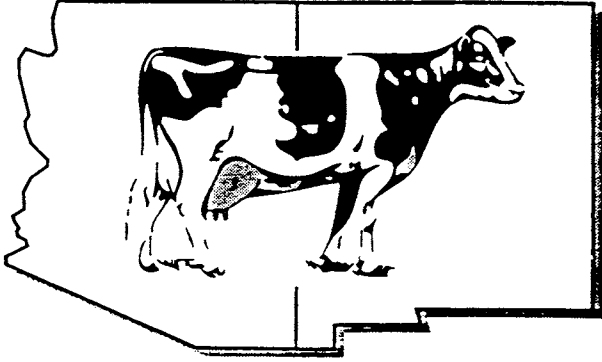


# ARIZONA & NEW MEXICO DAIRY NEWSLETTER



**COOPERATIVE EXTENSION**

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APRIL, 2002

This Month's Article:

**“Factors Influencing the Shedding of  
*Escherichia Coli* and *Samonella Spp.*  
in Holstein Cattle”**

**A.C. Fitzgerald, T.S. Edrington, T.R. Callaway, R.O Elder,  
J.D. Thomas, R.C. Anderson, D.J. Nisbet and M.L. Looper**  
New Mexico State University



Southwest Nutrition and Management Conference  
Proceedings are now available on our new website:

<http://animal.cals.arizona.edu/swnmc.php>

Check it out!

**New Mexico State University Extension Dairy Website:**  
**<http://www/nmsu.edu/~dairy>**

The following videos are available for checkout from Mike Looper, New Mexico State University. To obtain a video call Kathy Bustos, (505) 646-3325 or [kbustos@nmsu.edu](mailto:kbustos@nmsu.edu) and the video will be sent in the mail, pending availability. There is only one copy of each video available, so we request that videos be returned within two weeks. Note that four of the videos contain an English and Spanish version.

1. The Milking School. Utah State University. Spanish and English. 30 minutes
2. Fitting and Showing Your Dairy Animal....A Winning Experience. Department of Dairy Science, University of Wisconsin. 20 minutes
3. Proper Milking Procedure. University of Florida. Spanish and English. 12 minutes
4. Milking Machine Maintenance. University of Florida. Spanish and English. 16 minutes
5. The Basics of Vacuum and Milking Systems. DHIA Services, 1991. 53 minutes
6. Understanding Dairy Cattle Behavior to Improve Handling and Production. Livestock Conservation Institute, 1992
7. Managing Milking/Ordenar Lecheria. Spanish and English. 1999. 33 minutes
8. Get Milk! Joining A Dairy Crew. University of New Hampshire, 1999. 45 minutes

**Need to Calculate Production Costs?**

*University of Wisconsin dairy farm management specialist, Gary Frank, has developed a Excel spreadsheet to calculate variable cost of production and total cost of production. To access the spreadsheet, go to <http://www.wisc.edu/dairy-profit>, click on Decision Making Tools, then go to [costcwt.xls](#).*

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**FACTORS INFLUENCING THE SHEDDING OF *ESCHERICHIA COLI* AND *SALMONELLA SPP.*  
IN HOLSTEIN CATTLE**

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D. J. Nisbet<sup>2</sup> and M.L. Loofer<sup>1</sup>**

