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BEHAVIOR AND MILK YIELD RESPONSES OF DAIRY CATTLE TO SIMULATED JET AIRCRAFT NOISE

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Objectives were to evaluate the effects of simulated jet aircraft noise on behavior, milk production and composition, and residual milk of lactating dairy cows and release of Prolactin (Prl) and Cortisol (Gc) in response to the milking stimuli. Thirty-six lactating Holstein dairy cows were assigned to experiment when between 79 and 155 days in milk (DIM). Experiment was an incomplete block design with three treatments. Cows were assigned to receive two of the three treatments during consecutive 21-d periods, each of which was preceded by 5-7 d adjustment periods. Treatments were 1) control (no noise), 2) simulated jet noise (onset 107.7 dB/sec; Max dBA 113.6), and 3) simulated jet noise (onset 33.5 dB/sec; Max dBA 113.3). Cows were exposed to noise on 10-12 d/period with a frequency of 1-4 times/d. Milk yields, milk composition and residual milk were measured throughout the experiment. Dairy cows showed no signs of startle, freeze or retreat from noise at any time during the experiment and were not more agitated or aggressive during subsequent milking. There was no evidence to indicate failure to detect behavioral response occurred because cows acclimated to noise during the 21-d period. Milk yield, 3.5 % fat-corrected milk (FCM) yield, milk component percentages and residual milk were not affected significantly by exposure to simulated jet noise. Release of Prl and Gc at the time of milking in 12 of the cows (4 cows/treatment) did not differ due to exposure to noise. Effects on the different measures that were detected due to period, time of milking and other interactions were associated with advancing stage of lactation or management practices. Although there was no evidence that the cows became conditioned to the specific noise treatments evaluated, dairy cows may be acclimated to various noises associated with general dairy farm practices and the milking procedure which would affect their response to infrequent noises.

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1. INTRODUCTION

Many environmental factors and management practices can be considered as stressors which provoke physiological changes in domestic dairy cattle during growth and lactation. Physiological adjustments to environmental and management stressors have been used to characterize and/or identify the well-being of domestic animals. Measures often used to assess stress responses of animals include body temperature, heart rate, respiration rate, feed intake and eating pattern, digestibility of feedstuffs, weight loss or gain, immune function, secretion and peripheral concentrations of various hormones in plasma, milk yield, milk composition and quality, udder health, pregnancy status, and viability of offspring. Measure of several of these simultaneously permits critical evaluation of effects of stress on animal behavior and productivity.

Possible negative effects of noise on animal behavior and productivity has received attention during the past 50 years. Behavioral responses of mammals to noise greater than 90 dB likely will involve retreat from sound, freezing or a strong startle response (see review of Manci et al., 1988; Ames and Arehart, 1972). These aversive behaviors are less likely to occur where sound levels are less than 90 dB, but variation among animals within species is likely. Perhaps the most important behavioral response to unexpected noise, such as would occur by aircraft overflights, is startle. Responses may be expected to vary with noise type and levels and frequency of noise events, since domestic animals appear to acclimate to some sound disturbances. For dairy cows, noise of an exploding paper bag caused temporary cessation of milk production (Ely and Petersen, 1941), whereas general noise (105 dB) caused reduction in feed consumption, milk yield and rate of milk release by dairy cows (Kovalcik and Sottnik, 1971), but not when noise was at 80 dB. Exposure of 5 goats to jet noise caused a reduction in their milk yield (Sugawara et al., 1979). The intermittent exposure to noise, including jet noise, may have had a greater effect than continuous noise. Concentrations of metabolites (glucose and nonesterified fatty acids) were increased by tractor noise (97 dB; Broucek et al., 1983) and general noise (110 dB; Broucek et al., 1983).

Effects of jet aircraft noise and flyovers on milk production by dairy cows in herds located near existing air bases were evaluated by Parker and Bayley (1960). They found no evidence that proximity of dairy herd (3 miles) to an airbase or overflights by jet aircraft affected the milk production by the cows on these dairies. It was suggested that previous exposure to flyovers and/or sonic booms may have reduced startle impact from these overflights (Casady and Lehmann, 1974). Bond et al. (1974) found no evidence that sonic booms affected eating pattern or total feed intake of dairy cows. Reduction in food intake would be expected to be manifested by a corresponding reduction in milk yields.

Some physiological responses of lambs to sound were characteristic of those of the stress response (e.g., adrenal responses and acclimation to sound environment; Ames and Arehart, 1972). Sudden changes that startle domestic dairy cattle and cause tachycardia or bradycardia, through actions of catecholamines or the nervous system, could cause reduced milk production if they occurred around the time of milking. The milking process typically brings about multiple responses leading to release of oxytocin and the lactogenic hormones Prolactin and Glucocorticoids (Prl, Gc). This ensures efficient milk removal and maintenance of ongoing lactation (Blum et al., 1989; Mepham, 1987; Schams et al., 1984; Tucker, 1974, 1987). Startle response to loud and unexpected noise, such as jet aircraft overflights (> 110 dB), could reduce release of oxytocin, decrease efficiency of milk ejection and increase the residual milk and lead to overall reduction in milk production. Responses such as behavioral changes, amount of milk yield and percentage of residual milk following milking, or hormone release in response to milking would be good indicators of physiological response to noise that occurs around the time of milking.

Objectives of the experiment were to evaluate the effects of simulated jet aircraft noise on dairy cattle behavior, milk letdown, milk yield and composition and milking induced release of Prl and Gc around the time of milking. Two pre-recorded noise samples were used. These represented low-flying jet aircraft and the noise was generated via a loud-speaker cluster projected above the animals to simulate flyover conditions. The two chosen represented rapid (107.7 dB/sec) or more delayed onset (33.5 dB/sec) of noise with maximum A-weighted sound level of 113.6 and 113.3 dBA respectively for the two jet noise sources.

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2. MATERIALS AND METHODS

2.1 ANIMALS

Thirty-six Holstein dairy cows were assigned to experiment when between 79 and 155 d days in milk (DIM). One cow was removed from experiment after completing one period because she was ill; she was replaced. All cows were handled and managed as a single group. They were housed and fed in a free-stall barn equipped with electronic feeding gates (Calan Electronics, Northwood, NH), which were positioned open throughout the experiment. The free-stall barn was a covered building (shaded) equipped with fans; feed bunks and water were located under the roof of the free-stall barn.

All cows were fed twice daily at approximately 0800 h and 1500 h. Feed was offered to ensure at least a 5-10 % weighback each day, but individual cow feed intakes were not measured. Composition and analysis of the corn-based total mixed ration (TMR) fed is in Table 1.

2.2 DESIGN AND TREATMENTS

The experiment was an incomplete block design with three treatments. Each cow received three treatments (noise exposure conditions) during the experiment. The conditions were: 1) control, no simulated noise, ambient only; 2) simulated jet noise, onset rate 107.7 dB/sec, Max dBA 113.6; and 3) simulated jet noise, onset rate 33.5 dB/sec, Max dBA 113.3. The noise samples selected represent close to the worst case noise exposure conditions found in the field. Further description of the selected low-level aircraft flyover signals used are in Table 2. The physical layout of the observation area (holding area) where the cows were exposed to noise before entering the milking parlor is shown in Figure 1. The holding area was subdivided acoustically into two areas; each area represents a constant

Table 1. Composition and analysis of total mixed ration (TMR) fed to lactating Holstein dairy cows.[a]

Feedstuff	As Fed (Kg)	Percentage
Corn silage	27.70	62.05
Corn (ground)	5.30	11.87
Whole cottonseed	2.00	4.48
Distillers grain	3.15	7.06
Soybean meal	2.75	6.72
Alfalfa hay	3.00	6.16
Mineral premix	0.33	.74
Sodium bicarbonate	0.23	.52
Trace mineral salt	0.08	.18
Dicalcium phosphate	0.04	.09
Magnesium oxide	0.04	.09
Ammonium sulphate	0.02	.04
	44.64	100.00

Analysis	Actual	DM Basis
Moisture	42.4	---
Dry matter	57.6	---
Crude protein (%)	10.0	17.4
Available protein (%)	8.9	15.4
Unavailable protein (%)	1.1	2.0
Acid detergent fiber (%)	12.9	22.3
Total digestible nutrients (%)	44.0	76.0
NELactation (Mcal/kg)	.90	1.5

[a] Feed analyses done at the Forage Testing Laboratory, Northeast DHIA, Ithaca, NY.

Table 2. Summary of selected low level aircraft flyover signals for treatments.[1]

Treatment	Program Tape ID#	Aircraft Type	ALT (Ft)	Offset (Ft)	Speed (KTS)	Onset dB/sec	Area[c]	Descriptors (A-Weighted)[b] Leq[d]	Max[e]	SEL[f]
1 (control)	0	0	0	0	0	0	1	0	0	0
							2	0	0	0
2	10	F-4D	114	50	597	107.7	1	106.0	115.6	109.4
							2	102.0	109.6	105.4
3	12	B-1B	414	27	582	33.5	1	104.0	115.3	111.7
							2	100.0	109.3	107.7

[a] Data from Chavez et al., 1990.

[b] Noise level range in area (dBA) ± 2.00.

[c] See Figure 1 for location of areas.

[d] Leq or equivalent sound level is the steady A-weighted sound level which produces the same A-weighted sound energy over a stated period of time as a specified time varying sound. In this case, the time period starts when the aircraft noise exceeds a level of 70 dB and ends when the level falls below 70 dB.

[e] Max is the maximum root means squared dB level achieved during the flyover.

[f] SEL or Sound Exposure Level is the level in decibels of the time integral, relative to one second of the sound level.

