

Approval Date: October 28, 2004

FREEDOM OF INFORMATION SUMMARY
SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION
NADA 095-735

Monensin Sodium (RUMENSIN 80)

**Type A Medicated Article
for Dairy Cattle**

For increased milk production efficiency (production of marketable solids-corrected milk per unit of feed intake) in dairy cows.

Sponsored By:

**Elanco Animal Health
A Division of Eli Lilly & Co.
Lilly Corporate Center
Indianapolis, IN 46285**

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FREEDOM OF INFORMATION SUMMARY

RUMENSIN 80 Type A Medicated Article for Dairy Cattle

1. GENERAL INFORMATION:

- | | |
|----------------------------------|--|
| a. File Number: | NADA 095-735 |
| b. Sponsor: | Elanco Animal Health
A Division of Eli Lilly & Co.
Lilly Corporate Center
Indianapolis, IN 46285
Drug Labeler Code: 000986 |
| c. Established Name: | Monensin sodium |
| d. Proprietary Name: | RUMENSIN 80 |
| e. Dosage Form: | Type A medicated article |
| f. How Supplied: | 50 lb bag |
| g. How Dispensed: | OTC |
| h. Amount of Active Ingredients: | Monensin sodium – 80 grams per pound (176 g/kg) |
| i. Route of Administration: | Oral in feed |
| j. Species/Class: | Dairy Cows |
| k. Recommended Dosage: | Feed monensin sodium (RUMENSIN 80) at a dietary concentration of 11 to 22 g/ton of total mixed ration dry matter in lactating and dry cow rations. |
| l. Pharmacological Category: | Ionophore |
| m. Indications: | For increased milk production efficiency (production of marketable solids-corrected milk per unit of feed intake). |
| n. Effect of Supplement: | The supplement to the NADA provides for addition of a new class of animals (dairy cows) |

and for the use of monensin in dairy cows to increase milk production efficiency (production of marketable solids-corrected milk per unit of feed intake).

2. *EFFECTIVENESS:*

a. Dosage Characterization

Dose titration was performed as part of substantial evidence (see item 2.b below).

b. Substantial Evidence

Multi-location Study: Effect of Feeding Monensin on Lactation Performance of Dairy Cows

A study was conducted at 9 separate locations in the U.S. and Canada. The purpose of the study was to determine the animal safety and effectiveness of monensin, administered in feed at doses of 0, 8, 16 and 24 ppm of total mixed ration (TMR) dry matter (DM), in primiparous and multiparous dairy cows. For all cows and all locations, treatment began at 21 ± 3 days prior to anticipated calving date and extended through the entire first lactation, subsequent dry period, and ended at 7 days in milk (DIM) or at 200 ± 3 DIM of the second lactation, depending on the study location. At six study locations, all cows were to be treated through 7 DIM of the second lactation, while at two study locations, all cows were to be treated through 200 ± 3 DIM of the second lactation. At the ninth location, a portion of cows were to be treated through 7 DIM and a portion through 200 ± 3 DIM of the second lactation.

The investigators and study locations are listed in Table 1. Also identified in this table are the study locations that treated cows through 7 or 200 ± 3 DIM of Lactation 2.

Table 1. List of Principal Investigators and Study Locations

Study Identification	Principal Investigator(s)	Location
Indiana ^a	Michael J. Cecava, Ph.D. Judith N. Nielson, DVM	West Lafayette, Indiana
Ontario ^a	John J. Brennan, Ph.D.	Burford, Ontario, Canada
Quebec ^a	Elliot Block, Ph.D.	Ste. Anne de Bellevue, Quebec, Canada
Alberta ^a	John J. Kennelly, Ph.D.	Edmonton, Alberta, Canada
North Carolina ^a	Lon W. Whitlow, Ph.D. Alan H. Rakes, Ph.D.	Raleigh, North Carolina
Michigan ^a	Michael S. Allen, Ph.D.	East Lansing, Michigan
New York ^b	James E. Nocek, Ph.D.	Union Springs, New York
Florida ^c	H. Herbert Head, Ph.D.	Gainesville, Florida
California ^c	Terry Lehenbauer, DVM, MPVM, Ph.D. Mark van der List, BVSC, MPVM	Tulare, California

^aStudy conducted through 7 DIM of Lactation 2

^bA portion of study conducted through 7 DIM of Lactation 2 and another portion conducted through 200 DIM in Lactation 2

^cStudy conducted through 200 DIM in Lactation 2

The objective of this study was to determine, by dose titration, if monensin was safe for dairy cows, and effective for improving lactation performance. The indicator for lactation performance used was milk production efficiency, which was quantified as the amount of marketable milk (salable 4.0% solids-corrected milk) per unit of feed intake (as monitored via intake of net energy of lactation (NE_L)).

MATERIALS AND METHODS

Animals and Locations

Primiparous and multiparous Holstein dairy cows were used at each study location. Cows were assigned to each dose group (0, 8, 16 and 24 ppm of TMR DM) at each study location (Table 2).

Table 2. Number of Cows Initially Assigned to Treatment

Study Location	Parity ^a	Monensin Dose (ppm)				
		0	8	16	24	Total
Indiana	P	12	11	11	11	45
	M	18	16	16	15	65
Ontario	P	7	7	7	7	28
	M	13	13	13	13	52
Quebec	P	4	4	4	4	16
	M	12	13	13	12	50
Alberta	P	8	7	6	9	30
	M	10	8	8	9	35
North Carolina	P	7	6	7	7	27
	M	13	10	11	11	45
Michigan	P	9	11	11	9	40
	M	11	14	11	11	47
New York ^b	P	10	9	7	9	35
	M	17	24	17	21	79
New York ^c	P	14	18	17	14	63
	M	19	13	18	17	67
Florida	P	8	9	8	10	35
	M	22	20	21	19	82
California	P	10	9	10	9	38
	M	22	20	23	22	87
Total	P	89	91	88	89	357
	M	157	151	151	150	609

^aParity during Study Lactation 1: P = Primiparous; M = Multiparous

^bNew York cows to be observed through 7 DIM in Lactation 2

^cNew York cows to be observed through 200 DIM in Lactation 2

Selection Criteria

Holstein cows were assigned to treatment at 21±3 days prior to anticipated calving date. Multiparous cows (and primiparous cows where indicated) met the following selection criteria:

- No chronic mastitis or high (> 750,000 or a linear score of > 5.9) somatic cell count (SCC) for two of the last three test dates in the lactation prior to treatment
- No milk fever in any two previous lactations
- Four functional quarters
- Anticipated calving interval (CI) not more than 1.5 standard deviations greater than the mean CI of herd mates at the study location

- Expected dry period of > 45 days and < 100 days, based on actual dry-off date and anticipated calving date
- No quarters confirmed positive for *Staphylococcus aureus*, *Streptococcus agalactiae*, or *Mycoplasma spp.* from duplicate milk samples collected within 28 days of dry-off
- Serologically negative for Johne's disease, using an immunodiffusion test (AGID) for *Mycobacterium paratuberculosis* from a serum sample
- Body condition score of 2.5-4.5 (5 point scale, 0.25 increments) between 29-42 days pre-calving (also primiparous cows)
- Never treated with whey antibody products or vaccines or immunostimulants not approved by FDA or USDA (also primiparous cows)
- Never given investigational drugs for improving lactation performance
- Free of any known health conditions that would prevent completion of the study

Treatment Assignment

Within a study location, cows meeting selection criteria were assigned to the study in blocks of four cows (one cow for each dose) based on:

- Parity
- Anticipated calving date
- Previous lactation performance in multiparous cows: 305-day mature equivalent milk production or Breed Class Average
- Parent average in primiparous cows: Predicted Transmitting Ability of sire and dam
- Body weight
- Treatment with POSILAC® (sometribove zinc suspensions) in previous lactation (multiparous cows at California, Indiana, Michigan and New York)

Cows within blocks were randomly assigned to the 0, 8, 16, or 24 ppm dose groups.

Treatments

The test article for this study was monensin and originated from commercially available RUMENSIN 80 premix (monensin sodium, 80 g/454 g, Elanco Animal Health). The sponsor or sponsor's contract feed mill prepared a Type B supplement by mixing the premix with ground dry corn for monensin concentrations of 0, 160, 320 and 480 mg/kg of supplement dry matter. The supplement was packaged in 50 lb bags (color coded for each dose group) for shipment to the study locations. For masking of treatment concentrations, the respective concentrations associated with each color code were secured by the sponsor and not presented to the study locations.

The Type B supplement was mixed with TMR at an inclusion rate of 5% of TMR DM to achieve monensin concentrations of 0, 8, 16 and 24 ppm of TMR DM. Cows were

administered monensin in TMR at the appropriate concentration via once or twice daily feeding (for details on feeding, see section titled “Feeding and Nutrition”). Cows were given monensin continuously in TMR from 21 ± 3 days prior to anticipated calving, through Lactation 1, the subsequent dry period, and either 7 or 200 ± 3 DIM of Lactation 2, depending on the study location (see Table 2 above).

Removal from Study

Monensin feeding and data collection on cows continued until one of the following occurred:

- Cow calved after less than seven days or more than 40 days on treatment.
- A non-functional quarter was identified within seven days of calving (Lactation 1 only), or two or more non-functional quarters were identified at any time during the study.
- Normal study completion at either 7 or 200 ± 3 DIM of Lactation 2, depending on study location (see Table 2 above).
- Cow declared open and had completed 420 ± 3 DIM of Lactation 1.
- Cow met location-specific low milk production dry-off criterion, based on average daily yield during the first 10 days of a previous two week period.
- Cow experienced a severe or life-threatening health condition.

Housing

Housing of study cows was typical of systems used in the U.S. Locations used tie stalls, free stalls or dry lot housing. At locations with tie stalls, cows were fed individually in their stalls, while cows maintained in dry lot or free stall conditions wore transponders for individual feeding with Calan gates. Within a location, all cows were maintained under the same ambient conditions and similarly handled to avoid bias across treatment groups. The exception was at the New York location, where the replicate of cows assigned to be observed through 7 DIM of Lactation 2 was maintained in tie stalls, and the replicate of cows assigned to be observed through 200 DIM of Lactation 2 was maintained in a free stall barn and fed via Calan gates.

Milk Yield and Composition

Cows were milked twice daily except at the Florida and Michigan locations, where cows were milked three times daily. Milking intervals were similar for all study cows within a location. Milk weights were recorded daily at each milking for all study cows during Lactations 1 and 2. Milk production data were recorded manually (six locations), or captured electronically using the location’s milking system computers (three locations).

Cows were evaluated weekly to determine if they met dry-off criteria. Cows were dried off if their average daily milk production for the first ten days of the previous two week period fell below the selected production level established for their study location. In Lactation 1,

pregnant cows were dried off after meeting the low milk yield criterion, or at 223 ± 3 days of gestation, whichever occurred first.

Any milking in which a cow's milk should have been discarded because it was colostrum/transitional milk (defined as produced during the first 4 DIM), had an abnormal appearance (see Clinical Mastitis, below) and/or was produced during the withdrawal period for a therapeutic drug treatment was identified.

Each week, milk composition was determined from samples collected from each cow's milking during a 24 hr period, beginning 7 ± 3 days after calving. Sampling days were consistent within a study location. Samples were sent to the Northeast Dairy Herd Improvement (NEDHIA) laboratory in Ithaca, NY, for analyses. Samples were analyzed for percent fat, percent protein, percent lactose, percent total solids, concentration of milk urea nitrogen and SCC.

Three study locations (Alberta, California, and New York) evaluated milk components in more detail. Cows at the three locations were sampled in early (20-42 DIM), mid (130-170 DIM) and late (250-300 DIM) lactation. Samples were frozen and shipped to Alberta, where assays were conducted on individual cow samples collected at each stage of lactation for total solids, ash, total fat and fatty acid composition, free fatty acids, nitrogen fractions (total, non-protein, protein, casein, and whey fractions), casein proteins (total, kappa, alpha, and beta), whey proteins (lactalbumin and lactoglobulin) and minerals (aluminum, calcium, copper, iron, magnesium, phosphorous, potassium, sodium, sulfur, and zinc).

In addition to component testing, milk samples were collected at the Alberta location for organoleptic quality and growth of commercial starter cultures for yogurt and cheese. These samples were collected in early (20-90 DIM) and mid to late (160-230 DIM) lactation. To obtain sufficient volume for organoleptic evaluation and yogurt/cheese starter activities, samples were pooled for blocks of cows of the same treatment color code and stage of lactation for a given sampling period.

Feeding and Nutrition

All cows were fed TMR as per the 1989 National Research Council (NRC) Nutrient Requirements of Dairy Cattle, Sixth Edition, to support maintenance, milk production, and growth in primiparous cows. Cows were offered fresh feed once or twice daily throughout the study. Amount of feed offered at all locations was targeted to be approximately 105-110% of intake to allow feeding of cows for ad libitum consumption. Individual feed intake (feed offered and refused) was recorded daily for all study cows for the entire study period.

Feeds were formulated into five TMR for stages of lactation or the dry period at all study locations. The TMR designations and general nutrient specifications are provided in Table 3.

Table 3. Identification of Total Mixed Rations (TMR) and Associated Nutrient Specification Ranges.

TMR	NE _L ^{a,b} (Mcal/kg)	Crude Protein ^a (%)	Calcium ^{a,c,d} (%)	Phosphorous ^a (%)
Dry period, far off (FOD)	1.10-1.48	12.0-18.0	0.4-0.75	0.24-0.5
Dry period, close up (CUD)	1.50-1.68	13.0-16.5	0.4-0.75	0.35-0.5
High lactation TMR (TMR-1)	1.68-1.76	17.5-19.0	0.7-1.2	0.48-0.66
Medium lactation TMR (TMR-2)	1.55-1.67	15.0-17.5	0.6-1.2	0.4-0.5
Low lactation TMR (TMR-3)	1.40-1.54	13.0-16.5	0.6-1.2	0.35-0.5

^aRanges based on recommendation of the National Research Council (NRC, Nutrient Requirements of Dairy Cattle, Sixth Edition), 1989

^bNet energy for lactation

^cLactation rations with added fat contained a minimum of 0.9% calcium

^dCalcium specifications could be exceeded for locations using dietary cation-anion difference (DCAD) in the CUD TMR

In addition to the nutrient specifications listed in Table 3, TMR were targeted to have a minimum 19% acid detergent fiber (ADF) or 25% neutral detergent fiber (NDF).

The far-off dry period (FOD) TMR was fed during the dry period until 21±3 days before anticipated calving. At 21±3 days before anticipated calving, cows were fed the close-up dry period (CUD) TMR until calving. Two options relative to the special dietary mineral needs of the cow during the close up period were allowed at individual locations:

- a) low calcium (< 0.75%), or
- b) a diet formulated to have a negative cation-anion difference (DCAD) of < -10 meq, where DCAD is calculated by: $\text{meq}[(\text{Na} + \text{K}) - (\text{Cl} - \text{S})]/100 \text{ g DM}$.

The low calcium option was used at the Indiana, Ontario, North Carolina, and California locations. The DCAD option was used at the Michigan, Quebec, Florida, and Alberta locations. Due to concerns with reduced intake of the CUD TMR at the Alberta location, the DCAD was increased to -5 meq. At the New York location, the low calcium option was used at both calvings in the group of cows assigned to be observed through 200±3 DIM of Lactation 2. For the group of cows to be observed through 7 DIM of Lactation 2, the low calcium option was used for the first calving, while at the second calving, the low calcium option was used on the first 5 cows that calved, and the DCAD option was used on the remaining cows that calved.

Cows were assigned to lactation TMR based on criteria presented in Table 4.

Table 4. Criteria for Assignment of Lactation TMRs.

TMR Number	Instructions	BCS ^a	Milk Production
TMR-1	At calving and continuing for 84±3 DIM. Thereafter, change to TMR-2 when	≥ 3.0 ^b and	< 31.5 kg/day for multiparous cows and < 24.5 kg/day for primiparous cows
TMR-1 (exception)	At calving and continuing for 84±3 DIM. Thereafter, change to TMR-2 when	≥ 3.5 and	< 40 kg/day for multiparous cows and < 34 kg/day for primiparous cows
TMR-2	TMR-2 must be given for 28 days. Change to TMR-3 when	> 3.5	
TMR-3	Feed until the end of lactation.		

^aBCS = Body Condition Score on a 1-5 scale, 0.25 increments, where 1 = emaciated and 5 = obese

^bAmended to >3.0 at the California, Indiana, Michigan, New York, Ontario, and Quebec locations after the start of the study to avoid cows becoming too thin

TMR ingredients used during the study were common to the geographic locations of the study. Ingredients included but were not necessarily limited to alfalfa haylage, alfalfa hay, corn silage, cereal grain silage, high moisture ear corn, high moisture shelled corn, whole cottonseeds, dry corn supplements (containing test article), and concentrate mixes as needed to balance the TMR.

Nutrient specifications were maintained through periodic sampling and analysis of feeds, with adjustment of feed proportions as necessary. Analyses were performed by NEDHIA, Ithaca, NY. As a minimum, individual TMR ingredients were sampled and assayed 2-4 weeks prior to use primarily for DM, crude protein (CP), NDF (forages only), ADF (forages only), ether extract, calcium (Ca) and phosphorous (P). Samples were collected weekly and composited every four weeks during the early part of the study, but later were composited every two weeks to address concerns relative to variability in assay results. Samples of TMR were also collected on a similar schedule as the individual ingredients, but were collected at the time of mixing and feeding.