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**ABSTRACT:** Fecal samples were obtained in replicate from lactating (LAC; n = 120) and non-lactating (NLAC; n = 120) Holstein cows to determine the influence of time of day (AM vs PM), parity, and lactation phase [ ≤ 60 or > 60 d in milk (DIM)] on shedding of *Escherichia coli* O157:H7 (EHEC), *Escherichia coli* (EC), and *Salmonella spp.* (SM). Samples were collected at 0700 (AM) and 1700 (PM), placed on ice and shipped to laboratory for microbiological analyses. Mean ambient temperatures at time of collection were 24.9 and 31.8°C for AM and PM, respectively. Mean relative humidity was 50 and 24% for AM and PM, respectively, on the day(s) of collection. Temperature-humidity index was 72 for AM and 76 for PM. Incidence of EHEC, EC, and SM shedding was 56, 100, and 53%, respectively, in all cows. In LAC cows, SM shedding was increased (P < 0.05) in AM (60%) compared to PM (40%). Shedding of EHEC was increased (P < 0.05) in the AM, and SM shedding was increased (P < 0.05) in the PM in NLAC cows. Shedding of EHEC was more (P < 0.0001) prevalent in LAC (66%) than NLAC (34%) cows with no differences for EC and SM. More (P = 0.06) multiparous (67%) LAC cows shed SM than primiparous (33%) LAC cows. Parity did not influence (P > 0.10) shedding of bacteria in NLAC cows. Cows in early lactation (≤ 60 DIM) shed more (P < 0.05) SM and had a tendency (P = 0.10) to shed more EC than cows > 60 DIM. The most common *Salmonella* serotypes were Senftenberg (18%), Newport (17%) and Anatum (15%) of a total of 17 serotypes with several cows shedding more than one *Salmonella* serotype within a day. Time of day that fecal samples are collected may influence shedding of bacteria. In the current study, lactating cows shed more *Escherichia coli* O157:H7 than non-lactating cows. Cows in early lactation (≤ 60 DIM) shed more *Salmonella* and *Escherichia coli* compared to cows in later lactation.

Key Words: Dairy cow, *Escherichia coli*, *Salmonella*

### Introduction

*Escherichia* and *Salmonella spp.* in dairy cattle are zoonotic pathogens that pose health risks to humans via food-borne illness. Annually, pathogenic bacteria-related illnesses cost an estimated \$600 million to 3.5 billion for *Salmonella*, and \$200 to 600 million for *Escherichia coli* O157:H7 (Buzby et al., 1996). Approximately 500 deaths

occur each year in the U.S. due to Salmonellosis (CDC, 2002). Similarly, *Escherichia coli* O157:H7 can cause hemorrhagic colitis in humans with possible progression to hemolytic uremic syndrome (HUS), leading to acute renal failure (Martin et al., 1986; Ryser, et al., 1998). An estimated 61 fatalities occur annually due to HUS.

Calves and mature dairy cattle are reservoirs for opportunistic *Salmonella spp.* that cause fever and diarrhea in calves and abortion in mature cattle (Smith and House, 1992). *Escherichia coli* O157:H7 is most prominent in weaned calves (Ryser, 1998), and market cows shed significant amounts in their feces (Rice, 1997). Serotypes responsible for disease in cattle are Typhimurium, Dublin, Newport, Montevideo, and Anatum (Smith and House, 1992). Current studies focus on *Salmonella* and *Escherichia coli* O157:H7 fecal shedding in market cattle due to their potential contamination of meat and milk (Gay et al., 1994; Rice et al., 1997; Wells et al., 2001). Recently, The USDA-FSIS identified *Salmonella* as a target pathogen to be monitored in processing facilities (Kabagambe et al., 2000).

Objectives of this study were to determine effects of acute heat stress (time of day), parity, and stage of lactation on shedding of *Salmonella spp.*, *Escherichia coli* O157:H7 and generic *Escherichia coli* in dairy cattle.

### Materials and Methods

Lactating (LAC; n = 120) and non-lactating (NLAC; n = 120) Holstein cows were utilized in duplicate for fecal collection at 0700 (AM) and 1700 (PM) at a 3000 cow commercial dairy in Mesquite, New Mexico. All cows averaged 4.3 ± 0.1 yr and mean DIM for LAC cows was 150 ± 4 d. All cows were restrained in self-locking stanchions and approximately 15 g of fecal material was obtained via rectum using a separate veterinary palpation sleeve. Fecal samples were placed in Whirlpaks™ (Modesto, CA), packed on ice, and shipped to the Agricultural Research Service laboratory, to determine the prevalence of *Escherichia coli* O157:H7 (EHEC), *Escherichia coli* (EC), and *Salmonella spp.* (SM).