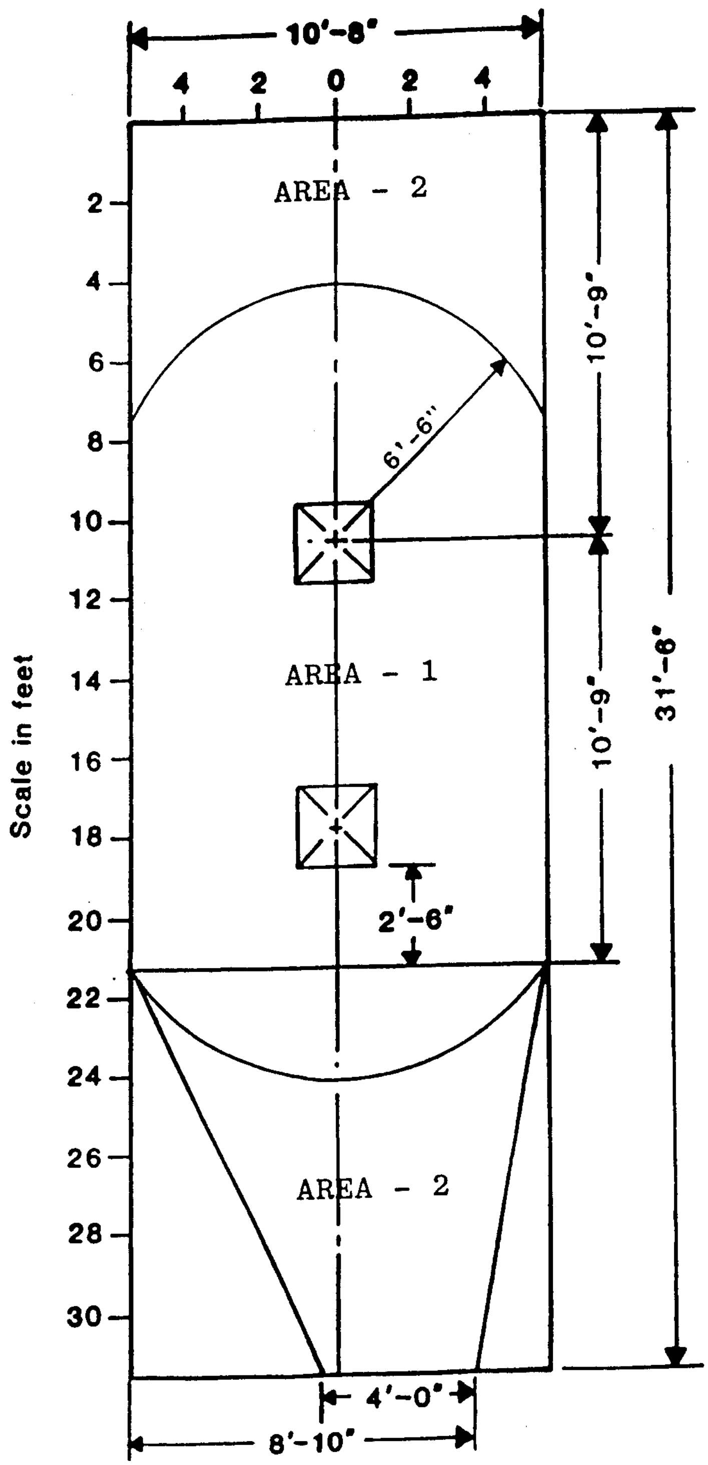

Figure 1. Observation Area Noise Sub-Area Map

noise exposure range. Thus, cows could stand anywhere within the holding area at the time of the aircraft noise simulations. Major difference in the two noise samples was the type of aircraft and the rate of onset rate (dB/sec) of the two treatments selected, since the maximum A-weighted sound level was essentially constant (Leq, Max dBA and SEL; Table 2).

Treatment periods were 21 d in length with a 5-7 d adjustment period preceding beginning of each period. Cows were assigned to treatment groups and blocked according to parity. Assignment of the 36 cows to a treatment sequence for the two periods is in Table 3.

Table 3. Assignment of lactating dairy cows to treatment and sequence of treatments during 21-d treatment periods.

Cow[a]	Treatment		
	Control[b](1)	Program[c] 10 (2)	Program 12[c] (3)
1,7,13,19,25,31	P_1[b]	P_2	
2,8,14,20,26,32		P_1	P_2
3,9,15,21,27,33	P_1		P_2
4,10,16,22,28,34	P_2	P_1	
5,11,17,23,29,35		P_2	P_1
6,12,18,24,30,36	P_2		P_1

[a] Cows were assigned in order 1 through 6, then assignment to treatments and sequence was repeated for groups of 6 cows until all 36 cows were assigned.

[b] P_1 = Period 1; P_2 = Period 2: Order of P_1, P_2 for cows described which treatment in a two-period sequence was given first.

[c] Program descriptions in Table 2.

Cows were exposed to appropriate simulated jet aircraft noise (program 10 or 12 ; Table 2) on a total of 10-12 d during a 21-d treatment period. Treatment noise exposures on a given day ranged from 1 to 4 times at either the morning (0500 h) or afternoon (1600 h) milking. On several days within the 21-d treatment period, exposure to noise event(s) occurred at both the AM and PM milkings. A typical pattern of aircraft noise exposures throughout the 21-d period is in Table 4. When there was more than a single noise exposure on the same day at a milking, the time lapse between exposure events on that day ranged from 30 sec to 10 min. This varied from day to day and for the two noise treatments (treatments 2 or 3). The presentation of noise events on a given day and across days was recorded on a data sheet and varied for cows within a 21-d treatment period in order to minimize the chance that cows would become accustomed to the treatment. This also better simulated the random exposure to sudden and unexpected jet aircraft flyovers that would occur under non-experimental conditions. Time intervals between multiple noise events on the same day were limited to 10 min so that cows would not be kept in the milking parlor holding area where noise events occurred (Figure 1) longer than typically would occur under non-experimental conditions. Cows were moved into the milking parlor and milking began within 1-3 min after the last noise event at that milking.

On each day, around the time the cows were exposed to simulated jet aircraft noise events, they were videotaped using an 8 mm Camcorder. This was done as carefully as possible to avoid conditioning the animal to this activity. Video records were accumulated and transferred to VCR tape along with verbal description of treatment and any response by the cows to the noise events.

Twelve cows assigned to experiment (2 cows/treatment/period) were used to evaluate the release of Prl and Gc in response to the

Table 4. Example of noise exposures, milk sampling, and residual milk measures during a treatment period.[a]

Day of Period[b]	Noise Events (#/d, AM or PM)[c]	Milk Samples (AM and/or PM)	Residual Milk Measured	Serial Blood Collections[d]
-2	0	AM - PM		
-1	0	AM - PM		
0	0	-	AM - PM	PM
1		1 P		
2	0	-		
3	2 P	-		
4	0	-		
5	3 P	-		
6	0	AM - PM		
7	0	AM - PM		
8	1 P, 2 A	-	AM - PM	
9	2 P	-		
10	0	-		
11	0	-		
12	4 P	AM - PM		
13	0	AM - PM		
14	3 P	-	AM - PM	PM
15	0	-		
16	0	-		
17	2 P, 1 A	-		
18	0	-		
19	3 P	AM - PM		
20	0	AM - PM		
21	2 P	AM - PM	AM - PM	

[a] Numbers of noise events per day and days on which cows were exposed to noise events varied. Cows were exposed to 0 to 4 noise events on 10 to 12 days during each of two treatment periods (program 10 or 12).

[b] Days 0, -1, -2 represent days during the adjustment period.

[c] A = AM ($\simeq$ 5:00 AM); P = PM ($\simeq$ 4:00 PM).

[d] Days of blood collection for 12 of the experimental cows to evaluate release of Prolactin and Glucocorticoid (Cortisol) in response to milking.

milking stimulus. This was done on d-0 (last day of the adjustment period) and again at d-14 of the period. Blood samples were collected from the cows at the time of the afternoon (PM) milking on these days (Table 4).

2.3 DATA COLLECTION

2.3.1 Milk Yields

Cows were milked in a double 8 Herringbone milking parlor equipped with 16 DeLaval milking units equipped with automatic cow identification and milk recording systems, automatic take-off units, and milk samplers. The milking parlor was connected directly to the holding area where noise events occurred just before the cows entered the milking parlor (Figure 1).

Twice daily milk yields were obtained throughout the adjustment and treatment periods. All milkings were done using the automatic take-off feature. This standardized the milking procedure and ensured that any delays in milk flow (milk ejection) would not be masked by the milker delaying removal of the milking machine if the cows milked out slower and to eliminate the possibility the milker would machine strip cows that did not let-down all their milk.

Collection of milk samples was at both the AM and PM milkings on two consecutive days in the adjustment and treatment periods (Table 4). Samples were collected using the automatic sampler devices, then they were well-mixed and transferred to one pint stoppered bottles and refrigerated. Measurement of residual milk during the experimental period was accomplished using injections of oxytocin. After the milking machine had automatically detached, at the end of milking, the cows were injected with 10-15 units of oxytocin (.5-.8 ml of solution, 20 U/ml), and after a 2-3 min wait, the milking machines were reattached to the cows. Milk obtained after oxytocin injection was measured and the residual milk was expressed as a percentage of the total milk yield at that milking [e.g. (lb residual milk/lb residual milk + lb milk) * 100 = percentage residual milk]. Collection of milk samples and measurement of residual milk most often was on days when noise events also occurred (Table 4).

2.4 CATHETERIZATION OF COWS AND COLLECTION OF BLOOD SAMPLES

The 12 cows were catheterized between 0700 and 0800 h on the day the blood samples were collected. Catheters were inserted in the jugular vein and retained between two layers of Elasticon tape (Johnson and Johnson) which was glued to the neck of the cow. Heparin solution (100 units/ml sterile saline) was used to keep the catheter free of blood clots. Just before the cows were taken to the holding area to prepare for milking the catheters were removed from between the tape, an 18 gauge needle was inserted in the end of the catheter to permit attachment of a luer-lok syringe and then catheters were flushed with a heparin solution (20 units/ml sterile saline). The needle then was closed with a luer-lok cap. Cows were moved to the holding area and blood samples (8 ml) were collected from the catheter at - 45, -30, -15 and 0 min relative to the time of the noise event. Heparin solution (20 units) was used throughout to keep the catheter open. Exposure to the noise events and the milking procedure were as for all other experimental cows. Once cows were in the milking parlor, samples of blood were collected at T_0 and at 1 min intervals for 10 min and at 15, 20, 25 and 30 min relative to the first sample (T_0) in the parlor. Immediately after collection blood samples were transferred into 15 ml polypropylene centrifuge tubes containing 200 units of heparin, mixed and stored on ice.

