Dairy Research 2005

Kansas Agricultural Experiment Station

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FOREWORD

Members of the Dairy Commodity Group of the Department of Animal Sciences and Industry are pleased to present this Report of Progress, 2005. Dairying continues to be a viable business and contributes significantly to the agricultural economy of Kansas. In 2004, dairy farms accounted for 2.9%, or $252 million, of all Kansas farm receipts, ranking 6th overall among all Kansas farm commodities. Kansas had the greatest percentage increase in milk produced between 1999 and 2004 (+57.7%) of all 50 U.S. states. During 2002, Kansas moved into the top 10 (#8) for milk production per cow. At the end of 2004, Kansas ranked #11 (19,611 lb), just 136 lb out of the #10 ranking. Wide variation exists in the productivity per cow, as indicated by the production testing program (Heart of America Dairy Herd Improvement Association [DHIA]). Nearly 105,000 cows were enrolled in the DHIA program from Kansas, Nebraska, Oklahoma, Arkansas, North Dakota, and South Dakota (including herds from Colorado and Missouri) beginning January 1, 2005. A comparison of Kansas DHIA cows with all those in the Heart of America DHIA program for the year 2004 is illustrated in the table below.

### Comparison of Heart of America (HOA) Cows with Kansas Cows - 2004

<table>
<thead>
<tr>
<th>Item</th>
<th>HOA</th>
<th>KS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of herds</td>
<td>718</td>
<td>235</td>
</tr>
<tr>
<td>No. of cows/ herd</td>
<td>152</td>
<td>148</td>
</tr>
<tr>
<td>Milk, lb</td>
<td>19,018</td>
<td>19,489</td>
</tr>
<tr>
<td>Fat, lb</td>
<td>704</td>
<td>720</td>
</tr>
<tr>
<td>Protein, lb</td>
<td>590</td>
<td>609</td>
</tr>
<tr>
<td>SCC x 1,000</td>
<td>378</td>
<td>416</td>
</tr>
<tr>
<td>Calving interval, mo.</td>
<td>14.5</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Most of this success occurs because of better management of what is measured in monthly DHI records. Continued emphasis should be placed on furthering the DHI program and encouraging use of its records in making management decisions. In addition, continued use of superior, proven sires in artificial insemination (AI) programs is essential. Emphasis on use of superior genetics through more use of AI sires is warranted.

The excellent functioning of the Dairy Teaching and Research Center (DTRC) is due to the special dedication of our staff. It has served us well since 1977. Our milk production with 200 cows has improved considerably according to our last test day in July 2005 (88 lb). Our rolling herd average for milk was 29,868 lb, with 1,059 lb of fat, and 915 lb of protein.

We acknowledge our current DTRC staff for their dedication: Michael V. Scheffel (Manager); Donald L. Thiemann; Daniel J. Umsheid; Glen Farrell, Kevin Good, Allen Hubbard, and Robert Fiest. Special thanks are given to Irene Vanderwerff and Cheryl K. Armendariz and a host of graduate and undergraduate students for their technical assistance in our laboratories and at the DTRC.

Each dollar spent for research yields a 30 to 50% return in practical application. Research is not only tedious and painstakingly slow but expensive. Those interested in supporting dairy research are encouraged to consider participation in the Livestock and Meat Industry Council (LMIC), a philanthropic organization dedicated to furthering academic and research pursuits by the Department of Animal Sciences and Industry (more details about the LMIC are found at the end of this publication).

J. S. Stevenson, Editor
2005 Dairy Research Report of Progress
It is a pleasure for the Dairy Commodity Group and the Department of Animal Sciences at Kansas State University to dedicate this Dairy Research Report to our friend and colleague, John Shirley. For 20 years, he served the Kansas Dairy Industry as a faculty member of the Department of Animal Sciences and Industry. He developed an outstanding teaching program in dairy cattle management and nutrition and was recognized by the College of Agriculture by receiving the Faculty of the Semester Award.

He advised hundreds of undergraduate and pre-veterinary students in their course of study in the College of Agriculture. He also mentored many graduate students in their dairy research projects. He coached many successful Dairy Cattle Judging Teams, advised the Kansas State University Dairy Club, and was named an Honorary Lifetime Member of the Kansas State University Dairy Club.

He served as Professor in Charge of the Kansas State University Dairy Teaching and Research Center. He conducted research to improve nutritional programs for dairy cattle and published research papers in national, regional, and state publications.

He spent many hours assisting dairy youth at the Kansas Junior Dairy Show and at the Kansas State Fair. He conducted many FFA and 4-H District and State Dairy Judging contests.

He received an Honorary State FFA Degree, the Honorary American FFA Degree, the VIP Award from the National FFA Association, and was selected as the Kansas Dairy Leader by members of the Kansas Interbreed Dairy Council in 2005.

He served on the Journal of Dairy Science Editorial Board and on the ADSA Midwest Section Board of Directors.

Dr. Shirley retired in August 2005. We will miss his unique stories and quick wit. But, most of all, we will miss his sincere concern for people and devotion to his family, friends, and colleagues.
CONTENTS

Serological Responses in Dairy Calves to Various Vaccines Administered via Needle-free or Conventional Needle-based Injections .............................................................. 1

Impact of Frequency of Feedline Soaking Combined with Evaporative Air Cooling in a Humid Environment ................................................................................................. 4

Using Vaginal Temperature to Evaluate Heat Stress in Dairy Cattle ......................................................... 9

Update on Ovulation-control Programs for Artificial Insemination of Lactating Dairy Cows ................................................................. 12

Kansas Farm Management Association Enterprise Analysis: Examining Differences Among High-, Medium-, and Low-profit Dairy Operations ............ 18

Index of Key Words ........................................................................................................................................ 23

Acknowledgments ........................................................................................................................................... 24

Biological Variability and Chances of Error ................................................................................................. 25

The Livestock and Meat Industry Council ................................................................................................. 26
SEROLOGICAL RESPONSES IN DAIRY CALVES TO VARIOUS VACCINES ADMINISTERED VIA NEEDLE-FREE OR CONVENTIONAL NEEDLE-BASED INJECTIONS

