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Food Safety of 'Organic,' Conventional Beef Not So Different, Ohio State Study Finds

WOOSTER, Ohio -- Consumers who buy ground beef labeled as "raised without antibiotics" don't always get what they bargained for and likely paid a higher price.

A study conducted by Ohio State University food-animal health researcher Jeff LeJeune found similar numbers of food-borne pathogens and antimicrobial-resistant bacteria in samples of ground beef from conventionally reared cattle and from those whose labels claimed to have come from cows that didn't receive any antimicrobial agents.

"At the microbiological level, there was little difference between both sample groups as far as

presence of pathogens or resistant organisms," said LeJeune, a scientist with the Food Animal Health Research Program (FAHRP) on the Ohio Agricultural Research and Development Center's (OARDC) Wooster campus. "It's incredible how close these numbers came out."

LeJeune analyzed 150 ground-beef samples (77 conventional, 73 antibiotic-free) bought at retail stores in Ohio, Florida and Washington, D.C. between Jan. 1 and Feb. 28, 2003. While some samples were frozen and others fresh at the time of purchase, all of them were frozen at minus-20 degrees Celsius prior to testing to ensure a uniform analysis; freezing can damage bacteria and result in a lesser pathogen count.

The beef was tested for coliforms, *E. coli*, *E. coli* O157, Shiga toxin 2-producing *E. coli*, *Salmonella*, and vancomycin-resistant enterococci. The results: 75.3 percent of conventional beef and 75.3 percent of antibiotic-free beef was contaminated with coliforms; 32.5 percent of conventional and 31.5 percent of antibiotic-free had *E. coli*; and 8.2 percent of conventional and 3.8 percent of antibiotic-free tested positive for Shiga toxin 2-producing *E. coli*.

Although the numbers vary somewhat, the differences are within the margin of sampling error, LeJeune said.

The level of contamination increased when the meat was cultured in a liquid medium overnight to detect even very low numbers of bacteria that may be present. Still, the difference between beef from conventional and antibiotic-free cattle

was still minimal - 87 percent and 89 percent had coliforms, and 77.9 percent and 76.7 percent had *E. coli*, respectively.

No *E. coli* O157, *Salmonella* or vancomycin-resistant enterococci were present in any of the 150 samples.

LeJeune said the percentage of contamination and concentration of coliforms found in this study are similar to those reported in the Nationwide Federal Plant Raw Ground Beef Microbiological Survey of 1994. By contrast, *E. coli* contamination in this study was detected in only half as many samples as reported 10 years ago, and *E. coli* concentration among positive samples was lower than in the federal survey.

"This data suggest that the magnitude and frequency of contamination of ground beef with *E. coli* has decreased over the past decade, possibly due to the proactive efforts of the processing industry to control microbial hazards," he pointed out.

Less *E. coli* in ground beef is good news. But the presence of antimicrobial-resistant bacteria, especially in the meat from cattle not fed antibiotics, is less encouraging.

Cattle in 83 percent of U.S. commercial feedlots routinely receive antibiotics for disease prevention and growth promotion during the finishing period. This practice, however, has been linked to the development of resistant bacteria, which can be transmitted through food and sicken people with infections that are more difficult or impossible to treat with those same antibiotics.

In the meantime, beef grown without antibiotics is being promoted as less likely to be tainted with antimicrobial-resistant bacteria, and a growing number of consumers are willing to pay higher prices for this assurance.

But LeJeune's research shows that at the grocery store, ground beef by any other name can still carry antibiotic-resistant bacteria.

LeJeune cultured the same 150 beef samples looking for resistance to 11 antibiotics commonly used in cattle. Again, the difference between

conventional and antibiotic-free beef samples was not significant. For example, bacteria resistant to tetracycline -- one of the most commonly used antimicrobial agents in cows -- was found in 18.2 percent of the conventional samples and in 19.2 percent of the antibiotic-free samples. Resistance to the antibiotic ampicillin was detected in 44.2 percent and 32.9 of the samples, respectively.

No data on resistant organisms is available from 1994 to know whether these numbers have increased, decreased or remained constant.

