A Rapid Screening Test for Hydrogen Peroxide Production by Lactobacilli

D. R. Martin and S. E. Gilliland

A dilution of a broth culture of *Lactobacillus lactis* was evenly spread with a sterile hockey stick on the surface of 15 ml of MRS lactobacilli agar (Difco Laboratories) containing 0.1 ml of peroxidase (0.2 mg/ml) and 0.1 ml of o-tolidine (20 mg/ml). Peroxidase in the presence of the chromogen o-tolidine reacts with hydrogen peroxide to produce a color change in the chromogen. It was assumed that any peroxide metabolically produced by the lactobacilli during colony formation on the agar medium described above would produce similar color changes. The plates were incubated 24 hr at 37 C. Three colony types were selected for isolation from the plates; those with no color zones surrounding them and those with intermediate and large brown zones.

The isolated cultures of lactobacilli were tested for the ability to produce peroxide in refrigerated sterile 10 percent NFMS (Gilliland and Speck, 1975). The cells in 10 ml of MRS broth were harvested by centrifuging in sterile centrifuge tubes at 12,000 x g for 10 min at 2 C. The pellet from each was resuspended in 5 ml of cold sterile 10 percent NFMS and transferred to a 50 ml Erlenmeyer flask containing 20 ml of cold sterile 10 percent NFMS. The flasks containing the samples were incubated for 22 hr at 5 C on a platform shaker to ensure continuous mixing during incubation. Hydrogen peroxide was measured by an enzymatic method described by Gilliland (1969). The number of viable organisms in the test cultures were determined at 0 hr and 22 hr by plating on MRS agar.

More peroxide was produced by the cultures isolated from colonies which produced the larger zones on the "peroxidase agar" than in those with smaller zones. The numbers of viable lactobacilli remained constant over the 22 hr period at 5 C. *L. lactis* cultures do not grow at 5 C and thus do not produce appreciable acid. The peroxide produced by the lactobacilli added to refrigerated foods can inhibit psychrotrophic spoilage organisms (Gilliland and

Speck, 1975). The "peroxidase agar" test described herein could be used as a rapid screening test for selecting cultures for preparing frozen concentrated cultures to be used for such control of psychrotrophic microorganisms in refrigerated food.

Literature Cited

Gilliland, S. E. 1969. Enzymatic determination of residual hydrogen peroxide in milk. J. Dairy Sci. 52:321.

Gilliland, S. E. and M. L. Speck. 1975. Inhibition of psychrotrophic bacteria by lactobacilli and pediococci in nonfermented refrigerated foods. J. Food Sci. 40:903.

MEAT and CARCASS EVALUATION

Conditions Associated With Net K^{40} Counting Using Animal Phantoms

D. D. Johnson, L. E. Walters, R. R. Frahm, R. D. Morrison and B. Lambert

The principle of K^{40} whole-body counting is currently being used at the Oklahoma Agricultural Experiment Station to evaluate both beef cattle and market weight swine. Previous studies at this station have shown that this method can be used to predict the lean body mass in both species, to within ±9 lb of fat free lean for beef cattle and ±2.5 lb of fat free lean for swine. These studies emphasized the need to identify and adjust for sources of variation in K^{40} counting where possible. Also these experiments brought to light other sources of variation not heretofore identified for which adjustments should be made in order to maximize the accuracy of the whole-body counting principle, especially where differences in live weight occur.

Several techniques to improve the relationship between net K^{40} count and lean muscle mass in live animals are presently being used. These techniques include washing the animal prior to counting for the purpose of removing fallout residue and foreign material high in potassium, in an effort to reduce animal contamination. Secondly, animals are held off feed for 24 hr prior to counting to adjust for fill. In addition, instrument fluctuations are continuously monitored by the use of a standard (known) reference source of radiation. This reference source is a container filled with potassium chloride which has been used for this purpose for an extended period of time.

Two other variables which until recently have been most difficult to identify and adjust for are (1) self-absorption and (2) background depression. *Self-absorption*, which is the scattering and absorption of radiation originating from the object being counted has been shown to be primarily associated with the weight (mass) of the animal. Such a phenomenon has been demonstrated in non-living masses called *phantoms* which are used to simulate animals. This condition occurs as the result of the inability of a certain part of the radiation originating within the animal or object to be counted, thus the term self-

absorption. For example, as animals increase in weight, it appears that some of the radiation may travel a distance great enough to increase its chance of being absorbed by body tissues and thus is unable to reach a detector and to be considered in net K^{40} count.

