

Effect of monensin supplementation on conjugated linoleic acid content in the milk of cows and buffaloes

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ABSTRACT

Multiparous Murrah buffaloes (16) and cows (16) in their early stage of lactation were randomly divided into 2 groups of 8 animals each (groups 1 and 2 for buffaloes and groups 3 and 4 for cows) to study the effect of monensin supplementation on conjugated linoleic acid content in their milk. Animals in groups 1 and 3 were offered maize forage, wheat straw and concentrate mixture and animals in groups 2 and 4 were supplemented with ionophore, i.e. 200 mg monensin sodium/d. Nutritional requirements of the animals were fulfilled as per standard requirements for 90 days of experimental period. Concentrate mixture contained more linoleic acid as compared to maize fodder (14.22 vs 3.54 mg/g dry sample); whereas linolenic acid was more in maize as compared to concentrate mixture (6.82 vs 1.32 mg/g dry sample). After an adaptation period of 30 days, the milk samples were collected at fortnightly intervals and analyzed for milk fat, solid not fat, total solids and milk protein. Milk sample (100 ml) from each buffalo (morning and evening milking) and cow (morning, afternoon and evening milking) was collected at fortnightly interval and pooled for fatty acid analysis including CLA. Daily milk yield averaged 7.19 and 6.91 kg in buffaloes and 10.96 and 10.67 kg in cows, in the two respective groups. There was no effect of monensin supplementation on the milk yield of cows and buffaloes. Broad milk composition was not affected by monensin supplementation in both the species. Total CLA content averaged 6.2 and 7.1 mg/g fat in buffaloes and 6.8 and 8.1 mg/g fat in cows in the 2 two respective groups showing an increase of 14.51 and 19.11% due to monensin supplementation in the two species, respectively, indicating the beneficial effect of monensin supplementation on CLA content.

Key words: Buffalo, Conjugated linoleic acid, Cow, Milk, Monensin supplementation

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (c-9, c-12 C_{18,2}) with two conjugated double bonds at various carbon positions in the fatty acid chain. It is formed as an intermediate during the biohydrogenation of linoleic acid to stearic acid by ruminal bacteria which help in disposing hydrogen. Monensin reduces methane production by inhibiting the gram-positive bacteria that produce hydrogen and therefore may interfere with the process of biohydrogenation. Interest in CLA has increased after the discovery of its health-promoting properties, including potent anticarcinogenic activity. Various studies have indicated that the diets rich in green fodder/pasture and certain oils have major influence on the CLA content of milk (Dhiman *et al.* 1999, Tyagi *et al.* 2006). Supplementation of monensin resulted in improvement in the CLA content of milk fat in some studies in cows (Bell *et al.* 2006, Odongo *et al.* 2007), but no information is available in its effect on CLA content of buffalo milk fat. Therefore,

the present studies were conducted to evaluate the effect of monensin supplementation on milk yield and its CLA content in both cows and buffaloes.

MATERIALS AND METHODS

Multiparous Murrah buffaloes (16) and cows (16) in their early stage of lactation were selected from the herd of Institute, were randomly divided in 4 equal groups of 8 each on the basis of body weight and milk yield (groups 1 and 2 for buffaloes and groups 3 and 4 for cows). Animals in groups 1 and 3 were offered maize forage, wheat straw and concentrate mixture (Table 1) and animals in treatment groups 2 and 4 were supplemented monensin sodium @ 200 mg/d. Nutritional requirements of cows were fulfilled as per NRC (1989) and that of buffaloes as per Kears (1982) for 90 days of experimental period. All the animals were housed in well-ventilated stalls having arrangements for individual feeding and watering. The buffaloes were milked twice at 600 and 1800 h, whereas cows were milked 3 times at 600, 1200 and 1800 hr daily. The proximate composition (AOAC 1995) and cell wall constituents (Van

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Soest et al. 1994) of various feedstuffs used in the experiment were estimated.