2.5 CHEMICAL ANALYSES

2.5.1 Milk

Milk samples were analyzed for content of milkfat by the Babcock method (AOAC, 1975). To measure the total solids content of milk (sum of lactose, protein, minerals, and milkfat), a well-mixed sample of milk was pipetted into tared aluminum pans (8-12 ml), weighed, dried in an oven at 50-70 °C for 16 to 24 h, then allowed to cool to room temperature, reweighed and residual dry matter obtained by difference. The solids-not-fat content of milk samples was the difference between total solids and milkfat content for individual samples. Milkfat was a single analysis, but total solids were done in duplicate with average total solids content used to calculate the solids-not-fat content for individual samples.

2.5.2 Blood Samples

Blood samples were centrifuged at 2500 x G for 30 min at 4°C and plasma decanted into duplicate polyproplene storage vials, capped and frozen (-20 C) until analyzed. Each sample was analyzed for concentrations of Prl and Gc by specific radioimmunoassays (Elvinger et al., 1991; Zoa-Mboe et al., 1989). Concentrations of hormones in plasma were expressed as ng/ml. All samples for an individual cow were assayed in a single assay to avoid interassay variation. Intraassay coefficients of variation for the two procedures were less than 10 %.

2.6 AMBIENT NOISE MEASUREMENTS

2.6.1 Cow Management Areas

The ambient noise levels in the milking parlor holding area, milking parlor and the free-stall barn were measured. Measurements were made with a Larson.Davis Sound Level Meter, Noise Dosimeter (Model 710) over a time period of 10 d when no simulated jet noise events also were occurring. The instrument was calibrated using a Precision Acoustic Calibrator (Larson.Davis; Model CA 250). The dosimeter used had a full 110 dB dynamic range with A-weighting frequency response. Measurement times in the three areas ranged from 45 min to 24 h and were replicated two to six times in each area.

2.6.2 FARM MACHINERY

Ambient noise is defined as all noise, near and far, other than the signal being studied; in this case, aircraft noise. In that sense, machinery, other than aircraft are included in the ambient noise. In order that the noise could be accurately described, various machines that cows were exposed to on the farm were measured. Noise of tractors, feed buggies, and front-end payloaders were measured at three distances from the machine (1, 3 and 10 meters) either outside or in the free-stall barn. Measurements were made so as to represent typical exposures cows would receive. All of these measures were at about 1.6 meters off the ground, approximately cow head height, and were replicated at least two times on separate days.

2.7 STATISTICAL ANALYSIS

2.7.1 Milk and Components

A series of statistical analyses was conducted to evaluate effects of treatment, period, time (AM or PM milking), day of period, and appropriate two and three-way interactions on milk yield, constituent percentages and yield and residual milk. The General Linear Model of SAS (1987) least squares analysis of variance was utilized. Regression analyses were performed to determine within-animal, within-treatment effects on measures of interest. Tests for significant linear, quadratic and cubic regressions were made. Highest order significant responses were tested for differences for individual measures. In addition, analyses were done to calculate three regression coefficients (a,b, and c) representing: a, the intercept (the milk yield at the beginning of the treatment period); b, the slope of the rise to peak production; and c, the slope of milk yield during the declining portion of the period. Arithmetic and least squares means of production measures were obtained from statistical analyses and means also were calculated using the a,b, and c coefficients. Mathematical models utilized for individual analyses are in the Results and Discussion section.

2.7.2 Plasma Hormones

A series of least squares analyses was conducted to evaluate the effects of cow, treatment, period, day of collection in the period, appropriate interactions and time (min). The General Linear Model of SAS (1987) was used. Regression analyses were performed to determine the within- animal effects of interest. Quartic regressions (the highest order significant) were used to describe the time trends of hormone response. Mathematical models utilized are in the Results and Discussion section.

3. RESULTS AND DISCUSSION

3.1 ANIMAL BEHAVIOR

Cows were exposed to simulated jet aircraft overflight noise events on 10-12 d during the 21 d of each of the two periods. Single and multiple events on the same day were recorded (1-4 events/d). There were no signs of startle, freeze or retreat by cows when they were exposed to either of the simulated jet noise treatments. Slight activity or movement by cows in the holding area before and after noise event(s) was typical of that of the control cows or nonexperimental cows when they were standing in the holding area prior to going into the herringbone milking parlor. Expected movements included changing location in the holding area, bumping adjacent cows, or infrequent head throwing in response to presence of flies. Similarly, there were no outward indications observed or recorded that cows were more agitated or aggressive during the 1-2 min after noise events while they were still in the holding area before they entered the milking parlor or during the time they were in the milking parlor. Lack of observed behavioral effects were equally true for the first and subsequent exposures on an individual day, as well as throughout the 21-d treatment period. Thus, there was no indication that cows responded differently later in the period because they had acclimated to repeated exposures to noise.

In the course of a normal day, dairy cows on large dairies are exposed to a wide variety of noises associated with general farm operation and the milking operation. These exposures include noises from farm tractors and machinery to deliver feed, trucks and maintenance and repair operations, as well as noises in the milking area from opening and closing access and confinement gates, vacuum pumps, and personnel in the holding area and milking parlor. The exposures of lactating dairy cows to noise in our experiment probably is typical of that on other large and medium sized dairies. The noise levels recorded and their distribution functions in the free-stall barn and in the holding and milking areas are in Tables 5 and 6 respectively. As shown, cows on the dairy farm were exposed to these noises daily in the free-stall barn and at times they were moved to the milking area. In addition to the noise in the feeding and holding areas, the cows were exposed intermittently to the noise of tractors, feed buggies and the payloaders used to clean the free-stall barn. A summary of results of such measures for these farm machines is in Table 7. Generally, these machines would pass within 1-10 meters of the cow. Although the Max dBA of noise in these areas was up to 90 dBA, it was significantly less than the simulated aircraft overflights by 23-29 dB. Unlike the jet noise, the onset of the noise from these sources are much slower and occur frequently during the day. Perhaps daily exposure to noise in the buildings and from farm machinery would minimize the outward response to unexpected noises such as that of jet aircraft overflights. Domestic animals appear to acclimate to some sound disturbances. Nonetheless, sound levels above 90 dB have been reported by other experimentors to be aversive with behaviors such as retreat from sound, freezing or startle a strong possibility. This type of behavior was not observed when cows were exposed to similar sound levels in the feeding and milking areas (Table 5). Trampling, moving, raising head, stampeding, jumping or running also may occur in response to sonic booms (Bell, 1972) with similar responses likely in response to low altitude subsonic airplane overflights, helicopters and sudden noises. Failure to detect effects of aircraft overflights on milk yields of dairy cows in a multiherd field study for herds located near air bases (Parker and Bayley (1960) may have resulted because of prior exposure to 4-8 sonic booms/day prior to collection of data (Casady and Lehmann, 1974). Kovalcik and Sottnik (1971) reported that noises at 105 dB caused reduced milk yield and feed consumption, but not if animals were exposed gradually to this noise level. Because the aircraft noise exposures were in the holding area and the

Table 5. Background noise levels for normal animal activity

Area	Sound Level Range dBA[a]	Leq
Free-stall barn[b]		
without machinery noise	51-79	62
with machinery noise[c]	59-92	73
Holding area[d]	63-84	68
Milking parlor[e]	67-90	73

[a] One second integration.

[b] Free-stall barn - cows were housed and fed here during time between milkings.

[c] Cleaning/feeding activity lasted approximately 2 hrs/d.

[d] Holding area - adjacent to milking parlor; noise events occurred here. Cows generally held here for 10-30 min preceding milking.

[e] Milking parlor - cows milked here; generally in parlor for 10-12 min, but milking lasted 4-8 min.

noise could not be heard above the noise typical of general dairy farm activities in the free-stall barn where cows were housed, they could not become acclimated in this way.

Since cows were managed as treatment groups, no other measures of response were attempted in the holding area where noise events occurred. This was done in order to eliminate, insofar as possible, other stresses and acclimation to treatment. Although

Table 6. Distribution function of ambient noise levels for dairy cow areas.

Level dBA	Free-stall Barn	Holding Area	Milking Parlor
	Percentages		
50	.00		
51	.01		
52	.04		
53	.24		
54	.60		
55	1.18		
56	2.01		
57	3.05		
58	4.03		
59	4.75		
60	5.99		
61	7.00		
62	7.20	.00	
63	5.81	.43	
64	4.65	13.57	
65	3.74	28.76	
66	4.67	25.87	
67	7.20	13.52	
68	7.71	5.97	
69	5.67	3.50	.00
70	4.11	2.13	.81
71	3.21	1.54	32.64
72	3.01	1.26	33.28
73	3.21	.96	16.22
74	2.40	.71	11.04
75	1.99	.63	2.55
76	2.36	.45	1.04
77	1.30	.29	.46
78	.57	.13	.65
79	.45	.09	.29
80	.44	.05	.22
81	.46	.05	.16
82	.41	.05	.15
83	.23	.02	.12
84	.19	.01	.12
85	.04	.00	.10
86	.01		.07
87	.01		.03
88	.01		.03
89	.01		.02
90	.00		.00

Table 7. Typical A-weighted sound levels for noise produced by several types of farm machines measured at three distances from dairy cows.[a]

Machine[b]	Distance (m)	Leq	Max (dBA)
(Diesel powered)			
Tractor	1	95.8	96.9
	3	89.1	89.9
	10	82.5	84.4
Front-end Loader	1	95.3	97.4
	3	84.3	82.4
	10	70.8	75.9
Articulated Payloader	1	94.8	95.4
	3	86.8	87.4
	10	76.9	77.4
(Gasoline powered) Data Ranger Feeder	1	95.8	96.8
	3	89.1	89.9
	10	82.5	84.4

[a] All measures were at approximately 1.6 M above ground level; about cows' head level.

