NUTRITIONAL REGIME, POST-SLAUGHTER CONDITIONING TEMPERATURE AND VACUUM PACKAGING EFFECTS ON CARCASS AND INSIDE CHUCK BACTERICLOGY

by

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B.S., Oklahoma State University, 1974

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

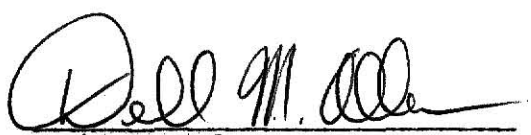
MASTER OF SCIENCE

Department of Animal Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

Approved by:



Major Professor

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ACKNOWLEDGMENTS

The author wishes to extend his sincere appreciation to Dr. Dell M. Allen, major professor for his guidance and encouragement during this study.

Appreciation is also extended to Dr. Melvin C. Hunt and Dr. Curtis L. Kastner for their guidance and assistance in sample collection and to Dr. George Milliken and Steve McGuire for their assistance in the statistical analysis of the data.

The author is grateful to fellow graduate students Mary Smith, Chainarong Kuntapanit, and George Gutowski for their aid in data collection.

The author extends very special appreciation to his wife Cathy, daughter Angela, and parents, Mr. and Mrs. Ralph L. Thomas, for their understanding and encouragement throughout his academic career.

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REVIEW OF LITERATURE

Growth Requirements of Microorganisms

Aerobic microorganisms can grow in an environment which provides: 1.) a supply of available nutrients; 2.) oxygen; 3.) water; and 4.) suitable pH and temperature.

Temperature

While most microorganisms can grow in a wide temperature range they can be broadly divided into 3 groups: psychrophiles, which grow from 20 to 30°C; mesophiles, 25 to 40°C; and thermophiles, 45 to 60°C (Lawrie, 1966 and Lechowich, 1971). Vanderzant and Johns (1972), define bacteria which can survive exposure to temperatures considerably above their maximal optimum temperature range for growth as themoduric and those that grow relatively rapidly at 7°C and below as psychrotrophic.

Meat is stored at low temperatures to control microbial growth (Lawrie, 1966). Stokes (1960) found that psychrophilic growth increases with increasing temperature. Cell numbers were maximum in 3 days when incubated at 20°C while it took 60 days at 0°C, even though larger final cell populations were obtained at the lower temperature ($\log_{10}$ 9.27 per ml vs 8.99 per ml). However, normally meat stored at low temperatures will have a longer shelf-life and lower microbial counts than meat stored at higher temperatures for a comparable time period. (Gardner, 1965; Birmingham *et al.*, 1966; Butler *et al.*, 1953; Rey *et al.*, 1970; Allen and Foster, 1960).

Controlling product temperature will influence types of microorganisms that can grow on it. Kirsch *et al.* (1952)

found most of the isolates from refrigerated ground beef were Pseudomonas and Achromobacter. Ayres (1960) held ground beef at 10°C and found mostly Psuedomonas. Other psychrotrophic bacteria associated to a lesser degree with spoilage of fresh, refrigerated meat are Achromobacter, Flavorbacterium, Lactobacillus, Microbacterium, and Micrococcus (Lechowich, 1971). The mesophilic and thermoduric microorganisms of major concern are the potential pathogens, e.g. Salmonella, Clostridium perfringens, Staphylococcus, and Clostridium botulinum (Lechowich, 1971).

Oxygen

Since oxygen availability can influence which types of microorganisms will grow, meat stored under vacuum will develop a different flora than meat stored in presence of oxygen (Pierson et al., 1970; Seideman et al., 1976). Microorganisms are divided into aerobes, which must have oxygen for growth; anaerobes, which grow only in absence of oxygen; and facultative, which grow in presence or absence of oxygen (Lawrie, 1966; Lechowich, 1971).

pH

Optimum pH for most microbial growth is 7 but some function best at a lower pH (Lawrie, 1966). Most microorganisms can grow within a range of pH 5 to 8, (Lechowich, 1971). Bacterial decomposition is slower in meat having a pH 6 or lower than in meat approaching neutrality (Brandly et al., 1966).

Water Activity

Most spoilage microorganisms require a high water

coefficient of water activity (a_w) for growth (Lechowich, 1971). Ayres (1965) noted that fresh meat has a a_w of 0.99. The a_w of pure water is 1.00. He also stated that most spoilage bacteria will not grow at a a_w of less than 0.96. However, fresh meat stored at this low a_w would be badly dessicated (Ayres, 1965).

Effect of Microbial Growth on Carcasses and Cuts

Microorganisms growing on fresh meat produce mainly slime, odor and color deterioration (Forrest et al., 1975). Ayres (1960) found the critical microbial number for slime production on refrigerated beef was $6x10^7$ organisms per cm^2 and Kraft and Ayres (1952) found definite odors when counts reached 10^7 per cm^2. Marriot et al. (1967) found increased microbial growth enhances meat discoloration. Product wholesomeness can also be affected with considerably fewer microorganisms if any are potential pathogens (Kastner et al., 1976). This obviously limits shelf-life of fresh meat and emphasizes the need to market fresh meat before spoilage problems arise.

Microorganisms act on fresh meat through carbohydrate, fat and protein metabolism (Lechowich, 1971). Off-odors, discoloration and slime production are the resultant end-products of this metabolism.

Microbial Carbohydrate Metabolism

Carbohydrate metabolism can occur aerobically, facultatively or anaerobically. Aerobic microorganisms metabolize carbohydrates to carbon dioxide and water. Facultative microorganisms

produce lactic, acetic or other organic acids (Lechowich, 1971). Under anaerobic conditions, two types of reactions can occur. Homofermentative organisms produce lactic acid while heterofermentative organisms produce equamolar amounts of lactic acid, ethyl alcohol and carbon dioxide (Stokes, 1960). Since little carbohydrate is present in meat (Merkel, 1971) these reactions yield only small amounts of undesirable end products. However, microorganisms can obtain some energy for growth by utilizing available carbohydrates (Lechowich, 1971).

Microbial Fat Metabolism

Microbial fat metabolism can occur by hydrolases or oxidases (Lechowich, 1971). Enzymatic oxidation yields peroxides which can be further degraded to aldehydes, ketones, carbonyls and condensation products of these compounds. These compounds give rise to the combination of flavors and odors known as rancidity. Generally, as rancidity increases, the microbial population decreases, because some products of fat metabolism are inhibitory to many microorganisms (Lechowich, 1971). Since most fat oxidation is non-enzymatic and non-microbial (autoxidation) and is due to O_2 in air reacting with fat under influence of heat, light, high energy radiation and various pro-oxidant catalysts (Dugan, 1971) rancidity is not normally a microbial problem.

Microbial Protein Metabolism

The most important reactions occuring in microbial spoilage of meat are metabolism of proteins. Stokes (1960) reported that microbial protein metabolism results in several reactions. Lechowich (1971) reported these reactions to be proteolysis, amino acid decarboxylation, amino acid deamination and metabolism

of specific amino acids. End products of these reactions are soluble peptides; carbon dioxide and amines; ammonia and fatty acids; hydrogen sulfide; mercaptans and indole, respectively. These end products generally have disagreeable odors, and overall breakdown of proteins by bacteria is referred to as putrefaction.