The milk samples collected at fortnightly intervals were analyzed for milk fat (BIS 1989), total solids (BIS 1989) and milk protein (AOAC 1995). Solid-not-fat content in milk was calculated by subtracting fat percentage from total solids. Another milk sample (100 ml) from each cow and buffalo was collected from the morning and evening milking at fortnightly intervals and pooled for fatty acid analysis including CLA as per Dhiman *et al.* (1999) using gas chromatograph fitted with ionization detector and 3396A integrator. Samples containing methyl esters of fatty acids in hexane (1 to 3 ml) were directly injected through the splitless injection port onto a supelco wax 10, fused silica capillary column (60 m × 0.32 mm i.d. 0.25 µm film thickness). Injector temperature of the gas chromatograph was maintained at 250°C. Initial oven temperature was 50°C which was programmed to increase @ 20°C per min up to 200°C and was held for 50 min. Thereafter, it was raised to 225°C @ 10°C per min and held for 20 min. Detector temperature of GC was 250°C. Fatty acids were identified by comparing the retention time with the methylated fatty acid standards including CLA (*cis*-9, *trans*-11 18:2). The percentage of each fatty acid was calculated by dividing the peak area under particular fatty acid by the total peak area under reported fatty acids.

Statistical analysis of the data for milk production and fatty acid composition of milk of different treatment groups was carried out through SPSS 10.0 software package using general linear model. The effect of different treatment (groups) was determined by analysis of variance (Snedecor and Cochran 1980). The statistical model used is described below:

$$Y_{ijkl} = \mu + B_i + D_j + A_k + e_{ijkl}$$

where, Y_{ijkl} , The *i*th observation of *k*th animal fed with *j*th diet and belonging to *i*th breed; μ , overall mean; B_i , effect

due to *i*th breed; D_j , effect due to *j*th diet; A_k , effect due to *k*th animal; and e_{ijkl} , random error.

RESULTS AND DISCUSSION

Intake, milk yield and milk composition: The proximate and cell wall composition of concentrate mixture, maize fodder and wheat straw are presented in Table 2. Fatty acid composition of maize fodder, concentrate mixture and wheat straw (Table 3) indicated that maize provided more of C 18:3 (6.99 mg/g), while concentrate mixture was high in C18:2 (14.22 mg/g). Elgersma *et al.*, (2003a) reported higher concentration of linolenic acid in fresh grass which varies with the plant and environmental factors such as stage of maturity as well as season. Tyagi *et al.* (2006) also reported higher content of C18:3 in berseem fodder (14.98 mg/g) as compared to concentrate mixture.

Body weight of buffaloes averaged 536.90, 544.30 and 374.64 and 334.48 kg in the two respective groups of each species (Table 4). DM intake/100 kg b.wt. was similar in both the groups of buffaloes, but it was higher in cows (group 4) supplemented with monensin. Mutsvangwa *et al.* (2003) reported that supplementation with monensin premix

Table 2. Chemical composition of offered feeds (% DM basis)

Component	Concentrate	Maize fodder	Wheat straw
OM	91.85	91.27	91.85
CP	23.06	8.37	5.51
EE	4.27	2.12	1.30
Total ash	8.15	8.73	9.56
NFE	55.22	35.27	40.36
NDF	40.12	62.76	79.68
ADF	13.79	36.40	55.21
Hemicellulose	26.33	23.36	27.47

Table 3. Fatty acid composition of forage and concentrate mixture

Fatty Acid ¹	Maize fodder	Concentrate mixture	Wheat straw
C12:0	0.05	0.00	0.03
C14:0	0.14	0.05	0.06
C14:1	0.34	0.00	0.00
C16:0	4.12	5.83	0.80
C16:1	0.08	0.17	0.03
C18:0	0.64	1.20	0.21
C18:1	0.91	16.07	0.69
C18:2	3.54	14.22	0.71
C20:0	0.10	0.42	0.71
C18:3	6.88	1.32	0.19
C22:0	0.17	0.64	0.09
C22:1	0.000	4.080	0.04
C24:0	0.19	0.44	0.06