The NE_L of each TMR was calculated as a weighted sum of the NE_L contributed by each TMR component. The NE_L value for concentrates and high moisture grains was based on 1989 Dairy NRC values, whereas NE_L for forages was calculated from the following equations from NEDHIA (with ADF percentages on a DM basis):

$$\begin{aligned} \text{Legume:} \quad & \text{NE}_L = 1.044 - (0.0123 \times \text{ADF}\%) \\ \text{Mixed:} \quad & \text{NE}_L = 1.044 - (0.0131 \times \text{ADF}\%) \end{aligned}$$

Grass: $NE_L = 1.085 - (0.0150 \times ADF\%)$
Corn Silage: $NE_L = 0.94 - (0.008 \times ADF\%)$

Body Weight and Body Condition Score

Body weight (BW) was measured within study locations on the same day(s) of the week and the same time of day, following the general schedule described below:

For Lactation 1, BW was determined:

- 29 to 42 days prior to anticipated calving date
- at the start of treatment (on two consecutive days)
- at 14 ± 3 days after calving, and at 14 day intervals thereafter until 112 ± 3 DIM
- at 28 day intervals beyond 112 ± 3 DIM
- dry-off (on two consecutive days)
- prior to change from the FOD to CUD TMR, and
- at the end of treatment (on two consecutive days)

For cows continuing to 200 ± 3 DIM of Lactation 2, BW was also determined:

- at 14 ± 3 after the second calving, and at 14 day intervals thereafter, and
- at the end of treatment (on two consecutive days).

Body condition score (BCS) was assessed on a 5-point scale in 0.25 unit increments (1 = emaciated and 5 = obese) by trained personnel at each location.

For Lactation 1, the subsequent dry period, and calving, BCS was determined:

- 29 to 42 days prior to anticipated calving
- one or two days prior to treatment start date
- within 48 hours of calving
- at 28 ± 3 days after calving, and at 28 day intervals thereafter
- at the beginning of the dry period
- at one or two days prior to change from the FOD to CUD TMR, and
- at the end of treatment.

For cows continuing to 200 ± 3 DIM of Lactation 2, BCS was also determined:

- at 28 ± 3 days after the second calving and at 28 day intervals thereafter, and
- at the end of treatment.

Clinical Mastitis

During both study lactations, each quarter of every cow was examined at every milking for clinical mastitis in milk fore strippings. Clinical mastitis was defined as the presence of abnormal milk (e.g., flakes, clots, discoloration or watery secretion). Detection of abnormal milk in strippings done between milkings was assigned to the next scheduled milking to be considered as part of the summary and analysis of clinical mastitis data. Observations of abnormal milk were recorded by quarter from initial observation to resolution. The end of a clinical case was defined as a return to normal milk for 21 days. If a different organism was cultured from the quarter during a mastitis case, that constituted a new mastitis case.

When clinical mastitis was first detected in a quarter, duplicate milk samples from the affected quarter were collected prior to administration of therapy. Affected quarters and cows were treated for clinical mastitis according to the standard therapeutic regimen(s) for that dairy farm. The duplicate quarter samples were sent to the farm's designated diagnostic laboratory and cultured for mastitis organisms. Culture results were used to ensure that appropriate therapy was applied, or where so indicated, adjust the approach to therapy in problematic cases of clinical mastitis.

Subclinical Mastitis

Cows designated to be observed through 200 ± 3 DIM of Lactation 2 were sampled for subclinical mastitis during Lactations 1 and 2. Duplicate quarter samples were collected within 7 ± 3 days post-calving (both lactations), at dry-off and within 7 days of removal from treatment. These cows were also sampled (single samples from each quarter) at intervals of 56 calendar days, beginning when the first cow at the location was 49 to 63 DIM during Lactation 1 until the last cow completed the study. All subclinical samples were frozen and shipped to the Mastitis Research Laboratory, Hill Farm Research Farm in Homer, Louisiana to culture for mastitis organisms.

Animal Health

Physical examinations were routinely conducted by veterinarians on all study cows. Observations included body temperature, general appearance, pulse, respiration, pregnancy status, and examination of animal systems for abnormalities. These systems included digestive, musculoskeletal, skin, cardiovascular, mammary, reproductive, urinary, respiratory, central nervous, eyes, and feet and legs. Abnormalities were recorded in the trial records.

Physical examinations were performed according to the following schedule:

- within 14 days prior to treatment start
- within 14 days prior to switching to the CUD TMR in the dry period between Lactations 1 and 2 (pregnant cows)

- within 7 days after calving at the beginning of Lactation 2 for cows observed through 200±3 DIM of Lactation 2

The study veterinarian also had the discretion to conduct a physical examination at any time in the study if a cow's health status so warranted.

Animals were also observed by study personnel twice daily at scheduled times throughout the study to document health abnormalities, locomotion, and reproductive events (estrus). Scheduled observation periods were a minimum of 30 minutes per observation period, except when cows were in box stalls, hospital pens or dry cow pens. In these instances, the observer determined the length of observation so as to be suitable for documenting an animal's health status. At each scheduled observation period, all study animals were documented as observed, and either as having no abnormalities or reproductive observations, or with a specific description of the abnormal observation or reproductive event. Unscheduled observations on health abnormalities or reproduction were made at any time. The observer recorded the cow ID, date, time of day, and the observation.

If an animal was observed with an abnormality or reproductive event, it was examined by a trained individual or veterinarian within 24 hours. The examiner would record supportive information and make a presumptive or final diagnosis. Cows were re-evaluated daily until the condition returned to normal. Observations, diagnostic findings, and health care products used (including dose and route of administration) were recorded for each health incident. Normal prophylactic treatments were administered to study cows according to location-specific standard operating procedures (SOPs), and were applied consistently to all study cows in all treatment groups. Observations on reproduction were handled as described in the "Reproductive Performance" section below.

Necropsy was conducted on animals that died or were euthanized to: 1) document the cause of death or morbidity; and 2) examine target tissues for ionophore toxicity. Gross examination was either conducted at the study location, or at the diagnostic laboratory that serviced that location. Gross lesions were documented in the necropsy records, and samples were collected as necessary for diagnostic purposes (e.g., histopathology, cultures, chemistries, etc.). In addition, samples of heart and skeletal muscle (diaphragm) were collected to evaluate for possible ionophore toxicity.

Reproductive Performance

Reproductive examinations were performed by rectal palpation at 30 days post-calving to determine the status of uterine involution and ovarian activity. Reproductive examinations were also performed at 70±3, 135±3 or 200±3 DIM in cows that had not been inseminated, or as needed to diagnose reproductive abnormalities.

Cows were observed for estrus during twice daily scheduled observation periods (minimum of 30 minutes at each period) and when other unscheduled observations on estrus were made. Dairy industry accepted aids for estrous detection such as marker crayons, patches, etc., were

used after 70 DIM when using location-specific SOPs. Hormonal treatments approved for synchronizing estrus could also be applied after 135 DIM, using location-specific SOPs. Primary (standing to be mounted by other cows) and secondary (e.g., mounting other cows, restlessness, vocalization, clear mucus from vulva, etc.) signs of estrus were recorded.

Cows were eligible for insemination from 50-200 DIM for both study lactations. Insemination occurred within 24 hours of observed estrous signs that met location-specific SOP criteria for insemination. Cows returning to estrus (failure to conceive or embryonic/fetal loss) prior to 200 DIM were eligible for re-breeding. Cows not pregnant by 200 DIM were considered open for study purposes. All cows were bred by artificial insemination. Insemination records included cow ID, date, time, sire ID, and inseminator ID.

Pregnancy status was determined by rectal palpation at 35 to 42 days after the last insemination. Cows diagnosed as pregnant at this examination were re-examined approximately 40 days later to confirm pregnancy. Pregnancy status was also confirmed on all pregnant cows during the last week of lactation or the first week of the dry period. Cows inseminated at 150 to 200 DIM of Lactation 2 and declared open at the end of treatment physical examination (197 ± 3 DIM) received a pregnancy examination approximately 40 days after insemination or 250 DIM to confirm final pregnancy status.

Calf Observations

For each cow, the following information was recorded at each calving: cow ID, date and time of calving, number of calves, ease of calving, and whether fetal membranes were expelled. Ease of calving used a 5-point scale: 1 = unobserved or no difficulty; 2 = slight difficulty; 3 = moderate difficulty; 4 = considerable force needed; and 5 = extremely difficult.

All calves born in both study lactations were uniquely identified, and within 24 hours of birth, the following data were recorded: birth status (live or dead), gender, weight, and physical condition (presence of abnormalities or birth defects). In addition to recording these observations, female calves born at the start of Lactation 2 were observed through 28 days of age. Daily observations on health and feed intake were recorded, along with any therapeutic or prophylactic treatments that were administered. Body weight was also recorded at 28 days of age.

DATA HANDLING

Milk Production Data

Milk yield data were summarized on a weekly basis for each cow. Cows were milked two or three times per day, depending on study location. A cow had to have at least 4 valid yields for milking #1, 4 valid yields for milking #2, and, where applicable, 4 valid yields for milking #3 for a specific study week, or else her average daily yield that week was considered missing.

For non-missing weeks, each valid yield of milk was designated as salable or unsalable. A cow's yield at a specific milking was considered unsalable if it was during the first 4 DIM (i.e., was colostrum or transition milk), had an abnormal appearance usually due to mastitis, or was collected during the milk withdrawal period for a mastitis or non-mastitis therapeutic drug. If a milking was designated as unsalable, the cow's yield of salable milk at that milking was considered to be a zero.

A cow's average yield of salable milk (including "zero" yields) for all valid #1 milkings was added to her average yield of salable milk for all valid #2 milkings (and average yield for all #3 milkings where applicable) to derive the cow's average daily yield of salable milk for the week.

Weekly yields of salable milk were corrected to 4.0% solids and 3.5% fat content. The following equation was used to calculate a cow's average daily salable 4.0% solids-corrected milk (SCM) yield each week of lactation:

$$\text{SCM (kg)} = \text{average daily salable milk yield (kg)} \times [(12.24 \times \text{fat \%} \times 0.01) + (7.10 \times \text{protein \%} \times 0.01) + (6.35 \times \text{lactose \%} \times 0.01) - 0.0345]$$

The following equation was used to calculate a cow's average daily salable 3.5% fat-corrected milk (FCM) yield each week of lactation:

$$\text{FCM (kg)} = \text{average daily salable milk yield (kg)} \times [(0.4324) + (16.218 \times \text{fat \%} \times 0.01)]$$

The fat, protein, and lactose percents used in these equations were derived from the milk composition data collected each week of the study while cows were lactating. As noted above, milk composition data were collected at each milking during a 24-hour period once a week. A weekly mean value was derived by weighting for the average yield of milk produced by that cow for each milking number that week (i.e., #1, #2, and where applicable, #3 milking of the day). If a fat, protein, or lactose percent value was missing from a specific milking number or was an outlier (see below), an interpolated value for calculating SCM and FCM was derived by averaging the values for that milking number during the previous and subsequent weeks. If a value for a milking number was missing for the first or last week of lactation, the value from that milking number from the nearest week was used as a replacement value in the calculation of SCM and FCM.

Salable SCM and salable FCM data were "standardized" to 308 DIM (44 weeks) in Lactation 1 and 203 DIM (29 weeks) in Lactation 2. For cows dried off after 308 DIM in Lactation 1, only the SCM and FCM data through 308 DIM were analyzed. If a pregnant cow was dried off before 308 DIM to allow for an adequate dry period (i.e., at 223 ± 3 days of gestation), the cow's salable SCM and FCM yields from dry-off until the equivalent of 308 DIM were considered missing in analysis. If a pregnant or open cow was dried off for low milk production and/or removed from study for a health problem before 308 DIM in Lactation 1,

the cow's salable SCM and FCM yields from dry-off until the equivalent of 308 DIM were set to equal zero in analysis. For cows dried off or removed from study for any reason before 203 DIM for Lactation 2, the cow's salable SCM and FCM yields from dry-off to the equivalent of 203 DIM were set to equal zero in analysis.

Also analyzed was average daily yield of unsalable milk, summarized on a weekly basis per cow. Unsalable milk was classified and analyzed as: 1) total unsalable milk; 2) unsalable milk due to abnormal appearance (e.g., clinical mastitis and colostrum/transition milk during first 4 DIM); 3) unsalable milk due to mastitis or mastitis therapy; and 4) unsalable milk due to non-mastitis therapy.

Milk Composition Data

Samples of milk collected weekly from each cow at each milking during a 24 hour period were analyzed for fat, protein, lactose, and total solids percent, urea nitrogen concentration, and SCC. Solids non-fat (SNF) percent was calculated for each sample by subtracting the fat percent from the total solids percent of the sample. Assay values outside of the following specified ranges were considered outliers and set to missing:

- Fat percent: 1.00-9.00%
- Protein percent: 1.00-7.00%
- Lactose percent: 2.00-7.00%
- Total solids percent: 5.00-18.00%
- Urea nitrogen concentration: 2.00-40.00 mg/dl

If the total solids and/or fat percent value for a milking was an outlier, the SNF percent for that milking was also considered to be an outlier. Because of the typically high variability in milk composition during the first week of lactation, milk composition values for all cows were considered to be missing during week 1 of both Lactation 1 and 2.

For purposes of analyzing milk composition data, if a milk composition value for one or more of the milkings during a specific week was missing, that week's average value for that variable was also considered missing.

For fat, protein, lactose, total solids, and SNF percent, a weekly mean value for each milk composition variable per milking number was derived for each cow by weighting for the average yield of milk produced by that cow at each milking number that week. For urea, a weekly mean value was derived for each cow as a simple average of the values for each milking number that week.

Somatic cell counts reported as < 12,500 per ml were set to 12,500, the limit of detection of instrumentation at the NEDHIA laboratory. Somatic cell counts were converted to linear scores by log₂ transformation of individual SCC values followed by subtracting 13.60964047. Linear scores were then summarized as an average linear score for each

animal at each week of a study period. Missing SCC values were handled as such in the statistical analysis.

Nutrition Intake and Balance Data

Average daily DM intake, NE_L intake, CP intake, NE_L balance, and CP balance were determined for each cow.

The DM content and NE_L and CP content (DM basis) of each TMR was determined from the most recent nutrient analysis of all TMR components and their proportions in the TMR on a DM basis. Each cow's daily intake of these nutrients was calculated by multiplying daily intake of the TMR assigned to the cow at that time by the most recent nutrient content of the TMR. Average daily intake of nutrients was then summarized per week.

Net energy balance (NEB) was defined as the difference between NE_L intake and NE_L required for maintenance and milk production. The following equations were used to calculate average daily NEB per week for each cow:

During lactation:

$$\text{NEB} = \text{NE}_L \text{ Intake (Mcal)} - [(\text{Body Weight}^{0.75}(\text{kg}) \times 0.08 \text{ (Mcal/kg)}) + (\text{Total 4.0\% SCM (kg)} \times 0.748 \text{ (Mcal/kg)})]$$

During dry period:

$$\text{NEB} = \text{NE}_L \text{ Intake (Mcal)} - [(\text{Body Weight}^{0.75}(\text{kg}) \times 0.104 \text{ (Mcal/kg)})]$$

(These equations were derived from the 1989 Dairy NRC and from Tyrrell and Reid, 1965, J. Dairy Science 48:1215.)

Net Protein Balance (NPB) was defined as the difference between dietary CP intake and protein required for maintenance and milk production. The following equations were used to calculate average daily NPB per week for each cow:

During lactation:

$$\text{NPB} = (\text{CP Intake (kg)} \times 1000) - [152.1111 \text{ (g)} + (0.421667 \text{ (g/kg)} \times \text{Body Weight (kg)}) + (\text{Total 3.5\% FCM (kg)} \times 84 \text{ (g/kg)})]$$

During dry period:

$$\text{NPB} = (\text{CP Intake (kg)} \times 1000) - [292.7556 \text{ (g)} + (1.514667 \text{ (g/kg)} \times \text{Body Weight (kg)})]$$

(These equations were derived from the 1989 Dairy NRC.)

Production Efficiency

Milk production efficiency was defined as kg of salable milk (4.0% SCM or 3.5% FCM) per Mcal NE_L intake for a given study period. Salable SCM per NE_L intake during Lactation 1 was considered to be the effectiveness variable of primary interest.

Weekly mean values for average daily SCM or FCM production and NE_L intake during the study period of interest were summed and then multiplied by 7 to derive the cow's total SCM/FCM production and NE_L intake during the period. Total NE_L intake was corrected for changes in body weight during the study period. The following equations summarize the calculation of production efficiency variables:

Salable 4.0% SCM Production Efficiency =

$$\frac{[\text{Sum of Salable 4.0\% SCM (kg)} \times 7]}{[(\text{Sum of NE}_L \text{ Intake (Mcal)} \times 7) - (\text{Weight Gain (kg)} \times 5.12 \text{ (Mcal/kg)} \text{ or } \text{Weight Loss}^* \text{ (kg)} \times 4.92 \text{ (Mcal/kg)})]}$$

Salable 3.5% FCM Production Efficiency =

$$\frac{[\text{Sum of Salable 3.5\% FCM (kg)} \times 7]}{[(\text{Sum of NE}_L \text{ Intake (Mcal)} \times 7) - (\text{Weight Gain (kg)} \times 5.12 \text{ (Mcal/kg)} \text{ or } \text{Weight Loss}^* \text{ (kg)} \times 4.92 \text{ (Mcal/kg)})]}$$

*Weight loss was expressed as a negative value.

Body Weight and Body Condition Score

Average BW and BCS during each specific study period were evaluated, as well as the change in BW and BCS from the beginning to the end of the study period.

For Lactation 1, the change in BCS between the initial BCS in lactation and the point of minimum BCS in the first 203 DIM was also determined.

Clinical Mastitis

Observations of clinical mastitis were summarized into the following variables in each study period where observations were recorded:

Animal Rate: Number of animals observed with clinical mastitis relative to the number of animals at risk during the study period in question

Quarter Rate: Number of quarters observed with clinical mastitis relative to the number of quarters at risk during the study period in question

Observation Rate: Number of quarter-days observed with clinical mastitis relative to the number of quarter-days at risk

Incident Rate: Number of incidents of clinical mastitis observed relative to the number of quarter-days at risk

Average Case Duration: Mean number of days that clinical mastitis incidents persisted

Subclinical Mastitis

Subclinical mastitis quarter samples were collected from cows at the California, Florida, and New York study locations. Evaluation of subclinical mastitis was based on two prospective sampling schemes: event-driven and calendar-driven sampling.