*Salmonella isolation.* Three to five grams of fecal material was enriched in 20 mL of tetrathionate broth for 24 h at 37°C. Post-enrichment of samples in Rapport-Vassilidis R10 broth was followed by streaking each sample on brilliant green agar with novobiocin for identification. Samples were characterized biochemically using lysine iron agar and triple sugar iron agar if they

displayed typical morphology of *Salmonella*. *Salmonella*-positive samples were confirmed by slide agglutination using *Salmonella* O antiserum poly A-I and Vi, and Group C1 factors. Samples were stored in pure culture on tryptic soy agar. All *Salmonella* positive isolates were sent to National Veterinary Services Laboratory (NVSL; Ames, IA) for confirmatory serotyping.

**Escherichia isolation.** A homogenous sample containing 10 g of feces, vancomycin (8 mg/L; Sigma Co.), cefixime (0.5mg/L; Lederle Laboratories), and cefsulun (10 mg/L; Sigma Co.) was prepared. The suspension was incubated at 37°C for 6 h followed by immunomagnetic bead enrichment consisting of 30-min incubation of 1 mL of GN enrichment broth with 20 µL of anti-O157 immunomagnetic beads on a rocker (60 cycles/min) at 25°C containing 0.05% Tween 20 (Dynal, Lake Success, NY). Immunomagnetic bead suspensions were washed three times in 1 mL of PBS containing 0.05% Tween 20 on a magnetic separation rack. After final wash, beads were resuspended in 100 µL of PBS/0.05% Tween 20. Fifty microliters of bead suspension was spread plated on sorbitol MacConkey (SMAC) plates containing cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L, Difco Laboratories; SMACct). Results were expressed as colony forming units (cfu; Elder et al., 2000.)

**Heat stress parameters.** Maximum ambient temperature and dew point were collected for each replicate from the Santa Teresa Weather Station in Santa Teresa, New Mexico. A temperature-humidity index (THI) was calculated for days of fecal collections. Briefly, THI was calculated as follows:  $THI = 0.45 T + 0.55 TH - 31.9 H + 31.9$  where T = dry bulb temperature expressed in °F and H = relative humidity/100 (Tarazon-Herrera et al. 1999).

**Statistical Analyses.** Chi-square analysis, using the FREQ procedure of SAS, was used to determine influence of time of day (heat stress) on shedding of SM, EHEC and EC. Influence of parity, lactation phase [ ≤ 60 or > 60 d in milk (DIM)] and lactation status (LAC vs. NLAC) on bacteria shedding also was analyzed by chi-square analysis.

## Results and Discussion

Incidence of bacteria shed in all cows was 56, 100 and 53% for EHEC, EC, and SM, respectively. Elder et al. (2000) reported an EHEC prevalence of 28% in feedlot cattle. Maximum EHEC prevalence from one fecal collection was 14 and 40% in lactating and non-lactating dairy cows, respectively (Mechie et al., 1997). A range of 5.4% in lactating cows to 18% in market dairy cows has been reported for SM prevalence (Wells et al., 2001).

Mean ambient temperatures at time of collection were 24.9 and 31.8°C for AM and PM, respectively. Furthermore, mean relative humidity was 50 and 24% for AM and PM, respectively, on collection days(s). Temperature-humidity index was 72 for AM and 76 for PM. Overall, time of day (acute heat stress) of fecal collection did not affect shedding of bacteria in both LAC and NLAC cows. Hancock et al. (1997) found prevalence of SM shedding for six of seven dairy herds peaked during

late summer and early fall. In the current study, heat stress between AM and PM collections may not have been severe enough to be associated with shedding of bacteria.