[b] Tractor = Ford 7700
Frontend Loader = Case Uniloader
Articulated Payloader = Allis Chalmers, Model 645M
Data Ranger Feeder = Self-propelled Data Ranger

no outward signs of stress or discomfort occurred with exposure to noise, it still was possible that some physiological responses (e.g., changes in respiratory rate, heart rate or hormonal secretions) occurred, but would not be assessed by visual observations.

Sudden or unfamiliar noise is believed to act as an alarm which activates the sympathetic nervous system and a short-term physiological stress reaction defined as the fight-or-flight response. A general pattern of response to stress would include activation of neural and endocrine systems causing changes such as increases in blood pressure, respiration rate, blood flow to various organs, amount of available glucose and blood concentrations of glucocorticoids. These measures of physiological response to sudden noise were not assessed but a consequence of activation of neural and endocrine responses, if it had occurred in the absence behavioral response could have important negative effects on milk production.

An important sequence of events provoked by presence of cows in the milking parlor, by preparation for milking and the attachment of the milking machines and the milking process itself is the secretion of oxytocin and lactogenic hormones. Oxytocin is important for the let-down of milk and the lactogenic hormones, Prl and Gc for the maintenance of cell numbers and their synthetic activity (Mepham, 1987, Schams et al.,1984; and Tucker, 1974, 1987). Failure to release adequate quantities of these hormones at the time of milking could result in decreased milk yields and/or greater percentage of residual milk. Indeed, an exploding paper bag at the time of milking unexpectedly resulted in short-term (< 1 hour) reduction in milk let-down, although total milk production was unaffected (Ely and Petersen, 1941). Therefore, effects of the transient and intermittent noise produced by simulated jet aircraft overflights on milk let-down, release of Prl and Gc to milking stimulus and total productivity also were assessed and possible effects on milk component percentages were evaluated.

3.2 MILK YIELD AND CONSTITUENTS

3.2.1 Milk Yield

A series of least squares analyses of variance evaluated effects of treatment. Included in the mathematical models utilized were treatment, period, cow, two and three-way interactions, time of milking (AM or PM), two, three and four-way interactions and day included as either a continuous or class variable. Least squares mean milk yields per milking for cows on the three treatments are in Table 8 and summary of a model used to evaluate effects is in Table 9. Analyses utilized 3954 milk yield measures (AM + PM) for the 36 cows assigned to experiment. Effects of time of milking on milk yield were considered in the model since noise events most often were limited to either the AM or PM milking. Using treatment by time interaction allowed us to evaluate association of noise event with milk yield.

Table 8. **Least squares mean yields of milk (lb/milking)[a].**

Source of Variation	Pounds of milk/milking	SEM[b]
Treatment (T)		
1	28.86	.24
2	29.16	.24
3	30.30	.24
Period		
1	30.75	.18
2	28.12	.18
Time		
1 (AM)	33.16	.18
(PM)	25.71	.18
T*Time		
1 1	32.07	.34
2 1	32.97	.34
3 1	34.46	.34
1 2	25.65	.34
2 2	25.36	.34
3 2	26.14	.34

[a] Model included day as a continuous variable (cubic, see Table 6).

[b] Standard error of mean.

Table 9.   **Least squares analysis of variance for milk yields (lb/milking).**

Source	df	MS	Probability
Treatment (T)	2	433.2519	> .18
Cow	36	3918.5791	< .0001
Period (Per)	1	5080.1060	< .0001
T*Per*Cow[a]	32	243.3886	< .0001
Time	1	41506.5804	< .0001
T*Time	2	170.0353	> .12
Cow*Time	36	201.8190	< .003
Per*Time	1	625.4030	< .008
T*Per*Cow*Time[b]	32	76.8330	< .007
Day	1	158.5048	< .06
Day*Day	1	151.1799	< .07
Day*Day*Day	1	119.5782	> .10
Residual	2877	44.5029	---

Model $R^2 = .6167$

[a] Error term for sources above it.

[b] Error term for the 4 sources above it.

No significant effects of treatment on mean yields of milk were detected. In fact, mean yields were numerically, but not significantly, greater for the two noise treatments (Table 8). Although no effects on mean milk yields were detected, it still was possible that there were time trends due to treatment during the 21-d treatment period. This was evaluated by calculating individual treatment regressions for milk yield and then testing for heterogeneity of regression to determine if three individual regressions better fit data than the pooled regression. Based upon this comparison, there was no evidence to indicate that the regression curves were not parallel. Although not proven to be different, the three treatment regressions for mean milk yield per day are graphed in Figure 2.

Similarly, the a, b and c coefficients, calculated as described in the Materials and Methods section, were used to evaluate initial, rise to peak and declining daily milk yields during the treatment period. The initial mean milk yield for cows was less than for the noise treatments and initial yields for the two noise treatments also differed (P<.05). Since this preceded exposure to simulated jet noise, difference in initial yields was not due to noise. The predicted daily milk yields for cows on the three treatments during the 21-d treatment period were calculated using these regression coefficients. These are presented in Table 10 along with the regression coefficients for the intercepts, and betas for each treatment. The daily means calculated were fairly constant throughout the 21-d period.

No treatment*time effects were detected for mean milk yield (Table 9), but significant effects due to period (P<.0001) and period*time (P<.008) were detected. Period effects were expected since all cows were at or beyond peak lactational milk yield (> 79 DIM) at time they were assigned to experiment and reduced mean production by cows represented the expected decline in production as lactation advanced. This typically may be 2-2½ %/wk for cows with good to moderate persistency (Whittemore, 1979). For cows producing 30.75 lb/milking (Table 8), reduction would be .7 to .8 lb/wk and over the 6 wk from beginning of first period to end of second period; this amounts to 3 to 5 lb/milking. However, the

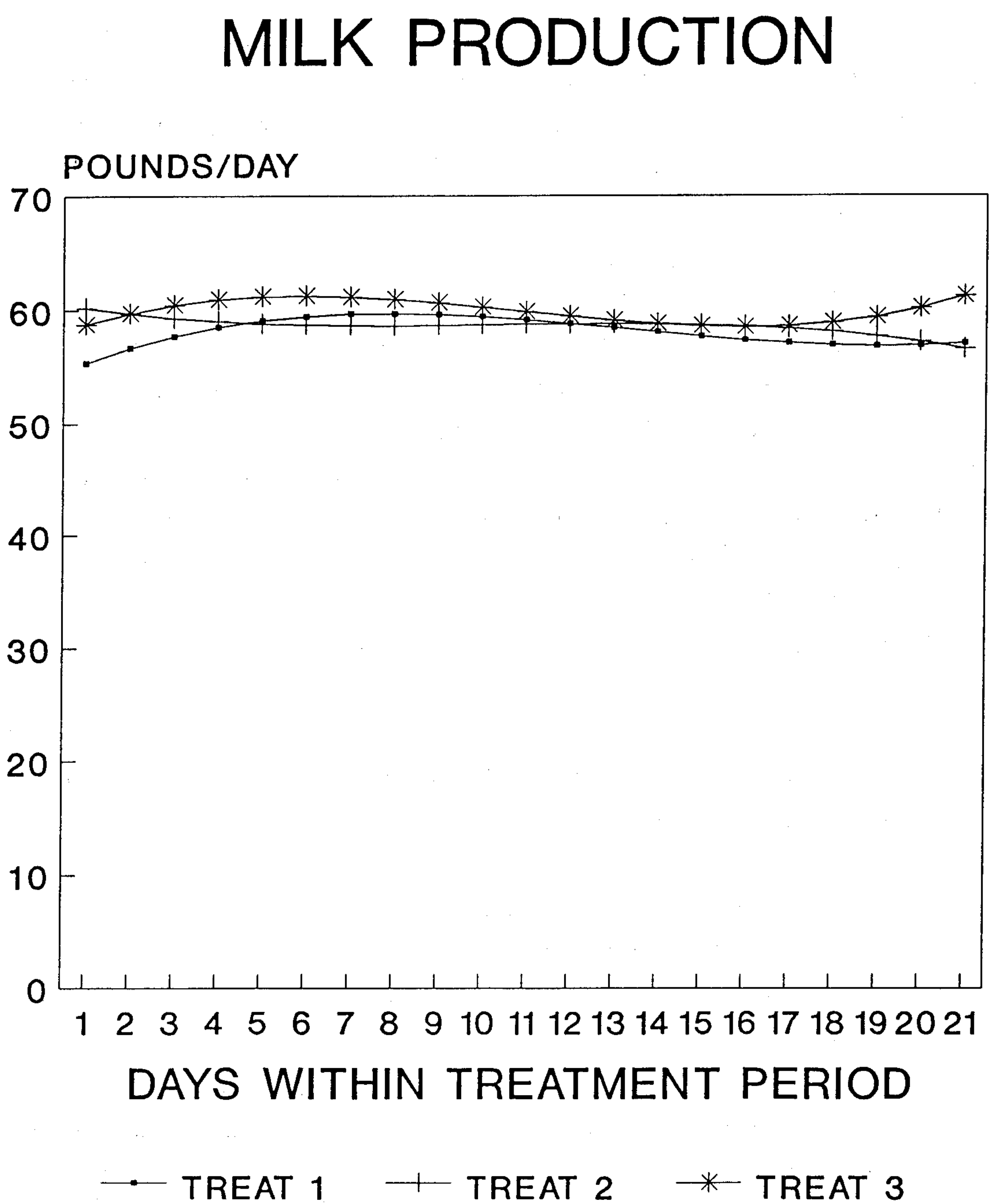

Figure 2. Milk production by dairy cows exposed to sudden jet aircraft noise just before milking

Table 10. **Multiple regression coefficients for milk yield and predicted daily milk yields during the 21-d period.**

	TREATMENT		
Intercept	53.65	60.85	57.40
Beta 1	1.78169	-0.71859	1.49125
Beta 2	-0.16016	0.07310	-0.17366
Beta 3	0.00395	-0.00232	0.00530

TREATMENT (lbs/day)		
55.27908	60.20335	58.71793
56.60794	59.68780	59.72529
57.66387	59.29061	60.44891
58.47055	58.99784	60.92059
59.05167	58.79559	61.17213
59.43093	58.66993	61.23531
59.63201	58.60694	61.14194
59.67861	58.59270	60.92380
59.59440	58.61330	60.61269
59.40308	58.65481	60.24040
59.12833	58.70332	59.83872
58.79385	58.74490	59.43946
58.42333	58.76564	59.07441
58.04045	58.75161	58.77535
57.66890	58.68889	58.57408
57.33238	58.56358	58.50240
57.05456	58.36174	58.59210
56.85914	58.06946	58.87497
56.76981	57.67281	59.38281
56.81025	57.15789	60.14741
57.00416	56.51076	61.20057

mean reduction would be about .2-.3 lb/wk, close to that found here (Table 8). Similarly, mean milk production by cows at the AM and PM milkings differed (Tables 8 and 9). This difference probably occurred for several reasons. First, daily milking times were at approximately 13 and 11 h splits. Secondly, cows are under greater environmental and management stress during the day, especially during the hotter times of the year, as when this experiment was conducted (April-June).

