Effect of different levels of monensin in diets containing whole cottonseed on milk production and composition of lactating dairy cows

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Summary

The objective of this study was to determine the effects of feeding different levels of monensin on feed intake, milk production and composition, and milk fatty acid profile in lactating Holstein cows. Four multiparous cows averaging 517 ± 47 (SD) kg in body weight and 101 ± 19.8 (SD) days in milk were housed individually in tie-stalls. The study was conducted as a 4 × 4 Latin square design for four periods (14-d for adaptation and 7-d for sampling). Cows were offered four dietary treatments (0, 10, 20, or 30 mg of monensin/kg of DM) as total mixed ration, twice daily. Dry matter (DM) intake was similar among treatments. Monensin supplementation significantly increased ($P<0.05$) milk yield and 4% fat corrected milk (FCM). Milk fat and protein percentages were not affected by monensin supplementation, but fat yield was increased. Monensin reduced the percentage of the short-chain and saturated fatty acids in milk fat, but had no effect on the percentages of medium- and long-chain fatty acids. Monensin supplementation increased ($P<0.05$) unsaturated fatty acids concentrations in milk fat. Based on the results of this study, feeding monensin was effective in inhibiting the biohydrogenation of unsaturated fatty acids in the rumen, and consequently increased the percentage of unsaturated fatty acids in milk fat, which improves the health characteristics of milk for human consumption.

Key words: Monensin, Whole cottonseed, Milk fatty acid composition, Dairy cow

Introduction

Positive effects of food components on human health have recently been recognized. Fat is the major energy containing component in milk and accounts for many of the physical properties, manufacturing characteristics, and organoleptic qualities of milk and milk products (Bauman and Griinari, 2003). Milk fat, due to its relatively high proportion of myristic ($C_{14:0}$) and palmitic acids ($C_{16:0}$), has been associated with human cardiovascular health problems (Noakes *et al*., 1996). Increasing dietary concentrations of unsaturated fatty acids decreases milk $C_{14:0}$ and $C_{16:0}$ levels (Palmquist *et al*., 1993). Increasing specific unsaturated fatty acids such as conjugated linoleic acid (CLA), linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) in milk would increase consumer interest and acceptance of milk due to the health benefits associated with these fatty acids (Ramaswamy *et al*., 2001).

The fatty acid content of the lactating cow diet affects the type and the proportion of the fatty acids in the milk fat (Grummer, 1991). Conjugated linoleic acid is an intermediate product of biohydrogenation of linoleic acid by the rumen bacterium, *Butyrivibrio fibrosolvens* (Harfoot and Hazelwood, 1988). Whole cottonseed is a unique feedstuff because of its high content of energy, mainly in the form of oil,

moderately high level of CP, and high quality fiber (Harvatine *et al*., 2002). Cottonseed oil is also a good source of unsaturated fatty acids and contains approximately 500 g/kg linoleic acid (NRC, 2001). Biohydrogenation of unsaturated fatty acids in the rumen is affected by the type and amount of fatty acid substrate, forage to grain ratio, and nitrogen content of the diet (Harfoot and Hazelwood, 1988).

Monensin is a carboxylic polyether ionophore antibiotic produced by fermentation of *Streptomyces cinnamonensis* (Russell, 2002) and has been used extensively in the diet of dairy cows (Da Silva *et al*., 2007; Odongo *et al*., 2007; Alzahal *et al*., 2008). The benefits of feeding monensin to dairy cattle include increased milk production and improved energy balance associated with reduced incidence of subclinical ketosis, clinical acidosis, and displaced abomasums (Duffield and Bagg, 2000). Monensin inhibits the growth of gram-positive bacteria. Several gram-positive bacteria, including *Butyrivibrio fibrosolvens*, are involved in rumen biohydrogenation (Ipharraguerre and Clark, 2003). Therefore, methods that decrease ruminal biohydrogenation would increase the transfer of polyunsaturated fatty acids from the diet to milk. Although there are many experiments measuring the effects of monensin in dairy cows, few studies have addressed the effects of dietary monensin on the profile of fatty acids in the milk fat of lactating dairy cows. Therefore, the present study was aimed at determining the effects of feeding different levels of monensin on feed intake, milk production, milk composition, and the milk fatty acid profile of lactating dairy cows.

Materials and Methods

Experimental design and data collection

Four multiparous Holstein cows with an average body weight of 517 ± 47(SD) kg, 101 ± 19.8 (SD) days in milk and 25.37 ± 6.8 (SD) kg/d of milk were assigned to a 4 × 4 Latin square design.

