/d) decreased ($P < 0.05$) and blood plasma glucose (mg/dl) concentration increased ($P < 0.05$) in treatment group as compared to control. The concentration of blood non-estrified fatty acid (NEFA), blood urea nitrogen (BUN), total protein (TP) and albumin were not affected ($P > 0.05$) by monensin supplementation. In conclusion, dietary monensin supplementation to non pregnant non lactating Murrah Buffaloes increased blood glucose concentration and reduced faecal nitrogen excretion which will reduce the contribution of buffaloes to nitrous oxide emissions and its negative impact on environment.

Keywords: Buffalo, Monensin, Blood metabolites, Faecal nitrogen, Environment impact

In agriculture sector, waste from animal production system contribute as much as 30–50% to the global N₂O emissions but relatively little attention has been given on developing mitigation options (Montes *et al.*, 2013). India is the largest producer of milk in the world and buffaloes contribute the highest (49.2 %) share to milk production in India (Basic Animal Husbandry Statistics, 2017). Buffalo is a triple purpose animal, being suitable for milk, meat and draught. Craine *et al.* (2010) reported that the crude protein concentration of ruminant diet essentially nitrogen concentrations of consumed feedstuffs often limit ruminant production. Dong *et al.* (2014) stated that intake of nitrogen identified as the main driver of ruminant nitrogen excretion. The efficiency of nitrogen retention and utilization by buffaloes play an important role in environmental relevance (Tamminga, 1996). In ruminant production systems it is beneficial to reduce the environmental release

of nitrogen in urine and faeces. Excess nitrogen release by ruminants can directly cause leaching and soil nutrient imbalance (Marini and Van Amburgh, 2005). Excess nitrogen can be converted to nitrous oxide which is a greenhouse gas with potential that is around 300 times that of carbon dioxide (Eckard *et al.*, 2010). Nitrogen excreted by ruminants is not utilized for growth and production and may negatively impact the environment. Monensin is a monovalent carboxylic polyether ionophore produced by *Streptomyces cinnamonensis* and most commonly used ionophore to improve the efficiency of production (meat and milk) in ruminants (Rodehutschord, 2013). Monensin supplementation improves nitrogen metabolism and reduced proteolysis of intake of feed protein because of its protein sparing characteristics (Poos *et al.*, 1979). The inclusion of monensin in ruminant's diets may benefit air quality by reducing methane (CH₄) and nitrogen emissions

and water quality by reducing nitrogen in manure, which can potentially leave the farm through leaching into ground water and through runoff into surface (Tedeschi *et al.*, 2003). Pambu- Gollah *et al.* (2000) stated that blood metabolites give rapid indication of an animal nutritional level at the particular point of time. Cinar and Sulu, (1995) reported that blood glucose level increased by monensin supplementation due to higher propionate production which is glucogenic in nature or could be due to shifting of digestion of starch and other soluble sugars from rumen to lower tract, from where it is absorbed as glucose (Haimoud *et al.*, 1995). Hence, this study was taken up to evaluate the effect of monensin on physiologically non productive buffaloes.

MATERIALS AND METHODS

The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC/03/16 dated 19.05.2016) of the National Dairy Research Institute, Karnal, India. The study was conducted in the experimental animal shed at Livestock Research Center of National Dairy Research Institute, 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-june to mid-august.

Animals, feeding and experimental design

Fourteen dry Murrah buffaloes having average body weight of 597.62 kg 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 = 4m² per animal) provided with uniform management practices and having facilities for individual feeding. Animals were dewormed using Fenbendazole (Panacur®, Intervet, India) at 10 mg/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 randomly divided into two groups of

seven animals in each on the basis of body weight (597.62 kg). Both groups were fed ration comprising of green sorghum fodder chopped at 2–3 cm length, 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) and wheat straw, to meet their nutrient requirements according to ICAR, (2013) without and with monensin supplementation (350 mg/head/day) 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.

Metabolism trial, Sampling, processing and analysis of feed, residue and faeces

The metabolism study with 3 days adaptation period followed by 7 days collection period was conducted after 55 days of experimental feeding trial, during which daily intake of feeds and output of faeces and urine were recorded. For nitrogen (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. Total daily voided of urine for 24 h was collected in plastic containers containing 25 ml of 25% sulphuric acid solution. An aliquot (0.5% of total urine output) was collected from the acidified urine for N estimation (Kjeldahl method).