L. C. Hollis, J. F. Smith, B. J. Johnson, S. Kapil\(^1\), and D. A. Mosier\(^1\)

Summary

One hundred and four, 5- to 10-month-old Holstein heifers and steers were blocked by age within gender and randomly assigned to treatments. Calves were vaccinated with 5-way modified-live respiratory viral vaccine, *Mannheimia hemolytic* bacterin/toxoid, and 5-way Leptospira bacterin, administered via either needle-free or conventional needle-and-syringe injection techniques. Blood samples were collected from all calves at the time of vaccination and 21 days later. Blood sera were analyzed for antibody titers to infectious bovine rhinotracheitis (IBR) virus as the indicator of serological response to the 5-way viral vaccine, to *Leptospira pomona* (LP) as the indicator of serological response to the 5-way Leptospira bacterin, and to *Mannheimia hemolytica* (MH) leukotoxoid. Responses of heifers on day 21 to the IBR fraction of the 5-way viral vaccine, MH bacterin, and LP fraction of the 5-way Lepto bacterin did not differ between methods of administration. Responses of steers on day 21 to the IBR fraction of the 5-way viral vaccine and MH bacterin, and LP fraction of the 5-way Leptospira bacterin did not differ between methods of administration. Responses of steers on day 21 to the IBR fraction of the 5-way viral vaccine and MH bacterin were greater for the needle-free method of administration, whereas serological response to the LP fraction did not differ between methods of administration. We conclude that needle-free injections can eliminate broken needles in the carcass, reduce needle-borne transmission of disease, and possibly produce greater serological responses to various antigens, compared with those obtained with conventional needle-and-syringe injection systems.

(Key Words: Needle-free Injection, Vaccines, Serology.)

Introduction

Beef and dairy cattle quality assurance guidelines recognize that inadequate animal restraint or use of small-diameter needles may result in needle breakage, with the broken fragment sometimes left in the tissue to pose a hazard to those who handle or eat the meat. They also recognize that blood-borne infectious diseases such as bovine leukosis or anaplasmosis may be transmitted animal-to-animal when a single needle is used to inject multiple animals. One technology that offers the potential to avoid these problems is the use of a pneumatically powered, needle-free injection device that uses air pressure to drive the vaccine through the skin and into the underlying subcutaneous tissue or muscle (Felton 250 Pulse™ Needle-Free Injector, Figure 1). The purpose of this study was to compare seroconversion when injecting a modified-live respiratory viral vaccine containing IBR vaccine, injecting a MH bacterin-leukotoxoid, and injecting a LP bacterin into Holstein heifer and steer calves by using either needle-free or conventional needle-and-syringe injection methods.

\(^1\)Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine. 
Procedures

Fifty-four, 5- to 10-month-old Holstein heifers, and 50 steers from the Kansas State University Dairy herd were used. Animals of similar age and the same gender were housed in groups of 4 to 5 per pen. Animals were blocked into pairs by age within each gender group, and the method of administration of products was randomly allocated to each calf of each pair in each age block. Treatment 1 (T1) consisted of a 2-mL dose of Bovi-Shield® Gold 5 modified-live viral vaccine administered by Felton Pulse™ 250 needle-free intramuscular (i.m.) injection in the right side of the neck, a 2-mL dose of One Shot® Mannheimia hemolytica bacterin-toxoid administered subcutaneously (s.c.) in the left side of the neck via a disposable 3-mL syringe and 18 gauge × 1 inch needle, and a 2-mL dose of Leptoferm-5® Leptospira bacterin administered i.m. in the left side of the neck via a syringe and needle as previously described. Treatment 2 (T2) consisted of a 2-mL dose of Bovi-Shield® Gold 5 administered i.m. in the right side of the neck via a syringe and needle, a 2-mL dose of One Shot® administered s.c. in the left side of the neck by needle-free injection, and a 2-mL dose of Leptoferm-5® administered i.m. in the left side of the neck by needle-free injection. Blood samples were collected from calves on day 0 (vaccination day) and 21 days later. All blood samples were forwarded to the Kansas State University Veterinary Diagnostic Laboratory for serological evaluation.

Results and Discussion

Serological responses to IBR virus, Mannheimia hemolytica, and Leptospira pomona are shown in Tables 1 and 2. In heifers (Table 1), method of administration had no effect on IBR, MH, or LP responses on day 21. In steers (Table 2), on day 21, IBR and MH titer responses were greater with needle-free administration. In contrast, no significant difference was detected between methods for LP responses.

Conclusions

These findings indicate that use of the needle-free injection system to vaccinate dairy heifers and steers results in similar or sometimes greater serological responses, when compared with those obtained with conventional needle-and-syringe injection systems. Needle-free injection can eliminate the possibility for broken needles being left in the carcass and reduce the possibility of needle-borne transmission of disease among animals.
## Table 1. IBR, *Mannheimia hemolytica*, and *Leptospira pomona* Serological Responses Associated with Route of Administration in Heifers

<table>
<thead>
<tr>
<th>Administration Method</th>
<th>Antigen</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 21</td>
</tr>
<tr>
<td>T1 needle-free</td>
<td>IBR</td>
<td>2.00 ± 0.7</td>
</tr>
<tr>
<td>T2 needle</td>
<td>IBR</td>
<td>0.52 ± 0.2</td>
</tr>
<tr>
<td>T1 needle</td>
<td><em>M. hemolytica</em></td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>T2 needle-free</td>
<td><em>M. hemolytica</em></td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>T1 needle</td>
<td><em>L. pomona</em></td>
<td>0.0</td>
</tr>
<tr>
<td>T2 needle-free</td>
<td><em>L. pomona</em></td>
<td>0.0</td>
</tr>
</tbody>
</table>

## Table 2. IBR, *Mannheimia hemolytica*, and *Leptospira pomona* Serological Responses Associated with Route of Administration in Steers

<table>
<thead>
<tr>
<th>Administration Method</th>
<th>Antigen</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 21</td>
</tr>
<tr>
<td>T1 needle-free</td>
<td>IBR</td>
<td>1.44 ± 0.27</td>
</tr>
<tr>
<td>T2 needle</td>
<td>IBR</td>
<td>1.12 ± 0.35</td>
</tr>
<tr>
<td>T1 needle</td>
<td><em>M. hemolytica</em></td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>T2 needle-free</td>
<td><em>M. hemolytica</em></td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>T1 needle</td>
<td><em>L. pomona</em></td>
<td>0.0</td>
</tr>
<tr>
<td>T2 needle-free</td>
<td><em>L. pomona</em></td>
<td>0.0</td>
</tr>
</tbody>
</table>

\(a, b\)Values having different superscript letters differ \((P<0.05)\).