Analysis of data by lactation status indicated that SM shedding was increased ( $P < 0.05$ ) in AM (60%) compared to PM (40%) in LAC cows. Shedding of EHEC was increased ( $P < 0.05$ ) in the AM, and SM shedding was increased ( $P < 0.05$ ) in the PM in NLAC cows. Chronic exposure to heat stress may influence bacteria shedding in dairy cows. *Escherichia coli* (Mechie et al., 1997) and *Salmonella* (Kabagambe et al., 2000; Wells et al., 2001) shedding was increased in months with increased temperatures. Although there were differences in shedding of bacteria in AM vs PM, no consistent patterns were detected.

Shedding of EHEC was more prevalent ( $P < 0.0001$ ) in LAC (66%) than NLAC (34%) cows with no differences for EC and SM (Figure 1). Of the more than 100 outbreaks of EHEC in humans in the last decade, 52% have been attributed to foods derived from cattle (CDC 2002) and milk products (Chapman et al., 1993; Upton and Coia, 1994).

More ( $P = 0.06$ ) multiparous (67%) LAC cows shed SM than primiparous (33%) LAC cows. Parity did not influence ( $P > 0.10$ ) shedding of bacteria in NLAC cows.

Cows in early lactation (≤ 60 DIM) shed more ( $P < 0.05$ ) SM compared to cows in later lactation (76 and 24% respectively; Figure 2). Likewise, cows in early lactation had a tendency ( $P = 0.10$ ) to shed more EC than cows in later lactation. Similarly, the number of fecal coliform bacteria increased after parturition in lactating cows (Pelan-Mattocks et al., 2000). Furthermore, 79% (11/14) of cows shed EHEC via milk within 90 d of calving (Todhunter et al., 1991).

Seventeen different serotypes were identified in the current study (Table 1). The most common SM serotypes shed were Senftenberg (18%), Newport (17%) and Anatum (15%). Several cows in the current study shed more than one SM serotype within a day. Serotypes responsible for disease in cattle are Typhimurium, Dublin, Newport, Montevideo, and Anatum. Furthermore, Dublin serotype is responsible for abortion in cattle (Smith and House, 1992).

Common serotypes of SM identified in the current study differ from published prevalence reports from other states. Sato et al. (2001) reported Typhimurium, Montevideo, and Muenster as the predominant serotypes in mature, diarrheic dairy cows in California. Typhimurium and Dublin were the predominant isolates in market dairy cows from Washington (Gay et al., 1994). Fecal samples collected from dairy cows (n = 3,640 cows) in 19 states indicated the most common serotype for SM were Montevideo, Cerro and Kentucky (Wells et al., 2001). Shedding of different serotypes of SM could indicate geographical and managerial differences among dairy cows (i.e., weather, immunity, health, etc.)

## Implications

Knowledge of the prevalence and factors that affect shedding of pathogenic bacteria in cattle is necessary to reduce the incidence of possible meat and milk contamination. Time of day and associated acute heat stress could influence shedding of bacteria, while differences in serotype shedding may be due to geographical and (or) management strategies. Stressors experienced by lactating cows 60 days in milk could influence bacteria shedding. Further investigation of the influence of stressors on bacteria shedding is warranted due to potential food-borne illnesses in humans.

### Acknowledgements

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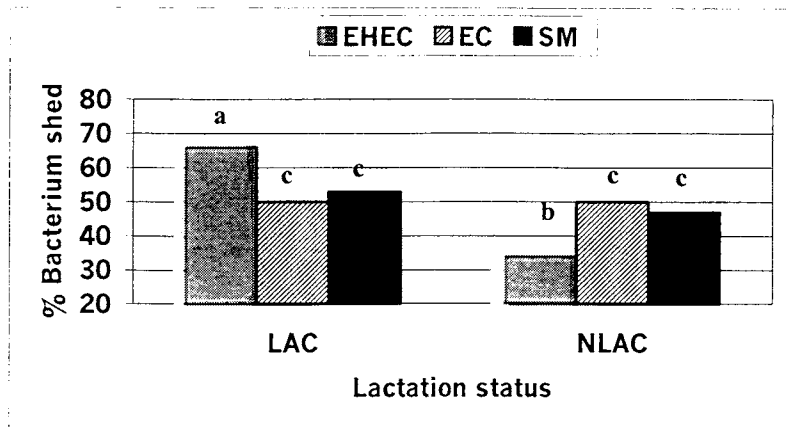