Each experimental period consisted of 14 days of adaptation to the diets and 7 day daily data collection. Control (basal) (without monensin supplementation) or monensin-supplemented diets (10, 20, or 30 mg/kg of DM) were fed individually twice daily at 0800 and 1600 h, and the daily allowance was adjusted to obtain 10% orts (Table 1). Monensin was mixed in the total mixed ration (TMR) as diluted premixes. The diets were formulated according to the NRC (2001) recommendations.

Table 1: Ingredients and chemical composition (DM basis) of the basal diet

Ingredient	% of DM
Alfalfa hay	17.99
Corn silage	26.09
Ground barley	19.26
Ground corn	12.35
Soybean meal	4.45
Whole cottonseed	11.47
Wheat bran	6.40
Salt	0.49
Mineral and vitamin mixture[1]	1.50
Chemical composition (%)	
DM	54.50
CP	15.80
Ether extract	4.70
NDF	31.90
Ash	6.60
NFC[2]	41.0
Ca[3]	0.79
P[3]	0.38
Mg[3]	0.19
NE_L[3], Mcal/kg	1.63

[1]Each kg of mineral and vitamin mixture contained: 180 g of Ca, 70 g of P, 35 g of K, 50 g of Na, 58 g of Cl, 30 g of Mg, 32 g of S, 5 g of Mn, 4 g of Fe, 3 g of Zn, 300 mg of Cu, 100 mg of I, 100 mg of Co, 20 mg of Se, 400,000 IU of vitamin A, 100,000 IU of vitamin D_3, and 245 IU of vitamin E. [2]NFC = Nonfiber carbohydrates, NFC = 100 – (%CP + %NDF + %EE + %ash). [3]Estimated using equations and values according to NRC (2001)

Cows were housed in tie stalls and milked twice daily at 0645 and 1530 h during the sampling period. Milk production and feed consumption were recorded at each milking and daily, respectively. Samples of TMR and orts were collected daily, stored at -20°C, and composited per cow for each experimental period. Composite samples were mixed thoroughly and sub-sampled for chemical analysis. Milk samples were

obtained from 4 consecutive milkings on day 16 and 17 of each experimental period and pooled within cow and period relative to production to obtain one composite milk sample per cow per period for chemical analysis. Milk samples were kept at room temperature, containing potassium dichromate as a preservative for determination of protein and fat concentrations (Milk-O-Scan 133B Foss Electric, Denmark). One milk sample without preservative was kept frozen for determination of the milk fatty acid profile. Feed and ort samples were dried in a forced-air oven at 60°C for 48 h. The dry weights were used to determine feed intake. Sub-samples of feed and orts were dried at 105°C for 24 h to correct the nutrient content to 100% DM. Feed samples were ground through a 1-mm screen. The TMR samples were analyzed (AOAC, 2000) for dry matter (DM), ash, crude protein (CP), ether extract (EE), and neutral detergent fiber (NDF).

Fatty acid analysis of whole cottonseed and TMR samples (Table 2) was carried out using the modified method of Sukhija and Palmquist (1988) and $C_{22:1}$ (erucic acid) was used as the internal standard. Milk (20 ml) was centrifuged at 8000 × g for 30 min to form a solid milk fat layer on top of the milk, and 100 mg of milk fat was used for analysis. Two milliliters of hexane was used as solvent. Methylation occurred by heating samples for 1.5 h at 50°C. After removal of the solvent layer, 1.0 ml of hexane was added to the original tube, and samples were mixed and centrifuged at 4000 × g for 20 min. The solvent layer was then removed and mixed with the first solvent layer. Approximately 0.5 g of anhydrous sodium sulfate was added to the composited sample, and the sample was vortexed and allowed to stand for 0.5 h before final centrifugation (Qui *et al*., 2004). The GC was equipped with a 30-m capillary column for analysis of fatty acids. The injector and detector ports were set at 280 and 300°C, respectively. Initially, the column temperature was kept at 160°C for 5 min, and then increased at a rate of 2°C/min to 180° when the column temperature was kept at 180°C for 5 min and then ramped (2°C/min) to 190°C. Fatty acid peaks were identified using pure methyl ester standards.