Microbiological Standards for Meat

Microorganisms can cause meat spoilage and render it asthetically unacceptable. Also, pathogens may be present. Because of this, some states and cities, have imposed laws to control sale of meat based on the microbial populations (Johnston, 1975).

Oregon's law (Oregon Administrative Rules 13-286 Meat Bacterial Standards) which became effective May 1, 1973 and is undoubtedly the most controversial law in effect to date, states there shall not be over 5 million microorganisms per gm in fresh or frozen meat and 1 million per gm in cooked, smoked or otherwise heat treated meat. Most probable number of *E. coli* shall not exceed 50 per gm in fresh or frozen meat and 10 per gm in cooked, smoked or otherwise heat treated meat. The state has the power to prosecute on the basis of these microbial standards. First offense usually results in a warning with possible prosecution after second offense.

Rhode Island's law is more moderate (1 billion aerobes and 200 coliforms for raw, fresh ground meats) and offenders cannot be prosecuted on the basis of microbial findings. Memphis, Tennessee has a very broad law, but inspection and microbial

results can be used in prosecution. Many other states, such as Virginia, North Dakota, Maryland, and Massachusetts have microbial standards for meat or are planning such.

Microbial standards for fresh meat are, even today, highly controversial. Weinzirle and Newton (1914) proposed a limit of 10 million microorganisms per gm of hamburger steak, even though they admitted poor agreement between the actual bacterial count and degree of spoilage. Carey (1916) reached a similar conclusion. LeFevre (1917) and Rogers and McCleskey (1957) said bacteria count could be useful in detecting use of improper materials and defects in meat product handling. Le Fevre (1917) proposed a limit of 1 million microorganisms per gm of hamburger steak. Rogers and McCleskey (1957) found determination of coliforms had doubtful significance in ground beef. Baltzer (1969) found that obvious spoilage was present when microbial numbers reached $5x10^7$ or 10^8 per gram, but there was no direct correlation between maximum bacterial count and acceptability. This same author concluded when maximum limit was reached there was still some time before sour or old taste occurred and that total count was useful only when taken as an initial count. Ayres (1960) stated the initial load of organisms determined the time requirement for development of slime and found critical value for slime production to be $6x10^7$ microorganisms per gram. This same worker, (Ayres, 1965) concluded that bacteria could reach 10^9 per gm without being visible to the naked eye. Kotula (1970) said that if meat is not temperature abused much of pathogen danger is avoided.

Methods of Sampling Used to Determine Microbial Populations on Carcasses and Cuts

Standardized microbial sampling techniques have not been established for carcasses or cuts so each worker must choose or develop their own.

Mallman et al. (1958) compared rinse and swab methods for bacterial sampling of poultry and concluded total immersion and rinsing was more accurate. Ayres (1960) used the same methods on refrigerated beef but found swabbing was more repeatable and easier to use. The rinse method was tedious due to the difficulty of obtaining an exact surface area. Berry et al. (1973) compared swab and rinse methods for sampling beef and pork adipose tissue and found the rinse method gave significantly higher results. The swab technique has been used by Stringer et al. (1969) for beef carcasses, by Schmidt and Gilbert (1970) for beef cuts, and Reagan et al. (1971) for lamb cuts. The rinse method has been used by Pierson et al. (1970) for beef cuts and Kastner et al. (1976) for beef carcasses. Templates of various kinds and areas have been used to outline the sample area (Ayres et al., 1950; Ayres and Adams, 1953; Walker and Ayres, 1956; Ayres, 1960; Eklund et al., 1961; May, 1971; Kinsley and Mountney, 1966; Stringer et al., 1969, and Kastner et al., 1976).

Effects of Nutritional Regime and Post-Slaughter Conditioning Temperature on Carcass Microbiology

When animal or carcass handling procedures are altered, differences in microbial growth on meat products may occur. Since procedure changes can effect both number and types of

microorganisms present, it becomes manditory to consider the microbial consequents of any proposed changes. New handling procedures or systems, no matter how desirable, will not be implemented if resultant products are microbiologically unsatisfactory.

Carcass Microbiology

Stringer et al. (1969) reported that Pseudomonas and Micrococcus were the predominant microorganisms on carcasses after chilling. Some Achromobacter, Flavobacterium, Bacillus and Aspergillus niger were also reported. These data support earlier work by Kirsch et al. (1952) who found Pseudomonas and Achromobacter to be predominant on refrigerated fresh beef and Ayres (1960) who found Pseudomonas to be predominant.

Microbial population of chilled beef carcasses slaughtered in a commercial meat packing plant were enumerated by Stringer et al. (1969). They found the $\log_{10}$ of mean carcass population to be 4.70 per 6.45 cm^2 immediately after slaughter and 5.08 per 6.45 cm^2 after an 18 hr chill. Fields et al. (1976) found $\log_{10}$ of mean microbial counts immediately after slaughter to range from 3.6 to 4.1 per cm^2 (4.4 to 4.9 per 6.45 cm^2) and 3.4 to 3.6 per cm^2 (4.2 to 4.4 per 6.45 cm^2) after a 48 hr chill at 0 to 1°C. Log_{10} of mesophilic and psychrotrophic counts ranges from 3.06 to 3.41 per 6.45 cm^2 for carcasses during a 10 hr chill at 2°C (Kastner et al., 1976).

Variations in counts due to carcass sampling location were noted by Stringer et al. (1969) and Kotula et al. (1975). Stringer et al. (1969) found that samples from moist areas (inside neck, exposed area of clod, and exposed muscle above

aitch bone) had higher microbial counts than samples from dryer areas (fat on outside of round or the chine bone area). Kotula et al. (1975) found forequarters more highly contaminated than hindquarters. The most contaminated area of the hindquarter was the outside flank where the $\log_{10}$ psychrotrophic count was 1.9 per cm^2 (2.8 per 6.45 cm^2).

Nutritional Regime

Theoretically differences in microbial growth due to nutrition might occur if there are changes in water activity (Ayres, 1965) or ultimate pH (Brandly et al., 1966), but this is not well documented in the literature.

Fields et al. (1976) reported only small differences in bacterial numbers at 48 hr post-mortem from youthful and mature (cow) beef carcasses. Assuming cows are leaner and fed on a lower plane of nutrition (maintenance vs growth) these data indicate that nutritional regime or fat cover has little effect on carcass microbiology.

Post-Slaughter Conditioning Period

Standard procedure for handling beef carcasses post-slaughter is to chill halves approximately 18 to 72 hr in 0 to 4°C coolers. However, due to the phenomenon of cold shortening (Merkel and Pearson, 1975) and/or possible economic benefits (Henrickson, 1975) considerable interest has developed in holding carcasses at an elevated temperature prior to conventional chilling and processing.