Blood parameter

Blood samples (10 ml) were collected at zero day and last day of animal trial (60th day) in sterile heparinised vacutainer tubes from jugular vein puncture, posing minimum disturbance to the animal. Immediately after collection, samples were kept in ice box and transported to the laboratory for further processing. The plasma was separated by centrifugation at 3000 rpm for 30 minutes and stored at 20°C in different aliquots analysed for glucose, blood urea nitrogen, total protein and albumin using diagnostic reagent kit provided by Recombigen Laboratories PVT. LTD (New Delhi). Plasma NEFA

concentration was estimated by copper soap solvent extraction method modified by Shipe *et al.* (1980).

Statistical Analysis

All the data collected during study were subjected to the statistical analysis as per Snedecor and Cochran, (1989). 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 of offered feedstuffs

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

Table 1: Chemical composition and energy contents of offered feedstuffs

Parameter (%DM)	Concentrate Mixture	Wheat straw	Sorghum Green fodder
DM	90.39	90.63	28.08
OM	94.54	89.87	89.25
CP	21.78	3.70	10.09
EE	3.90	1.30	1.93
TA	5.45	10.13	10.75
NFC	44.77	7.76	12.85
NDF	24.1	77.11	64.38
ADF	11.49	51.32	34.22
Hemicellulose	12.6	25.79	30.16
Cellulose	6.87	41.97	28.14
ADL	3.96	9.35	6.08
TDN	76.33	43.61	52.66
DE (MJ/kg DM)	14.08	8.05	9.71
ME (MJ/kg DM)	12.34	6.24	7.93

Nitrogen balance

Effect of dietary monensin supplementation on nitrogen (N) balance in dry buffaloes is presented in Table 2. Average N

intake (g/d) was 111.69 and 105.16 in control and treatment group, respectively and did not differ ($P>0.05$) between the two groups. There was no significant difference in N excretion in urine, total N out go, N absorption and N retention between the two groups. Thus efficiency of protein utilization of the rations was similar with and without monensin supplementation in non pregnant, non lactating buffaloes. However, faecal N excretion (g/d) was lower ($P<0.05$) in monensin supplemented group (42.76) than control (47.17), so despite the lower intake, overall N balance (g/d) did not differ ($P>0.05$) was between control and monensin supplemented group. Similar results were observed by Oliveira *et al.* (2007) who reported faecal nitrogen excretion decreased significantly in sheep fed on ration (65% signal grass hay and 35% concentrate mixture) supplemented with monensin (28 mg/kg DM). De and Singh, (2005) also found decreased ($p<0.05$) faecal N loss in the monensin supplemented group than in control. This improvement in N absorption might be due to improvement in lower tract digestibility (Haimoud *et al.*, 1995). Ruiz *et al.* (2001) stated that monensin may reduce fecal N output which supports our findings.

Table 2: Effect of dietary monensin supplementation on nitrogen balance in dry buffaloes

Parameter	Control	Treatment	P value
N Intake (g/d)	111.69±2.13	105.16±4.03	0.18
Faecal N outgo (g/d)	47.17 ^a ±1.25	42.76 ^b ±1.51	0.04
Urine N outgo (g/d)	60.24±1.65	57.46±3.66	0.50
Total N outgo (g/d)	107.41±2.06	100.23±3.98	0.14
N balance (g/d)	4.28±0.37	4.93±0.26	0.18
N absorption (% of intake)	69.40± 2.35	64.16±5.12	0.07
N retained (% of intake)	3.77±0.33	4.66±0.29	0.20
N retention (% of absorption)	6.26±0.62	10.01±2.69	0.37

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

Blood parameter

Blood glucose (mg/dl) concentration (Table 3) was similar in both the groups at the day of start of experiment and mean values were 50.84±0.42 and 50.77±0.33 for control and monensin supplemented group, However, there was increased ($P<0.05$) concentration of blood plasma glucose (mg/dl) in monensin supplemented group

