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Effect of Dietary Monensin Supplementation on Nitrogen utilization and Plasma metabolites in Lactating Murrah Buffaloes

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A B S T R A C T

The present study was conducted to evaluate the efficacy of monensin supplementation in nitrogen balance and blood metabolites in lactating murrah buffaloes receiving concentrate and sugar graze fodder. Twelve lactating Murrah buffaloes (567.50 ± 44.3 kg of live weight; initial days in milk = 52.83 ± 10.24; milk yield = 6-8 kg/d) were randomly allocated to two groups and were fed sugar graze and concentrate mixture as a total mixed ration feed at 70:30 ratio without supplementation (control) or supplemented with monensin 24 mg/kg of dry matter intake (monensin) for 60 days. Nitrogen utilization and plasma metabolites were measured after 50th day of monensin supplementation. Intake of nitrogen and outgo of urinary, faecal, milk and total nitrogen (g/d) were not ($P > 0.05$) affected by monensin supplementation. However, nitrogen retention (g/d) 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, blood urea nitrogen, total protein and albumin were not affected ($P>0.05$) by monensin supplementation. The results suggest that feeding 24 mg/kg dry matter intake of monensin on high forage diets has potential to improve nitrogen utilization and blood glucose concentration in lactating buffaloes which will help in improving profitability of dairy sector while reducing the environmental impact of milk production.

Introduction

In India, livestock rearing is a basic component of the agriculture production system, and plays an important role in the Indian economy (4.11% of the total GDP in 2012–2013 BAHS, (2014) and socio-economic development of millions of rural households (MOA, 2008). Indian livestock have the highest share of the world livestock population (FAO, 2006). 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). In agriculture sector, waste from animal production system contribute as much as 30–50% to the global N_2O emissions but relatively little attention has been given on developing mitigation options (Oenema *et al.*, 2005). Buffalo is a triple purpose animal,

being suitable for milk, meat and draught. The crude protein concentration of ruminant diet essentially nitrogen concentrations of consumed feedstuffs often limit ruminant production (Craine *et al.,* 2010). Dietary protein is used inefficiently by dairy animals compared to non-ruminants, with approximately 72% of nitrogen intake excreted in manure (Mills *et al.,* 2009). This low efficiency of dietary nitrogen utilization is attributable primarily to the effects of the rumen on dietary nitrogen utilization (Calsamiglia *et al.,* 2009). Nitrogen excretion in urine and faeces is a significant environmental concern due to nitrate (NO_3) leaching contributing to aquatic eutrophication, as well as effects on air quality and greenhouse gas emissions through gaseous losses as ammonia (NH_3) and nitrous oxide (N_2O). Dong *et al.,* (2014) stated that intake of nitrogen identified as the main driver of ruminant nitrogen excretion. 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. 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 by improve the efficiency of nitrogen utilization. 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 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 (Rodehutscord, 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 ruminants diet may benefit air quality by reducing CH_4 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). Therefore, various rumen modifiers including monensin have been used in ruminats to increase feed utilization, and production performance while reducing/maintaining environmental impact of milk production. 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). Therefore, the objectives of the present study were to evaluate the effects of monensin supplementation on nitrogen utilization and plasma metabolites of 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 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-December to mid-February. 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 randomly divided into two groups of six animals in each on the basis of body weight. Both groups were fed ration comprising of green sugar graze 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 concentrate mixture (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. 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 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 samples (10 ml) were collected at zero day and last day of animal trial (60^{th} 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 20^0C 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).

Results and Discussion

Chemical composition of ingredients of basal diet are 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).

Effect of dietary monensin supplementation on nitrogen utilization in lactating buffaloes is presented in Table 2. Average N intake (g/d) was 310.04±9.35 and 298.10±4.84 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 faeces, urine and milk, 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 lactating buffaloes. Overall N balance (g/d) was higher (P<0.05) in monensin supplemented group (9.05±0.23) in comparison to control (7.62±0.57). This improvement in N absorption might be due to improvement in lower tract digestibility (Haimoud *et al.*, 1995). According to Duffield *et al.*, (2008) monensin responsible for better nitrogen utilization as available amino acids in the intestine, resulting from changes in rumen fermentation. Monensin has the ability to select and decreases the population of the bacteria that degrade amino acids in the rumen environment and causes deamination. This increased flow of protein to the small intestine will up-regulate the amino acid uptake capacity of the small intestine (Gandra *et al.*, 2012).