Work supporting the need for rapid carcass chilling is abundant in the literature. Haines and Smith (1933) and Empey

and Scott (1939) (as reported by Stringer et al., 1969), stated that meat should be chilled as rapidly as possible to prevent growth of mesophilic bacteria. Stokes (1960) reported psychrophiles multiply faster at 20°C than at 0°C. Kotula (1970) reported that refrigeration of meat below 10°C will inhibit growth of Clostridium perfringens, Salmonella and Staphylococcus aureus. Kastner et al. (1976) reported that holding carcasses at elevated temperatures could support growth of spoilage and potentially pathogenic bacteria. Rey et al. (1970) found that beef carcasses aged at 16 or 22°C for 2 days then at 2°C for 2 days yielded cuts exhibiting statistically greater mesophilic and psychrophilic counts than carcasses held at 2°C for 4 days. Minks and Stringer (1972) found that cuts taken from conventionally chilled carcasses and aged at 4.4°C for 7 or 15 days had 5.34 times the increase in microbial counts as cuts aged at 0°C for a comparable time. Follett et al. (1974) found mean microbial counts for muscles excised pre-rigor and stored at either -5°C for 12 hr or 5 or 10°C for 24 hr were higher than those for corresponding muscles excised post-rigor (after 36 hr chill at 2 to 3°C) and held at 0 to 1°C.

In contrast to the above data, Bouton et al. (1974) indicated that on mutton carcasses innoculated and held at 0 to 1°C or 7 to 8°C, Pseudomonas and E. coli counts generally decreased at both temperatures over a 24 hr holding period. Fields et al. (1976) comparing conventionally chilled beef halves to halves held at 14 to 19°C for 12, 16, or 20 hr and then chilled at 2°C until 48 hr post-mortem, found significantly

higher microbial counts immediately after the high temperature storage. However, no difference was noted between control and treated halves at 48 hr post-mortem or between steaks removed from all sides immediately after fabrication or after 5 days of display. Kastner et al. (1976) found no significant difference in total aerobic mesophilic or psychrotrophic counts between beef halves held at 16°C for 6, 8 or 10 hr and conventionally chilled (2°C) halves.

Effect of Degree of Vacuum, Atmospheric Conditions and Storage Temperature on Microbiology of Vacuum Packaged Meat

Microbiology of Vacuum Packaged Meat

Warnecke et al. (1966) and Hodges et al. (1974) reported vacuum packaging provided a selective media for bacterial growth. Lactobacillus was the predominant microorganisms after vacuum storage (Jaye et al., 1963; Pierson et al., 1970; Seideman et al., 1976). Seideman et al. (1976) reported that Pseudomonas was only a small fraction of the microflora on vacuum packaged cuts.

Ulrich (1949) stated that vacuum packaging can reduce but not eliminate microbial growth. Many workers have reported fewer spoilage bacteria on vacuum packaged beef than aerobically packaged beef (Pierson et al., 1970; Baran et al., 1970; Minks and Stringer, 1972; Hodges et al., 1974). However, both aerobic and anaerobic counts increase during vacuum storage (Pierson et al., 1970; Baran et al., 1970; Minks and Stringer, 1972; Hodges et al., 1974; Seideman et al., 1976).

Degree of Vacuum

Ledward et al. (1971) and Baran et al. (1970) indicated that oxygen content in a vacuum package must be drastically reduced to prevent aerobic growth. Hodges et al. (1974) concluded that the degree of vacuum regulates growth rate of aerobic microorganisms, and that aerobic growth in vacuum packages could be due to: partial pressure of oxygen in the package, residual air trapped around or in meat, undetectable loss of vacuum, or presence of facultative anaerobes or aerobes. Seideman et al. (1976) found total aerobic psychrotrophic and mesophilic counts under high vacuum (29.4 in Hg) were generally lower after 35 days of storage than from cuts packaged under low (26 in Hg) or intermediate vacuum (28.5 in Hg). They also found aerobic bacterial counts were low (less than 10^4 per 6.45 cm^2) after 7 and 14 days of storage regardless of degree of vacuum.

Atmospheric Conditions

Baltzer (1969) and Hodges et al. (1974) found that anaerobic growth could be enhanced by microbial production of CO_2, which inhibits aerobes. Baltzer (1969) found anaerobiosis was reflected by: slow increase in total counts, souring instead of putrefaction and slime formation, and low final counts. Pierson et al. (1970) attributed the sour flavor of vacuum packaged beef cuts to the presence of lactic acid producing bacteria. A slow increase in total counts was also noted by Baran et al. (1970) who found that aerobic growth became stationary after 6 days vacuum storage. Anaerobes had increased up to 3 days, decreased to 20 days, and then increased.

Temperature

Reagan et al. (1971) reported that vacuum packaging does not compensate for improper refrigeration during storage of lamb. Jaye et al. (1962) found that Lactobacillus were greatly suppressed at 0 to 1°C but grew rapidly at 3°C or higher. Minks and Stringer (1972) found a significantly higher increase in microbial counts of vacuum packaged cuts held at high temperature.

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INTRODUCTION

Theotetically, differences in microbial growth on carcasses of animals fed differing rations might occur if there are changes in water activity (Ayres, 1965) or ultimate pH (Brandly et al., 1966). However, the effect of animal nutrition on carcass or cut microbiology is not well documented.

Holding bovine carcasses at an elevated temperature prior to conventional chilling has been of recent interest due to the phenomonen of cold shortening (Merkel and Pearson, 1975) and/or possible economic benefits (Henrickson, 1975).

Kotula (1970) reported that refrigeration of meat below 10°C will inhibit growth of Clostridium perfringens, Salmonella and Staphylococcus aureus. Kastner et al. (1976) reported that holding carcasses at elevated temperatures could support growth of spoilage and potentially pathogenic bacteria. Rey et al. (1970) found that beef carcasses aged at 16 or 22°C for 2 days then at 2°C for 2 days yielded cuts having statistically greater mesophilic and psychrophilic counts than carcasses held at 2°C for 4 days. Minks and Stringer (1972) found that cuts taken from conventionally chilled carcasses and aged at 4.4°C for 7 or 15 days had 5.34 times the increase in microbial counts as cuts aged at 0°C for a comparable time.

In contrast to the above data, Bouton et al. (1974) indicated that on mutton carcasses inoculated and held at 0

to 1°C or 7 to 8°C, Pseudomonas and E. coli counts generally decreased at both temperatures over a 24 hr holding period. Fields et al. (1976), comparing conventionally chilled beef halves to halves held at 14 to 19°C for 12, 16 or 20 hr and then chilled at 2°C until 48 hr post-mortem, found significantly higher microbial counts immediately after the high temperature storage. However, no difference was noted between control and treated halves at 48 hr post-mortem or between steaks removed from all sides immediately after fabrication or after 5 days of display. Kastner et al. (1976) found no significant difference in total aerobic mesophilic or psychrotrophic counts between beef halves held at 16°C for 6, 8 or 10 hr and conventionally chilled (2°C) halves.

Vacuum packaging of beef wholesale cuts has been widely used in the industry. Warnecke et al. (1966) and Hodges et al. (1974) reported vacuum packaging provided a selective media for bacterial growth. Lactobacillus was the predominant microorganism after vacuum storage (Jaye et al., 1962; Pierson et al., 1970; Seideman et al., 1976). Pseudomonas, the major spoilage microorganism of aerobically packaged or unpackaged fresh meat (Baltzer, 1969; Kirsch et al., 1975), was only a small fraction of the microflora which developed on vacuum packaged cuts (Seideman et al., 1976).