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**Figure 1. Felton Pneumatic System and needle-free injector.**
IMPACT OF FREQUENCY OF FEEDLINE SOAKING COMBINED WITH EVAPORATIVE AIR COOLING IN A HUMID ENVIRONMENT

M. J. Brouk, J. F. Smith, D. V. Armstrong, M. J. VanBaale, D. R. Bray, and J. P. Harner

Summary

Heat stress in hot and humid environments reduces milk production, decreases reproduction, and increases health-related problems. The summertime environment in north-central Florida is especially difficult because the combination of high relative humidity and high temperature results in a temperature-humidity index (THI) above the critical value of 72 for significant portions of the day. Previous work at Kansas State University had shown that the combination of soaking and evaporative air cooling could effectively cool heat-stressed cattle. Effectiveness of this feedline soaking, either in the afternoon and at night, or only at night, in combination with evaporative cooling was evaluated on a commercial dairy located in north-central Florida. A high-pressure fogging system and feedline soakers were installed in a typical 4-row freestall barn equipped with tunnel ventilation creating a north to south airflow of 6 to 8 mph at the cow level. Eight lactating Holstein cows in each of two, 292-stall pens were selected and fitted with vaginal temperature probes. Data on vaginal temperature and respiration rate were used to evaluate two cooling treatments. Barn temperature averaged 74.8 ± 5.4°F, relative humidity was 84.6 ± 15.4 %, and THI was 74.7 ± 5.3 during the study. The evaporative cooling system reduced average barn temperature by 0.9°F and reduced afternoon temperatures by a maximum of 9.2°F. Average respiration rates were less (58.5 vs. 66.9 breaths/min) in the afternoon and night soaking treatment, compared with the respiration rate of cattle in the night soaking treatment. Differences were greatest at the 10:00 p.m. observation (55.0 vs. 73.3 breaths/min). Average vaginal temperature was also less (102.0 vs. 102.6°F) in the afternoon and night soaking treatment. Our results indicate that the combination of cooling the air via a high-pressure fogging system and feedline soaking reduced heat stress experienced by dairy cattle. Using feedline soaking during the afternoon and night was more effective than soaking only at night.

(Key Words: Cow Comfort, Cow Cooling, Heat Abatement.)

Introduction

Heat stress causes a significant loss of milk production and income each summer in Kansas. Effects of heat stress continue to impact milk production, reproduction, and health into the fall and early winter. Impacts on reproduction and health also may negatively impact future lactations. Many Kansas State University studies have shown the positive benefits of heat abatement on milk production and dairy farm income. Other studies have shown that increasing the frequency of soaking and using supplemental airflow increases
heat loss from cattle and reduces body temperature and respiration rates. Amount of heat stress experienced by cattle is a function of air temperature, relative humidity, exposure to solar radiation, and airflow or wind speed. Relative stress levels are often described by the temperature-humidity index (THI), which combines the effects of temperature and relative humidity. It is generally accepted that dairy cattle begin to be stressed when THI exceeds 72.

The environment of north-central Florida is challenging. High temperature and relative humidity stress cattle and limit the effects of heat-abatement systems. High relative humidity reduces evaporation and, therefore, the degree to which water evaporation can be used to reduce air temperature in evaporative cooling systems or to reduce body surface temperature in soaking systems. Afternoon relative humidity, however, is generally reduced enough to gain some benefit from evaporative cooling of the air, and additional cooling may be possible from soaking. The purpose of this study was to evaluate the combination of evaporative cooling of the air with feedline soaking in the afternoon and at night or only at night.

**Procedures**

A 700-ft-long 4-row, head-to-head freestall dairy barn equipped with tunnel ventilation (north to south airflow) and a high-pressure fogging system was used to evaluate a combination cow-cooling system in north-central Florida. The fogging system operated when the temperature exceeded 80°F from 11:00 a.m. to 10:00 p.m. and when above 83°F from 10:00 p.m. to 11:00 a.m. the next day. Sidewall height was 12 ft, and the peak height of the roof was 13.2 ft with a 1/12 pitch. Curtain sidewalls were closed during the cooling study. A feedline soaking system also was installed in each of the two pens.

Eight lactating Holstein cows were selected from each of two pens and were fitted with a vaginal temperature recorder. In a replicated, switchback design, two soaking treatments were applied to the pens. Treatments were: 1) soaking in the afternoon and at night (10:00 p.m. to 6:00 a.m. the following morning; - A&N) and 2) soaking just at night (10:00 p.m. to 6:00 a.m. the following morning; - N). Feedline soakers were activated when the barn temperature exceeded 72°F, and the system soaked for 1.6 minutes (followed by 4.8 minutes off). Approximately 0.3 gal of water was applied to each cow-standing area per soaking. The 24-hour study day began at 10:00 a.m. and ended at 09:59 a.m. the next day. Respiration rates of the cattle fitted with the vaginal probes were observed and recorded at 6:00 a.m., 4:00 p.m., and 10:00 p.m. of each study day. Respiration rates were then averaged by day, treatment, pen, and time of observation before analysis. Vaginal temperature was recorded every minute and averaged into 15-minute periods. Barn and ambient temperature and relative humidity were recorded every 15 minutes with data loggers, and the data were averaged by hour of the day. A mixed-model procedure was used to analyze the data. Fixed effects included treatment and time of observation. Replicate was considered a random effect, and time of observation within pen was analyzed as a repeated measure.

**Results and Discussion**

Barn temperature averaged 74.8 ± 5.4°F, relative humidity was 84.6 ± 15.4 %, and THI was 74.7 ± 5.3 during the study. The evaporative cooling system reduced average barn temperature by 0.9°F and reduced afternoon temperatures by a maximum of 9.2°F. Average hourly variations in temperature, relative humidity, and THI are shown in Figures 1 through 3. Temperature differences were greatest between the barn and ambient condi-
tions in the afternoon hours when ambient relative humidity was least. Reduced afternoon ambient relative humidity increased water evaporation from the evaporative cooling system, and reduced barn temperature below that of ambient conditions. Evaporative cooling increased barn humidity, compared with ambient conditions, but barn THI was reduced.