Figure 1. Percent of *Escherichia coli* O157:H7 (EHEC), *Escherichia coli* (EC), and *Salmonella* (SM) shed in lactating (LAC) and non-lactating dairy (NLAC) cows (<sup>abc</sup> $P < 0.05$ ).

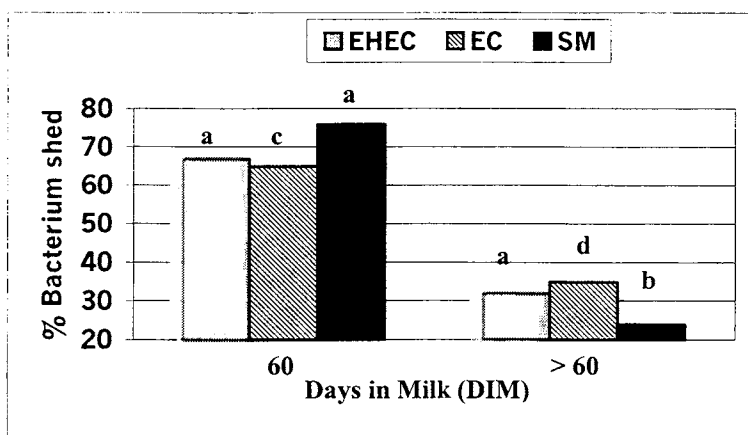


Figure 2. Percent of *Escherichia coli* O157:H7 (EHEC), *Escherichia coli* (EC), and *Salmonella* (SM) shed in early ( $\leq 60$  DIM) and later ( $> 60$  DIM) lactation by dairy cows. <sup>cd</sup>Different letters within a bacterium tend to differ ( $P = 0.10$ ). <sup>ab</sup>Different letters within a bacterium differ ( $P < 0.05$ ).

Table 1. Prevalence of *Salmonella* (SM) serotypes shed from lactating and non-lactating dairy cows

<i>Salmonella</i> serotype	%
Seftenberg	18
Newport	16.7
Anatum	14.7
Mbandaka	9.3
Meleagridis	9.3
Give	9.3
Tennessee	4.7
Alban	4
Cerro	3
Kentucky	2
Dublin	2
Untypable	2
Barranguilla	1
Havana	1
Agona	1
Cubana	1
Montevideo	1

# High Cow Report

## March, 2002

### MILK

<u>Arizona Owner</u>	<u>BarnNum.</u>	<u>Age</u>	<u>Milk</u>	<u>New Mexico Owner</u>	<u>BarnNum.</u>	<u>Age</u>	<u>Milk</u>
* Stotz Dairy West	12587	4-2	36,420	* Do-Rene Dairy	3862	4-03	38,790
* Rio Blanco Dairy	3016	4-4	35,860	* Do-Rene Dairy	4001	4-03	37,090
* Rio Blanco Dairy	2985	5-1	35,770	S.A.S. Dairy	4390	3-01	37,049
* Stotz Dairy West	12382	4-4	35,530	* Do-Rene Dairy	1275	6-06	36,840
* Red River Dairy	584	4-5	35,415	S.A.S. Dairy	1157	6-03	35,806
* Rio Blanco Dairy	4176	3-9	34,320	Red Roof Dairy	2102	5-11	35,644
* Red River Dairy	8398	5-6	34,291	* Hafliger Dairy	5355	6-06	35,250
* Red River Dairy	1222	3-3	34,225	* Desperado Dairy	7679	5-06	35,120
* Stotz Dairy West	13438	3-5	34,180	* Break Away Dairy	525	4-03	34,870
* Stotz Dairy West	19316	5-6	34,160	* Hafliger Dairy	5749	6-06	34,770