Ulrich (1949) stated that vacuum packaging reduced but did not eliminate microbial growth. Many workers have reported fewer spoilage bacteria on vacuum packaged beef than aerobically packaged beef (Pierson et al., 1970; Baran et al., 1970; Minks

and Stringer, 1972; Hodges et al., 1974). However, both aerobic and anaerobic counts have been found to increase during vacuum storage (Pierson et al., 1970; Baran et al., 1970; Minks and Stringer, 1972; Hodges et al., 1974; Seideman et al., 1976).

Ledward et al. (1971) and Baran et al. (1970) indicated that oxygen content of a vacuum package must be drastically reduced to prevent aerobic growth. Hodges et al. (1974) concluded that degree of vacuum regulates growth of aerobes. Seideman et al. (1976) found total aerobic psychrotrophic and mesophilic counts under high vacuum (29.4 in Hg) were lower after 35 days of storage than from cuts packaged under low (26 in Hg) or intermediate vacuum (28.5 in Hg). Aerobic counts were low (less than 10^4 per 6.45 cm^2) after 7 and 14 days storage regardless of degree of vacuum.

Reagan et al. (1971) reported that vacuum packaging does not compensate for improper refrigeration during storage of lamb. Jaye et al. (1962) found that Lactobacillus was suppressed at 0 to 1°C but grew rapidly at 3°C or higher. Minks and Stringer (1972) found a significantly higher increase in microbial counts of vacuum packaged cuts held at high temperatures.

When animal or carcass handling procedures are altered differences in microbial growth on meat products may occur. Since procedure changes can affect both number and types of microorganisms present, it becomes manditory to consider the microbial consequences of any proposed changes. New handling procedures or systems, no matter how desirable, will not be implemented if resultant products are microbiologically unsatisfactory.

The objective of this study was to evaluate the effects of nutritional regime and post-slaughter conditioning temperature on total aerobic psychrotrophic and mesophilic carcass bacterial counts and the effects of nutritional regime and vacuum packaging on total aerobic and anaerobic bacterial counts of vacuum packaged inside chucks.

EXPERIMENTAL PROCEDURES

Thirty-eight crossbred steers were randomly assigned to 4 nutritional regimes. All animals were initially fed on a brome and bluestem pasture supplemented with a wintering ration of protein and alfalfa. Ten grass-fed animals were slaughtered at end of summer directly off pasture. Ten steers were fed an additional 49 days on an 80% concentrate (short-fed), 8 were fed 98 days on an 80% concentrate (long-fed) and 10 were fed 98 days on a 40% concentrate 60% roughage ration (forage-fed).

Each carcass half was washed for 10 min with cold tap water before initial sampling. Right halves were chilled at 2°C and left halves were conditioned at 13°C for 8 hr then chilled at 2°C until carcass fabrication at 48 hr post-mortem.

Carcass bacterial samples were taken at 1, 8 and 46 hr post-mortem. One hr samples were taken immediately after washing and prior to placing the carcass in the 2°C or 13°C coolers. The 8 hr samples from the left halves were excised before transfer to the 2°C cooler.

Immediately prior to excising the 1 hr samples, 6 squares of 32.25 cm^2 each were outlined anterior to the 13^{th} rib and 6.0 to 8.0 cm dorsal to the ventral midline. The sample areas were outlined by using 2 sterile scalpels (scalpel-template) fixed 5.68 cm apart (figure 1) and making 3 horizontal and 2 vertical cuts (figure 2) through the muscle to the underlying fascia. At each sampling period 2 squares (64.50 cm^2) were aseptically removed by means of a sterile scalpel and forceps and placed in sterile phosphate buffer solution, pH 7.2 (Okey and Walter, 1972). Two sets of duplicate dilutions were made from each sample and plated with standard plate count agar (Difco). A psychrotrophic bacterial count was obtained by incubating 1 set for 10 days at 7°C and a mesophilic bacterial count was obtained by incubating the other set at 32°C for 48 hours. This procedure was repeated for each carcass half at each sampling period.

Inside chucks were removed from the right side of each carcass after a 48 hr chill at 2°C and prepared for sampling and vacuum packaging. Initial samples were taken immediately after fabrication and prior to vacuum packaging. The scalpel-template (figure 1) was used to outline two sampling areas (32.25 cm^2 each) on the subscapular surface. Samples were taken by rolling 4 sterile cotton swabs, moistened in sterile .1% peptone solution, over the outlined area (64.50 cm^2). Swabs were placed in sterile peptone solution and two sets of duplicate dilutions were prepared.

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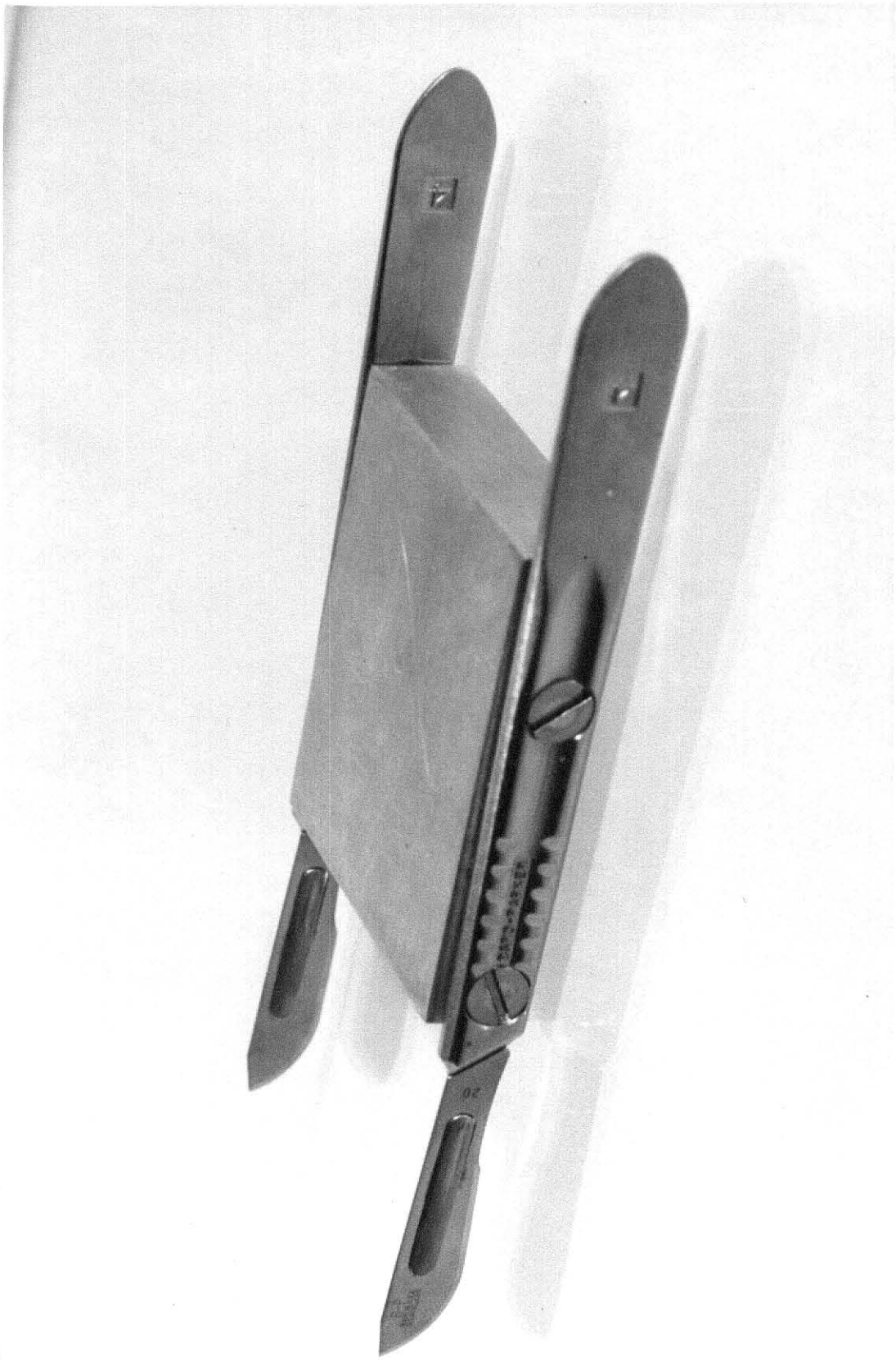