Significant period*time effects (Table 9) simply represented variability in yields throughout the two 21-d periods. Although differences were detected, basic time trend for daily milk yields did not differ (Figure 2) and effects were not associated with noise exposures. For each treatment, mean AM milk yields always were greater than PM yields (Table 8); difference was greatest for treatment 3 (8.32 lb), least for 1 (6.42 lb) and intermediate for 2 (7.61 lb) (overall difference was 7.1 lb). Daily differences for cows were variable. Some variability among cows may have occurred because it was necessary to milk cows approximately 30 min earlier on the 10-12 d during a period when they were exposed to noise in that period in order to avoid simultaneous exposure of cows on other treatments. In effect, this would have changed the milking interval and extended the daily split even further. It should not have affected total daily yields and when the statistical analyses were conducted using daily milk yields of cows the results were exactly the same.

3.2.2 3.5 % FCM and Constituents

Production of milk often is expressed on the energy content of the milk in order to correct for difference in fat and/or protein or solids-not-fat in milk. In this way, the amount of feed or tissue energy diverted to the mammary gland and secreted as milk components is accounted for. Small differences in the percentage composition of milkfat and/or daily milk yield could result in differences in 3.5 % FCM yields. Thus, effects of treatment on either milk yield or milkfat percent could be nonsignificant but the overall effect on milk yield corrected for energy content (3.5 % FCM) could be significant. A series of least squares analyses evaluated treatment effects on 3.5 % FCM and milk constituent percentages.

Mean percentages of milkfat and other milk constituents (protein, lactose, minerals, solids-not-fat) for treatments, periods or times (AM or PM milking) are in Table 11. For fat percentage, no significant effects of treatment ($P>.28$), treatment by time interaction ($P>.21$) or day by treatment by time interaction ($P>.33$) were detected. However, significant effects of time (AM or PM milking) and the three-way interaction day by time by period were significant. Similar results were obtained for solids-not-fat and total solids percentages (Table 12). Fat percentage of milk is the most variable of the constituents typically measured to characterize milk (Wilcox and Krienke, 1964). A Holstein cow averaging 3.5 % milkfat would be expected to have 68.2 % of her daily fat percentage fall between 3.05 and 3.95 % (one standard deviation) and about 94.6 % between 2.6 and 4.4 % (Wilcox and Krienke, 1964). Other constituents of milk are much less variable. Thus, variation in fat percentage observed was not unexpected (Table 13 and Appendix Table 1).

Percentages of milkfat and solids-not-fat are graphed in Figures 3 and 4. All percentages were within expected range for dairy cows following peak lactation and fed corn silage based TMRs. Effect of milking time (AM vs PM) was significant for total solids largely because this included the milkfat. Solids-not-fat did not vary due to time. Period effects on milk constituents agreed with expected changes that occur as milk yield declines; changes in milk yield and percentages of constituents are opposite.

Table 11. **Least squares means of percentage of milkfat, solids-not-fat (SNF), and total solids in milk.**

Source of Variation[a]		% Fat[b]	% SNF[c]	% Total solids
T	1	3.48 $\pm$ .068	8.75 $\pm$ .070	12.29 $\pm$ .069
	2	3.63 $\pm$ .066	8.65 $\pm$ .068	12.47 $\pm$ .067
	3	3.56 $\pm$ .067	8.69 $\pm$ .069	12.42 $\pm$ .068
PER	1	3.35 $\pm$ .050	8.81 $\pm$ .051	12.21 $\pm$ .051
	2	3.77 $\pm$ .049	8.58 $\pm$ .050	12.57 $\pm$ .050
TIME	1 (AM)	2.95 $\pm$ .049	8.71 $\pm$ .050	11.79 $\pm$ .050
	2 (PM)	4.16 $\pm$ .049	8.68 $\pm$ .051	12.99 $\pm$ .050
	N	824	824	824

[a] T = treatment; PER = period.

[b] Least squares mean $\pm$ standard error of mean (SEM).

[c] Solids-not-fat.

The yield of 3.5 % FCM was calculated according to Tyrrell and Reid (1965) using the following formula:

3.5 % FCM = (Milk yield X 0.4324) + (Fat yield X 16.22)

Fat yield and 3.5 % FCM are graphed in Figures 5 and 6 and the statistical analyses in Table 13. Arithmetic means for days on

Table 12. Least squares analysis of variance for percentages solids-not-fat (SNF) and total solids (TS) in milk.

Source	df	Solids-not-fat MS	Solids-not-fat Probability (P)	Total solids MS	Total solids Probability (P)
Treatment (T)	2	.1306	P>.84	.3320	P>.64
Cow	36	3.5381	P<.0001	2.2635	P<.001
Period (Per)	1	2.3570	P<.08	13.8348	P<.001
T*Per*Cow[a]	32	.7437	P<.08	.7543	P>.88
Time	1	.6726	P>.31	33.8421	P<.001
T*Time	2	.2117	P>.72	1.6635	P>.28
Cow*Time	36	1.6711	P<.003	.6751	P>.96
Per*Time	1	.2207	P>.56	9.4833	P<.01
T*Per*Cow*Time[b]	32	.6390	P>.89	1.2593	P>.19
Day	1	11.0279	P<.0005	.2608	P>.63
Day*T	2	.1076	P>.88	.0153	P>.21
Day*Per	1	.3100	P>.55	3.9339	P<.10
Day*Cow	36	3.1086	P<.0001	1.1533	P>.23
Day*Time	1	.3215	P>.55	.2284	P>.46
Day*T*Time	2	.0397	P>.95	1.3546	P>.53
Day*Per*Time	1	1.0861	P>.27	4.6252	P<.03
Day*Cow*Time	36	1.4875	P<.01	.6681	P>.94
Residual	600	.9096	----	1.0231	----
	Model R^2 = .3797			R^2 = .5895	

[a] Error term for sources above it.

[b] Error term for 4 sources above it.

Table 13. Least squares analysis of variance for fat yield and 3.5% FCM (lb/milking).

Source	df	Fat yield MS	Fat yield Probability (P)	3.5% FCM MS	3.5% FCM Probability (P)
Treatment (T)	2	.1353	> .37	81.0911	> .36
Cow	36	.3808	< .001	213.2270	< .002
Period (Per)	1	.9071	< .01	266.9004	< .07
T*Per*Cow[a]	32	.1320	> .57	77.7540	> .22
Time	1	.0551	> .59	806.6531	< .003
T*Time	2	.0043	> .66	11.1631	> .86
Cow*Time	36	.1361	> .82	64.8377	> .67
Per*Time	1	1.3788	< .01	567.9692	< .10
T*Per*Cow*Time[b]	32	.0020	< .11	75.3522	> .26
Day	1	.1338	> .33	100.9369	> .21
Day*T	2	.0498	> .70	25.5983	> .67
Day*Per	1	.8387	< .01	606.7865	< .002
Day*Cow	36	.1956	< .08	83.2095	> .14
Day*Time	1	.3414	> .12	192.4657	< .08
Day*T*Time	2	.0163	> .89	.5953	> .99
Day*Per*Time	1	1.3131	< .01	765.8489	< .001
Day*Cow*Time	36	.1124	> .80	59.6209	> .62
Residual	598	.1417	----	65.7096	----
	Model R^2 =	.5097		R^2 = .5997	

[a] Error term for sources above it.

[b] Error term for 4 sources above it.