Background depression is attributed to the absorption of environmental radiation by the object being counted. It is believed that self-absorption and background depression contribute to an underestimation of lean body mass in larger, heavier animals.

A study was undertaken in an effort to more thoroughly understand and identify the effects of self-absorption and background depression in the K^{40} whole-body counting procedure by the use of animal phantoms. These animal phantoms were constructed of one gallon and one quart plastic containers which were filled with a water solution of potassium and sodium chloride. The dimensions of each phantom were selected to approximate the length, width, and height dimensions of similar weight bred gilts involved in a companion nutrition study. Five phantoms: 200, 260, 320, 380 and 440 lb respectively were constructed by arranging the above mentioned containers in multiple layers placed on a mobile dolly, resembling the general shape of animals of these corresponding weights.

Each of these five phantoms weights were constructed using three concentrations of potassium, designated "high", "medium" and "low". The medium concentration was prepared to approximate the amount of potassium in the body of an "average" or "typical" bred gilt, where as the high concentration more closely approximated the amount of potassium expected in a very lean, heavily muscled bred gilt, and the low concentration approximated the amount of potassium in a fatter, lighter muscled bred gilt. The desired density of the solution (1.04 g/ml) was prepared to correspond with that of a typical gilt and was accomplished by adding specific amounts of sodium chloride in accordance with the concentration of potassium in the phantoms.

Mean counting efficiencies for each concentration and each weight are presented in Figure 1. These values represent the average of eight counts for each weight and each potassium concentration. Counting efficiency was calculated by dividing the mean net count of the phantom by the total counts possible from the known quantity of potassium in the phantom. These data indicate that as weight increases, counting efficiency decreases. This suggests that as the animals' weight increases there is a tendency to underestimate the amount of potassium in the animals' body by the K^{40} counter and therefore to underestimate the lean body mass of the animal. From these data prediction equations will be developed which will adjust for this decrease in counting efficiency.

These experiments using phantoms ranging in weight from 200 to 440 lb constitute the forerunner to another study currently being initiated using heavier weight phantoms corresponding in weight with yearling beef bulls

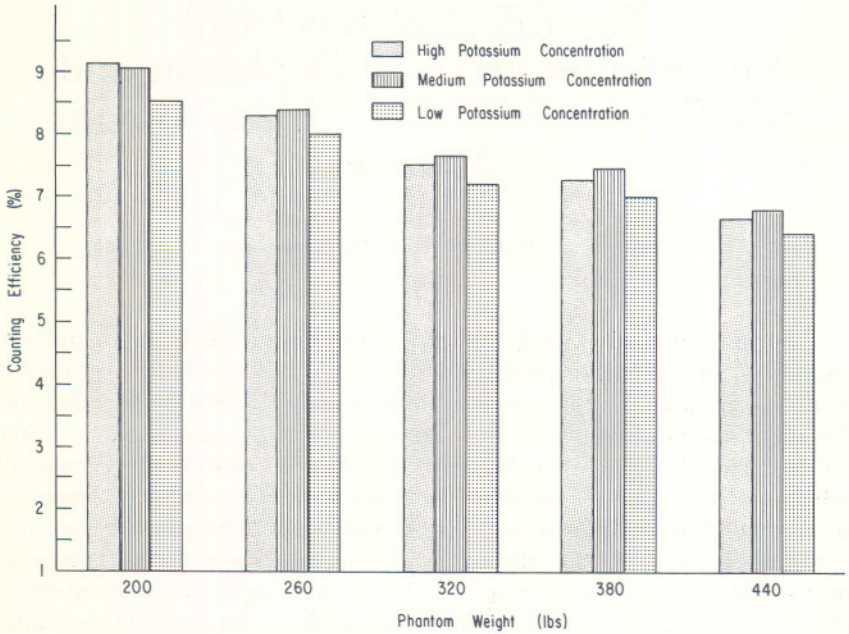

Figure 1. The relationship between counting efficiency and phantom weight at three potassium concentrations

ranging from 900 to 1300 lb. The prediction equation currently in use for the evaluation of beef bulls was developed from bulls weighing under 1000 lb. With new and meaningful information relating to the effects of weight on counting efficiency, it will be possible to more accurately evaluate beef bulls for lean content whose weights are heavier than those from which the present prediction equation was developed.