Figure 1. Scalpel-template

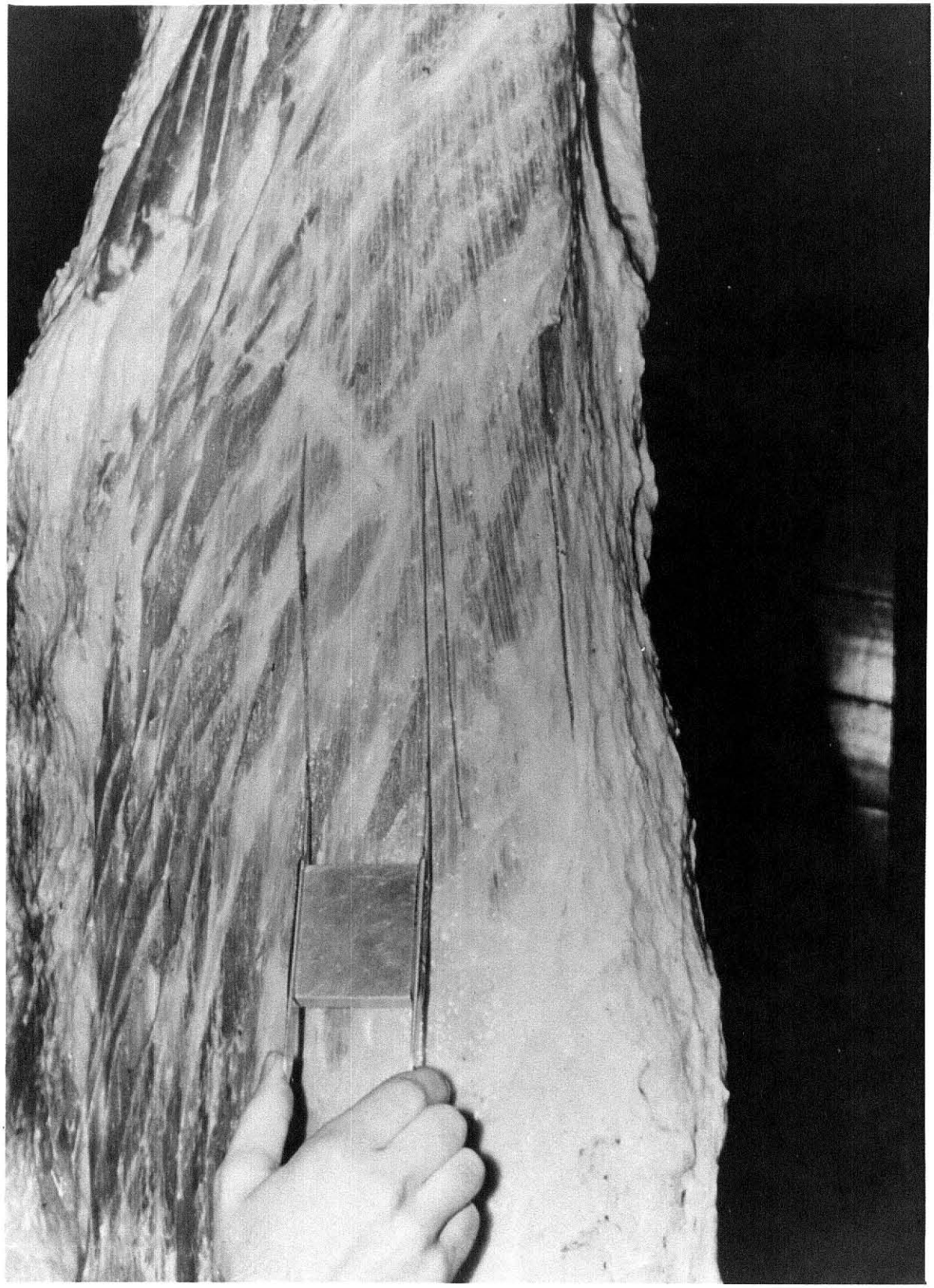

Figure 2. Use of scalpel-template

Aerobes were counted using 1 set of duplicates plated on standard plate count agar (Difco) and incubated at 21°C for 72 hours. Anaerobes were counted in the remaining duplicate set by plating with Schaedler agar (Bio Quest) and incubating at 32°C for 48 hours. The incubator was evacuted and flushed with oxygen free CO_2 3 times to insure a very low O_2 partial pressure.

After initial sampling, the cuts were vacuum packaged with heat shrinking and stored at 0 to 1°C for 21 days. Then, counts were repeated from adjacent areas on the inside chucks.

Colonies on each plate were enumerated according to Brazis et al. (1972) utilizing a Quebec colony counter. The average plate count of each set of duplicates was calculated and converted to its $\log_{10}$. Statistical analyses were preformed on the logarithmic values. Whenever discrepancies arose between counts of high and low dilutions, the lowest dilutions were considered to be the most accurate. All blank plates were arbitrarily given a count of one.

Data were analyzed by analysis of variance utilizing a split plot design. The technique of Duncan (1956) was employed for mean separation.

RESULTS AND DISCUSSION

Carcass Bacterial Counts

Analysis of variance for total psychrotrophs and mesophiles is presented in Table 1. For both incubation temperatures;

Table 1. Mean square values for feeding regime, chill temperature and sampling time on total psychrotrophic and mesophilic bacterial counts of beef carcasses

Source	d.f.	Mean Squares	
		Psychrotrophs	Mesophiles
Feed (A)	3	7.75*	26.39*
Temp (B)	1	11.45*	5.69*
FeedxTemp (B)	3	1.87	1.68
Time (C)	2	11.28*	7.68*
TempxTime (C)	2	.81	.40
FeedxTime (C)	6	1.57*	1.23*
FeedxTempxTime (C)	6	.40	.13
Error for (A)	34	1.23	.72
Error for (B)	34	.65	.70
Error for (C)	136	.39	.28

*Significant at ($P<0.05$)

feeding regime, chill temperature, sampling time, and feeding regime by sampling time interaction were significant.