### FAT

<u>Arizona Owner</u>	<u>Barn Num</u>	<u>Age</u>	<u>Fat</u>	<u>New Mexico Owner</u>	<u>Barn Num</u>	<u>Age</u>	<u>Fat</u>
* Red River Dairy	584	4-5	1578	* Hafliger Dairy	5355	6-06	1346
* Red River Dairy	552	4-5	1418	Pareo Dairy	1443	7-03	1317
* Red River Dairy	4193	4-4	1372	S.A.S. Dairy	1156	6-03	1299
Desert Ridge Dairy, L.L.C. 2	593	5-0	1362	* Hafliger Dairy	891	5-06	1279
* Red River Dairy	1357	3-3	1357	* Desperado Dairy	7679	5-06	1270
* Stotz Dairy West	13262	3-7	1340	S.A.S. Dairy	1157	6-03	1264
* Stotz Dairy West	13437	3-5	1336	Goff Dairy	13461	4-03	1264
* Stotz Dairy West	13438	3-5	1309	* Hafliger Dairy	6621	4-03	1258
* Horizon Dairy	3451	3-10	1307	* Do-Rene Dairy	1275	6-06	1253
* Red River Dairy	7788	5-6	1282	Goff Dairy	3841	6-06	1234

### PROTEIN

<u>Arizona Owner</u>	<u>Barn Num</u>	<u>Age</u>	<u>Protein</u>	<u>New Mexico Owner</u>	<u>Barn Num</u>	<u>Age</u>	<u>Protein</u>
* Rio Blanco Dairy	2985	5-1	1081	S.A.S. Dairy	1157	6-03	1135
* Stotz Dairy West	19316	5-6	1061	* Do-Rene Dairy	4001	4-03	1100
* Rio Blanco Dairy	3016	4-4	1044	* Do-Rene Dairy	3862	4-03	1088
* Rio Blanco Dairy	4176	3-9	1035	* Desperado Dairy	7679	5-06	1076
* Red River Dairy	1222	3-3	1013	S.A.S. Dairy	4390	3-01	1068
* Red River Dairy	9957	4-5	1011	* Hafliger Dairy	5355	6-06	1062
* Stotz Dairy West	11406	5-0	991	Goff Dairy	13461	4-03	1059
* Red River Dairy	3663	6-6	982	Goff Dairy	3841	6-06	1058
* Zimmerman Dairy	5179	3-8	981	* Do-Rene Dairy	3242	5-06	1052
* Stotz Dairy West	12382	4-4	979	* Desperado Dairy	8040	4-03	1047
				* Do-Rene Dairy	3356	5-06	1047

\* 3X day milking

**MARCH, 2002**  
**ARIZONA - TOP 50% FOR F.C.M.<sup>b</sup>**

<b>OWNERS NAME</b>	<b>Number of Cows</b>	<b>MILK</b>	<b>FAT</b>	<b>3.5 FCM</b>	<b>Days Open</b>
* Stotz Dairy West	2081	27,159	943	27,036	167
* Red River Dairy	3960	26,248	930	26,432	127
University of Arizona Holsteins	153	25,937	928	26,265	151
Martha Linda Dairy	1837	25,100	919	25,757	175
* Mike Pylman Dairy	2524	25,291	874	25,109	155
* Stotz Dairy East	1414	25,204	854	24,747	199
Paul Rovey Dairy	428	24,555	871	24,742	131
* Hillcrest Dairy	2439	25,006	838	24,403	165
Desert Ridge Dairy, LLC2	517	24,338	834	24,050	140
* Zimmerman Dairy	1160	23,779	835	23,823	199
University of Arizona Brown Swiss	137	22,253	863	23,618	139
* Arizona Dairy Company North	2638	23,489	829	23,602	157
DC Dairy, LLC	1084	22,635	828	23,215	158
* Arizona Dairy Company South	3146	23,083	808	23,083	152
* Del Rio Holsteins	1215	22,676	809	22,925	135
* Wigwam Dairy	1435	22,877	790	22,703	177
* Saddle Mountain Dairy	2205	23,609	765	22,613	137
Butler Dairy	612	22,391	785	22,413	168
* Dutch View Dairy	1554	22,027	771	22,028	183
* Danzeisen Dairy, LLC	1255	20,666	775	21,505	131
* Del Rio Brown Swiss	150	20,449	757	21,119	104
* Dairyland Milk Company	2497	20,971	739	21,052	133
* Gladtime West Holsteins	336	21,077	734	21,018	185