Event-driven quarter samples were collected from each animal at the following time points: 1) following Calving 1; 2) prior to dry-off or removal at the end of Lactation 1; 3) following Calving 2; and 4) prior to treatment end. The event-driven sampling times were common to all three study locations, and pooled analysis across the three locations was performed.

Calendar driven samples were collected from each animal on specific dates at approximately 56-day intervals. The schedule at a location was established when the first cow reached approximately 56 DIM. Since the sampling schedules were different at each of the three locations, pooled analyses across locations were not appropriate.

Subclinical results for both the event-driven and calendar-driven sampling schemes were summarized into observation rate as the number of quarter-days observed per quarter-days at risk.

Animal Health Data

Data were summarized into the main systems of digestive, metabolic, foot and leg, mammary, reproductive, skin, eye and lid, respiratory, urinary, musculoskeletal, cardiovascular, and central nervous system. Data were further summarized into subsystems and final diagnoses and/or specific abnormalities.

Data collected on daily observations, veterinary diagnoses, therapy, and physical and reproductive examinations were summarized by study period into the following variables:

Animal Rate: Number of animals observed with the characteristic of interest relative to the number of animals at risk during the study period in question

Observation Rate: Number of days observed with the characteristic of interest relative to the number of days at risk

Incident Rate: Number of incidents for the characteristic of interest relative to the number of days at risk

Average Case Duration: Mean number of days that incidents persisted for the characteristic of interest

Daily observations were summarized into all four listed variables. Veterinary diagnoses were summarized into animal rate, incident rate, and average case duration. Therapy data were summarized into animal rate and observation rate. Reproductive examination data were summarized into animal rate and observation rate. Physical examination data were only summarized for animal rate for each of the time points after initiation of treatment.

Reproduction data

Reproductive data were summarized for both lactations. Key variables evaluated (with definitions) are provided below.

Days to first standing estrus: Interval from calving to first detected standing estrus

Interestrous interval: Interval between estrous periods (standing estrous and/or secondary estrous signs) not separated by conception or pregnancy

Days to first service: Interval from calving to first artificial insemination

Services per animal: Number of artificial inseminations per animal during the breeding phase (50 to 200 DIM for both lactations)

Services per conception: Number of artificial inseminations per animal with a full-term pregnancy during the breeding phase (50 to 200 DIM for both lactations)

Average interval between services: Interval between artificial inseminations not separated by conception or pregnancy

First service conception rate: Proportion of cows that conceived to the first artificial insemination

Overall conception rate: Proportion of inseminations that resulted in a conception to any artificial insemination during the breeding phase

Pregnancy rate: Proportion of cows that conceived a full-term pregnancy (i.e., ≥ 250 days)

Days open to first conception: Interval from calving to conception from first artificial insemination

Days open for only cows with full-term pregnancies (Days Open A): Interval from calving to conception of full-term pregnancy, excluding cows that were open at 200 DIM

Days open for all cows (Days Open B): Interval from calving to conception of full term pregnancy, including open cows with days open censored at 200 DIM

Calving interval: Number of days between Calving 1 and Calving 2

Early pregnancy loss: Pregnancy loss prior to 90 days of gestation

Late pregnancy loss: Pregnancy loss between 90 and 250 days of gestation

Multiple birth rate: Number of births with > 1 calf as a proportion of all births

Stillbirth rate: Number of stillborn calves as a proportion of all births

Calving difficulty: Defined by 5-point scale where: 1 = unobserved or no difficulty; 2 = slight difficulty; 3 = moderate difficulty; 4 = considerable force needed; and 5 = extremely difficult

Calf birth weight: Weight of calves born to study cows, taken within 24 hr of birth

Female calf 28-day weight: Weight of female calves at 28 days of age (Calving 2)

Female calf 28-day average daily gain: 28-day weight minus birth weight of female calves divided by age in days (Calving 2)

Length of Lactation 1 and Length of Dry Period

The Length of Lactation 1 was calculated for cows that conceived full-term pregnancies in Lactation 1 as the difference in their dry-off date (following Lactation 1) minus their Calving 1 date. Cows removed from the study before dry-off were excluded from the calculation.

The Length of Dry Period between Lactations 1 and 2 was calculated for cows that conceived full-term pregnancies in Lactation 1 as the difference in their Calving 2 date minus their dry-off date (following Lactation 1). Cows with dry periods greater than 120 days were excluded from the analysis.

STATISTICAL PROCEDURES

Study periods used for statistical analyses of the data were:

- Treatment Start (21 days prior to anticipated calving) to Calving 1
- Calving 1 to 308 DIM (Standardized Lactation 1 Period)
- Calving 1 to Dry-Off or Removal
- Dry-off to Calving 2
- Calving 2 to 7 DIM (all study locations)
- Calving 2 to 203 DIM (Standardized Lactation 2 Period, California, Florida, and New York locations)
- Calving 1 to Calving 2
- Calving 1 to 203 DIM of Lactation 2 (California, Florida, and New York locations)

Table 5 shows which production variables were analyzed in each study period:

Table 5. Study periods in which production variables were analyzed.

Study Period	Milk Production & Composition	Production Efficiency	Nutrient Intake & Balance	BW/BCS
Treatment Start to Calving 1			✓	✓
Calving 1 to 308 DIM	✓	✓	✓	✓
Calving 1 to Dry-Off/Removal		✓	✓	✓
Dry-Off to Calving 2			✓	✓
Calving 1 to Calving 2		✓		✓ ^a
Calving 2 to 7 DIM	✓ ^b		✓	
Calving 2 to 203 DIM	✓	✓	✓	✓
Calving 1 to 203 DIM of 2 nd Lactation		✓		
^a Only change in BW and BCS				
^b Only milk production				

Cows that did not complete two-thirds (i.e., 203 DIM) of the Standardized Lactation 1 Period were excluded from statistical analyses of production data for all but the Treatment Start to Calving 1 Period. Cows that did not complete two-thirds (i.e., 133 DIM) of the Standardized Lactation 2 Period were excluded from analyses of production data for the Calving 2 to 203 DIM period and the Calving 1 to 203 DIM Period. Cows with short dry periods between Lactation 1 and 2 (i.e., less than 28 days) were excluded from analyses of production data for the Calving 2 to 7 DIM, Calving 2 to 203 DIM, and Calving 1 to 203 DIM Periods. Cows with dry periods greater than 120 days were excluded from analyses of production data for the Dry-Off to Calving 2, Calving 1 to Calving 2, Calving 2 to 7 DIM, Calving 2 to 203 DIM, and Calving 1 to 203 DIM Periods. A small number of cows were also excluded from

analyses of production data during study periods that they were switched to a specific TMR too soon or too late based on criteria described above, or that they were milked less frequently per day for a significant length of time compared to their herdmates while in a veterinary hospital.

All cows that started treatment were included in analyses of health and reproduction data for each study period that they remained on the study. Relative to reproductive performance, cows not at risk for a particular variable had that variable set to missing.

Milk production, length of lactation and dry period, milk composition, nutrition, production efficiency, body weight, and body condition score:

Mixed model analysis methods based on restricted maximum likelihood estimation were used to analyze all continuous variables related to milk production, milk composition, nutrition, body weight, body condition, and production efficiency. Two basic models were used. Model I was for multiple observations per animal per study period. Model II was for a single observation per animal per study period. Both models contained the same between-subjects fixed factors (the parity and treatment main effects), a baseline covariate, parity, treatment (four levels: 0, 8, 16 and 24 ppm of monensin), and the parity by treatment interaction. Parity was treated as a stratification variable in the analyses in order to account for differences between multiparous cows and primiparous cows. The covariate was expressed as a deviation from the animal's baseline value and the parity-specific mean of the covariate. The covariate was different for each level of parity. For multiparous cows, the covariate was previous lactation yield, and for primiparous cows, the covariate was pretreatment body weight.

Model I was used for multiple observations of the outcome variable per animal per study period. The repeated measures fixed factor was time (either weeks in milk or weeks dry). Random effects included the trial location effect, the location by treatment interaction, block within location effect (referring to the blocks used in randomization), and the random effect term for animal. Model II was used for single observations of the outcome variable per animal per study period, and consisted of the between-subject fixed factors and the baseline covariate.

The final form of the model for each variable was determined by preliminary testing and the use of standard diagnostics. This assessment determined the inclusion of interactions between fixed effects, the most appropriate terms involving the baseline covariate, the inclusion of certain random effects, and the need for weighted estimation. The final form of the model was used to estimate and test the effect of each monensin treatment group in comparison with the control group. A test level of $\alpha = 0.05$ was used to evaluate the statistical significance of these comparisons.

The minimum effective concentration for effectiveness was established from the dose-response relationship between monensin and salable 4.0% SCM production efficiency during the Standardized Lactation 1 Period. The general shape of the dose response curve was

determined first. Then the confidence region for the dose response curve was estimated, using results from the final form of the mixed model analysis. The minimum effective concentration was determined from this confidence region. The upper 95% confidence bound of the response at 0 ppm was compared to the lower 95% confidence bound of responses at non-zero concentrations. The lowest monensin concentration that produced a non-overlapping confidence bound was selected as the minimum effective concentration.

The effects of monensin treatment on salable SCM production efficiency during other study periods were also reviewed as supportive information.

The final form of the mixed model was also used to estimate best linear unbiased predictors (BLUPs) and their standard errors for location-specific outcomes for the indication variable, salable 4.0% SCM production efficiency. These estimates provided location-specific summaries and were not used in statistical tests.

Extensive Milk Composition, Sensory Evaluation, and Yogurt and Cheese Starter Cultures:

Extensive Milk Composition:

Extensive milk composition variables (total solids, ash, total fat, fatty acids, nitrogen fractions, casein proteins, whey proteins, and minerals) were analyzed with a repeated measures mixed effects linear model. Fixed effects between animals were parity and treatment. The repeated measure was stage of lactation. All interactions among the fixed effects were included in the analysis model. The random effects were location, block nested in parity by location, and treatment by block nested in parity by location. The final form of the model was used to estimate and test the effect of each monensin treatment group in comparison with the control group, using $\alpha = 0.05$.

Sensory Evaluation:

The sensory variables were analyzed with a mixed effects analysis of variance. Replicate and panelist were random effects, and treatment was a fixed effect. Follow-up contrasts on the treatment means were evaluated at $\alpha = 0.05$.

Yogurt Culture Evaluation:

For both early and mid to late lactation, the results were summarized by the number of replicate cultures in each treatment group that reached the endpoint pH value of 4.6 by 3.5 hours. A Kruskal-Wallis test was used to evaluate the effect of treatment group on the average rank of time to reach the endpoint pH value.

Cheese Starter Culture Evaluation:

The data were analyzed using a mixed effects linear model with treatment as a fixed factor and block as a random factor. Follow-up contrasts on the treatment means were evaluated at $\alpha = 0.05$.

Milk Somatic Cell Counts:

The average linear score for each animal at each week of a study period was analyzed with a repeated measures mixed model. The model included the fixed effects of treatment, parity, week and all interactions among these effects, and the random effects of location, location by treatment, block nested in parity by location and cow nested in location. The results of preliminary testing and other diagnostics were used to determine the final form of the model. The final form of the model was used to estimate and test the effect of each monensin treatment group in comparison with the control group. A test level of $\alpha = 0.1$ was used to identify results for further review.

Clinical mastitis, daily observations, veterinary diagnoses, physical and reproductive performance:

Reproductive and health data summarized as counts, proportions and ordered categorical responses (calving difficulty score) were treated as discrete variables, while variables such as average case duration, calf birth weight, female calf 28 day weight, and average daily gain, were treated as continuous variables.

There were three options for analyzing the discrete variables, depending on the number of occurrences of the variable in the data set. If there were 20 or more observations for both multiparous and primiparous parity groups, count and proportion variables were analyzed with a generalized linear mixed effects model (GLIMMIX). Model terms included the fixed effects of treatment, parity, and parity by treatment, and the random effects of location, location by treatment, and block nested in parity by location. Preliminary testing and other diagnostics were used to determine the final form of the GLIMMIX model. Ordered categorical data (calving difficulty score) were analyzed with generalized linear mixed effects model (PROBIT), with similar model terms as the GLIMMIX model, except that location was considered a fixed rather than a random effect. The final form of the models was used to estimate and test the effect of each monensin treatment group in comparison with the control group. Right-censored data (e.g., days to first standing estrus, days to first service, days open B) were evaluated using survival analysis (LIFEREG). A test level of $\alpha = 0.1$ was used to identify results for further review.

If there were fewer than 20 observations in at least one of the parity groups, then the two groups were analyzed separately. If a group had more than three observations, the Cochran-Armitage exact test for a linear trend was used, stratified by location. A test level of $\alpha = 0.1$

was used to identify results for further review. If a group had fewer than 3 observations, the data were not analyzed statistically and only the summary statistics were presented.

Average case duration was analyzed with a mixed effects linear model. Model terms included the fixed effects of treatment, parity, and parity by treatment, and the random effects of location, location by treatment, and block nested in parity by location. The number of cases of the health variable along with the results of preliminary testing and other diagnostics were used to determine the final form of the GLIMMIX model. The final form of the model was then used to estimate and test the effect of each monensin treatment group in comparison with the control group. A test level of $\alpha = 0.1$ was used to identify results for further review.

For offspring of study cows, a mixed effects linear model was used to analyze calf birth weight, female calf 28-day body weight, and female calf 28-day average daily gain. Model terms included the fixed effects of treatment, parity, and time (and their interactions), and the random effects of location, location by treatment, and block nested in parity by location. For analysis of birth weight, the fixed effect of gender (female or male calf) and interactions with gender were included as model terms.

RESULTS

Milk Production, Milk Composition, Nutrition, Body Condition Score, Body Weight and Production Efficiency Variables

The parity by treatment interactions were not significant in the analyses of production variables. Thus, all results presented are for pooled parity analyses.

Average daily salable 4.0% SCM and 3.5% FCM production during the Standardized Lactation 1 and 2 Periods are presented in Table 6. There were no significant differences among treatment groups during Lactations 1 and 2 for either variable ($P > 0.05$). There also was no significant effect of monensin treatment on salable 4.0% SCM and 3.5% FCM yields during the Calving 2 to 7 DIM Period (data not shown).

Unsalable milk yield was evaluated in the following categories: 1) total unsalable milk yield; 2) unsalable due to abnormal milk; 3) unsalable due to mastitis or mastitis therapy; and 4) unsalable due to non-mastitis therapy. Yields of these categories of unsalable milk did not differ significantly among dose groups during the Standardized Lactation 1 and 2 Periods and the Calving 2 to 7 DIM Period (data not shown).

Effect of monensin treatment on milk composition during the Standardized Lactation 1 and 2 Periods is presented in Table 7. During 1-308 DIM of Lactation 1, there was a significant linear decrease in fat percentage with increased dose of monensin. Protein percentage also decreased in a linear manner as the dose of monensin increased, but was seen mainly at the 24 ppm dose of monensin and was reduced only slightly from the 0 ppm dose group. There were no treatment effects on lactose percentage. Similar to results with protein percentage, SNF percentage decreased at the 24 ppm dose. Total solids percentage decreased in a significant linear fashion with increased dose of monensin, paralleling the reduction in fat percentage. Urea nitrogen was increased at the 8 vs. 0 ppm doses, though the 16 and 24 ppm dose groups did not differ from the 0 ppm dose.

During 1-203 DIM of Lactation 2 there were trends for reduced fat and total solids percentages at the 24 ppm dose, supporting the results observed for Lactation 1. However, due to fewer animals, no significant differences among doses of monensin were noted for these variables.

These results indicate that monensin treatment reduced fat percentage across the dose range evaluated. Thus, product labeling indicates that use of monensin in dairy cows reduces milk fat percentage, and this reduction increases with dose of monensin. Given that effects of monensin treatment on total solids percentage reflect the reduction in fat percentage, a labeling statement relative to reduced total solids percent is not necessary. There was a small but statistically significant reduction in protein (and SNF) percentage at the 24 vs. 0 ppm dose of monensin. This small reduction in protein percent is not of practical importance to dairy producers. The increase in milk urea nitrogen for the 8 ppm dose group compared to controls appears to be a spurious effect.

Table 6. Effects of Monensin Treatment on Salable 4.0% SCM and 3.5% FCM Production (kg/d) during the Standardized Periods of Lactation 1 (1-308 DIM) and Lactation 2 (1-203 DIM), Parities Combined.

	Dose of Monensin											
	0 ppm			8 ppm			16 ppm			24 ppm		
	LSMEAN ^a	SE ^b	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
1-308 DIM of Lactation 1												
Salable 4.0% SCM	26.4	0.6	202	26.7	0.6	204	26.3	0.6	211	26.3	0.6	201
Salable 3.5% FCM	28.6	0.6	202	29.0	0.6	204	28.5	0.6	211	28.5	0.6	201
1-203 DIM of Lactation 2^c												
Salable 4.0% SCM	30.3	1.5	58	29.7	1.5	56	31.4	1.5	47	30.7	1.5	52
Salable 3.5% FCM	33.4	1.7	58	33.0	1.7	56	34.8	1.8	47	33.7	1.8	52

^aLeast-squares mean

^bStandard error

^cIncludes cows only from California, Florida and New York locations

Table 7. Effects of Monensin Treatment on Milk Composition during the Standardized Periods of Lactation 1 (1-308 DIM) and Lactation 2 (1-203 DIM), Parities Combined.