¹Expressed as number of carbons: number of double bonds; *c*=*cis*

Table 1. Ingredient composition of concentrate mixture

Ingredient	Percentage
Maize	29
Groundnut cake (Expeller)	20
Barley	5
Mustard cake (expeller)	12
Wheat bran	20
De oiled Rice bran	11
Mineral mixture*	2
Common salt	1

Dicalcium phosphate 1.65 kg; chalk power 0.3312 kg; magnesium carbonate 0.0900 kg; sodium chloride 0.0900 kg; ferrous sulphate 0.01500 kg; copper sulphate 0.0021 kg; manganese oxide 0.0021 kg; cobalt chloride 0.0015 kg; potassium iodine 0.0003 kg; sodium fluoride 0.0003 kg; zinc sulphate 0.0075 kg.

Table 4. Effect of monensin on voluntary feed intake of lactating animals

Parameter	Buffaloes		Cows	
	Group 1	Group 2	Group 3	Group 4
Body weight (kg)	536.90	544.30	374.64	334.48
DMO (kg/animal/d)				
Maize fodder	5.22	5.10	4.38	4.38
Wheat straw	4.79	4.70	3.64	6.64
Concentrate mixture	5.55	5.44	6.13	6.07
TDMO	15.56	15.24	14.15	14.09
Residual feed	1.33	1.07	2.29	1.95
DMI	14.27	14.17	11.86	12.14
	±0.23	±0.33	±0.20	±0.09
%DMI	2.66	2.61	3.19	3.64

in Holstein cows receiving a total mixed ration increased DMI (21.3 vs. 19.8 kg/d).

The average milk yield in four respective groups did not show any difference due to monensin supplementation in both the species (Table 5). A number of investigations have demonstrated that monensin increases milk yield when fed to cows offered mixed grain and forage diets (Granzin and Dryden 1999) or pasture based diets (Hayes *et al.* 1996). Mutsvangwa *et al.* (2002, 2003) recorded significant increase in milk yield in monensin supplemented Holstein cows fed a total mixed ration. Monensin feeding typically increases milk to a larger extent in early lactation (Gallardo *et al.* 2005) compared to later lactation, where feed efficiency is improved by monensin (Ipharraguerre and Clark 2003, McGuffey *et al.* 2003), but this trend was not observed in the present experiment.

Monensin supplementation also did not affect the broad milk composition in both the species. Mutsvangwa *et al.* (2002, 2003) recorded depression in milk fat content, but little change in the yield of milk constituents in Holstein cows. Milk protein content remained unchanged due to monensin supplementation leading to increased protein yields (Granzin and Dryden 1999) due to increased milk yield. Milk fat content was higher ($P>0.01$) in buffalo milk as compared to cow milk (Table 5) due to species variation which has also been documented earlier by many workers (Bargo *et al.* 2002, Kathirvalen 2007). The higher fat content in buffalo

Table 5. Average milk production performance of animals in different groups

Particulars	Buffaloes		Cows	
	Group 1	Group 2	Group 3	Group 4
Milk yield (kg/d)	7.19±0.19	6.91±0.11	10.96±0.1	10.37±0.18
Milk fat (%)	6.06±0.12	5.93±0.09	3.27±0.04	3.28±0.04
Milk protein (%)	3.54±0.10	3.54±0.0	2.92±0.06	2.73±0.07
Total solids (%)	15.07±0.10	14.74±0.12	11.42±0.07	11.33±0.09
Soild-not-fat (%)	9.01±0.05	8.81±0.04	8.84±0.04	8.06±0.04

milk also resulted in higher total solids as well as solid-not fat content (Tyagi *et al.* 2006).

Fatty acid composition of milk: The average values for milk fatty acids are presented in Table 6. The levels of C14:0, C16:0 and C16:1 were significantly higher in buffalo milk as compared to cow milk, whereas, levels of C10:0, C12:0, C18:1, C18:2, C18:3 and CLA were higher in cow milk. Arumugam and Narayanan (1979) also reported similar pattern of medium chain fatty acids in cow and buffalo ghee. Tyagi *et al.* (2007) observed consistant values of fatty acids in the milk of buffalo and cow respectively. The total amount of unsaturated fatty acids was more in cow milk as compared to buffalo milk fat, while the amount of saturated fatty acids showed reverse trend (Table 6). The content of both mono- and poly-unsaturated fatty acids was higher in cow milk. The differences may be due to de novo synthesis at the mammary gland level which might also be responsible for more CLA content in cow milk fat as compared to buffalo milk fat (Baumgard *et al.* 2002).