Psychrotrophic and mesophilic mean counts tended to decrease from 1 to 46 hr even though grass- and long-fed psychrotrophic means and grass- and short-fed mesophilic means were lowest at 8 hours. Assuming the majority of the psychrotrophs to be Pseudomonas (Kirsch et al., 1952; Ayres, 1960; Stringer et al., 1969), this trend would agree with Bouton et al. (1974) who reported Pseudomonas to decrease on the exposed areas of inoculated mutton carcasses chilled at either 0 to 1°C or 7 to 8°C for 24 hours. Across all sampling periods, the grass-fed psychrotrophic and mesophilic mean count was highest followed in descending order by long-, short- and forage-fed for psychrotrophs and short-, long- and forage-fed for mesophiles.

The feeding regime by sampling time interaction means for total psychrotrophic bacterial counts are presented in Table 2. At 1 hr sampling period, the grass-fed mean count was significantly higher than all other groups and forage-fed mean was significantly lower than long-fed mean. At 8 hr no differences were significant and at 46 hr forage-fed mean count was significantly lower than other feeding regime means. Comparing within each feeding regime, there were no significant differences detected between any 8 and 46 hr sample means. Grass- and long-fed means at 1 hr were significantly higher than corresponding 8 and 46 hr sample means. One hr sample means from short- and forage-fed groups were significantly higher than corresponding 46 hr samples.

Table 2. Psychrotrophic bacterial counts of beef carcasses stratified according to sampling time and feeding regime [a]

Regime	Sampling Time (hours post-mortem)[b]			
	1	8	46	$\bar{x}$
Grass-fed	2.46^{c}	1.02^{c}	1.28^{c}	1.59
Short-fed	1.35^{de}	1.07^{c}	$.93^{c}$	1.12
Long-fed	1.80^{d}	1.27^{c}	1.31^{c}	1.46
Forage-fed	1.09^{e}	$.78^{c}$	$.47^{d}$	.78
$\bar{x}$	1.67	1.02	.98	

[a] Counts ($\log_{10}$) per 6.45 cm^2

[b] Means in same row underscored by a common line do not differ ($P>0.05$)

[c,d,e] Means in same column bearing different superscripts differ ($P<0.05$)

The feeding regime by sampling time interaction means for total mesophilic bacterial counts are presented in Table 3. At 1 hr sampling period grass-fed mean count was significantly higher and forage-fed was significantly lower than all other feeding regime means. At 8 hr, grass-fed mean was significantly higher than all others and short-fed was significantly higher than forage-fed. All feeding regime means were significantly different at 46 hr with grass-fed mean count being highest followed in descending order by short-, long- and forage-fed. Within each feeding regime, grass- and long-fed means at 1 hr were significantly higher than corresponding 8 or 46 hr means. No statistical differences were detected between the 3 sampling periods for short-fed carcasses. Forage-fed mean count at 46 hr was significantly lower than either the 1 or 8 hr means.

Conventionally chilled sides had significantly lower total psychrotrophic and mesophilic mean counts than treated sides (Table 4). This does not agree with Fields et al. (1976) who found no ultimate difference in bacterial counts between carcass halves held at 2°C for 48 hr post-mortem as compared to carcass halves held at 14 to 19°C for 12, 16 or 20 hr and subsequently at 2°C until 48 hr post-mortem. Kastner et al. (1976) found carcass halves held at 16°C for 8 hr and then 2°C for 48 hr post-mortem to have lower total psychrotrophic and mesophilic counts than halves held at 2°C for 48 hours.

Bacterial Counts of Vacuum Packaged Inside Chucks

Analysis of variance for total aerobes and anaerobes is presented in Table 5. For both, the effects of feeding regime

Table 3. Mesophilic bacterial counts of beef carcasses stratified according to sampling time and feeding regime [a]

Regime	Sampling Time (hours post-mortem)[b]			
	1	8	46	$\bar{x}$
Grass-fed	3.67[c]	2.58[c]	2.72[c]	2.99
Short-fed	2.32[d]	2.14[d]	2.17[d]	2.21
Long-fed	2.39[d]	1.76[de]	1.65[e]	1.93
Forage-fed	1.63[e]	1.49[e]	1.07[f]	1.40
$\bar{x}$	2.51	2.01	1.92	

[a] Counts ($\log_{10}$) per 6.45 cm^2

[b] Means in same row underlined by a common line do not differ ($P>0.05$)

[c,d,e,f] Means in same column bearing different superscripts differ ($P<0.05$)

Table 4. Psychrotrophic and mesophilic bacterial counts of beef carcass stratified according to sampling time and conditioning temperature[a]

Conditioning Temperature	Psychrotrophs				Mesophiles			
	Sampling Time				Sampling Time			
	1	8	46	$\bar{x}$	1	8	46	$\bar{x}$
2°C	1.56	.72	.72	1.00	2.42	1.84	1.69	1.99
16°C	1.78	1.33	1.24	1.45	2.59	2.17	2.14	2.30

[a]Count ($\log_{10}$) per 6.45 cm^2

Table 5. Mean square values for feeding regime and vacuum storage on total aerobic and anaerobic bacterial counts of inside chucks

Source	d.f.	Mean Square	
		Aerobic	Anaerobic
Feed (A)	3	18.85*	9.61*
Vacuum Storage (B)	1	1.37	7.16
FeedxVacuum Storage (B)	3	1.89*	2.60*
Error for (A)	34	.63	.43
Error for (B)	34	.47	.38

*Significant at ($P<0.05$)

and feeding regime by vacuum packaging interaction were significant.

The aerobes tended to decrease slightly during vacuum storage and is in opposition to earlier work (Minks and Stringer, 1972; Hodges et al., 1974; Seideman et al., 1976). The tendency for anaerobic mean counts to decrease during vacuum storage agrees with Baran et al. (1970) who found anaerobes increased for 3 days, then decreased to 20 days, then increased. However, Hodges et al. (1974) and Seideman et al. (1976) found anaerobes increased throughout vacuum storage. The decline in total aerobic and anaerobic counts or their lack of growth during vacuum storage could be due to storage holding temperature (0 to 1°C). This agrees with Jaye et al. (1962) who found that Lactobacillus growth was suppressed at 0 to 1°C.

The feeding regime by vacuum storage interaction means for total aerobes is presented in Table 6. For pre-vacuum samples, grass-fed mean count was significantly higher than all others and short-fed mean count was significantly higher than forage-fed. After vacuum storage, grass-fed mean count was still significantly higher than all others. Grass- and short-fed pre-vacuum packaged sample means were significantly higher than their respective post-vacuum storage sample means.

The feeding regime by vacuum storage interaction for anaerobes is presented in Table 7. Grass-fed beef had significantly higher mean counts than all other feeding regimes both before and after vacuum storage. Grass-fed mean counts decreased significantly during storage. No other differences were detected.