"The question is, why are they the same?" LeJeune said. "If the subtherapeutic (growth-promoting) use of antimicrobial agents is the sole driving force for the emergence and persistence of antimicrobial-resistant bacteria in the food supply, one would expect to find fewer antimicrobial-resistant counts in meat derived from cattle raised without the use of antibiotics for growth promotion."

The answer probably lies elsewhere, LeJeune said. Dissemination of antimicrobial-resistant bacteria from farm to farm can occur, possibly through contaminated feed, wildlife and other environmental sources. Cross-contamination can also take place during slaughter and processing.

"Meat is sterile in the cow," LeJeune explained. "The majority of coliforms and *E. coli* that contaminate cuts of beef do not necessarily originate directly from the intestinal tract of the animal from which the carcass is derived. But contamination from other carcasses being processed or processing equipment such as grinders and knives contributes significantly to the spread of bacteria. So if you slaughter and process conventionally reared animals and animals raised without antibiotics in the same place, cross-contamination can easily occur."

LeJeune said that raising cattle without the use of antibiotics will not by itself solve the problem of antimicrobial-resistant bacteria in beef.

"From a food safety perspective," he said, "taking away those antibiotics is not going to make a difference unless there is a concerted effort to minimize the spread of resistant bacteria among

live animals and reduce bacterial cross-contamination during slaughter and processing."

Food-borne pathogens cause an estimated 76 million cases of illness each year in the United States. Although most bacterial contaminants found in ground beef and other meat products can be destroyed by adequate cooking, 30 percent of Americans eat undercooked hamburger.

LeJeune's study was published in the July 2004 issue of the Journal of Food Protection.

OARDC is the research arm of Ohio State's College of Food, Agricultural, and Environmental Sciences.

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Monitoring Energy Balance in Transition and Early Lactation Cows

Retained placenta, metritis, ketosis, cystic ovarian disease, delayed conception, impaired neutrophil function, and mastitis have all been associated with negative energy balance in late gestation and/or early lactation. Energy balance has historically been monitored by daily observation of feed intake, manure consistency and volume, behavior, abdominal fill, body condition, and milk production. These were all accomplished by close observation by the producer, and were considered a part of good stockmanship. As herds increase in size, there is less opportunity to closely follow cows. Larger cow groups, loose housing, and reliance on hired labor contribute to difficulty in monitoring individual cows. Failure to recognize excessive energy deficit in a proportion of cows may lead to epidemic disease and a herd production breakdown.

One goal of production medicine is to quantify subclinical disease. Quantification is accomplished by using a test or set of tests, or monitors. Monitoring often relies on diagnostic tests to identify subclinical conditions affecting the herd or group. Monitoring programs are often formulated with the objective of assessing overall herd status. Therefore, monitors are taken from a set of target animals to allow measurement and assessment of the "control" the farm has over the parameter of interest. Control generally implies that some (high) proportion of cows is in some predetermined range compatible with acceptable production.

Monitoring Prepartum Energy Balance

The role of prepartum energy balance in promoting health and production of postpartum cows is well established. Energy deficits commonly occur near calving as a result of the well described decline in periparturient dry matter intake. The objective is to manage cows so that they cope well with this metabolic state. Maximizing dry matter intake of late gestation cows has been considered essential to minimize negative energy balance and associated postpartum health problems. However, recent debate has questioned whether the real need is to maximize dry matter intake or minimize relative intake depression in the dry period.

In any case, monitoring dry matter intake of dry cows on the farm remains difficult. Dry cow groups can be small and facilities marginal. Feed refusal is often not measured. Dry cow groups are dynamic, with cows entering and exiting with some frequency. This makes calculation of DMI difficult, since the denominator (number of cow-days) over time is not accurately computed. Additionally, pen averages may be deceiving, as some cows may be below average, while others are well above.

Body condition scoring is a useful long-term monitor of energy balance in cows, and correlates well to body fat. The 5 point BCS scale equates to about 75 lbs of empty body fat/BCS unit. However, due to short duration, it is unlikely that there will be any substantial change in BCS with each cow observed within the dry

period, unless extreme environmental conditions are present.