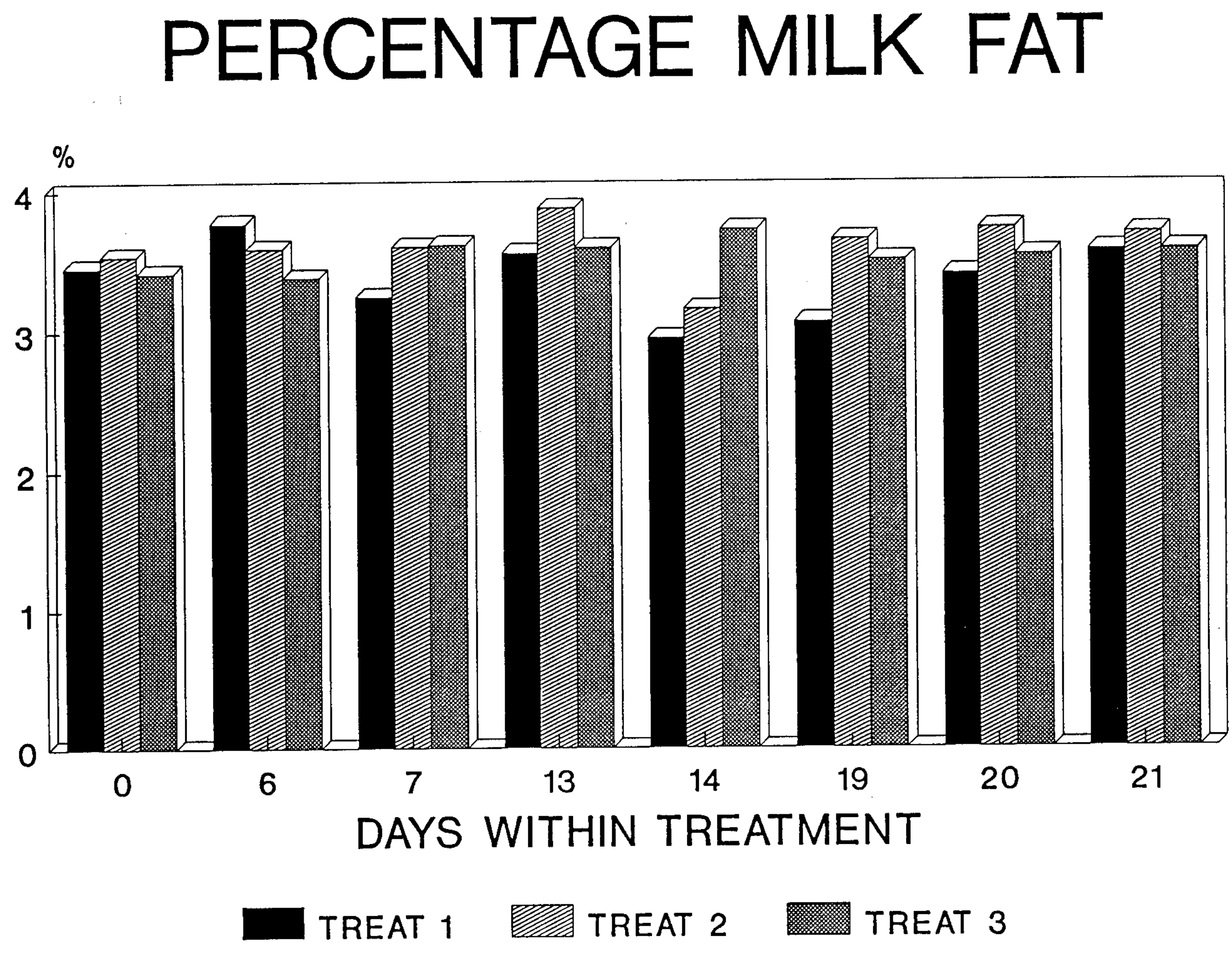

Figure 3. Percentage milk fat of cows exposed to sudden jet aircraft noise just before milking

PERCENTAGE SOLIDS-NOT-FAT

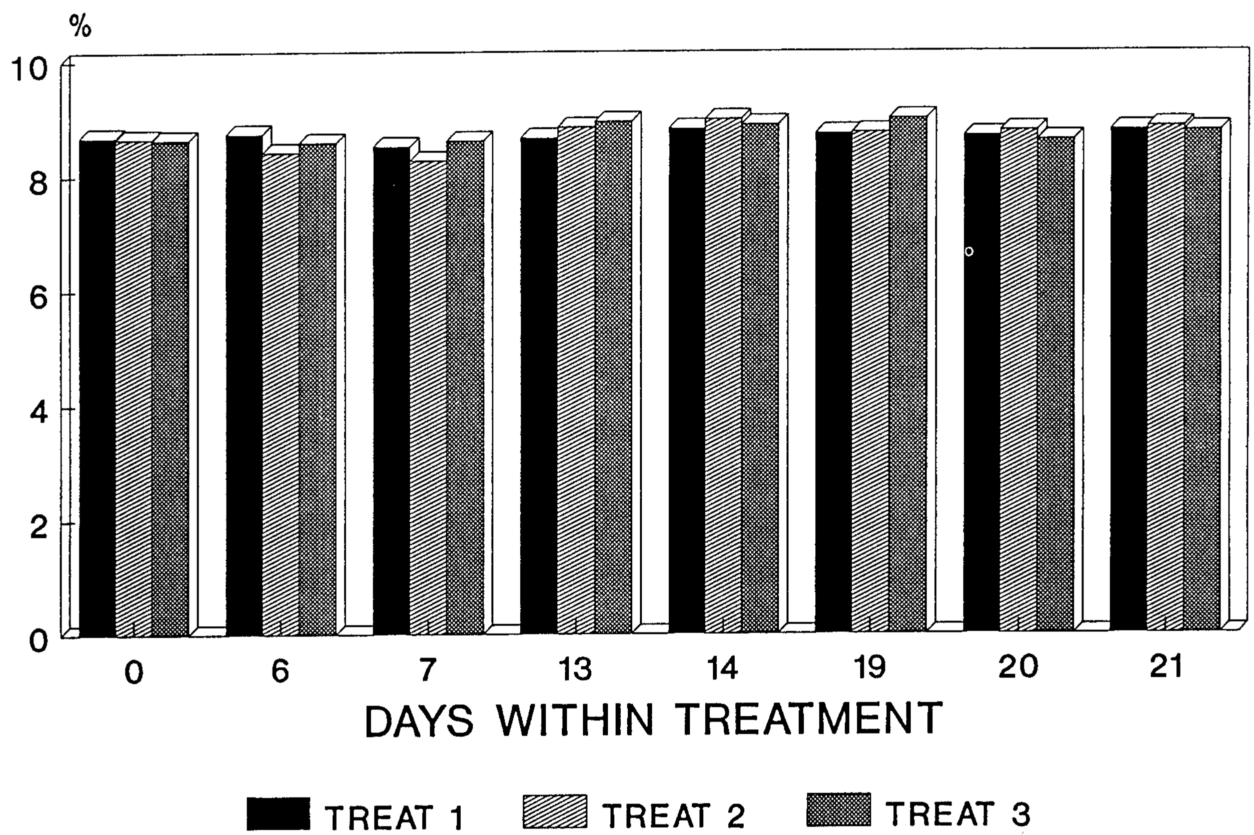

Figure 4. Percentage solids-not-fat of dairy cows exposed to sudden jet aircraft noise just before milking

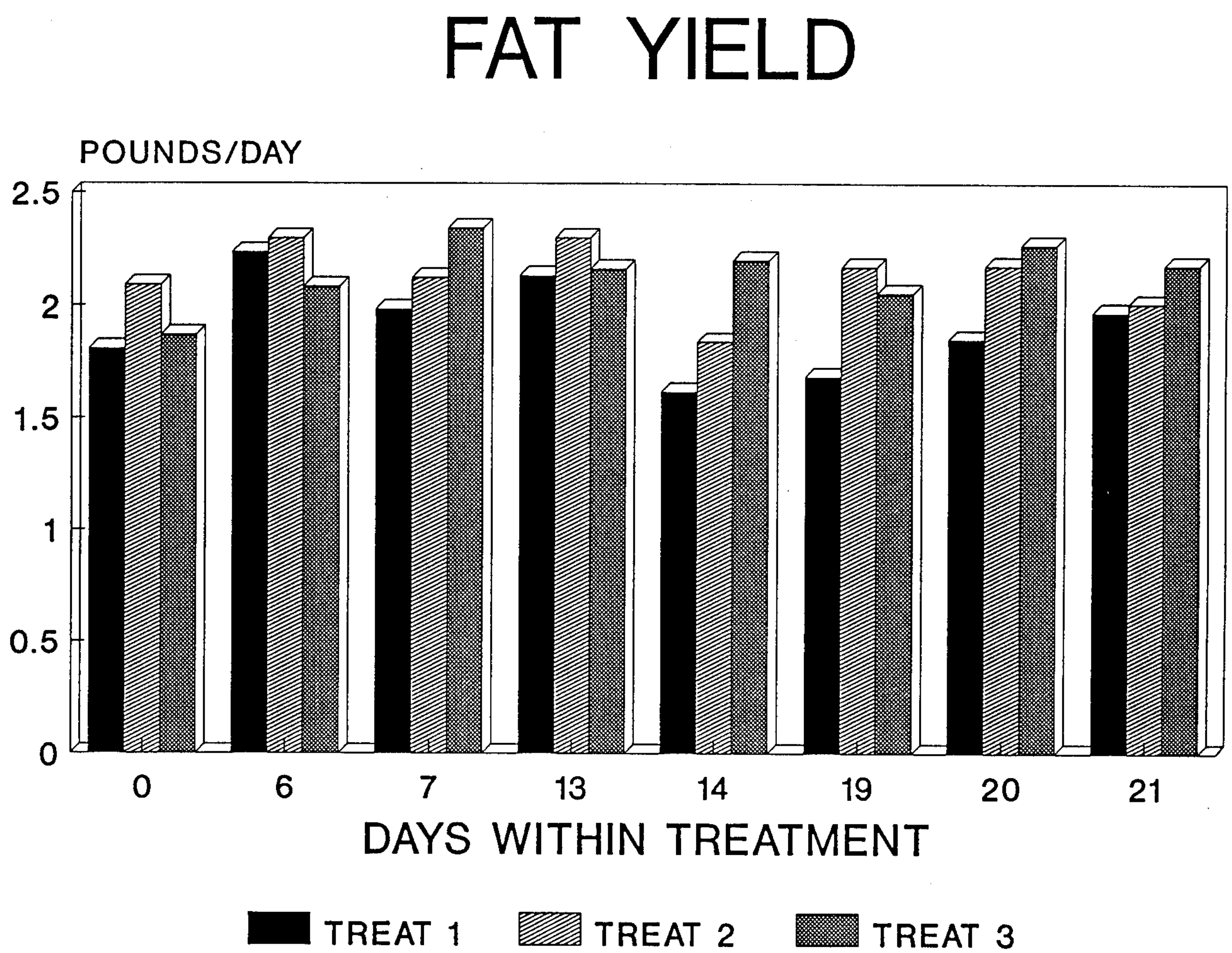

Figure 5. **Fat yield of dairy cows exposed to sudden jet aircraft noise just before milking**