Average respiration rates were less \( (P=0.05; \text{58.5 vs. 66.9 breaths/minute}) \) for cattle in the A&N treatment than for those in the N treatment. Differences (Figure 4) were greatest at the 10:00 p.m. observation (55.0 vs. 73.3 breaths/minute). Average vaginal temperature also was less \( (102.0 \text{ vs. } 102.6^\circ \text{F}) \) in the A&N treatment than in the N treatment. A significant drop in vaginal temperature was detected in the N treatment after the start of soaking at 10:00 p.m. (Figure 5). Our results indicate that the combination of cooling the air via a high-pressure fogging system and using feedline soaking reduced heat stress experienced by dairy cattle in a high-humidity environment. Using feedline soaking during the afternoon and night was more effective than soaking only at night. Soaking during the afternoon resulted in less body heat accumulation during the late afternoon and early nighttime, reducing heat stress experienced by cattle.

![Figure 1. Average Ambient and Barn Temperature by Hour of Day.](image1)

![Figure 2. Average Ambient and Barn Relative Humidity by Hour of Day.](image2)
Figure 3. Average Ambient and Barn THI by Hour of Day.

Figure 4. Average Respiration Rates of Cattle Exposed to Two Soaking Systems.
Figure 5. Average Vaginal Temperature of Cattle Soaked by Two Soaking Systems.
**Dairy Research 2005**

**USING VAGINAL TEMPERATURE TO EVALUATE HEAT STRESS IN DAIRY CATTLE**

*M. J. Brouk, B. Cvetkovic, J. F. Smith, and J. P. Harner*

**Summary**

A rise in body temperature is a signal that heat stress has exceeded the heat-exchange capacity of the dairy cow. Previous studies have shown a strong positive correlation between vaginal temperature and respiration rate, demonstrating a stress response to an increased body temperature. Vaginal temperature was collected by using temperature probes attached to an external data logger. Although these devices were very sensitive to changes in body temperature of cows housed in tie-stalls, the external data logger presented a significant application challenge for free-ranging animals housed in freestalls. A data logger was acquired that would be completely indwelling in the vagina. The U12 stainless steel model (Onset Computer Corporation, Pocasset, MA) was 0.5 × 4 inches and weighed about 2.6 oz. It was retained in the vagina with foam and a blank CIDR insert. These devices were used continuously to measure and record body temperature in free-ranging cattle for 5 to 7 days. Vaginal temperature was recorded at 1-minute intervals and then averaged into 5-minute blocks. Data were then graphed over a 24-hour period. Vaginal temperature increased with activity and amount of heat stress. Effective heat-abatement systems were shown to reduce vaginal temperature. On commercial farms, data were used to identify where heat abatement should be improved. Heat stress issues with milking parlor holding pens were easily identified. Producers and industry personnel could use data loggers to evaluate heat stress and the effectiveness of heat-abatement systems on free-ranging dairy cattle. Devices also could be used to validate the effectiveness of modifications to heat-abatement systems identified by the initial evaluation.

(Key Words: Cow Comfort, Cow Cooling, Heat Abatement.)

**Introduction**

Heat stress abatement is a critical management concern for dairy producers in Kansas. Many producers have installed heat-abatement systems and some have questioned the effectiveness of the systems. Methods to evaluate the effect of systems have been limited to evaluating respiration rates and milk response. An evaluation system that incorporated frequent measurement of body temperature would more accurately show where and when body temperature begins to rise in response to heat stress. Previous studies have used data loggers with an external temperature probe (model H08-031-08, Onset Computer Corporation, Pocasset, MA). The external probe was inserted into the vagina and held in place with foam, and the logger was then secured to the thurl with common duct tape. Data measurements were recorded over a period of 2 hours. Although these devices were

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1Dept. Biological and Agricultural Engineering.
very sensitive to changes in body temperature of cows housed in tie-stalls, the external data logger presented a significant application challenge for free-ranging animals housed in freestalls. A data logger was acquired that would be completely in-dwelling in the vagina. The purpose of this study was to validate the use of this logger for heat stress evaluation in lactating dairy cattle.

**Procedures**

To validate the system, 22 lactating Holstein cows were housed in a tie-stall barn for a period of 3 weeks. Body temperature was measured and recorded 4 days each week with the vaginal probes. The U12 stainless steel model (Onset Computer Corporation, Pocasset, MA) was 0.5 × 4 inches and weighed about 2.6 oz. It was held in the vagina with foam and a blank CIDR insert. Devices were programmed to measure continuously and record body temperature in free-ranging cattle for 5 to 7 days. Vaginal temperature was recorded at 1-minute intervals and then averaged into 5-minute blocks. Three days each week, rectal temperature, respiration rate, and skin surface temperature were measured and recorded at 6:00 a.m., 4:00 p.m., and 10:00 p.m. Respiration rate was visibly observed for 20 sec and recorded. Rear-udder skin surface temperature was measured with an infrared thermometer (Model 4KM98, Raytek®, Santa Cruz, CA). Before the start of the experiment, vaginal probes and rectal thermometers were validated in a water bath over the range of 85 to 110°F with a certified thermometer to ensure similar temperature responses in a controlled environment. Vaginal temperature data representing the same day and time as the rectal, respiration, and skin surface measurements were selected for analysis. Data were subjected to mixed-model procedures of SAS®.

In a separate study, 4 lactating cows in each of 2 pens were used in a switchback design to evaluate the effect of supplemental fans or no fans on vaginal temperature. The pen designs were similar in construction, and feedline fans were either operated (6:00 a.m. until 10:00 p.m.) or not operated on 2 similar summer days.

**Results**

Rectal and vaginal temperatures did not differ, and averaged 102.1 and 102.3°F, respectively. Rear-udder skin surface temperatures (94.3°F) were lower (P<0.01) than rectal and vaginal temperatures. Regression analysis of skin temperature on rectal temperature yielded an R² of 0.5, whereas vaginal temperature regressed on rectal temperature yielded an R² of 0.95. Results demonstrated that vaginal temperature was a good indication of body temperature and that rear-udder skin surface temperature was as accurate at predicting body temperature.