Currently, there is high interest in using serum non-esterified fatty acids (NEFA's) to monitor energy balance in dry cows. NEFA's are the fatty acid side chains derived from adipose fat stores. Serum NEFA levels reflect lipolysis, so they are a measure of energy balance at that time. Higher NEFA levels indicate mobilization of fat stores. Due to the decline in DMI, it is expected that late gestation cows will mobilize body fat stores. Of the metabolic measures examined (glucose, liver enzymes, urea, and ketone bodies) NEFA has shown the most utility to monitor prepartum energy balance.

Cows at risk of postpartum energy balance related health conditions tend to exhibit increased NEFA, compared to unaffected herd mates. It has been shown that cows with high prepartum NEFA levels are at increased risk for displaced abomasums (DA), and have decreased milk production. Recent work indicates that NEFA can be used to separate higher risk cows starting about 14 days prepartum. Surveys in Canadian dairy herds have indicated that about 15% of cows sampled between 2 and 14 days before calving had NEFA levels above 0.5 mEq/l. Including cows that calved within 2 days of sample acquisition, the prevalence NEFA levels > 0.5 mEq/l was 19%. Therefore, a suggested goal is to have no more than 20% of cows above 0.5 mEq/l, when sampled between -14 days and calving. A cut point of 0.4 mEq/l has been proposed by others. Compared to 0.5 mEq/l, the 0.4 mEq/l cutoff has lower specificity, resulting in a higher apparent prevalence of negative energy balance due to more false positives.

Process control charts have been suggested to aid interpretation of NEFA testing. They help to identify when a single sampling time result differs from a set goal (in this case 20%). Obviously, if one samples 10 cows and finds 9 above 0.5 mEq/l, there is something wrong that needs immediate investigation. But if one finds 4 of the 10 above 0.5 mEq/l, does that result alone suggest an immediate need to investigate? What if nothing is done, but the next month 4/10 are again above 0.5 mEq/l? Is the 2 month evidence

suggestive that we are "out of control" relative to our goal of 20%?

An example follows. Assuming that 10 cows are measured each time (i.e. a 240 cow herd visited monthly), then we can be about 95% sure the proportion of cows with high NEFA's is greater than 20% if:

any sample time reveals ≥ 5 of 10 cows > 0.5 mEq/l, or

2 consecutive times show ≥ 4 of 10 cows > 0.5 mEq/l, or

3 consecutive times show ≥ 3 cows > 0.5 mEq/l, or

6 or more times where ≥ 2 of 10 are > 0.5 mEq/l.

Previous work in Michigan has indicated that cows with NEFA levels ≥ 0.3 mEq/l between 3 and 35 days prepartum had a 2 fold increase in DA. The results of this study compare well with those of the previous Canadian study. Generally, the recommendation is that if dry cows are sampled between -30 to -14 days, then NEFA's should be below 0.35 mEq/l.

Urine or milk tests to detect ketone bodies have been investigated as a monitor of prepartum energy balance. While NEFAs are a direct measure of adipose tissue breakdown, ketone production is an indirect measure. Presumably, adipose tissue breakdown precedes ketone body formation, and cows can be in a negative energy balance state and not be ketotic.

To date, ketone tests, including urine dipsticks, have not been found to be particularly useful to monitor negative energy balance in the prepartum period.

Measures of Postpartum Energy balance

In distinction to prepartum, postpartum cows are expected to mobilize fat to produce milk. Therefore, NEFA's will be high in all healthy, productive postpartum cows. Postpartum, energy related disease is not regarded to be present unless cows enter the ketotic state. In contrast to prepartum, energy status postpartum is assessed using measures of ketone bodies. A

recent multi herd survey indicated that 20% of cows were affected with subclinical ketosis. This is probably an underestimation of the incidence of subclinical ketosis, since many cows may have short periods of subclinical ketosis that are not detected.

There are 3 principle ketone bodies. They are acetoacetate, acetone, and beta-hydroxybutyrate (BHB, also referred to as beta-hydroxybutyric acid or BHBA). Acetoacetate is formed in the liver and exported to tissues through blood. Levels increase dramatically in the ketotic state. It is not stable in tissues or in diagnostic samples, and readily decomposes to acetone and CO₂. There is good correlation between acetoacetate and acetone level in blood. Acetoacetate and acetone are found in the urine concentrated several times over blood or milk levels.