	Dose of Monensin											
	0 ppm			8 ppm			16 ppm			24 ppm		
Component	LSMEAN ^a	SE ^b	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
Lactation 1 (1-308 DIM)												
Fat (%) ^c	3.65	0.08	202	3.59	0.08	204	3.49 ^d	0.08	211	3.38 ^d	0.08	201
Protein (%) ^c	3.15	0.02		3.16	0.02		3.13	0.02		3.10 ^e	0.02	
Lactose (%)	4.83	0.03		4.79	0.03		4.80	0.03		4.81	0.03	
Solids Non-Fat (%) ^c	8.73	0.04		8.69	0.04		8.68	0.04		8.66 ^e	0.04	
Total Solids (%) ^c	12.38	0.09		12.29	0.09		12.17 ^e	0.08		12.04 ^d	0.09	
Urea N (mg/dL)	16.95	0.69		16.41 ^e	0.69		16.56	0.69		16.52	0.69	
Lactation 2 (1-203 DIM)												
Fat (%)	3.65	0.14	58	3.65	0.14	56	3.66	0.15	47	3.50	0.15	52
Protein (%)	3.03	0.04		3.01	0.04		3.01	0.04		3.00	0.04	
Lactose (%)	4.73	0.07		4.65	0.07		4.75	0.07		4.77	0.07	
Solids Non-Fat (%)	8.50	0.09		8.41	0.09		8.48	0.10		8.49	0.10	
Total Solids (%)	12.15	0.20		12.07	0.20		12.14	0.21		11.99	0.21	
Urea N (mg/dL)	16.45	1.08		16.83	1.08		17.18	1.09		16.67	1.09	

^aLeast-squares mean

^bStandard error

^cLinear decrease with increasing dose of monensin (P < 0.05)

^dDifferent from 0 ppm dose group (P ≤ 0.01)

^eDifferent from 0 ppm dose group (P < 0.05)

Effects of monensin treatment on dry matter (DM) and net energy of lactation (NE_L) intake are presented in Table 8. Effects on crude protein intake were similar (data not shown). During most study periods, there was a reduction in DM and NE_L intake, particularly at the 24 ppm dose. In the Treatment Start to Calving 1 Period, there was a reduction in DM and NE_L intake at the 24 ppm dose, while during 1-308 DIM of Lactation 1 and the Dry-Off to Calving 2 Period, there was a significant linear reduction in DM and NE_L intake with increasing dose of monensin. (Results for the Calving 1 to Dry-Off/Removal Period were similar; data not shown.) No significant differences were noted during the Calving 2 to 7 DIM Period (data not shown). There were trends for reduced DM and NE_L intake during 1-203 DIM of Lactation 2 (Table 8), which supported the results observed for Lactation 1, but these effects were not significant.

Based on these results, product labeling indicates that reduced voluntary feed intake is associated with monensin treatment of dairy cows, and there is a greater reduction in voluntary feed intake as the dose of monensin increases.

Net energy balance (NEB) analysis results are presented in Table 9. Similar to the reduction in DM and NE_L intake, monensin treatment decreased NEB. There was a linear decrease with dose during 1-308 DIM of Lactation 1 and the Dry-Off to Calving 2 Period (Table 9) and the Calving 1 to Dry-Off/Removal Period (data not shown). When making pairwise comparisons with the 0 ppm dose, significant differences were noted at the 16 and 24 ppm doses during 1-308 DIM of Lactation 1, and at the 24 ppm dose for the Treatment Start to Calving 1 and Dry-Off to Calving 2 Periods. There were no significant differences in NEB across treatment groups during the Calving 2 to 7 DIM Period (data not shown). There also were no significant differences during 1-203 DIM of Lactation 2 (Table 9), although NEB for the 24 ppm group tended to be lower than controls. Overall, reductions in NEB reflected the reduced feed intake in monensin-treated cows. In each of the study periods, changes in net protein balance (NPB; data not shown) paralleled the changes in NEB. Across doses of monensin, cows maintained similar levels of FCM and SCM milk production (see Table 6), such that the modest reduction in NEB and NPB did not appear to have a deleterious effect on production.

Effects of monensin treatment on average body condition score (BCS) and change in BCS are presented in Table 10. Average BCS did not differ among dose groups during any study period. In addition, the treatment by week interaction was not significant for any period, indicating that patterns in BCS during each study period were not different among treatment groups.

During 1-308 DIM of Lactation 1, the change in BCS was significantly different between the 0 ppm and 8, 16, and 24 ppm dose groups, with monensin treated cows losing less body condition (Table 10). However, these differences (less than 0.10 units) are not biologically meaningful because they are below the smallest discernable difference in BCS in this study, which was 0.25 units (1-5 scale). These results did demonstrate that monensin-treated cows maintained similar amounts of body condition compared to

control cows. There were no significant differences in the change in BCS among treatment groups during other study periods (see Table 10).

Effects of monensin treatment on average body weight (BW) and change in BW are presented in Table 11. There were no significant differences among dose groups for average BW and change in BW during any study period.

Table 8. Effects of Monensin Treatment on Dry Matter Intake (DM; kg/day) and Net Energy of Lactation Intake (NE_L; Mcal/day) during: Treatment Start to Calving 1; 1-308 DIM of Lactation 1; Dry-Off to Calving 2; and 1-203 DIM of Lactation 2, Parities Combined.

	Dose of Monensin											
	0 ppm			8 ppm			16 ppm			24 ppm		
	LSMEAN ^a	SE ^b	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
Treatment Start to Calving 1												
DM Intake	11.0	0.4	230	11.0	0.4	228	10.9	0.4	226	10.5 ^e	0.4	227
NE _L Intake	17.2	0.7		17.1	0.7		17.0	0.7		16.3 ^e	0.7	
1-308 DIM of Lactation 1												
DM Intake ^c	19.9	0.4	202	20.0	0.4	204	19.4 ^e	0.4	211	19.2 ^f	0.4	201
NE _L Intake ^c	33.8	0.9		33.9	0.9		32.9 ^e	0.9		32.6 ^f	0.9	
Dry-Off to Calving 2												
DM Intake ^c	12.8	0.4	152	12.5	0.4	144	12.5	0.4	136	12.0 ^f	0.4	146
NE _L Intake ^c	18.7	0.7		18.1	0.7		18.2	0.7		17.5 ^f	0.7	
1-203 DIM of Lactation 2^d												
DM Intake	21.9	0.9	58	22.2	0.9	56	21.9	0.9	47	21.0	0.9	52
NE _L Intake	38.0	1.9		38.6	1.9		38.1	1.9		36.4	1.9	

^aLeast-squares mean

^bStandard error

^cLinear decrease with increasing dose of monensin (P < 0.01)

^dIncludes cows only from California, Florida, and New York locations

^eDifferent from 0 ppm dose group (P < 0.05)

^fDifferent from 0 ppm dose group (P < 0.01)

Table 9. Effects of Monensin Treatment on Net Energy Balance (NEB; Mcal/d) during: Treatment Start to Calving 1; 1-308 DIM of Lactation 1; Dry-Off to Calving 2; and 1-203 DIM of Lactation 2, Parities Combined.

	Dose of Monensin											
	0 ppm			8 ppm			16 ppm			24 ppm		
	LSMEAN ^a	SE ^b	N	LSMEA N	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
Treatment Start to Calving 1												
NEB	3.9	0.7	230	3.8	0.7	228	3.7	0.7	226	3.0 ^e	0.7	227
1-308 DIM of Lactation 1												
NEB ^c	3.6	0.7	202	3.4	0.7	204	2.7 ^f	0.7	211	2.4 ^f	0.7	201
Dry-Off to Calving 2												
NEB ^c	4.5	0.7	152	4.0	0.7	144	4.0	0.7	136	3.3 ^f	0.7	146
1-203 DIM of Lactation 2^d												
NEB	2.9	2.0	58	4.0	2.0	56	3.1	2.0	47	2.2	2.0	52

^aLeast-squares mean

^bStandard error

^cLinear decrease with increasing dose of monensin ($P < 0.01$)

^dIncludes cows only from California, Florida, and New York locations

^eDifferent from 0 ppm dose group ($P < 0.05$)

^fDifferent from 0 ppm dose group ($P < 0.01$)

Table 10. Effects of Monensin Treatment on Average Body Condition Score (BCS) and Change in BCS during: 1-308 DIM of Lactation 1; Dry-Off to Calving 2; and 1-203 DIM of Lactation 2, Parities Combined.

	Dose of Monensin											
	0 ppm			8 ppm			16 ppm			24 ppm		
	LSMEAN ^a	SE ^b	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
1-308 DIM of Lactation 1												
Average BCS	3.01	0.05	200	3.01	0.05	204	3.05	0.05	210	3.04	0.05	200
Change in BCS	-0.13	0.08	194	-0.04 ^d	0.08	201	-0.05 ^d	0.07	204	-0.06 ^d	0.08	195
Dry-Off to Calving 2												
Average BCS	3.39	0.07	148	3.36	0.07	141	3.42	0.07	136	3.39	0.07	144
Change in BCS	0.09	0.04	149	0.06	0.04	140	0.08	0.04	135	0.04	0.04	142
1-203 DIM of Lactation 2^c												
Average BCS	2.89	0.06	57	2.88	0.06	56	2.97	0.06	47	2.91	0.06	51
Change in BCS	-0.32	0.08	56	-0.27	0.08	55	-0.22	0.08	47	-0.30	0.08	49

^aLeast-squares mean

^bStandard error

^cIncludes cows only from California, Florida, and New York locations

^dDifferent from 0 ppm dose group (P < 0.01)

Table 11. Effects of Monensin Treatment on Average Body Weight (BW; kg) and Change in BW during: 1-308 DIM of Lactation 1; Dry-Off to Calving 2; and 1-203 DIM of Lactation 2, Parities Combined.

	Dose of Monensin											
	0 ppm			8 ppm			16 ppm			24 ppm		
	LSMEAN ^a	SE ^b	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
1-308 DIM of Lactation 1												
Average BW	598	7	202	600	6	204	601	6	211	603	6	201
Change in BW	61	9	200	71	9	204	65	9	211	70	9	200
Dry-Off to Calving 2												
Average BW	723	11	149	722	11	141	727	11	135	725	11	146
Change in BW	-7	5	151	-8	5	140	-6	5	134	-14	5	144
1-203 DIM of Lactation 2^c												
Average BW	601	5	58	597	5	56	611	6	47	611	6	52
Change in BW	-24	8	58	-16	8	56	-8	8	47	-14	8	52

^aLeast-squares mean

^bStandard error

^cIncludes cows only from California, Florida, and New York locations

Effects of monensin treatment on production efficiency (kg salable 4.0% SCM per Mcal NE_L intake) are presented in Table 12. Effects of treatment on production efficiency measured as kg salable 3.5% FCM per Mcal NE_L intake were similar to the results observed for SCM per NE_L intake during all study periods (data not shown).

Results for individual locations (least-squares means and standard errors only) during 1-308 DIM of Lactation 1 are presented in Table 13.

In order for treatment comparisons in milk production efficiency to be valid, change in BCS in monensin-treated cows during the main study periods of evaluation could be no worse than control cows. Given that average BCS and change in BCS were similar among all dose groups during all study periods, consideration of milk production efficiency was valid.

There was a linear increase in milk production efficiency with dose of monensin during 1-308 DIM of Lactation 1, with the 16 and 24 ppm doses greater than the 0 ppm dose (Table 12). Similar effects were observed during the Calving 1 to Dry-Off/Removal and Calving 1 to Calving 2 Periods (data not shown). Similar trends, albeit not statistically significant, were also noted during 1-203 DIM of Lactation 2 (Table 12) and the Calving 1 (Lactation 1) to 203 DIM of Lactation 2 Period (data not shown).

Milk production efficiency increased linearly with dose of monensin. To determine the minimum effective dose, the confidence region for the dose response curve was estimated. The minimum effective dose was defined as the lowest non-zero concentration for which the lower limit of the 95% confidence interval did not overlap with the upper limit of the 95% confidence interval for the 0 ppm dose. This approach was performed with non-overlapping confidence intervals around the 0 ppm dose group and 16 ppm dose group, the minimum effective dose that was a defined dose in the study design. The minimum effective dose was determined to be 12 ppm (see Figure 1).

The results demonstrate that monensin is effective for use in dairy cows to increase milk production efficiency (production of marketable solids-corrected milk per unit of feed intake) at the dose range of 12 to 24 ppm of TMR on a 100% DM basis.

Table 12. Effects of Monensin Treatment on Production Efficiency (kg Salable 4.0% SCM per Mcal NE_L) during the Standardized Periods of Lactation 1 (1-308 DIM) and Lactation 2 (1-203 DIM), Parities Combined.

	Dose of Monensin											
	0 ppm			8 ppm			16 ppm			24 ppm		
	LSMEAN ^a	SE ^b	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
1-308 DIM of Lactation 1^c	0.822	0.021	200	0.831	0.021	204	0.843 ^d	0.021	211	0.854 ^e	0.021	200
1-203 DIM of Lactation 2	0.798	0.030	58	0.759	0.030	56	0.824	0.031	47	0.840	0.031	52

^aLeast-squares mean

^bStandard error

^cLinear increase with increasing dose of monensin ($P < 0.05$)

^dDifferent from 0 ppm dose group ($P < 0.05$)

^eDifferent from 0 ppm dose group ($P < 0.01$)

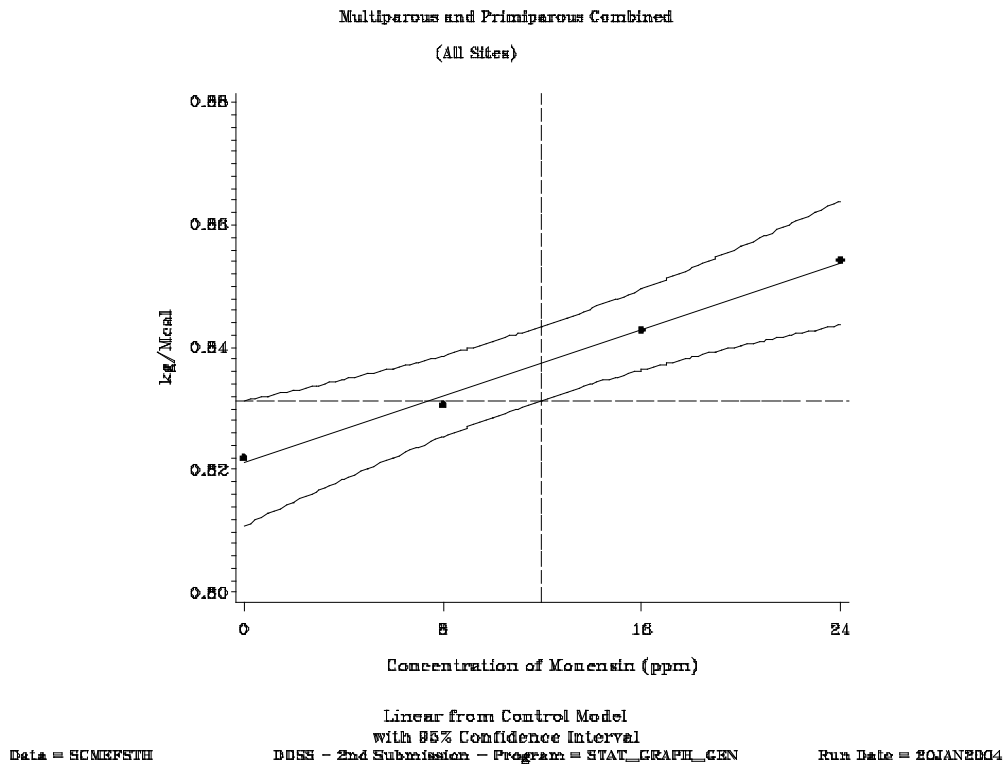
Table 13. Effects of Monensin Treatment on Production Efficiency (kg Salable 4.0% SCM per Mcal NE_L Intake) During the Standardized Period of Lactation 1 (1-308 DIM), Presented by Study Location, Parities Combined. Treatment Contrasts Within Location Were Not Performed.

	Dose of Monensin											
	0 ppm			8 ppm			16 ppm			24 ppm		
Location	LSMEAN ^a	SE ^b	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
Indiana	0.796	0.012	21	0.805	0.011	23	0.817	0.011	24	0.828	0.012	21
New York	0.871	0.009	51	0.880	0.009	54	0.892	0.009	53	0.904	0.009	51
Florida	0.754	0.012	20	0.762	0.011	26	0.775	0.012	21	0.786	0.011	24
Ontario	0.884	0.012	18	0.893	0.012	19	0.905	0.012	18	0.916	0.012	19
Quebec	0.882	0.014	15	0.890	0.014	12	0.902	0.014	16	0.914	0.014	15
Alberta	0.851	0.014	12	0.859	0.014	11	0.872	0.014	14	0.883	0.014	13
North Carolina	0.755	0.014	15	0.764	0.014	11	0.776	0.014	16	0.787	0.014	10
California	0.749	0.011	29	0.757	0.011	29	0.770	0.011	29	0.781	0.011	29
Michigan	0.857	0.012	19	0.865	0.012	19	0.878	0.012	20	0.889	0.012	18

^aLeast-squares mean

^bStandard error

Figure 1. Milk Production Efficiency (kg salable 4.0% SCM per Mcal NE_L Intake) from Calving 1 to 308 DIM (Lactation 1)



Conclusions on production, nutrition, BCS, BW, and efficiency related variables:

- 1) Monensin treatment of dairy cows does not affect production of 4.0% SCM or 3.5% FCM.
- 2) Monensin treatment is associated with a reduction in milk fat percentage. Product labeling indicates that use of monensin in dairy cows is associated with reduced milk fat percentage, and the reduction in milk fat percent increases as the dose of monensin increases.
- 3) Effects of monensin treatment on BCS are not biologically meaningful. There are no significant effects of treatment on BW.
- 4) Monensin treatment reduces voluntary feed intake. Product labeling indicates that reduced voluntary feed intake is associated with monensin treatment of dairy cows, and there is a greater reduction in voluntary feed intake as the dose of monensin increases. Product labeling also recommends that users rule out monensin as the cause of reduced feed intake before attributing to other causes such as illness, feed management, or the environment.
- 5) Monensin is effective for use in dairy cows to increase milk production efficiency (production of marketable solids-corrected milk per unit of feed intake) between the doses of 12 to 24 ppm of TMR on a 100% DM basis. To be consistent with other approved uses of monensin in cattle, the approved dose range on product labeling for milk production efficiency in dairy cows was converted from ppm to grams per ton: 11 to 22 grams per ton of TMR on a 100% DM basis.