A decrease in saturated FA concentration and an increase in $C_{18:1}$ content of milk fat in dairy cows fed monensin was observed in cows, but this trend was not observed in buffaloes (Table 6). Sauer *et al.* (1998) also reported a decrease in saturated FA content and increase in $C_{18:1}$ in milk fat of dairy cows fed monensin. Fellner *et al.* (1997) indicated that monensin interferes with ruminal lipid metabolism by inhibiting lipolysis and biohydrogenation of dietary unsaturated fatty acids, thereby increasing the content of unsaturated fatty acids in ruminal bacteria and the flow of unsaturated FA to post-ruminal sites (Kobayashi *et al.* 1992) resulting in decrease in saturated FA concentration of milk fat in dairy cows fed monensin.

The CLA content in milk fat was increased by 14.51 and 19.11% in buffaloes and cows, respectively (Table 6), due to monensin supplementation @ 200 mg/d/animal. Odongo *et al.* (2007) reported that monensin reduced the percentage of short and medium-chain saturated fatty acids C7:0, C9:0, C15:0 and C16:0 in milk fat by 26, 35, 19 and 6%, respectively and increased the long-chain saturated FA by 9% and total conjugated linoleic acid (CLA) by 43%. However, the increase in CLA content was comparatively less in the present finding. Mutsvangwa *et al.* (2003) studied the effect of a monensin premix on milk fatty acid content during grain-induced subacute ruminal acidosis (SARKA) in Holstein cows receiving a total mixed ration. The administration of a monensin premix elevated milk fat proportion of total short-chain saturated fatty acids (sum of C_4 to C_{15}), but conjugated linoleic acid isomers and linoleic acid were unaffected. Linolenic acid ($C_{18:3}$) in milk fat of monensin-treated cows was lower when compared with placebo-treated cows during the SARA period which was not the case in the present experiment. Bell *et al.* (2006) evaluated the effect of supplementation of safflower oil, flaxseed oil, monensin, and vitamin E on the concentration

Table 6. Average fatty acid composition of milk (mg/g fat)

Fatty acid	Buffalo milk			Cow milk		
	Group 1	Group 2	SEM	Group 3	Group 4	SEM
C _{10:0}	15.3	15.8	0.5	21.6	23.4	0.5
C _{12:0}	19.2	19.8	0.6	27.6	24.8	0.7
C _{14:0}	108.3	107.4	1.8	98.7	99.4	2.0
C _{16:0}	309.3	310.6	4.0	262.6	273.6	3.8
C _{16:1}	17.2	18.6	0.7	10.2	11.6	0.5
C _{18:0}	137.9	136.7	3.3	147.5	146.7	3.6
C _{18:1}	215.4	212.7	4.6	235.6	242.3	5.4
C _{18:2}	23.2	23.6	0.1	28.2	30.6	0.5
CLA	6.2	7.1	0.4	6.8	8.1	0.6
C _{18:3}	7.1	7.4	0.1	7.8	8.4	0.5
SFA	590.0	590.3	6.5	558.0	567.9.3	5.5
UFA	269.1	269.4	3.6	288.6	301.00	3.7

SFA, Sum of C_{10:0}, C_{12:0}, C_{14:0}, C_{16:0} and C_{18:0}; UFA=Sum of C_{16:1}, C_{18:1}, C_{18:2} CLA and C_{18:3}.

of conjugated linoleic acid in bovine milk fat and found that the combination of safflower oil with monensin instead of monensin alone was effective in increasing milk fat CLA.

It is concluded that daily supplementation of 200 mg monensin to buffaloes and cows in their early lactation did not affect the milk production, but increased the CLA content to the extent of 14.51 and 19.11%, respectively, indicating the potential of monensin to enhance the nutritional properties of milk with regard to human health.

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