Data collected on a commercial farm are displayed in Figure 1. Cows with access to supplemental fan cooling had lower vaginal temperatures than did those without supplemental fan cooling. A dramatic drop in vaginal temperature was observed within 1 hour after the fan cooling system began to operate, compared with a rise in vaginal temperature when fans were not used. Lack of fan cooling in the evening resulted in a rise in vaginal temperature until the fans resumed operation the next day.

Data collected and summarized show the efficiency of the evaluation system to detect heat stress in dairy cattle. This evaluation tool was useful in evaluating heat-abatement treatments and in identifying needed changes in heat-abatement protocols. Further evaluation and use of this technology will aid producers, allied industry partners, and researchers in identifying heat stress issues on farms and in research projects.
Figure 1. Average Vaginal Temperature of Cows, With and Without Supplemental Fan Cooling.
UPDATE ON OVULATION-CONTROL PROGRAMS FOR ARTIFICIAL INSEMINATION OF LACTATING DAIRY COWS

J. S. Stevenson

Summary

Use of timed AI programs has become commonplace on most dairy farms either because cows are not watched sufficiently to detect estrus, or because expression of estrus is limited by confinement housing. A number of programs are available to set up first-postpartum inseminations that include some timed AI or timed AI of all cows once the end of voluntary waiting period (VWP) is reached. One approach may include a period of heat detection and AI until, for example, 100 days in milk, when a timed AI protocol is applied to all previously non-inseminated cows. Another approach includes injections of prostaglandin F$_{2\alpha}$, followed by periods of heat detection and AI, until a timed AI protocol is applied to all previously non-inseminated cows. Another approach may use a timed AI protocol that is applied so all cows can be first inseminated after the end of the VWP. The most sophisticated system involves presynchronizing estrous cycles during the latter part of the VWP and then applying a timed AI protocol. When protocols are applied correctly, ensuring that each cow is injected and inseminated appropriately, conception rates are either equal to, or slightly less, than those achieved when inseminations are based solely on behavioral signs of estrus (i.e., standing estrus). In contrast, pregnancy rates are almost always greater because more cows are inseminated (PR = AI submission rate × conception rate). Early application of Ovsynch before pregnancy status is known can allow all open cows to be re-inseminated by 2 to 3 days after their nonpregnant status is confirmed. This last program can essentially eliminate heat detection; when heats are observed, however, it becomes a bonus to the system.

(Key Words: Ovulation Control, Artificial Insemination.)

Introduction

In 1995, Kansas ranked 30th in total milk production and dairy cow numbers and 36th in milk production per cow in the United States. Since that time, a major revitalization of the industry has occurred. Kansas has experienced an 88% increase in total milk production, 38% increase in dairy cow numbers, and a 36% increase in milk yield per cow during the past 10 years. At the end of 2004, Kansas ranked 18th in total milk production, 19th in dairy cows, and 11th in milk production per cow. With this growth, we find that the majority of our cows are housed in confinement, in which they are nearly always on concrete except during the dry period.

Expression of estrus is greater when cows are housed on surfaces other than concrete. Given a choice between a grooved concrete surface and dirt, most cows choose to mount and stand on dirt where footing is more sure. Cow barns that are flushed, despite having adequately grooved concrete floors, become slick with age, and may suppress heat expression. Not only is heat expression reduced in
such environments, but with fewer people to manage more cows, time spent observing cows for sexual behavior is often nonexistent. Heat detection is now the same as reading tail-chalk rubs, coupled with occasional palpation to verify uterine tone and presence of mucus. As a consequence, timed AI programs have become popular to replace watching cows for heat and replace inseminations based on sexual behavior.

The objective of this update is to review several programs that can be used to set up cows for first inseminations after calving, to supplement or entirely replace once-standard heat-detection and AI programs.

Heat Detection + Cleanup Timed AI

For those who want to use heat detection and artificially inseminate as many as cows as are detected in heat, the program described in Figure 1 fits that objective. At the end of the VWP, all cows detected in heat are inseminated according to conventional procedures and the a.m./p.m. rule.

Once cows reach so many days in milk and are not yet bred (e.g., by 100 days in milk), the Ovsynch protocol is applied to these non-inseminated cows to ensure that they are bred within 10 days. If cows show estrus during that protocol, they should be inseminated according to the a.m./p.m. rule and the remaining protocol should be discontinued.

Prostaglandin + Heat Detection + Cleanup Timed AI

If your objective is to breed more cows at standing heat after the VWP, a PGF injection can be given near the end of the VWP to induce heats before AI. If desired, after 14 days, all non-inseminated cows can be reinjected, followed again by heat detection and AI (Figure 2). As in the previous protocol, once cows reach so many days in milk and are not yet inseminated, a timed AI protocol is applied.

Timed AI (Ovsynch)

A timed AI protocol such as Ovsynch can be used if you want to use limited heat detection before first services (Figure 3). When the Ovsynch protocol is applied, a few cows may show heat early during the protocol. They should be inseminated according to the a.m./p.m. rule, and the remaining hormone injections should be discontinued. It is not necessary to inject GnRH if the cow shows good heat before AI. The best time to do a timed insemination is between 0 and 24 hours after the second GnRH injection. Conception rates generally are slightly better when cows are inseminated at 16 hours, but 16 hours is impractical in most large herds, and cows are generally inseminated at either 0 or 24 hours after the second GnRH injection. Inseminations at 24 hours tend to be slightly better than at 0 hours, but that means those cows must be handled twice on consecutive days, rather than once when AI is done at the same time as the second GnRH injection.

Presynch + Ovsynch

Presynchronizing estrous cycles before applying Ovsynch generally improved conception rates achieved after timed AI (Figure 4). Several published studies indicate that conception rates are improved by about 10 to 15 percentage points. In the original studies, the interval between the two Presynch PGF injections was 14 days, but the interval between the second Presynch injection and the first GnRH injection of Ovsynch was 12 days. Some have changed that second interval to 14 days. If using a 12-day interval, the Presynch injections can be administered on Wednesdays and Ovsynch begins 12 days later, on a Monday. For the 14-day interval, the Pre-
synch injections are administered on Mondays, and Ovsynch begins 14 days later, on a Monday.