Length of Lactation 1 and Length of Dry Period

Effects of monensin treatment on the lengths of Lactation 1 and the subsequent dry period are presented in Table 14. Parity by treatment interaction was not significant. Monensin treatment had no significant effects on the lengths of either period.

Table 14. Effects of Monensin Treatment on the Length of Lactation 1 and the Subsequent Dry Period (Days), Parities Combined.

	Dose of Monensin											
	0 ppm			8 ppm			16 ppm			24 ppm		
	LSMEAN ^a	SE ^b	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
Lactation 1 Length	319	3.5	153	318	3.5	151	316	3.5	145	326	3.4	157
Dry Period Length	61.6	1.4	151	62.2	1.5	145	61.4	1.5	137	62.0	1.4	151

^aLeast-squares mean

^bStandard error

Extensive Milk Composition, Starter Cultures and Milk Flavor

Milk dry matter, ash, and mineral contents are presented in Table 15. Dry matter and ash percentages were similar among the four dose groups. There was a small increase in the content of calcium, magnesium, and zinc for the 24 vs. 0 ppm dose groups, though these findings are likely of limited biological relevance. Based on these results, monensin treatment had no negative effect on milk dry matter, ash, and mineral composition.

Milk crude protein (total) and crude protein fractions are presented in Table 16. There were no significant differences among dose groups for total protein or for the nonprotein nitrogen, protein, casein, and whey fractions. There was a trend for total protein to be reduced at the higher doses of monensin, but similar to protein results from study-wide analyses, the decrease was minimal and not of practical concern.

Results for short and medium-chain fatty acids content of milk are presented in Table 17. There were decreases in some of these fatty acids associated with monensin treatment. Similarly, there were slight reductions in free fatty acids (Table 18). These changes in fatty acid composition are not likely to affect quality, stability or manufacturing qualities of milk from monensin-treated cows.

Based on results from organoleptic testing, monensin treatment of cows did not affect the smell or taste of pasteurized fresh milk samples (data not shown). For cheese starter cultures, mean pH and titratable acidity at 0 and 24 hours of culture, and lactic acid production at 24 hours, are presented in Table 19. Mean pH at 0 hours and titratable acidity at 0 and 24 hours decreased with monensin treatment. Lactic acid production increased in the 24 vs. 0 ppm dose group. With respect to yogurt starter cultures, there were no effects of monensin treatment on pH values (data not shown). These results indicate that cheese and yogurt starter culture activities were not negatively affected by monensin treatment of cows.

Table 15. Effects of Monensin Treatment on Dry Matter, Ash, and Mineral Content in Milk.

	Dose of Monensin				
	0 ppm	8 ppm	16 ppm	24 ppm	SEM ^a
Dry Matter (%)	11.73	11.49	11.53	11.57	0.180
Ash (%)	0.72	0.73	0.72	0.73	0.005
Mineral Content (per 100 ml of milk)					
Aluminum (µg)	45.11	44.05	48.19	43.48	3.990
Calcium (mg)	110.98	111.70	111.66	115.13 ^b	1.305
Copper (µg)	7.03	6.85	7.50	6.82	1.085
Magnesium (mg)	10.50	10.54	10.73	10.92 ^b	0.135
Manganese (µg)	2.19	2.07	2.20	2.22	0.164
Phosphorous (mg)	87.67	87.48	87.31	87.94	1.682
Potassium (mg)	135.45	138.63	136.45	138.18	1.982
Sodium (mg)	35.45	35.76	35.83	35.20	1.517
Sulphur (mg)	28.42	28.24	28.00	28.28	0.391
Zinc (µg)	369.80	375.12	376.55	393.23 ^b	14.466

^aPooled standard error of the least-squares means

^bDifferent from 0 ppm dose group (P < 0.05)

Table 16. Effects of Monensin Treatment on Crude Protein Fractions in Milk.

	Dose of Monensin				
	0 ppm	8 ppm	16 ppm	24 ppm	SEM ^a
Crude Protein Fraction: ^b					
Total	3.14	3.13	3.11	3.11	0.047
Nonprotein Nitrogen	0.17	0.16	0.17	0.17	0.006
Protein	2.85	2.84	2.81	2.80	0.042
Casein	2.33	2.30	2.30	2.29	0.037
Whey	0.51	0.54	0.51	0.53	0.012

^aPooled standard error of the least-squares means

^bPercentage of whole milk

Table 17. Effects of Monensin Treatment on Total Short and Medium-Chain Fatty Acids in Milk.

	Dose of Monensin				
	0 ppm	8 ppm	16 ppm	24 ppm	SEM ^a
Fatty Acid (%) ^b					
C4:0	3.05	2.98	2.98	2.87 ^c	0.195
C6:0	2.94	2.84	2.85	2.75 ^c	0.106
C8:0	1.20	1.16	1.14	1.11 ^c	0.071
C10:0	2.61	2.59	2.52	2.48	0.217
C12:0	2.91	2.91	2.79	2.80	0.260
C14:0	9.70	9.69	9.50	9.59	0.421
C14:1	0.92	0.91	0.89	0.95	0.124
C16:0	26.67	26.70	26.72	26.77	1.113
C16:1	1.19	1.23	1.23	1.33 ^c	0.103
C18:0	12.62	12.12	12.16	11.58 ^c	1.084
C18:1 ω 9	22.25	21.76	21.75	21.58	1.105

^aPooled standard error of the least-squares means

^bNo differences were noted for linoleic (C18:2) or linolenic (C18:3) fatty acids

^cDifferent from 0 ppm dose group (P < 0.05)

Table 18. Effects of Monensin Treatment on Short and Medium-Chain Free Fatty Acids in Milk.

	Dose of Monensin				
	0 ppm	8 ppm	16 ppm	24 ppm	SEM ^a
Fatty Acid (mg/mL) ^b					
C4:0	1.10	1.04	1.03	1.00 ^c	0.110
C6:0	1.06	0.99	0.98	0.96 ^c	0.078
C8:0	0.43	0.41	0.39	0.39 ^c	0.037
C10:0	0.95	0.91	0.87	0.87	0.104
C12:0	1.05	1.02	0.96	0.98	0.119
C14:0	3.49	3.35	3.27	3.30	0.244
C14:1	0.33	0.31	0.31	0.33	0.032
C16:0	9.61	9.20	9.20	9.23	0.836
C18:0	4.51	4.14	4.17	3.98 ^c	0.483
C18:1 ω 9	7.91	7.38	7.42	7.33 ^c	0.221

^aPooled standard error of the least-squares means

^bNo differences were noted for linoleic (C18:2) or linolenic (C18:3) fatty acids

^cDifferent from 0 ppm dose group (P < 0.05)

Table 19. Effects of Monensin Treatment on pH, Titratable Acidity, and Lactic Acid Production in Cheese Starter Cultures from Milk in Late Lactation.

	Dose of Monensin				
	0 ppm	8 ppm	16 ppm	24 ppm	SEM ^a
pH:					
0 hours	6.65	6.78 ^b	6.73	6.75 ^b	0.030
24 hours	4.72	4.68	4.67	4.68	0.023
Titratable Acidity (mL 0.1 N NaOH/10 mL of milk)					
0 hours	6.23	5.45 ^b	5.18	4.83 ^b	0.219
24 hours	9.23	8.58	8.13 ^b	8.73	0.275
Lactic Acid production (mg/100 ml)					
24 hours	0.27	0.28	0.27	0.35 ^b	0.027

^aPooled standard error of the least-squares means

^bDifferent from 0 ppm dose group ($P < 0.05$)

Mastitis

The effects of monensin treatment on incidence of clinical mastitis are presented in Table 20 (Calving 1 to Dry-Off/Removal; Lactation 1) and Table 21 (1-203 DIM; Lactation 2). During Lactation 1, no differences among dose groups were noted for animal rate, quarter rate, observation rate, incident rate, and case duration. Similarly, for Lactation 2, no differences were noted among dose groups for animal rate, quarter rate, observation rate, and incident rate. For case duration in Lactation 2, the treatment by parity interaction was significant and, thus, separate parity analyses are presented in Table 21. No difference in case duration associated with monensin treatment was noted in multiparous cows. For primiparous cows, case duration was extended in the 8 vs. 0 ppm dose groups. There was a non-significant decrease in case duration for cows in the 16 or 24 ppm groups vs. 0 ppm group. Because of the inconsistent results across dose groups, plus the small number of animals contributing to the analysis of case duration for primiparous cows in Lactation 2, the longer duration of cases seen for the 8 ppm dose is considered spurious. There were few observations of clinical mastitis during the Treatment Start to Calving 1 and Dry-Off to Calving 2 periods. There were no treatment-related differences during these two periods (data not shown).

Similar to results for clinical mastitis, mastitis therapy did not increase with dose of monensin in Lactations 1 and 2 (data not shown). Thus, use of monensin in lactating dairy cows is not associated with increased incidence of, or therapies for, clinical mastitis.

Effects of monensin treatment on milk somatic cell count (SCC) are presented in Table 22. For Lactation 1 (1-308 DIM), SCC was increased for the 8 ppm dose, but not the 16 and 24 ppm doses. The overall treatment effect was not significant ($P > 0.10$). For

Lactation 2 (1-203 DIM), there were no treatment related effects on SCC. Given that the results in Lactation 1 did not appear to be dose related, and there was no associated increase in clinical mastitis (see Table 20), it was concluded that the increase in SCC at the 8 ppm dose was not of concern.

Table 20. Effects of Monensin Treatment on Clinical Mastitis during Lactation 1
(Calving 1 to Dry-Off/Removal from Study), Parities Combined.

	Monensin Dose (ppm)			
	0	8	16	24
Animal Rate:				
No. of Cows Observed	120	112	102	107
No. of Cows at Risk	236	238	234	234
Rate	0.472	0.451	0.405	0.428
Quarter Rate:				
No. of Quarters Observed	215	203	179	193
No. of Quarters at Risk	944	952	936	936
Rate	0.200	0.197	0.175	0.185
Observation Rate:				
No. of Quarter-Days Observed	1713	1638	1518	1532
No. of Quarter-Days at Risk	279204	281144	290116	287752
Rate (per 1000 Quarter-Days at Risk)	4.375	5.164	4.665	5.011
Incident Rate:				
No. of Incidents Observed	309	286	244	250
No. of Quarter-Days at Risk	279204	281144	290116	287752
Rate (per 1000 Quarter-Days at Risk)	0.893	0.865	0.749	0.764
Case Duration:				
No. of Cases	308	286	244	250
No. of Cows with Cases	120	112	102	107
Average Duration	7.34	8.82	7.48	8.12

Table 21. Effects of Monensin Treatment on Clinical Mastitis during Lactation 2 (1-203 DIM)^a, Parities Combined.

	Monensin Dose (ppm)			
	0	8	16	24
Animal Rate:				
No. of Cows Observed	37	35	31	29
No. of Cows at Risk	63	64	60	62
Rate	0.589	0.521	0.490	0.478
Quarter Rate:				
No. of Quarters Observed	70	67	58	50
No. of Quarters at Risk	252	256	240	248
Rate	0.279	0.239	0.213	0.200
Observation Rate:				
No. of Quarter-Days Observed	900	1327	890	622
No. of Quarter-Days at Risk	47584	48424	44308	48056
Rate (per 1000 Quarter-Days at Risk)	13.570	15.713	13.317	7.804
Incident Rate:				
No. of Incidents Observed	98	98	103	77
No. of Quarter-Days at Risk	47584	48424	44308	48056
Rate (per 1000 Quarter-Days at Risk)	1.900	1.716	1.907	1.511
Case Duration:^b				
<u>Multiparous Cows:</u>				
No. of Cases	59	58	65	43
No. of Cows with Cases	24	23	21	18
Average Duration	14.12	8.50	10.06	10.93
<u>Primiparous Cows:</u>				
No. of Cases	32	31	29	30
No. of Cows with Cases	13	12	9	11
Average Duration	16.78	34.54 ^c	14.36	9.95

^aIncludes cows only from California, Florida, and New York locations

^bSeparate parity analyses due to significant treatment by parity interaction

^cDifferent from 0 ppm dose group (P = 0.01)

Table 22. Effects of Monensin Treatment on Somatic Cell Count (SCC; linear score^a and cells/ml^b) during the Standardized Periods of Lactation 1 (1-308 DIM) and Lactation 2 (1-203 DIM), Parities Combined.

	Monensin Dose											
	0 ppm			8 ppm			16 ppm			24 ppm		
Component	LSMEAN ^c	SE ^d	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
1-308 DIM of Lactation 1												
Linear Score	2.61	0.20	221	2.85 ^e	0.20	224	2.73	0.20	223	2.60	0.20	224
SCC (cells/ml)	76138			90274			82802			76040		
1-203 DIM of Lactation 2												
Linear Score	2.77	0.44	61	2.98	0.44	62	2.93	0.44	58	2.43	0.44	62
SCC (cells/ml)	85259			98498			95455			67168		

^aLinear score = $\text{Log}_2(\text{cells/ml}) - 13.60964047$

^bBack-transformed from least-squares means of linear scores

^cLeast-squares mean

^dStandard error

^eDifferent from 0 ppm dose group ($P < 0.10$)

Observation rates for subclinical bacteriology results are summarized in Table 23 (event-driven sampling) and Table 24 (calendar-driven sampling). Because of overall sparseness of data and a general lack of treatment-related effects at specific sampling periods (data not shown), data were pooled across events (calvings, dry-off, study removal) and locations for event-driven sampling, and across locations and calendar dates for calendar-driven sampling.

There were no treatment-related increases in prevalence of quarters with positive cultures for the following microorganism groupings: 1) all microorganisms; 2) environmental pathogens; 3) coagulase-negative Staphylococci; 4) contagious pathogens; and 5) other microorganisms.

Conclusions on Mastitis:

Based on these results, monensin treatment at the recommended doses does not increase the incidence of, or therapeutic treatment for, mastitis in dairy cows.

Table 23. Effects of Monensin Treatment on Observation Rate for Subclinical Mastitis Bacteriology, by Microorganism Grouping, from Event-Driven Quarter Sampling during Lactations 1 and 2^a.

	Monensin Dose (ppm)			
	0	8	16	24
Quarter-Days at Risk	1101	1104	1087	1097
All Microorganisms:				
Quarter-Days Observed	110	117	107	98
Observation Rate ^b	0.076	0.082	0.065	0.063
Environmental:				
Quarter-Days Observed	27	16	31	23
Observation Rate	0.005	0.003	0.007	0.004
Coagulase Negative Staphylococci:				
Quarter-Days Observed	61	71	43	55
Observation Rate	0.020	0.030	0.014	0.021
Contagious:				
Quarter-Days Observed	10	23	17	12
Observation Rate	0.009	0.021	0.016	0.011
Other Microorganisms:				
Quarter-Days Observed	12	7	16	8
Observation Rate	0.011	0.006	0.015	0.007

^aOnly California, Florida, and New York locations

^bQuarter-days observed per quarter-days at risk

Table 24. Effects of Monensin Treatment on Observation Rate for Subclinical Mastitis Bacteriology, by Microorganism Grouping, from Calendar-Driven Quarter Sampling during Lactations 1 and 2^a.

	Monensin Dose (ppm)			
	0	8	16	24
Quarter-Days at Risk	2655	2815	2711	2800
All Microorganisms:				
Quarter-Days Observed	268	294	289	262
Observation Rate ^b	0.104	0.107	0.107	0.096
Environmental:				
Quarter-Days Observed	38	42	61	45
Observation Rate	0.013	0.014	0.021	0.015
Coagulase Negative Staphylococci:				
Quarter-Days Observed	176	190	146	172
Observation Rate	0.057	0.065	0.051	0.059
Contagious:				
Quarter-Days Observed	32	40	46	29
Observation Rate	0.011	0.009	0.013	0.006
Other Microorganisms:				
Quarter-Days Observed	22	22	36	16
Observation Rate	0.004	0.003	0.006	0.004

^aOnly California, Florida, and New York locations

^bQuarter-days observed per quarter-days at risk

Animal Health and Therapy:

Of the abnormalities observed within the main systems (digestive, metabolic, foot and leg, mammary, reproductive, skin, eye and lid, respiratory, urinary, musculoskeletal, cardiovascular and central nervous system), only abnormalities and therapy associated with the reproductive system were adversely affected by monensin treatment. As such, only those notable health abnormalities and therapy from the reproductive system that were both statistically significant and biologically relevant are presented.

The effect of monensin treatment on metritis, based on veterinary diagnosis, is presented for Lactation 1 in Table 25. There was a linear increase in the animal rate for metritis with dose of monensin. There was no effect of monensin treatment on incident rate or average case duration. During Lactation 2, cows that initiated the study as primiparous had an increase in the animal rate and incident rate for metritis during the first seven days of lactation (data not shown).

The effect of monensin on cystic ovaries, based on veterinary diagnosis, is presented for Lactation 1 in Table 26. There were increases in the animal and incident rates for cystic ovaries in primiparous cows during Lactation 1, while average case duration was not affected by monensin treatment. During Lactation 2, cows that initiated the study as primiparous cows had an increase in the incident rate of cystic ovaries (data not shown).