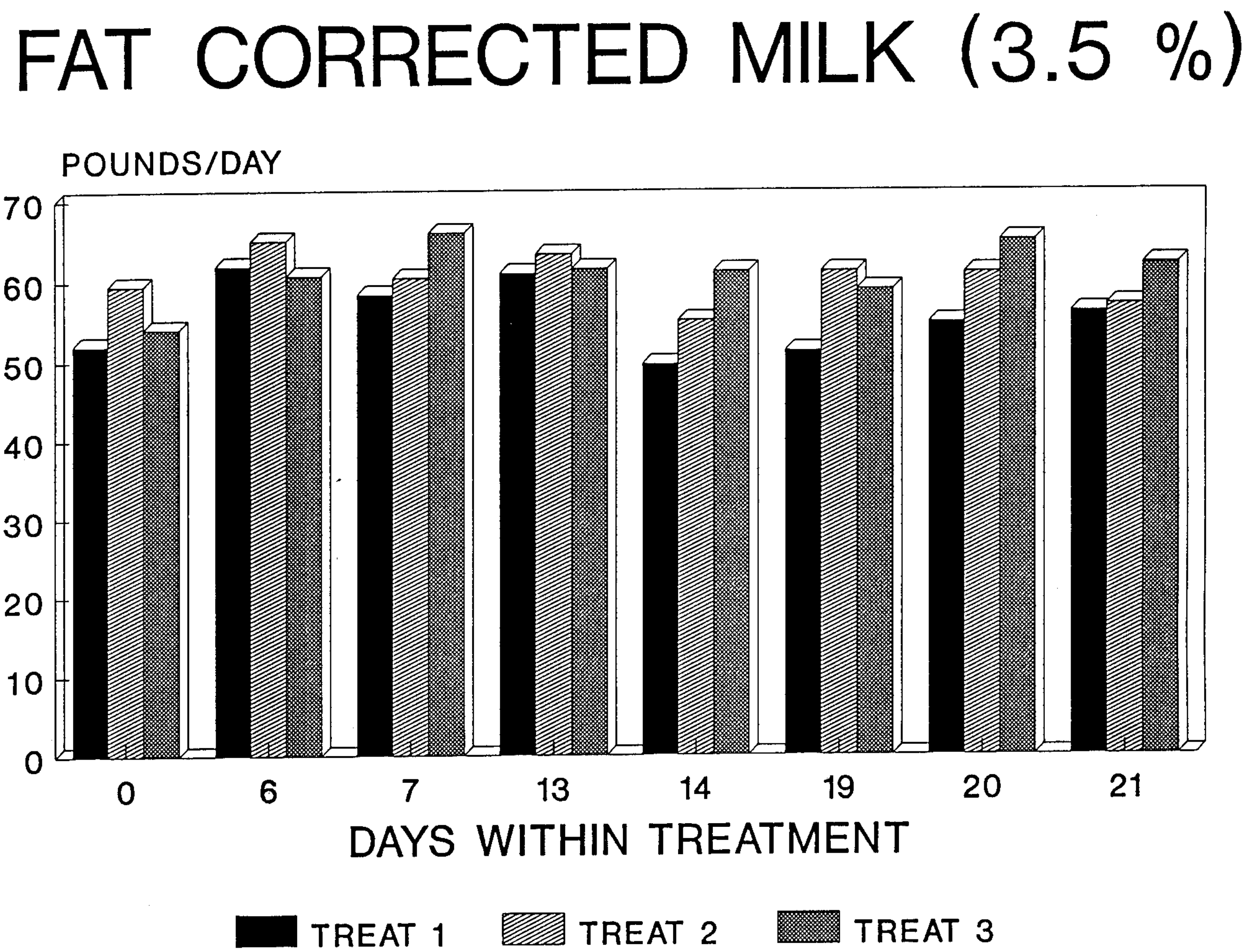

Figure 6. Fat corrected milk of cows exposed to sudden jet aircraft noise just before milking

which milk composition analyses were made are in Appendix Table 1. No significant effects of treatment or treatment by time interaction were detected (Table 13). Effects of period were significant, as for percentage fat. These results, although based on fewer observations, agreed with those for actual milk yield. A change in fat percentage, fat yield and 3.5 % FCM would logically be expected if there had been stress provoked by exposure to simulated jet noise which decreased amount of milk harvested at each milking and increased the residual milk. Residual milk is that not obtained by normal milking procedures and may represent 5-15 % of total milk yield/milking. Further proof that treatment did not affect milk yield or composition was provided by measuring residual milk. Residual milk obtained after injection of oxytocin ranged from 0.1 to 11.3 lb/d. Residual milk was variable; CV was greater than 50 %. Mean daily residual milk during the 21-d period is graphed in Figure 7. Although mean lb/d declined with advancing day in period ($P<.01$) and a significant treatment*time interaction was detected ($P<.01$), there was no significant trend in residual milk for treatments across days (day*treatment*time interaction, $P>.26$).

Failure to detect significant treatment effects on residual milk agreed with results of milk yield, 3.5 % FCM and fat percentage and yield. If exposure to jet noise treatments had resulted in acute or chronic physiological responses, in spite of absence of behavioral response, it logically would involve release of hormones. Important changes would involve hormones associated with milk let-down (Oxytocin) and maintenance of lactation (Prl and Gc), presumably through action of catecholamines, such that yield of milk at that milking and at later milkings would be less.

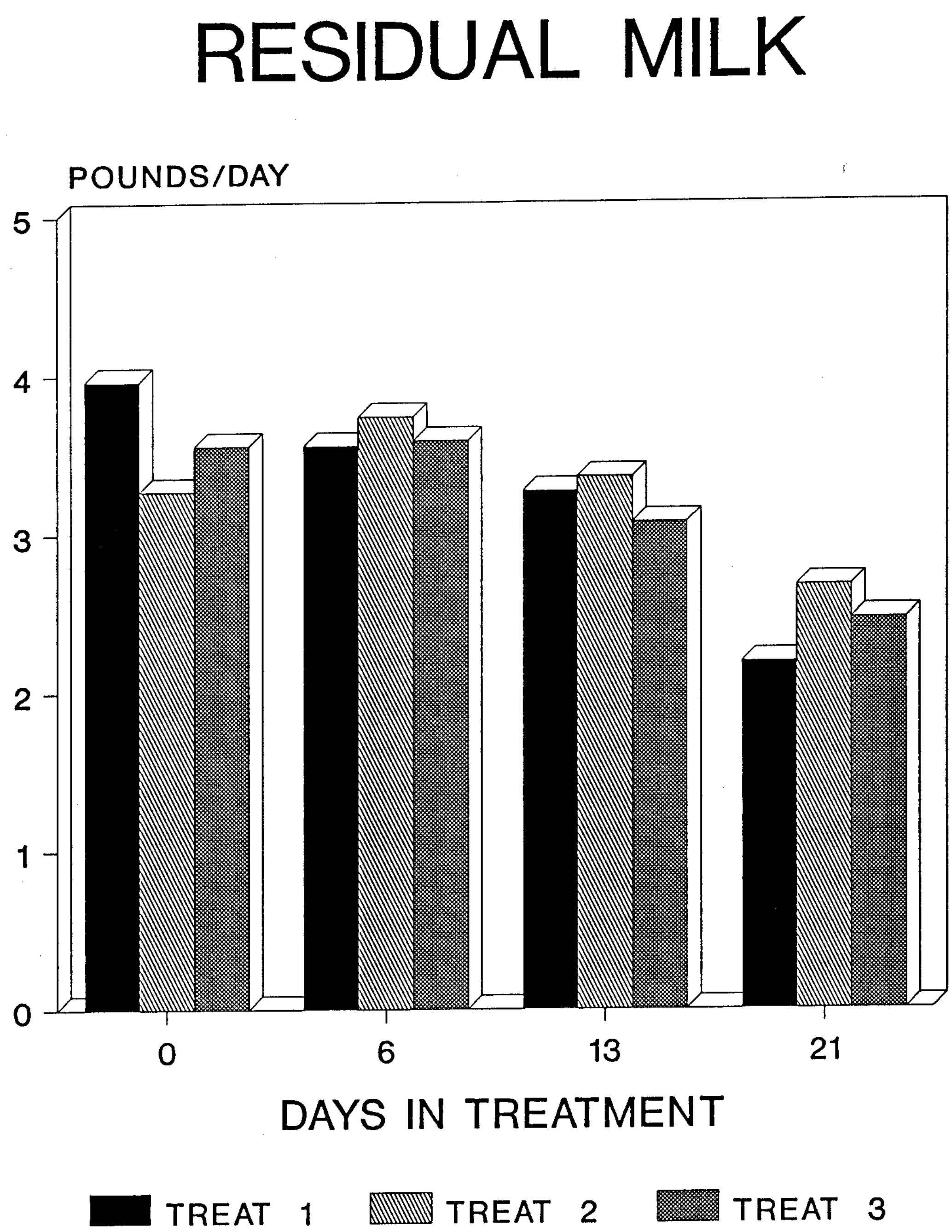

Figure 7. **Residual milk of dairy cows exposed to sudden jet aircraft noise just before milking**

3.2.3 Hormone Release

The importance of Oxytocin to elicit milk ejection is well accepted. Oxytocin causes contraction of myoepithelial cells, an increase in intramammary pressure and expulsion of milk from the mammary glands (Mepham, 1987). Exploding a paper bag delayed milk removal by cows for < 1 hr (Ely and Petersen, 1941), presumably by causing release of epinephrine, although this hormone could not be measured at that time. Since a standardized milking procedure was used in our study, which involved automatic take-off of machines when milk flow decreased to .7 lb/30 sec, a decrease in the efficiency of the milk-ejection mechanism should have been detectable, if it had existed. Mayer et al.,(1984) emphasized the importance of standardizing the milking procedure, including washing the udder before attaching milking machines, in order to minimize residual milk. Even when carefully standardized they found great variation in the secretion of Oxytocin and milking characteristics of lactating dairy cows.

Differences in residual milk within and among treatments in the present study would not be unexpected (Figure 7). Although we did not measure concentrations of Oxytocin during this experiment there is no reason to suspect that differences due to noise treatments existed. Many other factors can affect efficiency of milk removal from the mammary glands. Furthermore, Blum et al., (1989) showed that milk removal could be inhibited by factors that did not affect release of oxytocin. For example, catecholamines transported directly to the mammary gland in the blood, reduced mammary blood flow and inhibited milk removal. This likely was through interaction of catecholamines with alpha adrenergic receptors in the smooth muscle cells of ducts and teats of the mammary gland. Local release of inhibitors in the mammary gland under stress would be inhibitory to milk let-down and would result in an increase in residual milk. We saw no evidence of this type of response to jet noise since milk yields were unchanged and residual milk was not different across treatments.