Table 2: Fatty acid profile (g/100 g of total fatty acids) of the basal diet and whole cottonseed

Fatty acid[1]	Basal diet	Whole cottonseed
$C_{14:0}$	0.73	0.75
$C_{16:0}$	23.21	23.56
$C_{16:1}$	0.27	0.40
$C_{18:0}$	2.58	3.07
$C_{18:1}$ (cis-9)	19.87	10.91
$C_{18:2}$ (cis-9 cis-12)	51.65	49.71
$C_{18:3}$ (n-3)	0.66	0.25
Others[2]	1.03	11.35
Saturated[3]	26.52	27.38
Unsaturated[4]	72.45	61.27
U: S ratio[5]	2.73	2.24

[1]Expressed as number of carbons: number of double bonds. [2]Unidentifiable peaks. [3]Sum of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{17:0}$, and $C_{18:0}$. [4]Sum of $C_{14:1}$, $C_{15:1}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$. [5]Unsaturated: saturated fatty acids ratio

Statistical analysis

All statistical analyses were performed using PROC MIXED of SAS (1999). Data on DM intake, milk production, milk composition, and milk fatty acids profile were analyzed using the following model:

$Y_{ijk} = \mu + P_i + C_j + T_k + e_{ijk}$

Where,

Y_{ijk} = the dependent variable

μ = overall mean

P_i = effect of period

C_j = random effect of cow

T_k = effect of treatment

e_{ijk} = residual error

Statistical significance was declared at $p<0.05$

Results

The DM intake of cows was not significantly different ($P>0.05$) among treatments (Table 3). The addition of monensin to the TMR (especially 10 to 20 mg/kg of DM) increased ($P<0.05$) milk yield and 4% FCM (Table 3). Milk fat and protein percentages were not significantly affected by monensin supplementation, but milk fat yield (kg/day) increased by monensin supplementation compared to the control diet (Table 3). Monensin supplementation decreased concentrations of $C_{10:0}$, $C_{12:0}$ and $C_{18:0}$ and increased those of $C_{18:1}$ (cis-9), $C_{18:1}$ (trans-9) and $C_{18:2}$ (trans-10, cis-12),

but had no significant effect on concentrations of $C_{14:0}$, $C_{14:1}$, $C_{15:1}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:2}$ (cis-9, cis-12) and $C_{18:3}$ (n-3) in milk fat (Table 4). Feeding monensin decreased the concentrations of short-chain and saturated fatty acids but increased the concentrations of unsaturated fatty acids, however, it had no significant effect on the medium- and long-chain fatty acids in milk fat (Table 4). The ratio of the unsaturated to saturated fatty acids in milk fat was significantly (P<0.05) higher for cows fed monensin compared to the control group (Table 4).

Discussion

Feed consumption was not affected by feeding monensin. Published data on feed consumption are not consistent; while feeding monensin at 22 mg/kg of DM (Osborne *et al*., 2004), 24 mg/kg of DM

Table 3: Effects of different levels of monensin on feed intake, milk production and milk composition of dairy cows

Item	Monensin (mg/kg of DM)				SEM[1]	P Contrast[2]
	0	10	20	30		C vs. M
DMI (kg/d)	21.52	21.62	21.53	21.35	0.54	0.14
Milk yield (kg/d)	25.33^{c}	31.78^{a}	31.03^{a}	29.58^{b}	0.62	0.003
4% FCM (kg/d)	22.35^{b}	26.22^{a}	25.80^{a}	24.79^{ab}	0.96	0.005
Fat (%)	3.23	2.83	2.97	2.95	0.17	0.16
Fat (kg/d)	0.77^{b}	0.90^{a}	0.92^{a}	0.91^{a}	0.04	0.02
Protein (%)	3.10	3.05	3.00	3.08	0.09	0.63
Protein (kg/d)	0.78	1.13	1.00	0.91	0.12	0.14

$^{a, b, c}$Means within a row without common superscript differ (P<0.05). [1]Standard error of the mean. [2]P-values for contrast: control vs. monensin

Table 4: Effects of different levels of monensin on the fatty acid concentrations (g/100 g fatty acids) of milk fat of dairy cows