Hormone therapies for Lactation 1 (Calving 1 to Dry-Off/Removal) are presented in Table 27. There was an increase in animal rate with monensin treatment, likely associated with increases in metritis and cystic ovaries in monensin-treated cows (see Tables 25 and 26).

Increases in metritis, cystic ovaries, and hormone therapies are addressed in product labeling.

Table 25. Effect of Monensin Treatment on Metritis (Veterinary Diagnoses) during Lactation 1 (Calving 1 to Dry-Off/Removal from Study).

	Monensin Dose (ppm)			
	0	8	16	24
Animal Rate:				
Number of Cows Observed	38	50	60	57
Number of Cows at Risk	236	238	234	234
Rate ^a	0.149	0.208	0.237 ^b	0.226
Incident Rate:				
Number of Incidents Observed	38	53	60	57
Number of Days at Risk	69801	70286	72529	71938
Rate (per 1000 Days at Risk)	0.527	0.762	0.860	0.812
Case Duration:				
Number of Cases Observed	37	52	60	57
Number of Animals with Cases	37	49	60	57
Average Duration	12.37	15.00	11.08	11.66

^aLinear increase with increasing dose of monensin ($P < 0.10$)

^bDifferent from 0 ppm dose group ($P < 0.10$)

Table 26. Effect of Monensin Treatment on Cystic Ovaries (Veterinary Diagnoses) during Lactation 1 (Calving 1 to Dry-Off/Removal from Study).

	Monensin Dose (ppm)			
	0	8	16	24
Animal Rate^b:				
Multiparous Cows:				
Number of Cows Observed	22	21	23	24
Number of Cows at Risk	152	150	150	147
Rate	0.129	0.116	0.130	0.136
Primiparous Cows:				
Number of Cows Observed	6	8	13	15
Number of Cows at Risk	84	88	84	87
Rate ^c	0.061	0.073	0.133 ^d	0.147 ^d
Incident Rate^b:				
Multiparous Cows:				
Number of Incidents Observed	25	24	30	25
Number of Days at Risk	44600	43984	47736	44427
Rate (per 1000 Days at Risk)	0.414	0.361	0.414	0.355
Primiparous Cows				
Number of Incidents Observed	6	10	17	19
Number of Days at Risk	25201	26302	24793	27511
Rate ^c (per 1000 Days at Risk)	0.168	0.246	0.422 ^d	0.432 ^d
Case Duration^a:				
Number of Cases	30	33	47	44
Number of Animals with Cases	27	28	36	39
Average Duration	19.90	14.06	14.67	20.46

^aPooled parity analysis

^bSeparate parity analyses due to significant treatment by parity interaction

^cLinear increase with increasing dose of monensin ($P < 0.10$)

^dDifferent from 0 ppm dose group ($P < 0.10$)

Table 27. Effect of Monensin Treatment on Hormone Therapies during Lactation 1
(Calving 1 to Dry-Off/Removal from Study).

	Monensin Dose (ppm)			
	0	8	16	24
Animal Rate:				
No. of Cows Observed	127	129	138	142
No. of Cows at Risk	236	238	234	234
Rate ^a	0.555	0.562	0.609	0.642 ^b
Observation Rate:				
Number of Days Observed	530	504	631	554
Number of Days at Risk	69801	70286	72529	71938
Rate (per 1000 Days at Risk)	6.805	7.412	8.507	7.723

^aLinear increase with increasing dose of monensin ($P < 0.10$)

^bDifferent from 0 ppm dose group ($P < 0.10$)

Reproductive Performance:

Effects of monensin treatment on days to first standing estrus and interestrous interval are presented in Table 28. No differences among treatments were seen for days to first standing estrus in both study lactations, indicating that the postpartum interval from calving to first standing estrus was not extended with monensin treatment. In addition, the interestrous interval did not differ among treatments in both study lactations. Thus, expression of estrus and/or an observer's ability to detect estrus in dairy cows was not hindered by monensin treatment.

Days to first service and interval between services are presented in Table 29. Days to first service was not different among treatments in Lactation 1. For Lactation 2, there was a significant treatment by parity interaction, so separate parity results are presented. In separate parity analyses, days to first service did not differ among treatments in Lactation 2. In addition, interval between services did not differ among treatments in Lactation 1. For Lactation 2, there was a significant treatment by parity interaction, so separate parity results are presented. No differences in interval between services were noted in the parity-specific analyses for Lactation 2. Thus, monensin did not extend the postpartum interval from calving to first service or the interval between services. These findings parallel those on estrus provided in Table 28.

Results for the services per animal variables are presented in Table 30. During Lactation 1, there was a treatment by parity interaction for services per animal (all animals), so separate parity results are provided. There were no treatment differences in multiparous cows, while in primiparous cows the 24 ppm dose group had a greater number of services per animal than primiparous cows in the 0 ppm dose group. For Lactation 2, there was a linear increase in services per animal, with services per animal greater in the 16 and 24 vs. 0 ppm dose groups.

In Lactation 1, the treatment by parity interaction was significant for services per conception for cows that conceived a full-term pregnancy, so separate parity results are presented. No difference among treatments was noted in multiparous cows, while in primiparous cows, there was an increase in services per animal in the 24 vs. 0 ppm dose groups. For Lactation 2, there were no significant differences among treatment groups, though there was a numerical trend for an increase in services per conception in the 16 and 24 ppm dose groups. Taken together, results from the two services per animal variables, either when considering all cows or only those that experienced a full-term conception, indicate that monensin treatment may reduce fertility in dairy cows.

Table 28. Effects of Monensin Treatment on Days to First Standing Estrus and Interestrous Interval During Lactation 1 and Lactation 2.

	Monensin Dose							
	0 ppm		8 ppm		16 ppm		24 ppm	
	LSMEAN	N	LSMEAN	N	LSMEAN	N	LSMEAN	N
Days to First Standing Estrus								
Lactation 1	74.5	209	73.8	217	68.6	216	73.0	205
Lactation 2	87.8	50	75.5	55	81.1	57	79.4	57
Interestrous Interval								
Lactation 1	23.1	104	23.6	110	22.7	120	23.7	116
Lactation 2	27.3	21	30.4	23	29.8	29	30.1	34

Table 29. Effects of Monensin Treatment on Days to First Service and Interval Between Services in Cows During Lactation 1 and Lactation 2.

	Monensin Dose							
	0 ppm		8 ppm		16 ppm		24 ppm	
	LSMEAN	N	LSMEAN	N	LSMEAN	N	LSMEAN	N
Days to First Service								
Lactation 1	84.1	218	84.7	211	86.9	219	83.6	221
Lactation 2 ^a								
Multiparous	90.4	37	85.2	36	92.5	33	86.2	38
Primiparous	89.0	23	109.9	25	85.5	22	79.9	22
Interval Between Services								
Lactation 1	32.9	106	32.2	119	35.0	119	31.2	129
Lactation 2 ^a								
Multiparous	27.7	19	33.5	14	28.9	23	27.8	17
Primiparous	28.3	12	27.6	9	22.9	12	35.4	14

^aSeparate parity analyses due to significant treatment by parity interaction

Table 30. Effects of Monensin Treatment on Services per Animal for All Cows Inseminated, or Only Cows with Full-Term Pregnancies, During Lactation 1 and Lactation 2.

	Monensin Dose							
	0 ppm		8 ppm		16 ppm		24 ppm	
	LSMEAN	N	LSMEAN	N	LSMEAN	N	LSMEAN	N
Services per Animal^a								
Lactation 1 ^c								
Multiparous	1.98	142	2.09	134	2.24	143	2.06	141
Primiparous	1.95	76	1.99	77	1.76	76	2.41 ^d	80
Lactation 2 ^e	1.86	60	1.71	61	2.40 ^d	55	2.37 ^d	60
Services per Conception^b								
Lactation 1 ^c								
Multiparous	1.82	101	1.97	97	1.88	93	1.84	101
Primiparous	1.68	59	1.71	58	1.52	56	2.13 ^d	63
Lactation 2	1.86	48	1.64	47	2.09	44	2.01	46

^aAll cows that were inseminated

^bOnly cows that conceived a full-term pregnancy

^cSeparate parity analyses due to significant treatment by parity interaction

^dDifferent from 0 ppm dose group (P < 0.10)

^eLinear increase with increasing dose of monensin (P < 0.10)

Effects of monensin treatment on first service and overall conception rates are presented in Table 31. For both variables in Lactation 1, there was a significant treatment by parity interaction, so separate parity results are provided. In multiparous cows, first service conception rate decreased linearly with dose of monensin, with conception rates lower at the 16 and 24 ppm doses vs. the 0 ppm dose. In primiparous cows, first service conception rate was less in cows in the 24 vs. 0 ppm dose groups. First service conception rate did not differ among treatments during Lactation 2. Overall conception rate in Lactation 1 did not differ among treatments in primiparous cows, while for multiparous cows, overall conception rate was less in cows in the 16 vs. 0 ppm dose groups. Overall conception rate decreased linearly with dose of monensin in Lactation 2. Similar to results with services per animal, reduced conception rates indicate that monensin may reduce fertility in dairy cows.

Pregnancy rate results are presented in Table 32. Pregnancy rate did not differ among treatments during either study lactation. Even though fertility appeared to be reduced in monensin-treated cows as reflected in results for services per animal and conception rate, overall pregnancy rate did not differ, indicating that any reduction in fertility is likely a manageable effect of monensin use in dairy cows.

Days open variables are presented in Table 33. Days open to first conception was extended in the 24 vs. 0 ppm dose groups in Lactation 1. Since the treatment by parity interaction was significant for days open to first conception during Lactation 2, separate parity results are presented. There were no significant differences among treatment groups for either multiparous or primiparous cows. Days Open A (includes only cows with full-term conception) in Lactation 1 was extended in the 24 vs. 0 ppm dose groups. Since the treatment by parity interaction was significant for Days Open A during Lactation 2, separate parity results are presented. There were no significant treatment differences in Days Open A during Lactation 2 in multiparous or primiparous cows. Days Open B (includes data from all cows) did not differ among treatments during both study lactations. Calving interval increased linearly with dose of monensin, seen primarily in the 24 vs. 0 ppm dose groups. Results related to an extension in the days open and calving interval variables were likely related to increased services per animal and reduced conception rate as noted previously.

Calving difficulty scores (data not shown) did not differ among treatments for either study lactation.

Table 31. Effects of Monensin Treatment on Conception Rates During Lactation 1 and Lactation 2.

	Monensin Dose (ppm)			
	0	8	16	24
First Service Conception Rate:				
Lactation 1^a				
Multiparous:				
No. of Cows Conceiving	71	56	53	55
No. of Cows Inseminated	138	132	141	136
Conception Rate ^b	0.521	0.432	0.380 ^c	0.413 ^c
Primiparous:				
No. of Cows Conceiving	34	30	36	25
No. of Cows Inseminated	75	77	72	80
Conception Rate	0.461	0.401	0.511	0.318 ^c
Lactation 2:				
No. of Cows Conceiving	25	31	18	26
No. of Cows Inseminated	58	56	54	59
Conception Rate	0.439	0.560	0.345	0.424
Overall Conception Rate:				
Lactation 1^a				
Multiparous:				
No. of Conceptions	117	111	109	112
No. of Inseminations	293	292	331	306
Conception Rate	0.417	0.397	0.341 ^c	0.386
Primiparous:				
No. of Conceptions	64	66	61	67
No. of Inseminations	154	162	140	201
Conception Rate	0.424	0.420	0.449	0.350
Lactation 2:				
No. of Conceptions	48	47	44	46
No. of Inseminations	118	107	137	144
Conception Rate ^b	0.424	0.443	0.334	0.318

^aSeparate parity analyses due to significant treatment by parity interaction

^bLinear decrease with increasing dose of monensin (P < 0.10)

^cDifferent from 0 ppm dose group (P < 0.10)

Table 32. Effects of Monensin Treatment on Pregnancy Rates During Lactation 1 and Lactation 2.

	Monensin Dose (ppm)			
	0	8	16	24
Pregnancy Rate:				
Lactation 1				
Number of Pregnant Cows	160	155	149	164
Total Number of Cows	246	242	239	239
Pregnancy Rate	0.646	0.631	0.620	0.686
Lactation 2:				
Number of Pregnant Cows	48	47	44	46
Total Number of Cows	95	89	97	91
Pregnancy Rate	0.529	0.520	0.456	0.492

Table 33. Effects of Monensin Treatment on Days Open to First Service, Days Open A (Only Cows that Conceived a Full-Term Pregnancy), Days Open B (All Cows) and Calving Interval During Lactation 1 and Lactation 2.

	Monensin Dose							
	0 ppm		8 ppm		16 ppm		24 ppm	
	LSMEAN	N	LSMEAN	N	LSMEAN	N	LSMEAN	N
Days Open to First Conception								
Lactation 1	99.8	181	104.6	177	100.4	170	107.7 ^a	179
Lactation 2 ^b								
Multiparous	105.4	27	101.6	28	125.4	26	99.7	28
Primiparous	98.7	21	101.2	19	98.8	18	112.3	18
Days Open A								
Lactation 1	101.6	160	104.2	155	102.1	149	109.6 ^a	164
Lactation 2 ^b								
Multiparous	107.6	27	107.5	28	124.7	26	99.7	28
Primiparous	102.4	21	100.7	19	102.4	18	113.1	18
Days Open B								
Lactation 1	134.0	218	138.5	211	145.0	219	137.1	221
Lactation 2	127.0	60	135.5	61	134.6	55	138.9	60
Calving Interval^c	380.8	160	383.6	155	381.8	149	389.6 ^a	164

^aDifferent from 0 ppm dose group (P < 0.10)

^bSeparate parity analyses due to significant treatment by parity interaction

^cLinear increase with increasing dose of monensin (P < 0.10)

Effects of monensin treatment on rates of early and late pregnancy loss, stillbirths and multiple births are presented in Table 34. There were no differences among treatments during both study lactations for each of these variables. Given the shortened duration of observation in Lactation 2, a determination of late fetal loss was not performed. In Lactation 2, there appeared to be an increase in stillbirth rate in monensin-treated cows. The stillbirth rate in the 0 ppm dose group was very low, with the stillbirth in the 8, 16, and 24 ppm dose groups of a comparable level to all dose groups in Lactation 1. Given this, increased stillbirth rate is not likely of concern in dairy cows given monensin.

Calf birth weights (by gender) for both study lactations, and female calf 28-day weight and average daily gains for Lactation 2, are presented in Table 35. Birth weights of male and female calves for Lactation 1 did not differ among treatments. During Lactation 2, female calves of monensin treated cows were significantly heavier than female calves of control cows, though these modest differences are not likely of concern. As expected, birth weights of male calves were heavier than birth weights of female calves. Body weight of female calves at 28 days of age and average daily gain from birth to 28 days of age did not differ among treatment groups.

The negative effects of monensin treatment on services per animal, conception rate, days open, and calving interval are addressed in product labeling.

Table 34. Effects of Monensin Treatment on Early and Late Pregnancy Loss, Stillbirth, and Multiple Birth Rates During Lactation 1 and Lactation 2.

	Monensin Dose (ppm)			
	0	8	16	24
Early Pregnancy Loss^a:				
Lactation 1:				
Number of Losses	19	9	16	10
Number of Cows	181	177	170	179
Loss Rate	0.105	0.051	0.094	0.056
Lactation 2:				
Number of Losses	3	2	2	1
Number of Cows	48	47	44	46
Loss Rate	0.063	0.043	0.045	0.022
Late Pregnancy Loss:^{bc}				
Lactation 1:				
Number of Losses	5	13	9	6
Number of Cows	181	177	170	179
Loss Rate	0.028	0.073	0.053	0.034
Stillbirth Rate:				
Lactation 1:				
Number of Losses	28	23	20	25
Number of Cows	236	238	234	234
Stillbirth Rate	0.125	0.101	0.089	0.109
Lactation 2:				
Number of Losses	5	17	12	10
Number of Cows	160	155	149	164
Stillbirth Rate	0.031	0.110	0.081	0.061
Multiple Birth Rate:				
Lactation 1:				
Number of Multiple Births	9	11	7	7
Number of Cows	236	238	234	234
Multiple Birth Rate	0.038	0.046	0.030	0.030
Lactation 2:				
Number of Multiple Births	5	10	8	9
Number of Cows	160	155	149	164
Multiple Birth Rate	0.031	0.065	0.054	0.055

^aPregnancy loss prior to 90 days of gestation

^bPregnancy loss between 90 and 250 days of gestation

^cStudy was not of sufficient duration to determine late pregnancy loss in Lactation 2

Table 35. Effects of Monensin Treatment on Calf Birth Weights (Lactation 1 and Lactation 2) and Female Calf Weights and Average Daily Gain at 28 Days of Age (Lactation 2 only).

	0 ppm			8 ppm			16 ppm			24 ppm		
	LSMEAN ^a	SE ^b	N	LSMEAN	SE	N	LSMEAN	SE	N	LSMEAN	SE	N
Lactation 1												
Birth Weight (kg)												
Male Calves	43.5	0.9	119	43.7	1.0	116	43.8	1.0	119	44.0	0.9	118
Female Calves	39.2	0.9	125	40.2	0.9	133	40.3	0.9	122	40.5	0.9	123
Lactation 2												
Birth Weight (kg)												
Male Calves	45.6	1.1	85	46.6	1.1	77	45.9	1.1	82	45.3	1.1	92
Female Calves	40.3	1.1	79	42.0 ^c	1.1	86	42.3 ^c	1.2	74	43.0 ^c	1.1	81
Female 28-Day Weight (kg)	49.4	1.2	71	49.6	1.2	79	51.1	1.3	63	52.3	1.2	69
Female 28-Day Average Daily Gain (kg/day)	0.31	0.04	71	0.29	0.04	78	0.30	0.04	63	0.32	0.04	69

^aLeast-squares mean

^bStandard error

^cDifferent from 0 ppm dose group (P < 0.10)

3. *TARGET ANIMAL SAFETY:*

Study Title:

Acute Target Animal Safety Study in Lactating Dairy Cows Given an Oral Bolus Dose of Monensin at 0, 1, or 10 mg/kg of Body Weight Per Day for up to 21 Consecutive Days.