BHB is the predominate ketone in blood. Milk BHB levels are much lower than blood levels, but both are directly correlated. BHB, because it is stable, is an excellent direct measure of ketone status of an animal. The mammary gland extracts BHB so that mammary vein BHB levels may be less than jugular vein samples. Diurnal variation is present with BHB, with peak levels occurring 4 hours post-feeding, due to rumen production of BHB. The significance of diurnal variation in cows affected with clinical ketosis is questionable, but it may be a consideration when collecting samples for monitoring subclinical ketosis in the herd.

Postpartum serum BHB level is useful in diagnosing cows with subclinical ketosis, and has become the gold standard. Serum BHB levels of $\geq 1400 \mu\text{mol/L}$ (equivalent to 14.4 mg/dl) have been considered diagnostic for ketosis and have been associated with increased odds of DA when measured at postpartum week 1 (3X odds of subsequent DA) and when measured at postpartum week 2 (8X odds of subsequent DA). Measurement of serum BHB can be performed at reasonable cost at many diagnostic labs. As an alternative, a BHB semi-quantitative cowside milk dipstick test (KetoLac) is available in Canada. This test has been shown to correlate well with serum BHB. The optimal cutoff point for diagnosis of subclinical ketosis using the

KetoLac was reported to be $\geq 100 \mu\text{mol/L}$, and had reasonable sensitivity (73%) and good specificity (93%) compared to blood BHB. KetoLac sticks can be obtained from the Canadian distributor CDMV by calling 450 771-2368. The cost per test is currently approximately CA\$2.20, and the minimum purchase is 300 tests (www.cdmv.com).

Recently, the use of urine Ketostix in diagnosing subclinical ketosis has been reported. Urine ketone levels in samples collected between 2-15 day postpartum compared favorably with serum BHB (the gold standard). Dipstick readings \geq "small" were 78% sensitive and 96% specific, in identifying cows with serum BHB $\geq 1400 \mu\text{mol/L}$. Use of KetoCheck powder for determination of ketone level in "bucket" milk resulted in low sensitivity, and did not appear to be a viable diagnostic for subclinical ketosis. Results from this report differ from previous reports that indicated urine dipsticks were of limited value in diagnosing subclinical ketosis. Differences in studies may have arisen as a result of different dipstick methodology or procedures. Use of Ketostix and other "dipsticks" require attention to detail and prompt interpretation, as color changes progress with time.

First test milk component analysis has been used as a measure of fresh cow energy balance. Cows at increased odds of a DA up to 3 weeks postpartum had a first test fat:protein ratio of 1.4 or greater (equivalent to a protein:fat ratio of 0.71). Therefore, if fat % is more that 1.5 times the protein percentage, cows may be in negative energy balance and may be at increased risk of DA. Unfortunately, this measure has low sensitivity (58%) and specificity (69%), which greatly limits its utility.

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The variability in the interval between estrus and ovulation in cattle and its determinants

Over the years there has been a reduction in fertility of Holstein cows and some minor reduction in Holstein heifers. One of the possible reasons for this reduction in fertility may be due to the chronology of events leading to ovulation. It is possible that this process is different in cattle nowadays compared to cattle used in previous studies. If this is the case, there could be implications on the time in which artificial insemination (AI) takes place. The use of the AM/PM rule for AI makes the assumption that the time between estrus and ovulation is somewhat constant among all cattle. A study was performed in France to examine the variability in the interval between estrus and ovulation in cattle and its determinants. This study used two experiments to examine the relationships among estrus behavior, follicular growth, hormonal events, and time of ovulation. Some of the methods used in these experiments are not approved or appropriate for use in the United States; however, the results and findings are still very applicable.