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

Table.2 Effect of dietary monensin supplementation on nitrogen utilization in lactating Murrah buffaloes

Parameter	Control	Treatment	P value
N Intake (g/d)	310.04±9.35	298.10±4.84	0.24
Faecal N outgo (g/d)	122.26±6.91	115.02±2.15	0.23
Urine N outgo (g/d)	119.63±4.22	112.00±3.81	0.26
Milk N (g/d)	60.52±1.41	62.04±2.11	0.65
Total N excretion (g/d)	302.42±9.82	289.06±4.94	0.21
N balance (g/d)	7.62^{b}±0.57	9.05^{a}±0.23	0.03
Absorption (% of intake)	60.55±1.06	61.23±0.62	0.57
N retained (% of intake)	2.30±0.28	2.60±0.15	0.33

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

Table.3 Effect of Monensin supplementation on plasma metabolites in lactating buffaloes

Parameter	Days	Control	Treatment	P value
Blood glucose (mg/dl)	Zero day	49.56±0.41	49.51±0.65	0.96
	60th day	51.93 ±0.84 [b]	55.39 ±0.71 [a]	0.02
BUN (mg/dl)	Zero day	23.07±0.20	23.03±0.10	0.79
	60 th day	22.90±0.91	21.29±0.84	0.87
NEFA (µmol/l)	Zero day	141.50±3.12	142.67±2.39	0.77
	60 th day	138.16±0.90	137.67±0.84	0.70
Total Protein (mg/dl)	Zero day	6.87±0.13	6.85±0.14	0.93
	60 th day	7.11±0.13	7.15±0.15	0.86
Albumin (mg/dl)	Zero day	3.01±0.23	3.02±0.12	0.98
	60 th day	3.11±0.13	3.15±0.15	0.97

Means bearing different superscripts [a, b] in same row differ significantly (P<0.05)

Based on these findings, it appeared that monensin altered the nitrogen excretion pattern and may increase the proportion of amino acids absorbed positively by increasing the quality and quantity of nitrogen absorbed.

The concentration of plasma metabolites for both the groups of lactating buffalo at zero day and at the end of experimental trial (60thday) are presented in Table 3.

Ndlovu *et al.,* (2007) reported that plasma concentrations of metabolites such as glucose, BUN, NEFA, Total protein, albumin, globulin and minerals reflect nutritional status of dairy cows. Blood glucose (mg/dl) concentration was similar at the day of start of experiment for control (49.56±0.41) and treatment group (49.51±0.65). However, there was increased (P<0.05) concentration of blood plasma glucose (mg/dl) in monensin supplemented group (55.39±0.71) as compared to control ((51.93±0.84) till the end of the experiment. These values are within normal physiological range and comparable with findings of previous study (Hagawane *et al.,* 2009; Kalasariya *et al.,* 2017 and Yogi, 2017). In consonance with our finding, Helal and Lasheen (2008), also reported significant increase in blood glucose concentration with monensin (400 mg/d) supplemented feed in lactating buffaloes. Propionate are the precursor for gluconeogenesis and its supply is increased by the action of ionophores in the rumen (Bauman and Elliot, 1983), this might be the possible explanation for increase in blood glucose in the monensin supplemented group. Blood urea nitrogen (mg/dl) concentration was similar for both the group at the day of start of experiment. At the end of animal trial concentration of blood urea nitrogen (BUN) was not differed in control (22.90±0.91 mg/dl) and monensin supplemented (21.29±0.84 mg/dl) group. These values are within normal physiological range and comparable with findings of Yogi (2017). Similar to present findings Lamba *et al.,* (2013) observed that concentration of BUN was unaffected by monensin (300 mg/d) supplementation to lactating cows and monensin (24 mg/kg DM) supplementation did not altered blood profile in mid lactation Holstein cows reported by Vendraminia *et al.,* (2016). Plasma NEFA (µmol/l) concentration was similar in both the groups at the day of start of experiment. At the end of the animal trial plasma level of NEFA (µmol/l) did not differ (P>0.05) in control (138.16±0.90) and monensin supplemented (137.67±0.84) group. In agreement to present study findings

Mullins *et al.*, (2011) reported no significant effect on plasma NEFA concentration in transition dairy cow fed ration supplemented with monensin (400mg/cow/day). On other hand Duffield *et al.*, (2008) reported that monensin decrease NEFA concentration by a meta-analysis including 24 studies. Different response for blood NEFA concentration could be attributed to variation in type and composition of offered feedstuffs and species of animal. This difference might be due to animal species and their physiological stages. Initial blood plasma concentration of total protein (6.87±0.13; 6.85±0.14 mg/dl) and albumin (3.01±0.23; 3.02±0.12 mg/dl) concentrations was also similar in both the groups and at the end of the experimental trial blood plasma concentration of total protein (7.11±0.13; 7.15±0.15) and albumin (3.11±0.13; 3.15±0.15 mg/dl) concentration was also not affected (P>0.05) by monensin supplementation. These values are within normal physiological range and comparable with findings of Yogi, (2017). Similar to findings of present study Helal and Lasheen, (2008) who reported no significant effect on plasma total proteins and albumin concentration in monensin (400mg/day) supplemented lactating buffaloes.

It was concluded that supplementation of monensin (24 mg/kg of dry matter intake) to lactating Murrah Buffaloes increased blood glucose concentration indicating more available energy and could improve nitrogen utilization which will reduce the contribution of lactating buffaloes to green house gases emissions and their impact on the environment.

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