Location: Greenfield, Indiana

Investigators: J. M. Buck and M. N. Novilla

Objective: To characterize the toxic syndrome of monensin in lactating dairy cows after oral bolus dosing at 0, 1, or 10 mg/kg of body weight per day for up to 21 days duration.

MATERIALS AND METHODS

Test Animals

This study was conducted in compliance with Good Laboratory Practice (GLP) regulations, 21 CFR 58.21, except where otherwise noted.

Thirty clinically normal Holstein dairy cows, 15 primiparous and 15 multiparous weighing 446 - 544 kg and 480.5 - 548 kg, respectively, were obtained as candidate animals for the study. The primiparous cows were two years old, 50 to 72 days in milk (DIM), and were producing approximately 27 to 38 kg/d of milk. The multiparous cows were three years old (second lactation), 44 to 74 DIM, and were producing 34 to 54 kg/d of milk. The cows were either verified not pregnant when they arrived at the study site, or if cows had recent breeding dates, they were given 25 mg of prostaglandin F₂ α (dinoprost tromethamine) two days after arrival to terminate potential pregnancy.

Test Animal Housing and Care

Upon arrival at the study site, cows were milked and individually identified with an identically-numbered tag in each ear. Neck chains with transponders were placed around cows' necks. Cows were weighed and given routine preventative health procedures (vaccinations, de-lousing, rumen magnets) and blood was drawn for Johne's disease testing by Agar Gel Immuno Diffusion (AGID). Tests were done at the Animal Health Diagnostic Laboratory, East Lansing, Michigan (non-GLP lab).

The cows were ranked by weight and assigned to a tie stall in the housing facility. The day after arrival, and every day thereafter, the cows were returned to their assigned tie stalls after the morning milking. The cows were released in the morning for approximately one hour of exercise in a dry lot with shade. Following the exercise period, the cows returned to their assigned tie stalls until the afternoon milking, after which they were again returned to their assigned tie stalls.

During the pre-study and study periods, the cows were fed a single total mixed ration (TMR) to allow for *ad libitum* consumption of feed. Rations were adjusted weekly according to the dry matter (DM) content of the different silage and high moisture corn components of the TMR. Cows were fed as per the 1989 National Research Council (NRC) recommendations for Dairy Cattle to support maintenance, milk production, and growth in primiparous cows, and the general nutrient specifications were as follows on a DM basis:

Crude Protein - 17-19%
Net Energy of Lactation (NE_L) - 1.63-1.76 Mcal/kg
Calcium - 0.9-1.3%
Phosphorous - 0.48-0.58%

Cows were milked twice daily at approximately 11 and 13 hour intervals in a double six herringbone milking parlor. Milk weights were collected throughout the live phase of the study. The daily milk production was calculated by adding the production from the AM milking and the previous evening milking. Starting on the day after arrival, milk composition samples were collected at the PM milking and the following AM milking and were collected weekly, thereafter. The milk composition samples were analyzed by Northeast DHIA (a non-GLP lab), Ithaca, New York, for percent fat, protein, lactose, total solids, and somatic cell count.

Test Article

Monensin sodium was used to prepare individual animal doses of 1 or 10 mg•kg body weight (BW)⁻¹•day⁻¹ administered orally by gelatin capsule. A non-medicated milk replacer was used to fill the control capsules, and added to the monensin to fill the 1 mg•kg BW⁻¹•day⁻¹ dose capsules. The 10 mg•kg BW⁻¹•day⁻¹ dose capsules were filled only with monensin.

Treatment Groups and Study Duration

On the day of arrival, cows were weighed and ranked by weight and parity. Twenty-four of the available 30 cows were randomly selected and assigned to three treatment groups (0, 1, and 10 mg•kg BW⁻¹•day⁻¹) as follows. Four body weight blocks were formed, each consisting of three primiparous cows and three multiparous cows. Blocks 1 through 4 were formed from the lightest to heaviest animals, respectively. Specifically, the sequence 1 through 15 was formed for each parity as the heaviest animal to the lightest animal, then animals 1 through 3 were assigned to block 4, 5 through 7 to block 3, 9 through 11 to block 2, and 13 through 15 to block 1. The fourth, eighth, and twelfth animals in the sequence were assigned as replacement animals. Blocks were randomly assigned to a location in the building with respect to side (east/west) and end (north/south). Parity was randomly assigned to location within each block (north/south) and treatment was randomly assigned to animals within each block-parity group. The extra cows were held until all cows assigned to treatment groups had started dosing.

Treatment groups were color-coded so that the clinical veterinarians and all animal technicians were masked to the treatment assignments.

The day of treatment initiation was designated as Study Day 1, and events were identified as study days before treatment initiation (negatively numbered; either acclimation or pretreatment periods) or during treatment (positively numbered; treatment period).

Cows from blocks 1 and 3 started the treatment period one week before the cows in blocks 2 and 4. Therefore, cows in blocks 1 and 3 had approximately a two-week acclimation period and cows in blocks 2 and 4 had approximately a three-week acclimation period. Cows in all blocks underwent a 14 day pretreatment period after the acclimation period. If the TMR consumption during the treatment period for a cow dropped below 10% of the pretreatment period average TMR consumption for three consecutive days (anorexia criteria), the cow was removed from the study and necropsied. All cows from blocks 2 and 3 plus the high dose cows from blocks 1 and 4 were originally scheduled to be necropsied on Study Day 21. Since all of the high dosed cows were removed by Study Day 8 for meeting the anorexia criteria, and no other cows showed signs of toxicity, the cows in the 0 and 1 mg•kg BW⁻¹•day⁻¹ dose groups in Blocks 2 and 3 were necropsied on Study Day 14 as per the study protocol.

Preparation of Test Article in Gelatin Capsules

The dose was prepared on an individual cow basis by a technician who was not involved with the live animal phase, using the mean of body weights recorded on Study Days -14, -7, and -1 of pretreatment. Each capsule was individually identified with the cow number and capsule number using colored permanent markers to reflect the treatment color code. Capsules for the control animals were filled with the nonmedicated milk replacer. The dose capsules were prepared within four days of dosing and stored at ambient temperatures.

Administration of Dose

During each morning of the treatment period, the cows were bolus dosed with a gelatin capsule containing either 0, 1, or 10 mg•kg BW⁻¹•day⁻¹ monensin using a balling gun. Cows in the 0 and 1 mg•kg BW⁻¹•day⁻¹ groups were dosed for 14 consecutive days. The 10 mg•kg BW⁻¹•day⁻¹ treatment group was dosed until the cows were removed from study and necropsied due to the anorexia criteria.

Survival and Clinical Signs

Body temperatures were collected daily throughout the acclimation, pretreatment, and treatment periods. A veterinary health exam was performed on each cow on Study Days -1, 3, 7, and 14 (± one day). Body temperature, pulse, respiration, rumen motility, and abnormal observations were recorded.

The cows were observed twice daily throughout the acclimation period and the pretreatment period. During the treatment period, all cows were observed approximately every four hours as long as any cow displayed clinical signs of toxicity. When no signs of toxicity were observed, health observations returned to the twice a day schedule for all cows.

Body Weight, Body Condition Score, and Feed Consumption

The cows were weighed upon arrival, on Study Days -28, -21, -14, -7, -1, 7, and 14, or just prior to necropsy for the animals that were removed prematurely. Body condition score (BCS) was collected on Study Days -28, -7, and prior to necropsy or study termination using a 1 through 5 scale with 0.25 increments. The amounts of TMR issued and refused were measured daily per cow throughout the live phase of the study and recorded on an as-fed basis.

Terminal Procedures

All of the cows from blocks 2 and 3 and the high dosed cows from blocks 1 and 4 were necropsied. The cows were transported from the barn to the necropsy facility in a stock trailer. The cows were euthanized by captive bolt concussion followed by exsanguination. At the end of the live phase of the study, the remaining cows were transferred to an on station stock herd.

Clinical Pathology

Blood samples for hematology and clinical chemistry determinations were obtained from each animal in the 0 and 1 mg•kg BW⁻¹•day⁻¹ groups on Study Days -14, -7, -1, 3, 7, and 14. For the animals in the 10 mg•kg BW⁻¹•day⁻¹ group, blood samples for hematology and clinical chemistry determinations were obtained from each animal on Study Days -14, -7, -1, and 3, and once on Study Days 4 to 7 just prior to euthanasia for meeting anorexia criteria.

Urine samples for myoglobin determinations were obtained from each animal in the 0 and 1 mg•kg BW⁻¹•day⁻¹ groups on Study Days -14, -7, -1, 3, 7, and 14. For the animals in the 10 mg•kg BW⁻¹•day⁻¹ group, urine samples were obtained from each animal on Study Days -14, -7, -1, and 3, and once on Study Days 4 to 7 just prior to euthanasia for meeting anorexia criteria.

Hematology

Blood samples were collected into tubes containing EDTA anticoagulant. Peripheral blood smears were prepared for each sample and were evaluated microscopically. Values for the following parameters were determined.

Parameter (Abbreviation)	Unit/Result
Erythrocyte count (RBC)	Million/microliter (mil/μL)
Hemoglobin (HGB)	Grams/deciliter (g/dL)
Hematocrit (HCT)	Percent (%)
Mean corpuscular volume(MCV)	Femtoliters (fl)
Mean corpuscular hemoglobin (MCH)	Picograms (pg)
Nucleated red blood cells (NRBCS)	Number of erythrocytes/100 leukocytes (N ERY/100 LEUKS)
Mean corpuscular hemoglobin concentration (MCHC)	Grams/deciliter (g/dL)
Erythrocyte morphology:	Normal erythrocytes (Norm Erys)
Anisocytosis (Aniso)	Slight (Slt); Moderate (Mod); Marked (Mkd)
Poikilocytosis (Poik)	Slight (Slt); Moderate (Mod); Marked (Mkd)

Leukocyte count, total (WBC)	Thousands/microliter (thous/ μ L)
Leukocyte differential:	Thousands/microliter (thous/ μ L)
Lymphocytes (LYMS)	
Neutrophils (NEUTS)	
Monocytes (MONOS)	
Eosinophils (EOS)	
Basophils (BASOS)	
Large unstained cells (LUCS)	
Leukocyte morphology	Normal Leukocytes (Norm Leuks)
Platelet count (PLT)	Thousands/microliter (thous/ μ L)
Platelet morphology	Normal Platelets (Norm Plts)

Blood samples were collected into tubes containing sodium citrate anticoagulant. Plasma was obtained by centrifugation and assayed for fibrinogen (FBGN; mg/dL).

Clinical Chemistry

Blood samples were collected into tubes containing no anticoagulant. Serum was obtained by centrifugation and values for the following parameters were determined.

Parameter (Abbreviation)	Unit
Glucose (GLU)	Milligrams/deciliter (mg/dL)
Blood urea nitrogen (BUN)	Milligrams/deciliter (mg/dL)
Creatinine (CREAT)	Milligrams/deciliter (mg/dL)
Total bilirubin (T BILI)	Milligrams/deciliter (mg/dL)
Alkaline phosphatase (ALP)	International units/liter (IU/L)
Alanine transaminase (ALT)	International units/liter (IU/L)
Aspartate transaminase (AST)	International units/liter (IU/L)
Creatine phosphokinase (CPK)	International units/liter (IU/L)
Lactate dehydrogenase (LDH)	International units/liter (IU/L)
Calcium (CA)	Milligrams/deciliter (mg/dL)
Inorganic phosphorus (IP)	Milligrams/deciliter (mg/dL)
Sodium (NA)	Milliequivalents/liter (mEq/L)
Potassium (K)	Milliequivalents/liter (mEq/L)
Chloride (CL)	Milliequivalents/liter (mEq/L)
Total protein (TP)	Grams/deciliter (g/dL)
Albumin (ALB)	Grams/deciliter (g/dL)
Globulin (GLOB)	Grams/deciliter (g/dL)
Albumin/globulin ratio (AGRTO)	Ratio
Free fatty acids (FFA)	Milliequivalents/liter (mEq/L)

Urinalysis

Urine samples were collected for measurement of myoglobin (MYGLBN; negative, trace, 1+, 2+, 3+).

Necropsy

A necropsy was conducted on each animal by a board certified pathologist and appropriately trained personnel. The necropsy included examination of all external body surfaces and orifices; the thoracic, abdominal, and pelvic cavities and their viscera; and cervical tissues and organs.

Organ Weights

Kidneys, liver and heart from each killed animal were weighed after removing extraneous adjacent tissue.

Tissue Preservation

Samples of the following sets of organs and tissues, as well as tissues containing gross lesions, were collected from each animal and preserved in 10% neutral buffered formalin:

Thyroid glands	Kidneys
Adrenal glands	Urinary bladder
Mammary gland	Bone marrow
Pancreas	Tongue
Ovaries	Esophagus
Uterus	Stomach (rumen, reticulum, omasum, abomasum)
Thymus	
Lymph nodes (cervical, mediastinal, and mesenteric)	Duodenum
Spleen	Jejunum
Lungs	Ileum
Liver	Colon
Gallbladder	Cecum
Heart	Sciatic nerve
right atrium	Abdominal muscle
left atrium	Pectoral muscle
septum	Quadriceps femoris muscle
right ventricle	Gastrocnemius
left ventricle	Diaphragm

Histopathology

Preserved tissue specimens were trimmed, processed through graded alcohol and clearing agent, infiltrated and embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The tissue sections were examined by light microscopy by a board certified veterinary pathologist. Histologic changes were described, when applicable, according to their distribution, severity, and morphologic character. Tissues from all animals were examined microscopically. The pathologist assigned diagnosis status to specific histologic lesions or groups of lesions. Diagnosis status was reserved for lesions that were treatment-related or nontreatment-related lesions that were judged significant to the health status of the animal in question. Special attention was directed to cardiac

and skeletal muscle tissues, known targets of monensin toxicity. Morphologic changes attributable to systemic and local effects of the high monensin dose were the basis for the whole animal diagnosis of toxicosis in this study.

Following completion of the primary tissue evaluation by the study pathologist, an independent peer review evaluation was conducted. The purpose of this peer review was a pathology data review and quality assessment of the study pathologist's evaluation of the histopathological findings. Attention was directed to the completeness, accuracy, and consistency of the original evaluation. The final pathology report is the consensus of the study and peer review pathologists.

STATISTICAL METHODS

Quantitative results for BW, BCS, average daily body temperature, average daily DM intake, average daily milk yield, milk composition, hematology, and clinical chemistry were analyzed statistically with mixed model methods based on restricted maximum likelihood estimation. Due to the various sampling schemes and time points included in the analyses for the different parameters, two different models were used. One model accommodated a repeated measures analysis and the other was used for analyses of a single time point. Both models included a pretreatment covariate (the average value of the data collected during the pretreatment period), parity, and treatment group as between subject fixed main-effect factors, while the repeated measures model additionally included the number of days or weeks after treatment initiation. All computations for the two models were conducted using PROC MIXED of SAS.

RESULTS

There were no treatment-related clinical signs of toxicity or mortality in the 0 and 1 mg•kg BW⁻¹•day⁻¹ treatment groups. In contrast, all eight cows in the 10 mg•kg BW⁻¹•day⁻¹ treatment group exhibited signs of monensin toxicity including severe anorexia, lethargy, diarrhea, sunken eyes, and a gaunt appearance. Average body temperature was increased in the 10 vs. 0 or 1 mg•kg BW⁻¹•day⁻¹ treatment groups (101.6 vs. 101.2, and 101.0 degrees, respectively; $P < 0.10$).

All eight cows on the 10 mg•kg BW⁻¹•day⁻¹ treatment were terminated early in the study because they met the anorexia criteria and were euthanized: two cows on Study Day 4, three cows on Study Day 5, two cows on Study Day 6, and one cow on Study Day 8. All cows in the 0 and 1 mg•kg BW⁻¹•day⁻¹ treatment groups continued through Study Day 14. Thus, production results will be presented for all three treatment groups only through Study Day 5, and clinical chemistry and hematology results through Study Day 3, the last day in which all 10 mg•kg BW⁻¹•day⁻¹ cows had blood collected. (Only one of eight cows on the 10 mg•kg BW⁻¹•day⁻¹ treatment was sampled on the scheduled Study Day 7 blood collection.)

Dry matter intake, NE_L intake, milk production, BW, and BCS results are presented in Table 36. There were no significant differences in DM and NE_L intake, milk production, BCS, and BW for cows in the 0 and 1 mg•kg BW⁻¹•day⁻¹ treatment groups for any of the time periods considered. Since all 10 mg•kg BW⁻¹•day⁻¹ cows were terminated from treatment prior to Study Day 14, results to this time point are not available for this treatment group. Milk production and intake of DM and NE_L for Study Days 1-5 were markedly reduced in the 10 vs. 0 and 1 mg•kg BW⁻¹•day⁻¹ treatment groups. Similarly, BCS and BW were reduced in the 10 vs. 0 and 1 mg•kg BW⁻¹•day⁻¹

treatment groups at study termination (Study Day 14 for 0 and 1 mg•kg BW⁻¹•day⁻¹ group; when euthanized for 10 mg•kg BW⁻¹•day⁻¹ group). At least some of these observations were likely related to anorexia noted in cows in the 10 mg•kg BW⁻¹•day⁻¹ treatment group.

Weights of kidneys, liver, and heart were reduced in the 10 vs. 0 and 1 mg•kg BW⁻¹•day⁻¹ treatment groups at a magnitude similar to the reduction in BW (approximately 15%). When organ weights were expressed on a g/kg of BW basis, no treatment-related differences were noted (data not shown).