We measured the release of two important lactogenic hormones (Prl and Gc) in a sub-sample of 12 cows assigned to the experiment. Data collected on hormone concentrations around the time of milking were subjected to statistical analyses. A series of analyses was conducted to evaluate effects due to treatment and other sources of variation and a summary of results for the two hormones is in Appendix Table 2. Analyses used 585 and 588 hormone measures for Prl and Gc.

No significant effect of treatment was detected for mean concentration of Prl but effect was significant for Gc. This indicated that mean responses of Gc for both collections within a treatment period (d 0 and d 14) differed across treatments. Since only the second of the two collections (d 14) within a period could have been affected by exposure to noise treatments, we evaluated the two way interactions of collection and cow, period or treatment. In no case were there significant effects detected (Appendix Table 2). This indicated that within a period there was no difference within cow in the release of either of the hormones to the milking stimulus. Significant differences due to cow and period were detected. These differences may have resulted, in part, because of management and environmental effects. Cows would be expected to become accustomed to more frequent handling associated with catheterization and blood collection over time. Differences in ambient temperature, humidity and/or windspeed are effects that could increase variation within and between periods. However, these management and environmental effects would not bias results since cows were assigned to receive different treatments in different sequences throughout the three experimental periods (Table 3).

Although no important effects of simulated jet noise on mean response of Prl and Gc were detected it still was possible trends in hormone release differed due to noise treatments. This was evaluated, as for milk yields, by calculating the highest order regression which was significant (quartic) for collections within a treatment. These were graphed and are in Figure 8. For each of the hormones we also tested for heterogeneity of regression to see if individual regressions fit the data better than the pooled regressions. There was no evidence to indicate that regression curves for the collections within treatment for the individual hormones were not parallel. Response curves for both Prl and Gc showed the expected increase at the time of udder wash and attaching the milking machine to the cow (Mepham, 1987).

Concentrations increased then returned to essentially pre-milking concentrations by 30 min post-milking. These responses and results agreed with results for production, milk composition, and residual milk in that no effects of noise treatment were detected. Differences due to cow and period have been detected and reasons discussed. In no instance are results inconsistent.

3.3 IMPLICATIONS

No measure of production response (milk yield, 3.5 % FCM, milk composition, amount of residual milk) or hormone release in response to milking was affected significantly by exposure of cows to two types of simulated jet aircraft noise just before time of milking. Although this logically is a time period when dairy cattle may be susceptible to this stressor, none was apparent. Video recordings of all cows at time of noise exposure showed no startle response nor other behavioral manifestations. Because dairy cattle were managed as a group and efforts were made to avoid conditioning them to treatment or handling that greatly differed from normal, we were unable to make acute or chronic measures of physiological responses such as heart and respiration rates, or secretion of stress hormones such as

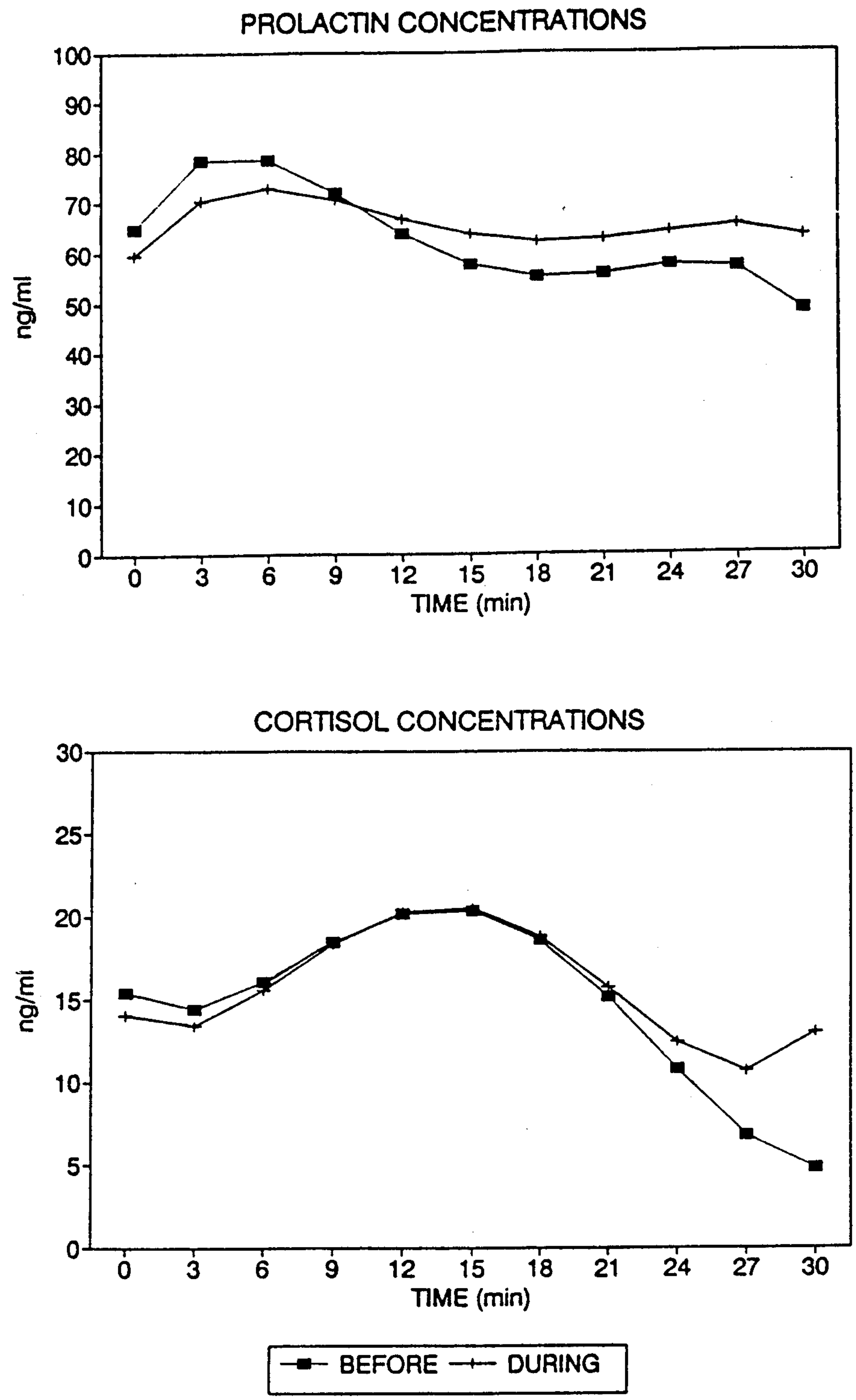

Figure 8. Pooled regressions of Prolactin and Cortisol response to milking in Holstein cows before (d 0, last day of adjustment period) and during exposure (d 14 of period) to simulated jet noise. Udder wash (30 sec) began at 0 min, milking machines were attached at 1 min and milking was completed by 9 min.

catecholamines or cortisol around the time stress was applied. If such physiological changes occurred, they were not detrimental to production during the course of a 21-d exposure period to simulated jet noise. Daily exposure of dairy cows to noise in the locations where they are housed and fed and milked and to farm machinery used to feed them, to clean the free-stall barn and to perform other activities around the farm may acclimate them such that unexpected noise, such as jet aircraft noise, causes no behavioral or physiological responses resulting in decreased productivity.

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Appendix

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Appendix Table 1. Arithmetic means for fat and SNF percentages, fat yield and 3.5% FCM.

Treatment	Day	Fat %	SNF %	Fat Yield	3.5% FCM
1	0	3.46	8.69	1.81	52.06
	6	3.78	8.75	2.23	61.91
	7	3.25	8.52	1.98	58.36
	13	3.56	8.67	2.13	60.94
	14	2.95	8.82	1.62	49.59
	19	3.07	8.74	1.69	51.23
	20	3.41	8.70	1.85	54.81
	21	3.57	8.79	1.97	56.18
2	0	3.54	8.66	2.09	59.47
	6	3.59	8.42	2.30	64.99
	7	3.60	8.27	2.12	60.43
	13	3.88	8.85	2.30	63.31
	14	3.16	8.99	1.84	55.09
	19	3.65	8.76	2.17	61.16
	20	3.73	8.78	2.17	61.01
	21	3.70	8.86	2.01	57.02
3	0	3.42	8.64	1.87	54.07
	6	3.38	8.59	2.08	60.67
	7	3.62	8.62	2.34	65.92
	13	3.60	8.94	2.16	61.46
	14	3.73	8.89	2.20	61.17
	19	3.51	8.99	2.05	58.89
	20	3.54	8.62	2.27	65.02
	21	3.58	8.77	2.18	62.08

Appendix Table 2. Least squares analysis of variance for prolactin and cortisol concentrations during milking.

Source	df	Cortisol MS	Cortisol Probability (P)	Prolactin MS	Prolactin Probability (P)
Cow (C)	11	6656.12	<.0012	7725.95	<.4483
Period (Per)	1	7852.29	<.0074	34481.25	<.0559
Treatment (T)	2	2480.51	<.0627	3584.64	<.6140
T*Per*Cow[a]	8	620.90	<.0001	6909.10	<.0001
Collection (Coll)	1	6.46	<.9481	2359.59	<.5421
C*Coll[b]	11	1456.87	<,6042	5959.83	<.4123
Per*Coll	1	590.23	<.5719	2835.71	<.4733
T*Coll	2	729.53	<.6652	1627.29	<.7317
C*Per*T*Coll[c]	8	1699.67	<.0001	5007.65	<.0001
Min	1	239.60	<.1398	10401.29	<.0001
Min^2	1	838.63	<.0059	9326.93	<.0001
Min^3	1	1084.03	<.0018	7061.55	<.0005
Min^4	1	1090.51	<.0017	5591.18	<.0001
Residual	539[d]	109.63	---	321.95	---
	Model R^2 = .6788			R^2 = .6429	

[a] Error term for sources above it.
[b] Error term for 4 sources above it.
[c] Error term for 3 sources above it.
[d] Residual df = 536 for Prolactin.

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