Fatty acid[1]	Monensin (mg/kg of DM)				SEM[2]	P Contrast[3]
	0	10	20	30		C vs. M
$C_{10:0}$	3.72^{a}	3.27^{b}	2.75^{b}	3.31^{ab}	0.23	0.06
$C_{12:0}$	3.81^{a}	3.04^{b}	2.67^{b}	3.19^{b}	0.18	0.007
$C_{14:0}$	10.73	11.97	10.91	12.04	0.73	0.32
$C_{14:1}$	0.77	1.17	0.84	0.73	0.23	0.59
$C_{15:1}$	0.16	0.15	0.33	0.11	0.08	0.69
$C_{16:0}$	33.66	31.61	32.00	34.29	0.85	0.33
$C_{16:1}$	1.33	1.58	1.34	1.48	0.28	0.69
$C_{17:0}$	0.50	0.48	0.54	0.58	0.04	0.46
$C_{18:0}$	15.73^{a}	10.68^{b}	12.71^{b}	11.22^{b}	0.89	0.007
$C_{18:1}$ (cis-9)	22.89^{b}	28.48^{a}	27.62^{a}	25.24^{ab}	1.36	0.04
$C_{18:1}$ (trans-9)	1.22^{b}	1.71^{ab}	2.28^{a}	2.37^{a}	0.27	0.03
$C_{18:2}$ (cis-9, cis-12)	2.06	2.51	2.66	2.80	0.22	0.06
$C_{18:2}$ (trans-10, cis-12)	0.03^{b}	0.09^{a}	0.07^{a}	0.08^{a}	0.007	0.01
$C_{18:3}$ (n-3)	0.32	0.32	0.47	0.42	0.12	0.58
Others[4]	2.13	2.75	2.88	2.35	0.52	0.37
Short[5]	7.53^{a}	6.31^{b}	5.42^{b}	6.49^{b}	0.33	0.02
Medium[6]	43.83	46.94	45.75	49.29	2.32	0.24
Long[7]	40.88	43.90	45.86	41.82	1.77	0.19
Unsaturated[8]	29.05^{b}	36.10^{a}	35.46^{a}	33.45^{a}	1.26	0.006
Saturated[9]	68.15^{a}	61.06^{b}	61.57^{b}	64.11^{ab}	1.45	0.01
U:S ratio[10]	0.42^{b}	0.60^{a}	0.58^{a}	0.52^{ab}	0.04	0.05

$^{a, b, c}$Means within a row without common superscript (s) differ (P<0.05). [1]Expressed as number of carbons: number of double bonds. [2]Standard error of the mean. [3]P-values for contrast: control vs. monensin. [4]Unidentifiable peaks. [5]Short-chain fatty acids ($C_{10:0}$-$C_{12:0}$). [6]Medium-chain fatty acids ($C_{14:0}$-$C_{17:0}$). [7]Long-chain fatty acids ($\geq C_{18:0}$). [8]Sum of $C_{14:1}$, $C_{15:1}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$. [9]Sum of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{17:0}$, and $C_{18:0}$. [10]Unsaturated: saturated fatty acids ratio

(Bell *et al*., 2006), 300 mg/d (Van der Werf *et al*., 1998; Phipps *et al*., 2000) or 320 mg/d (Da Silva *et al*., 2007) did not have a significant effect on DM intake of dairy cows, other researchers (Ali Haimoud *et al*., 1995; Green *et al*., 1999) reported a reduction in DM intake due to monensin feeding.

Monensin (especially at 10 and 20 mg/kg of DM) increased the milk yield and 4% FCM although the DM intakes were similar for all diets (Table 3). The effect of monensin on milk yield has also been inconsistent. Lean and Wade (1997), Phipps *et al*. (2000), and Ruiz *et al*. (2001) found increased milk production, but according to Osborne *et al*. (2004), Bell *et al*. (2006), and Da Silva *et al*. (2007) feeding monensin (20 to 24 mg/kg DM) had no significant effect on milk yield. These discrepancies could be related to factors such as stage of lactation, diet composition, and the length of the experimental period. Sauer *et al*. (1998) suggested that some adaptive changes in the rumen microflora can also affect the response of dairy cows to monensin supplementation. The response to monensin also differs with the genetic backgrounds of the cows. Cows with a high capacity for milk production showed a better response to monensin supplementation (Van der Werf *et al*., 1998). A supply of glucogenic precursors, resulting from changes in the pattern of rumen fermentation, can also be a likely mechanism of supporting additional milk yield by monensin supplementation.

Milk fat percentage was not significantly affected by monensin supplementation. This is in agreement with the results of Martineau *et al*. (2007), but Phipps *et al*. (2000), Da Silva *et al*. (2007), Odongo *et al*. (2007), and Alzahal *et al*. (2008) reported a reduction in milk fat percentage with monensin supplementation. The milk-fat-depressing effect of monensin has been attributed to the reduced ruminal production of acetate and butyrate, which might result in a shortage of lipogenic precursors for the synthesis of fatty acids in the lactating mammary gland (Dye *et al*., 1988; Van der Werf *et al*., 1998). Alternatively, data from *in vitro* experiments indicated that monensin may inhibit ruminal biohydrogenation of long chain fatty acids (Fellner *et al*., 1997), which in turn might enhance the supply of trans-10, cis-12 CLA to the mammary gland (Bauman and Griinari, 2001). Increased availability of this trans fatty acid in the mammary gland, which appears to be a potent inhibitor of the *de novo* synthesis of fatty acids (Bauman and Griinari, 2001), might be part of the mechanism responsible for the reduced milk fat output of monensin-treated dairy cows. The higher milk yield and 4% FCM with monensin treated diets might be due to the higher milk yield with these diets compared to the control diet.