Table 6. Mean aerobic bacterial counts for vacuum packaged inside chucks stratified according to pre- and post-vacuum storage and feeding regime[a]

Regime	Vacuum Storage[b] Pre	Post	$\bar{x}$
Grass-fed	3.51^{c}	2.86^{c}	3.18
Short-fed	2.22^{d}	1.32^{d}	1.77
Long-fed	1.31^{de}	1.44^{d}	1.37
Forage-fed	$.71^{e}$	1.13^{d}	.92
$\bar{x}$	1.97	1.70	

[a]Count ($\log_{10}$) per 6.45 cm^2

[b]Means in same row underscored by a common line do not differ ($P>0.05$)

[c,d,e]Means in same column bearing different superscripts differ ($P<0.05$)

Table 7. Mean anaerobic bacterial counts for vacuum packaged inside chucks stratified according to pre- and post-vacuum storage and feeding regime[a]

Regime	Vacuum Storage[b]		$\bar{x}$
	Pre	Post	
Grass-fed	3.05[e]	1.41[c]	2.23
Short-fed	.91[d]	.93[cd]	.92
Long-fed	1.45[d]	.96[cd]	1.21
Forage-fed	.80[d]	.48[d]	.64
$\bar{x}$	1.56	.95	

[a]Counts ($\log_{10}$) per 6.45 cm^2

[b]Means in same row underlined by a common line do not differ ($P>0.05$)

[c,d]Means in same column bearing different superscripts differ ($P<0.05$)

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CONCLUSIONS

Regardless of reported statistical differences, it must be stated that all counts were well below the limit of $10^7/cm^2$ defined by Kraft and Ayres (1952) as the surface count when definite odors could be detected. Changes that did occur usually involved $\log_{10}$ changes of less than 1 and are of doubtful microbiological significance (Kotula et al., 1975).

Some variation in counts could be due to seasonal effects and carcass sampling location (Stringer et al., 1969). In addition, the relative sample size in relation to total carcass or inside chuck surface area was quite small and could be responsible for some variation. Based on this data, it is doubtful whether any detected differences can be construed as being practical due to the relative small differences.

These data indicate that carcasses from animals of differing nutritional backgrounds can be held at 13°C for 8 hr then conventionally chilled and remain within acceptable microbial limits. Also, inside chucks from conventionally chilled carcasses of animals with differing nutritional background can be stored under vacuum for 21 days at 0 to 1°C and be within acceptable microbial limits.

The scalpel-template technique for outlining sampling areas avoided making templates from metal or paper and sterilzation was simple since the blades could be flamed immediately prior to sampling. This sampling method was considered superior to methods used by previous workers.

SUMMARY

Thirty-eight crossbred steers were used to evaluate the effects of nutritional regime (grass-, short-, long-, and forage-fed) and post-slaughter conditioning temperature (2°C vs. 13°C) on carcass psychrotrophic and mesophilic bacterial counts. Inside chucks from right halves of the carcasses chilled at 2°C for 48 hr were used to evaluate the effects of nutritional regime and vacuum packaging on total aerobic and anaerobic bacterial counts. Psychrotrophic and mesophilic mean bacterial counts tended to decrease from 1 to 46 hr post-mortem regardless of conditioning temperature. At 46 hr post-mortem, the forage-fed group mean psychrotrophic count was significantly lower ($P<0.05$) than all other feeding regimes. All had significantly different ($P<0.05$) mesophilic mean counts at 46 hr post-mortem (grass-fed>short-fed>long-fed>forage-fed). Carcass halves chilled at 2°C for 46 hr had lower total psychrotrophic and mesophilic mean bacterial counts than corresponding halves chilled at 13°C for 8 hr then at 2°C for 36 hours. Total aerobic and anaerobic counts tended to remain constant or decrease slightly during vacuum storage for 21 days at 0 to 1°C. Both aerobic and anaerobic counts on grass-fed carcasses after vacuum storage were significantly higher ($P<0.05$) than all other feeding regime means. Aerobic and anaerobic counts on short-, long-, and forage-fed carcasses after vacuum storage were statistically similar. All carcass and inside chuck bacterial counts were well within acceptable limits. Scalpel-template

sampling was considered to be a significant improvement compared to previous methods used by other workers.

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Appendix A. Feed Rations and Slaughter Schedule

Thirty-eight crossbred steers were used. These steers were born at the U.S. Meat Animal Research Center in the Spring, 1974 and were castrated at birth. The calves grazed with their dams until weaning at 6 months of age. After weaning, the steers were fed rations which contained approximately 65% corn silage, 15% alfalfa haylage and 20% grain and soybean supplement for 75 days until start of the experiment in mid-December. At that time, each steer was implanted with Ral-Gro (36 mg zeranol).

All steers were fed on a winter growing ration (ration 1) for 134 days, then 133 days of grazing on cool and warm season grasses. Ten grass-fed animals were slaughtered at this time. After a 6 day adjustment period; 10 animals (forage-fed) were fed 98 days on a 60% forage ration (ration 2), 10 animals (short-fed) were fed 49 days on a 20% forage ration (ration 3), and 8 animals (long-fed) were fed 98 days on a 20% forage ration (ration 3).

Ration Ingredients and approximate composition

Ingredient	International ref. no.	Ration 1	Ration 2	Ration 3
Corn silage, %	3-02-824	48.0	40.0	0.0
Alfalfa haylage, %	3-08-151	50.0	20.0	20.0
Cracked corn, %	4-02-932	0.0	36.0	75.2
Supplement[a], %		2.0	4.0	4.8
Approximate ration composition, dry matter basis[b]				
Dry matter, %		44.9	60.0	81.2
Crude protein, %		14.6	13.0	13.0
Metabolizable energy, Mcal/kg		2.18	2.84	3.11

[a]Soybean meal (ref. no. 5-04-604) supplement containing calcium, phosphorus, vitamin A and chlortetracycline. Steers also had free choice access to both block salt and a mixture of 1/3 loose salt, 1/3 limestone and 1/3 dicalcium phosphate.

[b]Nutrient composition based on tabular values (N.R.C., 1963) supplemented with limited proximate analyses.

Appendix B. Detailed Procedures

A. Variables

1. Total aerobic mesophiles and psychrotrophs for 76 sides chilled at 2°C for 48 hr or 13°C for 8 hr then 2°C for 36 hr and sampled at 1, 8 and 46 hr post-mortem.

2. Total aerobic and anaerobic microorganisms for 38 inside chucks from conventionally chilled halves pre- and post-vacuum packaging.