(53.51±0.57) as compared to control (51.26±0.44) till the end of the experiment. Hagawane *et al.* (2009) reported blood glucose level in dry buffaloes (52.72±4.22 mg/dl) which is comparable to present findings. Similar to these findings Broderick, (2004) reported increased (P<0.05) blood glucose concentration in lactating dairy cows fed ration based on alfalfa silage supplemented with monensin (10 mg/kg DM). Helal and Lasheen, (2008) also found that blood glucose concentration was increased (p<0.05) with monensin supplementation in lactating buffaloes, respectively. This might be due to monensin supplementation increased glucose synthesis due to higher propionate production in rumen (Van Maanan *et al.*, 1978). Initial blood urea nitrogen (mg/dl) concentrations (19.36±0.37 and 19.04±0.05) were similar in both the groups and at the end of the animal trial concentration of BUN (mg/dl) did not (P>0.05) differ between control (21.02±0.17) and monensin supplemented group (20.35±0.45) (Table 3). Similar to present study findings Anassori *et al.* (2015) found no effect (P>0.05) on BUN concentrations in ruminally fistulated rams fed ration supplemented monensin (33 mg/kg DM) as compared to control. Rowghani *et al.* (2006) also found that no significant effect on BUN concentration in growing lambs fed on corn silage, hay and barley grain based diet supplemented with monensin (22 mg/kg DM). Lamba *et al.* (2013) also observed that concentration of BUN was unaffected by monensin (300 mg/d) supplementation to lactating cows. Initial concentrations of Non-Esterified Fatty Acids (µmol/l) (Table 3) was similar in both the groups at the day of the start of the experiment and mean values were 124.83±1.19 and 125.17±0.48 for control and monensin supplemented group, respectively. At the end of the animal trial concentration of NEFA (µmol/l) did not differ (p>0.05) in control (122.33±1.74) and monensin supplemented group (120.83±2.21). Juchem *et al.* (2004) observed plasma NEFA concentration was increased (p<0.01) in Holstein dairy cows treated prepartum with Sodium monensin (335mg/d). On other hand Anassori *et al.* (2017) found significant reduction (P<0.05) in blood NEFA concentrations in monensin (33 mg/kg DM) supplemented 5-6 month fattening lambs compared to control. Initial blood plasma concentration of total protein (7.48±0.09; 7.47±0.08 mg/dl) and albumin (3.05±0.10; 2.86±0.18 mg/dl) concentrations was also similar in both the groups and at the end of the experimental trial blood plasma concentration of total protein (7.47±0.07;

7.51±0.10) and albumin (3.12±0.11; 2.97±0.12 mg/dl) (Table 3) concentration was also not affected (P>0.05) by monensin supplementation. Anassori *et al.* (2017) found no significant effect on monensin in blood plasma concentration of total protein and albumin in monensin (33 mg/kg DM) supplemented 5-6 month fattening lambs group compared to control. Different response for blood NEFA, TP and Albumin concentration could be attributed to variation in type and composition of offered feedstuffs and species of animal.

Table 3: Effect of Monensin supplementation on blood parameters in dry buffaloes

Parameter	Day	Control	Treatment	P value
Blood glucose (mg/dl)	Zero day	50.84±0.42	50.77±0.33	0.89
	60 day	51.26 ^b ±0.44	53.51 ^a ±0.57	0.001
BUN (mg/dl)	Zero day	19.36±0.37	19.04±0.05	0.86
	60 day	21.02±0.17	20.35±0.45	0.84
NEFA (µmol/l)	Zero day	124.83±1.19	125.17±0.48	0.80
	60 day	122.33±1.74	120.83±2.21	0.61
Total Protein (mg/dl)	Zero day	7.48±0.09	7.47±0.08	0.89
	60 day	7.47±0.07	7.51±0.10	0.86
Albumin (mg/dl)	Zero day	3.05±0.10	2.86±0.18	0.39
	60 day	3.12±0.11	2.97±0.12	0.36

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

CONCLUSION

It was concluded that supplementation of monensin ((350 mg/head/day) to non pregnant non-lactating Murrah Buffaloes increased blood glucose concentration indicating more available energy and could reduce faecal nitrogen excretion which will reduce the contribution of buffaloes to green house gases emissions and their impact on the environment. However, concentration of blood non-esterified fatty acid, blood urea nitrogen, total protein and albumin were not affected (P>0.05) on monensin supplementation.

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