Monensin had no significant effect on milk protein percentage or milk protein yield, which is consistent with previous studies (Van der Werf *et al*., 1998; Da Silva *et al*., 2007; Alzahal *et al*., 2008).

In the current study, feeding monensin decreased concentrations of short-chain and saturated fatty acids and increased concentrations of unsaturated fatty acids, but had no effect on medium- and long-chain fatty acids in milk fat. In the study by Da Silva *et al*. (2007) feeding monensin at 20 mg/kg of DM had no effect on short-, medium- and long-chain fatty acids concentrations, but decreased saturated fatty acids concentrations in milk fat. Fatty acids in milk arise from two sources; uptake from circulation and the *de novo* synthesis within the mammary epithelial cells (Neville and Picciano, 1997). Short- and medium-chain fatty acids arise almost exclusively from the *de novo* synthesis using circulating acetate and butyrate originating from the rumen, whereas long-chain fatty acids are derived from the uptake of circulating lipids. Palmitic acid ($C_{16:0}$) originates from both the *de novo* synthesis and uptake from circulating lipids (Mansbridge and Blake, 1997). In the current study, lower concentrations of short-chain fatty acids in milk fat might, in part, be due to a reduction in ruminal production and supply of acetate and butyrate, as reported previously (Van der Werf *et al*., 1998; Martineau *et al*., 2007). The increased proportion of trans-10, cis-12 CLA with monensin supplementation might also have been involved in decreasing the *de novo* synthesis of the short-chain fatty acids in mammary gland (Baumgard *et al*., 2000). It is generally accepted that ionophores, such as monensin, are partially

effective in inhibiting the biohydrogenation of linoleic acid, thus reducing the rate of stearic acid production (Fellner *et al*., 1997). Feeding monensin inhibits the last step of biohydrogenation because it increases the rumen outflow of trans $C_{18:1}$ fatty acids and reduces the outflow of stearic acid (Bauman and Griinari, 2003).

The higher concentration of $C_{18:1}$ (cis-9) in milk of monensin treated cows in the present experiment, at least in part, may suggest that monensin partially inhibited microbial biohydrogenation of $C_{18:1}$ (cis-9). According to Van Nevel and Demeyer (1995), monensin inhibits lipolysis and subsequently reduces the formation of free carboxyl groups necessary for the hydrogenation of double bonds.

Addition of monensin to TMR increased $C_{18:1}$ (trans-9) concentration in milk fat compared to the control group in our work. Fellner *et al*. (1997) observed a higher concentration of trans $C_{18:1}$ in continuous cultures of ruminal bacteria following the infusion of monensin. Ruminal microorganisms capable of fatty acid hydrogenation are often divided into groups A (e.g., *B. fibrisolvens* and *Ruminococcus albus*) and B (e.g., *Fusocillus* spp.) based on their products and patterns of isomerization during biohydrogenation (Harfoot and Hazelwood, 1988). Bacterial species in group A hydrogenate linoleic acid to $C_{18:1}$ (*trans*) but appear incapable of hydrogenating monoenes. Group B bacteria, on the other hand, can hydrogenate a wide range of monoenes to stearic acid, including $C_{18:1}$ (trans-9). Although monensin is known to inhibit gram-positive bacteria (e.g., *B. fibrisolvens*; Van Nevel and Demeyer, 1995), it seems from the results of the present study that monensin might also inhibit the activities of group B bacteria, responsible for the last step of biohydrogenation of $C_{18:2}$ (cis-9, cis-12) to $C_{18:0}$. As a consequence, more $C_{18:1}$ (trans-9) can escape biohydrogenation, thus resulting in a higher level of $C_{18:1}$ (trans-9) in the milk fat. In terms of human health, these alterations can be considered as an improvement in the fatty acid profile of milk fat because saturated fatty acids have been reported to constitute the hypercholestrolemic portion of milk fat (Ney, 1991).

Addition of monensin to the diet increased the milk production, but had no significant effect on milk fat and protein percentages. Monensin increased the ratio of unsaturated to saturated fatty acids in milk fat. Therefore, monensin can be considered as an effective inhibitor of biohydrogenation of unsaturated fatty acids in the rumen, and consequently as a tool for increasing the supply of unsaturated fatty acids to the mammary gland for milk fat synthesis, thus enhancing the nutritional properties of the milk in terms of human health.

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