**No Heat Detection, Timed-AI System**

For those wanting to eliminate heat detection, the system illustrated in Figure 5 will fit that objective. This protocol sets up all cows for first services, and the Ovsynch protocol is initiated by administering GnRH to all cows 7 days before they are to be pregnancy diagnosed [assuming that cow has not been re-bred based on recurring heat at the first eligible cycle after first AI (20 to 25 days after timed AI)]. For those cows found open 7 days later, the remaining injections of Ovsynch are given (PGF, followed by GnRH in 48 hours and timed AI). This system is currently being applied on dairy farms with success.

**Available Products for Use in Timed-AI Programs**

A number of prostaglandin (Table 1) and GnRH (Table 2) products are available for use in these programs. All products are effective if used at the appropriate dosages. Use at least 1-inch needles when administering (i.m.) these products. A 1.5-inch needle is even better to ensure that all of the product is placed deep in the muscle and does not flow back out through the injection site. Flow back is a problem when using larger than 18-gauge needles. Ensure that injection sites are clean before injecting product. To prevent transmission of blood-borne diseases (e.g., bovine leukosis or anaplasmosis), use needles only once.

<table>
<thead>
<tr>
<th>Product</th>
<th>Supplier</th>
<th>Recommended Dose</th>
<th>Labeled Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutalyse®</td>
<td>Pfizer Animal Health</td>
<td>25 mg i.m.</td>
<td>Dairy heifers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5 cc)</td>
<td>Lactating dairy cows</td>
</tr>
<tr>
<td>Estrumate®</td>
<td>Schering-Plough Animal Health Hoechst Roussel Vet.</td>
<td>0.5 mg i.m.</td>
<td>Dairy heifers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2 cc)</td>
<td>Lactating dairy cows</td>
</tr>
<tr>
<td>Prostamate® (generic of Lutalyse)</td>
<td>IVX Animal Health/Phoenix Sci.</td>
<td>25 mg i.m.</td>
<td>Dairy heifers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5 cc)</td>
<td>Lactating dairy cows</td>
</tr>
<tr>
<td>In-Synch® (generic of Lutalyse)</td>
<td>Agri Labs</td>
<td>25 mg i.m.</td>
<td>Dairy heifers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5 cc)</td>
<td>Lactating dairy cows</td>
</tr>
</tbody>
</table>

1These are prescription products only available from a licensed veterinarian.
### Table 2. Current GnRH Products Available for Ovulation

<table>
<thead>
<tr>
<th>Product</th>
<th>Chemical Form</th>
<th>Dose</th>
<th>U.S. Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystorelin®</td>
<td>Gonadorelin diacetate hydrochloride</td>
<td>100 µg i.m. (2 cc)</td>
<td>Merial Limited, Iselin, NJ</td>
</tr>
<tr>
<td>Factrel®</td>
<td>Gonadorelin hydrochloride</td>
<td>100 µg i.m. (2 cc)</td>
<td>Fort Dodge Labs</td>
</tr>
<tr>
<td>Fertagyl®</td>
<td>Gonadorelin</td>
<td>100 µg i.m. (2 cc)</td>
<td>Intervet, Inc.</td>
</tr>
<tr>
<td>OvaCyst®</td>
<td>Gonadorelin diacetate hydrochloride</td>
<td>100 µg i.m. (2 cc)</td>
<td>IVX Animal Health/Phoenix Scientific</td>
</tr>
</tbody>
</table>

¹These are prescription products only available from a licensed veterinarian.

---

**Figure 1. Heat Detection Plus Cleanup Timed AI.**
Figure 2. Prostaglandin (PGF)-induced Heats, Followed by Heat Detection Plus Cleanup Timed AI.

Figure 3. A Timed-AI Protocol (Ovsynch).
Figure 4. Presychronized Estrous Cycles (Presynch) Before a Timed-AI Protocol (Ovsynch) to Set Up First Postpartum Inseminations.

Figure 5. No-heat-detection System that Applies Either Presynch + Ovsynch or Ovsynch to Cows to Set Up First Services, and Then Begins the Ovsynch Protocol (first GnRH injection) 7 Days Before Cows are Checked for Pregnancy. The Ovsynch Protocol is Only Completed in Open Cows.
Summary

Thirty-one dairy producers participated in the Kansas Farm Management Association (KFMA) dairy enterprise analysis each year from 2002 to 2004. The dairy farms were sorted based on 3-year average returns over total costs and were categorized as high-, medium-, and low-profit farms. The highest-profit farms earned an average of $795 more per cow ($4.20 per cwt of milk) than the low-profit farms earned. High-profit farms averaged $521 more milk sales per cow than low-profit farms did. This difference in profitability was due entirely to greater milk production, inasmuch as milk prices among profit groups did not differ from each other. High-profit farms produced almost 4,000 lb more milk per cow per year and had slightly lower costs than low-profit farms had. Returns for the mid-profit farms were more than $400 per cow less than returns of the top farms, but were more than $350 per cow greater than those of low-profit farms. The mid-profit farms had production levels similar to those of the high-profit farms, but their costs were significantly greater. Over the 3 years analyzed, it was better to have high production and high costs than to have low production and low costs. But these 3-year averages indicate that dairies can achieve high production levels while keeping costs in check, and these operations are significantly more profitable than other dairies.

Introduction

The U.S. dairy industry has been downsizing in terms of the number of dairy operations for more than 50 years. In recent years, however, it seems that the rate of consolidation has been occurring at a faster pace. For dairies to be competitive and survive in the future, it is imperative that managers understand what their strengths and weaknesses are. By recognizing business strengths and weaknesses, dairy managers can better focus their management efforts in areas in which they will be most beneficial. The best way for an individual dairy to identify its strengths and weaknesses is to benchmark the operation against other dairies. Related to this, producers also can benefit by simply understanding why some dairy producers are more profitable than others. Thus, the objective of this study is to examine differences in profitability that exist among Kansas dairy operations in Kansas and attempt to identify the major determinants of these differences.