Both experiments used Holstein cows and heifers housed in a free stall barn and fed a balanced ration. The Crestar method without eCG was used for estrus synchronization. This method consists of an ear implant of progestagen for 10 days, and an injection of estradiol valerate and a progestagen at the time of implant insertion. A luteolytic dose of prostaglandin was injected 2 days prior to implant removal. Animals were observed for standing heat every 4 hours following implant removal. In experiment 1, 12 cyclic heifers were treated twice with 2 weeks between treatments. Their ovaries were scanned (real-time ultrasonography) daily and every 4 hours from the time of observed estrus until ovulation. Images were recorded onto videotape and the growth of follicles larger than 5 mm were followed. Ovulation was determined to have occurred at the midpoint of the last observation of a large follicle and the time when the follicle was no longer detected. Blood samples were

obtained every 4 hours between implant removal and ovulation. In experiment 2, two groups of cows (15 and 20) and 12 pubertal heifers were used. Blood samples were collected every 4 hours between 12 and 68 hours after implant removal for LH assay in 2 groups of cows (3rd month of lactation) and 12 pubertal heifers. Blood was taken on the day of estrus, day 7, and day 14 for a progesterone measurement in order to assess the presence of a functional CL.

In experiment 1, all heifers displayed estrus, had an LH surge, and ovulated. The results showed that the intervals between estrus & ovulation, estrus & LH peak, estrus & estradiol peak, and LH peak & ovulation were positively correlated. The variability in the intervals from estrus to LH peak (80.6%) and from LH peak to ovulation (68.7%) explained the majority of the total variability in the interval from estrus to ovulation.

The variation in the interval between estrus & LH peak was positively correlated to the number of follicles larger than 5 mm at implant removal and negatively correlated to the size of the preovulatory follicle. The interval from estrus to ovulation was also positively correlated with the number of follicles larger than 5 mm at implant removal, and negatively correlated with the size of the preovulatory follicle. In experiment 2, 12 of 15 cows displayed estrus and 13 of 15 had an LH surge. In the 2nd group, 18/20 cows showed estrus, but all had an LH surge. All heifers showed estrus and had an LH surge. The LH peak occurred before estrus in 7.7% of the heifers and 43.3% of the cows. The duration of the interval between estrus and the LH peak was longer in heifers than in cows, but the variation was higher in cows than in heifers.

The results concerning the hormonal patterns and sequence of events were similar to previous findings, but the intervals between the onset of estrus or the LH surge and ovulation were longer than those reported in previous findings. The interval between the onset of estrus and ovulation was more variable than the interval between the LH peak and ovulation. The duration of the intervals between estrus to LH peak and LH peak to ovulation may be related to ovarian function. The researchers indicate that the time and magnitude of the preovulatory surge

of LH are regulated by factors associated with follicular growth. The results also suggest that the time of occurrence of ovulation after the LH peak may be regulated by these factors. The implications are that techniques to control follicular waves could be beneficial to improve fertility in timed AI. Since the interval between estrus and the LH surge causes the main variability between estrus and ovulation, there could be fertility benefits associated with synchronizing the LH surge when using timed AI. This study showed that the overall interval between estrus and ovulation is longer than previously reported, which may indicate that late ovulation is more frequent today. These findings need to be confirmed on a larger number of animals from studies in which the females are not synchronized, or have been synchronized by different treatment modalities.

Saumande J. & Humblot P. (2005). The variability in the interval between estrus and ovulation in cattle and its determinants. *Animal Reproduction Science*, 85, 171-182.

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Poultry Health Management School

Ohio State University Extension in collaboration with Michigan State University, Purdue University, the University of Wisconsin, and Penn

State University are pleased to announce the 2005 Poultry Health Management School. This year's school will be held at Michigan State University in East Lansing, Michigan and will focus on poultry management strategies for each of the three major production species. The Turkey & Broiler School will be held on May 9-10 and the Layer School will be held on May 11-12. The Schools are designed to provide continuing education through in-depth lecture presentations and hands-on training to those individuals working in the poultry industry. For more information, please contact Mr. Jeff Workman, Registration Coordinator, at (614) 292-9453 or workman.45@osu.edu



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On-Line Newsletter

The Veterinary Extension unit at The Ohio State University first published the *Ohio Veterinary Newsletter* in 1974 to relay current information of interest to Ohio veterinary practitioners in food animal and general practice. This is our first "on-line only" newsletter. We are currently updating our e-mail list and will send an electronic link whenever a new issue is posted to our website. Those without e-mail access who would like to continue to receive a paper copy by mail may subscribe for a fee of \$10 per year. If you have any further questions, please do not hesitate to contact Mr. Jeff Workman, Extension Program Assistant; or Dr. Teresa Morishita, Extension Unit Coordinator.

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