The Effect of Electrical Stimulation on ATP Depletion and pH Decline in Delay Chilled Bovine Muscle

P. A. Will and R. L. Henrickson

For optimum processing efficiency a carcass should be fabricated immediately after being dressed. However, there are metabolic activities in muscles which should proceed while the muscle remains on the skeleton. Recent research dealing with the removal of muscle systems before initial chilling of the bovine carcass has suggested that this process offers economic advantages to the meat industry. The application of an electrical current to freshly slaughtered beef carcasses has been shown to induce an increased rate of glycolysis, and to reduce the time for the onset of rigor mortis. This rapid onset of rigor mortis has great protential for preventing the detrimental effects of cold and thaw shortening and permits muscles to be removed soon after slaughter.

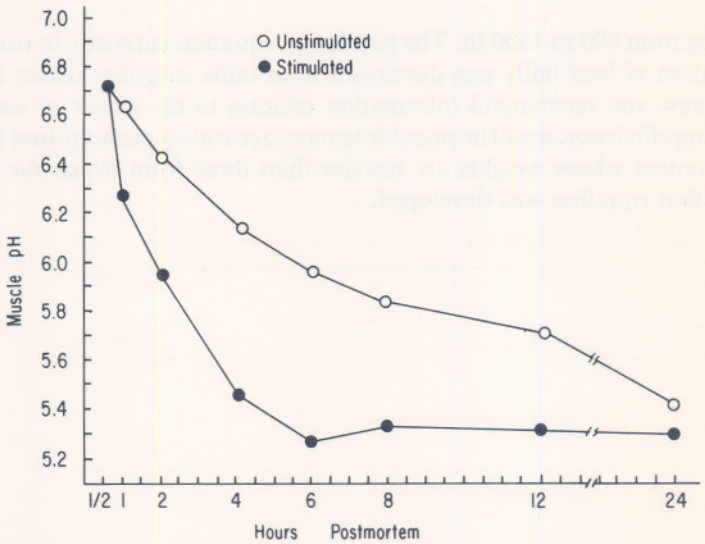

Figure 1. Effect of electrical stimulation on pH decline

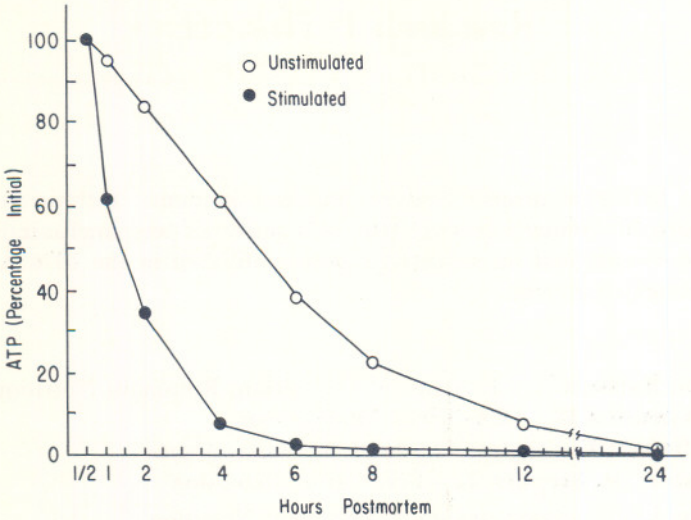

Figure 2. Effect of electrical stimulation on ATP depletion

This study was undertaken to assess the effectiveness of electrical stimulation as a means of speeding postmortem metabolism as measured by ATP (adenosine triphosphate) depletion and pH decline in delay chilled bovine carcasses.

Six animals of similar weight and age were used in this study. Electrical stimulation was initiated 30 min post mortem. The stimulated side received a square wave pulse of 300V., 400c/s with a duration of 0.5 msec and a current of 1.9 amps for a period of 15 min, while the control side received no electrical stimulus. ATP and pH measurements were taken at eight time periods. (0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 24.0 hr) postmortem. Muscles from the electrically stimulated sides of beef exhibited significantly faster reductions of ATP and pH than unstimulated controls (Figures 1 and 2).

Research Personnel

This is a listing of project leaders, graduate students, technicians and herdsmen in the Animal Science Department and other personnel as indicated who have co-authored the research reports published in the 1978 Animal Science Research Report.

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