Procedures

Income, cost, and a limited amount of production data for individual producers participating in the Kansas Farm Management Asso-
ciation *Enterprise PROFITCENTER Summary* for the years 2002 through 2004 were collected for analysis. Multiple years were used because returns for an individual producer can vary considerably from year to year due to factors beyond their control (e.g., prices and weather); thus a multi-year average should be a better indication of the dairies’ long-run expected profits relative to other dairies. The number of farms with data in the KFMA database for the years 2002 to 2004 ranged from 56 to 63 in any individual year, but this analysis only considered those operations that had participated during all 3 years. In addition, some farms were dropped from the analysis due to missing or incomplete data. After these criteria were met, 34 dairy operations had complete data for all 3 years. A similar analysis was done using the last 5 years (2000 to 2004). This reduced the number of operations for analysis to 31. Results for the 5-year analysis were similar, so only the 3-year average results are reported herein.

After all farms meeting data requirements were identified (34 dairies), 3-year averages for relevant income, cost, and production measures were calculated. These measures were calculated per dairy, per cow, and per cwt of milk produced. In addition, economic-return measures, such as returns above variable cost (VC), returns above total cost (TC), and returns to labor and management, were calculated. Fixed costs represent depreciation, unpaid labor, taxes on real estate, and an assigned interest charge. Variable costs represent all other costs, with the major expense categories being feed, hired labor, repairs, vet, breeding, and dairy supplies (for a listing of all expenses, see the *Enterprise PROFITCENTER Summary 2004* report). To see the *Enterprise PROFITCENTER Summary 2004* report, go to:

http://www.agmanager.info/farmmgt/income/

Three-year averages for all income, cost, and production measures were sorted from high to low on the basis of returns over total costs per cwt, such that profit categories could be identified. The 11 farms with the highest returns over total cost were classified as being the High 1/3, the next 12 farms were classified as being the Mid 1/3, and the 11 farms with the lowest returns over total cost were classified as the Low 1/3. It is important to recognize that the reported averages for all measures were based on the sort by returns over total cost. Thus, by definition, the High 1/3 farms will have the highest profit, but this does not necessarily hold for other income and cost measures.

To determine if profit-category averages of the various measures differed statistically from one another, a two-tailed *t*-test was used, along with a 90% confidence level. For example, this *t*-test indicated if the average profit of the 11 best farms was statistically different from the average profit of the 11 worst farms, and likewise for the middle grouping.

**Results and Discussion**

Figure 1 shows the return over total cost plotted against herd size for the 34 different farms, by profit category. A number of things can be seen from this figure. First, returns over total cost differed by approximately $7/cwt from the most to the least profitable dairies. Second, the number of cows in the herd for this group of 34 dairies ranges from 37 to 237 cows, indicating that the data represent the traditional family operation compared with the large commercial dairies that are becoming more prevalent in the industry. Finally, Figure 1 reveals a positive relationship between profitability and farm size. But there are dairies that are counter to this trend (i.e., the most profitable dairy was a small herd, and some of the larger herds have below-average profits).
Table 1 shows the 3-year averages for selected economic measures of the dairy producers, by profit category. Reinforcing the trend in Figure 1, the data show that high-profit dairy farms had larger herd sizes, and this was statistically different from both the mid- and low-profit dairies. The high- and mid-profit groups produced more milk than did low-profit dairies. Milk prices were not different among profit categories and, thus, differences in gross income per cow were driven principally by production (other income also had a small impact).

The mid-profit group had higher costs than the other groups had, whereas little difference existed in costs per cow between the high- and low-profit groups. Because the high-profit farms had high production and relatively low costs per cow, they had the lowest costs per cwt of milk produced. No differences were detected between feed and variable costs per cwt for the mid- and low-profit groups, due to the trade-off between production and costs (i.e., mid-profit farms had higher costs and higher production). But the mid-profit farms had lower fixed costs per cwt that resulted in lower total costs per cwt as well. High-profit dairies had a cost-per-cwt advantage of $3.64, compared with low-profit dairies ($1.95 advantage over mid-profit farms), indicating that they can withstand low milk prices much better.

There was almost an $800 difference in profits per cow ($4.20 per cwt of milk) between the high-profit dairies and the low-profit dairies. The low-profit dairies had an average return of -$386 per cow, indicating that these dairies likely are losing equity over time or are relying upon outside income to help support the dairy. These dairies show a positive return to labor and management of $116 per cow, but this is somewhat misleading because they paid $178 per cow for hired labor. Thus, even though the dairy owner(s) may be willing to work for low labor returns, their employees are not likely to do the same and, therefore, this positive return to labor and management offers little consolation.

Figure 2 shows the relationship between profitability (returns over total cost per cwt of milk) and annual costs per cow. The lack of a strong relationship in these data indicates that being a low-cost operator, in terms of dollars per cow (compared with dollars per cwt) does not necessarily ensure higher profitability. The high-profit dairies tended to have lower costs per cow than did the mid-profit farms with comparable production. The low-profit farms also generally had lower costs than the mid-profit farm, but their production was significantly lower. Thus, with these data, it seems that striving for high production is preferred to being low cost (i.e., comparing mid-profit farms with low-profit farms). The high-profit farms indicate that it is not an either-or decision (i.e., either high production or low costs). This group of dairies was able to attain both high production and relatively low costs over this 3-year period (this result held true in the 5-year analysis). This indicates that dairy producers wanting to be competitive selling commodity milk need to strive for high production levels, but cost control is still extremely important.
Figure 1. Relationship Between Return over Total Costs per Cwt and Herd Size, by Profit Category.

Figure 2. Relationship Between Return over Total Costs per Cwt and Total Cost per Cow, by Profit Category.
Table 1. Selected Average Economic Measures of Dairy Producers, by Profit Category\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Profit Category</th>
<th>Difference between High 1/3 and Low 1/3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High 1/3</td>
<td>Mid 1/3</td>
</tr>
<tr>
<td>Number of farms</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Number of dairy cows</td>
<td>140(^a)</td>
<td>96(^b)</td>
</tr>
<tr>
<td>Pounds of milk per cow</td>
<td>20,998(^a)</td>
<td>20,994(^a)</td>
</tr>
</tbody>
</table>

**INCOME**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High 1/3</td>
<td>Mid 1/3</td>
<td>Low 1/3</td>
</tr>
<tr>
<td>Milk sales, $/cow</td>
<td>$2,835(^a)</td>
<td>$2,845(^a)</td>
<td>$2,314(^b)</td>
</tr>
<tr>
<td>Gross income, $/cow</td>
<td>$3,370(^a)</td>
<td>$3,363(^a)</td>
<td>$2,636(^b)</td>
</tr>
<tr>
<td>Milk price, $/cwt</td>
<td>$13.51</td>
<td>$13.55</td>
<td>$13.66</td>
</tr>
<tr>
<td>Gross income, $/cwt</td>
<td>$16.09</td>
<td>$16.01</td>
<td>$15.53</td>
</tr>
</tbody>
</table>