**TOP 50% ACTUAL MILK - OFFICIAL & UNOFFICIAL HERDS FOR NEW MEXICO**

<b>OWNERS NAME</b>	<b>Number of Cows</b>	<b>MILK</b>	<b>FAT</b>	<b>3.5 FCM</b>	<b>Days Open</b>
* Pareo Dairy #1	1320	25,209	936	26,080	153
* Hafliger Dairy	1756	24,163	939	26,109	158
Ken Miller Dairy	308	24,855	835	24,289	170
* Do-Rene Dairy	2396	24,835	847	24,475	130
McCatharn North Dairy	1087	24,662	843	24,336	145
* Pareo Dairy #2	2609	24,362	917	25,406	141
Price's Roswell Farm	2751	24,160	885	24,800	135
* S.A.S. Dairy	1863	24,112	855	24,292	151
* Tallmon Dairy	525	23,697	845	23,951	164
* Vaz Dairy	1506	23,295	803	23,096	155
Just Fine Dairy	294	22,480	842	23,376	177
Desperado Dairy	1453	22,071	832	23,037	156
* Wayne Palla Dairy	3300	21,855	805	22,506	139
* Break-Away Dairy	1289	21,832	731	21,296	139

\*3X a day milking

<sup>b</sup> Average Milk & Fat figure may be different from monthly herd summary; figures used are last day/mo.



**ARIZONA & NEW MEXICO HERD IMPROVEMENT SUMMARY FOR  
OFFICIAL HERDS TESTED MARCH, 2002**

		ARIZONA	NEW MEXICO
1.	Number of Herds	55	28
2.	Total Cows in Herd	81,038	44,032
3.	Average Herd Size	1,473	1,573
4.	Percent Days in Milk	91	86
5.	Average Days in Milk	190	193
6.	Average Milk - All Cows Per Day	65.0	60.1
7.	Average Percent Fat - All Cows	3.6	3.7
8.	Total Cows in Milk	73,745	37,903
9.	Average Daily Milk for Milking Cows	72.7	69.7
10.	Average Days in Milk 1 <sup>st</sup> Breeding	85	77
11.	Average Days Open	153	148
12.	Average Calving Interval	13.9	13.9
13.	Percent Somatic Cell - Linear 0-4	83	78
14.	Percent Somatic Cell - Linear 5-6	12	14
15.	Percent Somatic Cell - Linear 7 & above	5	6
16.	Average Previous Days Dry	63	70
17.	Percent Cows Leaving Herd	31	33.9
	*****	*****	*****
		STATE AVERAGE	
	MILK	21,418	21,853
	Percent Butterfat	3.5	3.7
	Percent Protein	2.9	3.0
	Lbs. Fat	762	792
	Lbs. Protein	623	662

ARIZONA COOPERATIVE EXTENSION  
U.S. DEPARTMENT OF AGRICULTURE  
THE UNIVERSITY OF ARIZONA  
TUCSON, ARIZONA 85721

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OFFICIAL BUSINESS  
PENALTY FOR PRIVATE USE, \$300

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