Table 36. Effects of Monensin Treatment at 0, 1 or 10 mg•kg BW⁻¹•day⁻¹ on Dry matter (DM) Intake, Net Energy of Lactation (NE_L) Intake, Milk Production, Body Condition Score (BCS), and Body Weight (BW) in Lactating Dairy Cows.

Variable	Monensin (mg•kg BW ⁻¹ •day ⁻¹)		
	0	1	10
DM Intake (kg/day)			
Pretreatment period (2 weeks)	21.8	20.6	20.5
Average intake, Study Days 1-5	21.2	20.9	3.0 ^a
Average intake, Study Days 1-14	21.6	21.2	N/A ^b
NE_L Intake (Mcal/day)			
Pretreatment period (2 weeks)	38.5	36.4	36.2
Average intake, Study Days 1-5	37.2	36.7	5.3 ^a
Average intake, Study Days 1-14	38.0	37.3	N/A
Milk Yield (kg/day)			
Pretreatment period (2 weeks)	35.5	30.7	33.1
Average yield, Study Day 5	33.7	34.1	1.6 ^a
Average yield, Study Days 1-5	33.5	33.5	13.0 ^a
Average yield, Study Days 1-14	33.2	33.6	N/A
BCS			
Pretreatment	2.77	2.99	2.83
Study termination (Study Day 14 or euthanasia)	2.90	2.88	2.76 ^a
BW (kg)			
Pretreatment	545	554	544
Study Day 7 (or prior to euthanasia)	546	552	468 ^b
Study Day 14, termination of study	549	557	N/A

^aDifferent from 0 mg•kg BW⁻¹•day⁻¹ dose group (P < 0.10)

^bN/A = Not Applicable

Clinical chemistry and hematology results are presented in Table 37. There were no significant differences in clinical pathology measurements in cows in the 0 vs. 1 mg•kg BW⁻¹•day⁻¹ treatment groups. Changes of clinical pathology importance related to monensin treatment were limited to cows given 10 mg•kg BW⁻¹•day⁻¹.

Compound-related changes of clinical pathologic importance (all statistically significant) consisted of increases in enzymes of muscle origin (CPK, AST, and LDH); increases in parameters indicative of hemoconcentration (BUN, CREAT, TP, and ALB); increases in the sentinel proteins of inflammation (FBGN and GLOB); increases in FFA and T BILI; decreases in electrolytes (NA, K, and CL); and alterations in minerals (decreases and increases, respectively, in CA and IP). Also of clinical pathologic significance were: slight increases in the erythrogram parameters (RBC, HGB, and HCT) secondary to hemoconcentration, and slight to moderate decreases in the leukogram parameters (WBC, LYMS, NEUTS, MONOS, and EOS). All of the clinical pathological changes of importance are consistent with acute monensin toxicity. There were no differences among treatments in the urinalysis for myoglobin (data not shown).

Table 37. Effects of Monensin Treatment at 0, 1 or 10 mg•kg BW⁻¹•day⁻¹ on Clinical Signs of Toxicity on Study Day 3.

Parameter	Monensin (mg•kg BW ⁻¹ •day ⁻¹)		
	0	1	10
Blood Erythrogram parameters			
Erythrocyte Count (RBC), mil/μL	7.06	6.84	8.32 ^a
Hemoglobin (HGB), g/dL	11.1	10.9	13.1 ^a
Hematocrit (HCT), %	27.9	27.2	33.0 ^a
Serum Clinical Chemistries			
Blood Urea Nitrogen (BUN), mg/dL	14.32	14.12	19.70 ^a
Creatinine (CREAT), mg/dL	1.07	1.07	1.22 ^a
Albumin (ALB), g/dL	3.79	3.72	4.05 ^a
Total Proteins (TP), g/dL	7.88	7.91	8.90 ^a
Blood Leukogram Parameters			
Leukocyte Count, Total (WBC), thous/μL	8.40	9.20	5.49 ^a
Lymphocytes (LYMS), thous/μL	3.47	3.79	2.82 ^a
Neutrophils (NEUTS), thous/μL	3.67	3.88	1.79 ^a
Monocytes (MONOS), thous/μL	0.87	0.78	0.5 ^a
Eosinophils (EOS), thous/μL	0.17	0.14	0.04 ^a
Serum Enzymes			
Creatinine Phosphokinase (CPK), IU/L	174	192	672 ^a
Aspartate Transaminase (AST), IU/L	87	87	117 ^a
Lactate Dehydrogenase (LDH), IU/L	1086	1044	1261 ^a
Inflammation Proteins			
Plasma Fibrinogen (FBGN), mg/dL	204	226	443 ^a
Serum Globulin (GLOB), g/dL	4.09	4.19	4.86 ^a
Serum Electrolytes/Minerals			
Sodium (NA), mEq/L	141.3	140.1	136.9 ^a
Potassium (K), mEq/L	4.42	4.35	3.54 ^a
Chloride (CL), mEq/L	101.9	100.9	97.6 ^a
Calcium (CA), mg/dL	9.60	9.14	8.13 ^a
Inorganic Phosphorous (IP), mg/dL	6.76	6.54	7.92 ^a

^aDifferent from 0 mg•kg BW⁻¹•day⁻¹ dose group (P < 0.10)

Morphologic pathology changes of toxicologic importance were limited to the 10 mg•kg BW⁻¹•day⁻¹ group. At necropsy, treatment-related findings included pale areas on the heart from four cows; increased fluid in the pericardial sac of two cows; distended gallbladder in seven cows; and reddened rumenal mucosa of three cows. There were ingesta in the rumens of all these cows, but ingesta was semi-fluid in one cow. The four cows necropsied in each of the 0 and 1 mg•kg BW⁻¹•day⁻¹ treatment groups had no treatment-related gross pathological findings.

Microscopically, the most frequent striated muscle alterations occurred in the heart of all eight cows from the 10 mg•kg BW⁻¹•day⁻¹ group. Changes consisted of multifocal areas of myocardial degeneration (sarcoplasmic vacuolation with swelling and eosinophilia) and necrosis (sarcoplasmic hypereosinophilia and nuclear pyknosis, contraction bands, and fragmentation and/or lysis of myofibrils) with or without cellular infiltrates (lymphocytes, macrophages, and a few neutrophils). The cardiotoxicosis was moderate in the cow that survived to Study Day 8, with lesions in the atria, ventricles, and interventricular septum in decreasing order of involvement. In cows terminated earlier, cardiotoxicosis was slight in four cows and minimal in the other three cows.

Five cows in the 10 mg•kg BW⁻¹•day⁻¹ group had skeletal muscle lesions attributed to monensin toxicity. Toxic myopathy was graded minimal in three cows and slight in two cows. One cow experienced more muscles with toxic myodegeneration and/or necrosis than the other cows. Seven cows from the 10 mg•kg BW⁻¹•day⁻¹ group had necrotizing mucosal inflammation of the rumen, reticulum, and omasum which correlated with necropsy findings. This lesion was consistent with the local irritant effect of toxic doses of monensin in the rumen. The severity of mucosal inflammation decreased with time based on Study Day when euthanized. Rumenitis was moderate in the three cows examined on Study Days 4 and 5, slight in the four cows examined on Study Days 5 and 6, and not significant in the one cow examined on Study Day 8. The above effects were not present in cows in the 0 or 1 mg•kg BW⁻¹•day⁻¹ treatment groups.

In conclusion, the toxicity of monensin was limited to the 10 mg•kg BW⁻¹•day⁻¹ treatment group. The clinical signs and pathology were consistent with those observed in previous experimental studies and confirmed field cases of toxicosis in cattle. No signs of monensin toxicosis were noted for cows in the 0 and 1 mg•kg BW⁻¹•day⁻¹ treatment groups.

Animal Safety Information from 9-Herd Study:

See Section 2.b above for results from the 9-herd study on mastitis, somatic cell counts, animal health, drug therapy and reproductive performance.

4. HUMAN SAFETY:

This supplemental application is for the addition of a new class of animals (Dairy Cows) and for new indications related to the use of monensin in dairy cows. FDA determined that the supplemental application needed only to address the total residue depletion, metabolism studies, and comparative metabolism studies. All other human food safety information was derived from the original application and subsequent applications.

A. Toxicity:

In vivo toxicity studies were conducted to support the original approval for Rumensin® 80 Type A medicated article for beef cattle (NADA 095-735) and Coban (monensin) for poultry (NADA 38-878). These studies are covered in the Freedom of Information Summary that accompanied the original approvals for these applications. No additional toxicity studies were required for this approval.

Based on toxicological studies conducted in five species, and submitted in support of NADA 38-878, FDA determined that the most sensitive species was the dog. Based on these studies FDA determined a no observed adverse effect level of 1.25 mg/kg/day.

On February 3, 1999, FDA published an ADI for monensin of 12.5 micrograms per kilogram of body weight (as codified in 21 CFR 556.420). The established ADI will not change with this supplemental approval.

Safe concentrations of 1.5, 3.0, 4.5 and 6.0 ppm are established for muscle, liver, kidney and fat of cattle, respectively. FDA has established a safe concentration of 200 ppb for milk. The safe concentration was derived by setting aside 40% of the ADI, or 5 µg/kg/day, for milk. The safe concentration for milk (SCmilk) was calculated as follows:

$$\begin{aligned}\text{SCmilk} &= (5 \mu\text{g/kg/day} \times 60 \text{ kg}) \div 1.5 \text{ kg/day (consumption value for milk)} \\ &= 200 \mu\text{g/kg or 200 ppb}\end{aligned}$$

B. Total Residue Depletion and Metabolism Studies:

The levels of total drug-related residues of monensin in the edible tissues and milk of dairy cattle treated with [¹⁴C] monensin were determined in a tissue residue study conducted by Elanco Animal Health.

Investigator: Allison S. Kennington, Ph.D.

Dose: Animals were administered doses of 1.8 mg/kg body weight for 9 ½ days. Dosing was by gelatin capsules administered via rumen cannula twice daily at 12-hour intervals. This dose corresponds to 1.5X the proposed use level of 24-ppm dietary monensin.

Test Animals: Five Holstein cows in their second lactation (at the earliest) weighing between 510 and 625 kg.

Withdrawal Schedule: Practical zero withdrawal (6 hours)

Milk samples were collected from each of the animals twice daily at 12-hour intervals and radioassayed for total drug residues. Milk residues were found to reach steady state concentrations after approximately five days of dosing.

Six hours after the final dose, the animals were sacrificed and samples of liver, kidney, loin muscle, and subcutaneous fat were collected for radioassay for total drug-related residues. The results of the study are shown in **Table 38** and **Table 39**.

Table 38: Summary of the Mean Total Radioactive Residues (ppm) in the Primary Edible Tissues of [¹⁴C] Monensin-Treated Dairy Cows

Liver	Kidney	Muscle	Fat
1.28	0.07	NDR	0.02

NDR=No Detectable Residue

Table 39: Summary of the Mean Total Radioactive Residues in Milk (ppb) of [¹⁴C] Monensin-Treated Dairy Cows

Day / Time	Residue (ppb)	Day / Time	Residue (ppb)
Day 1 / a.m.	0.3	Day 5 / p.m.	48.8
Day 1 / p.m.	2.2	Day 6 / a.m.	45.9
Day 2 / a.m.	11.4	Day 6 / p.m.	47.4
Day 2 / p.m.	22.6	Day 7 / a.m.	45.7
Day 3 / a.m.	31.4	Day 7 / p.m.	50.6
Day 3 / p.m.	40.0	Day 8 / a.m.	43.5
Day 4 / a.m.	39.9	Day 8 / p.m.	48.1
Day 4 / p.m.	46.7	Day 9 / a.m.	41.7
Day 5 / a.m.	45.5	Day 9 / p.m.	44.5
		Day 10 / a.m.	43.8

The [¹⁴C] metabolite profile in liver was determined using solvent extraction with subsequent characterization by high performance liquid chromatography-mass spectrometry/liquid scintillation counting (HPLC-MS/LSC).

Approximately 75% of the total [¹⁴C] monensin residue present in liver was extractable into methanol/water (80/20) at a practical zero withdrawal. The extraction and fractionation of the radioactive residues in the liver indicated that monensin and three metabolites, O-demethylated monensin (M-1), O-demethylated monensin with an added site of hydroxylation (M-2), and decarboxylated monensin with oxidation of the –OCH₃ group to a ketone (M-6), were the main constituents of the total radioactive liver residue. M-6, monensin, M-2, and M-1 represent approximately 24%, 9%, 6%, and 6% of the total [¹⁴C] activity in the sample extract respectively. Fecal samples were also extracted and fractionated, and demonstrated the presence of the same three metabolites and parent monensin. Monensin, M-6, M-2, and M-1 represented 50%, 5%, 4% and 4% of dairy cow fecal extract respectively.

Fractionation of milk by two different methods that utilized one or more wet chemistry procedures (solvent extraction, liquid-liquid partitioning, large bore column chromatography, or HPLC-MS) demonstrated that monensin is present in the milk, but at extremely low levels (approximately 2% of the total radioactivity, or less than 1 ppb).

C. Comparative Metabolism Studies:

Donoho et al., (1978)¹ showed that the most abundant metabolites in liver fractions of steers dosed with monensin were M-6, M-1, monensin, and M-2. They estimated that these compounds represented 6%, 5%, 3%, and 1% of the [14C] radioactivity in liver, respectively, as compared with 24%, 9%, 6%, and 6% of M-6, monensin, M-2 and M-1, respectively, that were seen in the liver extracts of dairy cows. Fecal extracts from steers contained monensin, M-1, M-6, and M-2 at 50% 5%, 2%, and 2% of the total fecal radioactivity, respectively. This compares favorably with the percentages seen in dairy cow extracts of 50%, 5%, 4%, and 4% of monensin, M-6, M-1, and M-2, respectively. All the metabolites identified in dairy cow liver, milk, or fecal extracts were also identified in chicken liver extracts (Donoho et al., 1982)², and rat fecal samples by Donoho et al., (1978)¹.

¹ Donoho, A., Manthey, J., Occolowitz, J., Zornes, L. *J Agrc. Food Chem.* **26**, 1090-1095, (1978).

² Donoho, A.L.; Herberg, R.J.; Zornes, L.L.; Van Duyn, R.L. *J Agrc. Food Chem.* **30**, 909, (1982).

D. Withdrawal Period and Milk Discard Time, Milk Tolerance, and Analytical Methods:

Monensin is already approved for use in various classes of cattle, excluding lactating dairy cows, with a zero withdrawal period. The data in Table 38 confirm the applicability of the zero withdrawal period for lactating cows.

The data summarized above show that the total residue of monensin in milk from cows treated at 1.5x the intended dose peaked at approximately 50 ppb, which is well below the safe concentration of 200 ppb for milk. These data support the assignment of a zero milk discard. Furthermore, the metabolism data demonstrate that monensin is extensively metabolized, making it impractical to develop a regulatory method for monensin in milk. FDA, therefore, has waived the need for the requirement of a regulatory method.

Although the requirement for a regulatory method has been waived, the determinative HPLC method, which was used in support of the original monensin sodium applications, is available from the Center for Veterinary Medicine.

The results of other analytical testing, specifically antibiotic screening assays, also were considered by FDA. Milk samples were collected from cows given 1 or 10 mg of monensin/kg body weight and subjected to antibiotic milk screen used by the milk industry for detection of antibiotic presence in milk. The screens were the Delvo Test P (Gist Brokades) and the Bacillus stearothermophilus Tablet Disk Assay for Penicillin G. Amoxicillin, Ampicillin and Cephapirin (Charm Sciences, Inc.). Milk from the 1 mg treatment group was assayed at 1, 7, and 14 days after initiation of treatment, and none of the samples tested positive. Milk from all the cows on the 10 mg/kg body weight were assayed on SD 1 and were found negative for both the Delvo and Charm tests. Milk from the one cow alive on SD 7 in the 10 mg/kg body weight group also tested negative for both tests. Residues of monensin in milk should not cause positives in the Delvo P and Charm Tests when monensin is used properly in lactating cows. These results supported FDA's decision to waive the need for a regulatory method for monensin in milk.

E. User Safety Concerns:

User safety concerns associated with the effects of accidental inhalation or direct contact have been satisfactorily addressed by establishing label warnings. The bags of Type A medicated article, Type B medicated feed, and Type C medicated feed contain the following warning:

When mixing and handling Rumensin® 80, use protective clothing, impervious gloves and a dust mask. Operators should wash thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse with water.

5. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that monensin sodium fed to dairy cows (11 to 22 g/ton of total mixed ration dry matter in lactating and dry cow rations) is safe and effective for the claims indicated in section 1 of this FOI Summary.

Under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the date of the approval. The three years of marketing exclusivity applies only to the use of the product (RUMENSIN 80) (11 to 22 g/ton of total mixed ration dry matter in lactating and dry cow rations) in dairy cows for increased milk production efficiency (production of marketable solids-corrected milk per unit of feed intake) for which this supplement is approved.

Pursuant to 21 CFR 514.106 (b)(2)(vi), this supplemental NADA approval is regarded as a Category II supplemental change which required a reevaluation of safety and efficacy data in the parent NADA.

The drug is to be fed in Type C medicated feeds in accordance with section 1 of the FOI Summary and the Blue Bird labeling that is attached to this document.

The Center for Veterinary Medicine has concluded that, for this product, adequate directions for use by the layperson have been provided and the product will have over-the-counter (OTC) status. Label directions provide detailed instruction in plain language. The drug product is not a controlled substance. Thus, the drug product is assigned OTC status, and the labeling is adequate for the intended use.

No patent information was submitted by the sponsor with this application.

6. ATTACHMENTS:

Facsimile Labeling is attached as indicated below:

RUMENSIN 80 Type A Medicated Article Label
Monensin Type B Dry Dairy Cattle Medicated Feed Label
Monensin Type B Liquid Dairy Cattle Medicated Feed Label
Monensin Type C Dry Dairy Cattle Medicated Feed Label