**COSTS**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>High 1/3</td>
<td>Mid 1/3</td>
<td>Low 1/3</td>
</tr>
<tr>
<td>Variable costs, $/cow</td>
<td>$2,419(^a)</td>
<td>$2,817(^b)</td>
<td>$2,421(^a)</td>
</tr>
<tr>
<td>Feed costs, $/cow</td>
<td>$1,415(^a)</td>
<td>$1,654(^b)</td>
<td>$1,428(^a)</td>
</tr>
<tr>
<td>Fixed costs, $/cow</td>
<td>$542</td>
<td>$560</td>
<td>$601</td>
</tr>
<tr>
<td>Total costs, $/cow</td>
<td>$2,961(^a)</td>
<td>$3,376(^b)</td>
<td>$3,022(^a)</td>
</tr>
<tr>
<td>Variable costs, $/cwt</td>
<td>$11.57</td>
<td>$13.41(^b)</td>
<td>$14.21(^b)</td>
</tr>
<tr>
<td>Feed costs, $/cwt</td>
<td>$6.73(^a)</td>
<td>$7.86(^b)</td>
<td>$8.51(^b)</td>
</tr>
<tr>
<td>Fixed costs, $/cwt</td>
<td>$2.62(^a)</td>
<td>$2.73(^a)</td>
<td>$3.63(^b)</td>
</tr>
<tr>
<td>Total costs, $/cwt</td>
<td>$14.19(^a)</td>
<td>$16.14(^b)</td>
<td>$17.83(^c)</td>
</tr>
</tbody>
</table>

**RETURNS**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High 1/3</td>
<td>Mid 1/3</td>
<td>Low 1/3</td>
</tr>
<tr>
<td>Returns above VC, $/cow</td>
<td>$951(^a)</td>
<td>$545(^b)</td>
<td>$216(^c)</td>
</tr>
<tr>
<td>Returns over TC, $/cow</td>
<td>$409(^a)</td>
<td>-$14(^b)</td>
<td>-$386(^c)</td>
</tr>
<tr>
<td>Returns to labor and mgt, $/cow</td>
<td>$847(^a)</td>
<td>$452(^b)</td>
<td>$116(^c)</td>
</tr>
<tr>
<td>Returns above VC, $/cwt</td>
<td>$4.52(^a)</td>
<td>$2.60(^b)</td>
<td>$1.32(^c)</td>
</tr>
<tr>
<td>Returns over TC, $/cwt</td>
<td>$1.90(^a)</td>
<td>-$0.13(^b)</td>
<td>-$2.30(^c)</td>
</tr>
<tr>
<td>Returns to labor and mgt, $/cwt</td>
<td>$4.01(^a)</td>
<td>$2.12(^b)</td>
<td>$0.66(^c)</td>
</tr>
</tbody>
</table>

\(^1\)Profit categories were based on sorting 3-year average (2002 to 2004) of Return over Total Cost ($/cwt).

\(^a,b,c\)Values having different superscript letters differ (P<0.10).
Dairy Research 2005

INDEX OF KEY WORDS

Indexer’s note: The numbers indicate the first pages of each article that uses the listed key word.

| Artificial Insemination (12) | Management (18) |
| Cost (18) | Needle-free Injection (1) |
| Cow Comfort (4, 9) | Ovulation Control (12) |
| Cow Cooling (4, 9) | Profitability (18) |
| Economics (18) | Serology (1) |
| Heat Abatement (4, 9) | Vaccines (1) |
Dairy Research 2005

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Fort Dodge Animal Health, Fort Dodge, IA
Felton International, Lenexa, KS
Grain State Soya, West Point, NE
Heart of America Dairy Herd Improvement Association (DHIA), Manhattan, KS
Hubbard Feed, Mankato, MN
Intervet, Inc., Millsboro, DE
Iowa Limestone, Des Moines, IA
Kansas Agricultural Experiment Station, Manhattan, KS
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Kansas Dairy Commission, Wamego, KS
Kansas Farm Management Association, Manhattan, KS
Linn Willow Creek Dairy, Linn, KS
Meier Dairy of Palmer, Inc., Palmer, KS
Merial Limited, Iselin, NJ
Monsanto Company, St. Louis, MO
Ohlde's Dairy, Linn, KS
Pfizer Animal Health, Kalamazoo, MI
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Rota-Mix, Dodge City, KS
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Contribution No. 06-46-S, from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan 66506. Trade names are used to identify products. No endorsement is intended, nor is any criticism implied of similar products not named.
BIOLOGICAL VARIABILITY AND CHANCES OF ERROR

Variability among individual animals in an experiment leads to problems in interpreting the results. Although the cattle on treatment X may have produced more milk than those on treatment Y, variability within treatments may indicate that the differences in production between X and Y were not the result of the treatment alone. Statistical analysis allows us to calculate the probability that such differences are from treatment rather than from chance.

In some of the articles herein, you will see the notation "P<.05". That means the probability of the differences resulting from chance is less than 5%. If two averages are said to be "significantly different," the probability is less than 5% that the difference is from chance, or the probability exceeds 95% that the difference resulted from the treatment applied.

Some papers report correlations or measures of the relationship between traits. The relationship may be positive (both traits tend to get larger or smaller together) or negative (as one trait gets larger, the other gets smaller). A perfect correlation is one (+1 or -1). If there is no relationship, the correlation is zero.

In other papers, you may see an average given as 2.5 ± .1. The 2.5 is the average; .1 is the "standard error." The standard error is calculated to be 68% certain that the real average (with an unlimited number of animals) would fall within one standard error from the average, in this case between 2.4 and 2.6.

Using many animals per treatment, replicating treatments several times, and using uniform animals increase the probability of finding real differences when they exist. Statistical analysis allows more valid interpretation of the results, regardless of the number of animals. In all the research reported herein, statistical analyses are included to increase the confidence you can place in the results.
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