B. Materials

1. Incubators
   a. 7C
   b. 21C
   c. 32C

2. Sterile swabs - 240 for all 4 feeding regimes

3. Sterile templates
   a. Two scalpels fixed 5.68 cm apart
   b. Use for carcass and chucks

4. Peptone blanks for swabs from chucks
   a. Pre- and post-vacuum samples
      10 - 100 ml blanks (0.1% peptone in screw-top dilution bottles) per feeding regime

5. Rinse bottles for carcass data
   60 - 250 ml erlenmeyer flasks with rubber stoppers per feeding regime

6. Rinse solution
   a. 6,000 ml per feeding regime
   b. 24,000 ml for all feeding regimes

7. Peptone broth solution
   a. 1,100 ml per feeding regime
   b. 4,400 ml for all feeding regimes
   c. Dry peptone - 4.4 gm

8. Pipettes
   Total for all feeding regimes 400 1.1 ml pipettes

9. Petri dishes
   Total for all 4 feeding regimes 2,320

10. Water baths
    1 (42-45°C)

11. Bunsen burner and ethanol lamp

12. Agar
Total for all 4 feeding regimes 840 gm of dry agar

13. Ethanol - 95%
1 pt.

14. Dilution bottles
Total per feeding regime (pre- or post-vacuum only) 20

15. Dilution solution
Total per feeding regime (pre- or post-vacuum only)
1,980 ml of sterile buffered or distilled, deionized water.

16. Forceps and scalpels of 4 each

17. Screw-top dilution bottles

18. 1 bottle pure CO_2

C. Preparation of Materials for Carcasses

1. Phosphate buffered dilution blanks
   a. Stock solution
      1) Dissolve 34 gm potassium acid phosphate (KH_2PO_4) in 500 ml distilled, deionized water
      2) Adjust to pH 7.2 with N sodium hydroxide
      3) Dilute to 1 liter with distilled, deionized water

   b. Working solution
      1) Take 1.25 ml of stock phosphate buffer solution
      2) Dilute to 1 liter with distilled, deionized water

2. Preparation of dilution blanks
   a. Using a diluter calibrated to dispense 104 gm, dispense 104 gm of working solution into each 250 erlenmeyer
   b. Stopper with No. 6 rubber stopper and cover with aluminum foil
   c. Autoclave for 15 min at 121°C
      1) Place empty tray on top of bottles in autoclave to prevent stoppers from popping out
      2) When cooling after autoclaving, periodically loosen stoppers to prevent them from being pulled into bottle

3. Agar preparation
   a. Melt 23.5 gm of standard plate count agar (Difco) in 1 liter distilled, deionized water
   b. Dispense into screw-top dilution bottles and cap loosely
   c. Autoclave for 15 min at 121°C
   d. If for immediate use

1) Allow to air cool slightly
2) Place in water bath
3) Don't use until agar is same temperature as water bath

e. If for later use
1) Allow to solidify
2) Remelt in autoclave when needed (remelt 1 time only)

D. Procedures - Carcasses

1. Upon splitting carcasses, wash each half for 10 min. beginning at the hind shank and continuing anteriorally.

2. Use scalpels fixed 5.69 cm apart to outline area to be sampled. Make 3 horizontal and 3 vertical cuts anterior to the 13th rib and 6.0 to 8.0 cm dorsal to the ventral midline.

3. Aseptically excise two 5.69x5.69 cm strips as outlined by scalpel-template from each side at three time periods 1, 8 and 46 hr post-mortem.

4. Place excised tissue (64.5 cm^2) in 100 ml of buffered, sterile rinse solution.

5. Store sample not over 1 hr in the rinse solution. Prior to dilution and plating, shake samples by striking bottom of bottle against palm of hand 30 times in 7 seconds.

6. Prepare 1/10 and 1/100 dilutions and plate in duplicate using Standard Plate Count Agar for incubation at TWO temperatures.

7. Incubate 1 set of duplicate plates at $7^{\circ}C$ for 10 days and 1 set of duplicates at $32^{\circ}C$ for 48 hr.

8. Identification for rinse bottles: Sample No.
Identification for plates: Sample No., Date, Dilution, time

9. Count plates according to Standard Methods to represent total aerobic mesophilic and psychrotrophic organisms.

10. Convert actual to $\log_{10}$ for computer analysis.

E. Preparation of Materials for Vacuum Cuts

1. Dilution preparation
a. Peptone dilution blank
1) Dissolve 1.5 gm peptone to 1.5 liter distilled, deionized water
2) Put 104 ml into 10 screw-top dilution bottles
3) Autoclave 15 min at $121^{\circ}C$

2. Agar preparation
   a. Aerobic agar
      1) Use standard plate count agar (Difco)
      2) Prepare as described for carcass
   b. Anaerobic agar
      1) Melt 41.9 gm of Schaedler anaerobic agar in 1 liter distilled, deionized water
      2) Dispense into screw-top dilution bottles
      3) Autoclave 15 min at 121°C

F. Procedures - Vacuum Cuts

1. If pH is determined on the inside chuck, this should be done prior to the micro sampling. The inside chuck should also be weighed after the micro sampling and just prior to packaging.

2. Swab (pre-vacuum packaging and after 21 days storage) 64.50 cm^2 of the top side of the inside chuck using area outlined by scalpel-template. Score with a knife the area sampled so as to not re-swab the same area. Use 4 swabs per 64.50 cm^2 surface area.

3. Place swabs in 100 ml of sterile peptone broth.

4. Shake swabs, dilute to 1/10 and 1/100 and plate as above.

5. Incubate duplicate plates at 21°C for 72 hrs for aerobic organisms.

6. Incubate duplicate plates at 32°C for 48 hr for anaerobic organisms
   a. Set plates in anaerobic incubator and draw vacuum of 27 in. Hg
   b. Flush with pure CO_2 (analyzed by Servomex Oxygen Analyzer)
   c. Repeat 3 times (will leave approximately .008% residual oxygen)

7. Count and record total aerobic and anaerobic organisms.

8. Convert actual counts to $\log_{10}$ for computer analysis.

NUTRITIONAL REGIME, POST-SLAUGHTER CONDITIONING TEMPERATURE AND VACUUM PACKAGING EFFECTS ON CARCASS AND INSIDE CHUCK BACTERIOLOGY

by

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B.S., Oklahoma State University, 1974

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

Thirty-eight crossbred steers were used to evaluate the effects of nutritional regime (grass-, short-, long-, and forage-fed) and post-slaughter conditioning temperature (2°C vs. 13°C) on carcass psychrotrophic and mesophilic bacterial counts. Inside chucks from right halves of the carcasses chilled at 2°C for 48 hr were used to evaluate the effects of nutritional regime and vacuum packaging on total aerobic and anaerobic bacterial counts. Psychrotrophic and mesophilic mean bacterial counts tended to decrease from 1 to 46 hr post-mortem regardless of conditioning temperature. At 46 hr post-mortem, the forage-fed group mean psychrotrophic count was significantly lower ($P<0.05$) than all other feeding regimes. All had significantly different ($P<0.05$) mesophilic mean counts at 46 hr post-mortem (grass-fed>short-fed>long-fed>forage-fed). Carcass halves chilled at 2°C for 46 hr had lower total psychrotrophic and mesophilic mean bacterial counts than corresponding halves chilled at 13°C for 8 hr then at 2°C for 36 hours. Total aerobic and anaerobic counts tended to remain constant or decrease slightly during vacuum storage for 21 days at 0 to 1°C. Both aerobic and anaerobic counts on grass-fed carcasses after vacuum storage were significantly higher ($P<0.05$) than all other feeding regime means. Aerobic and anaerobic counts on short-, long-, and forage-fed carcasses after vacuum storage were statistically similar. All carcass and inside chuck bacterial counts were well within acceptable limits. Scalpel-template sampling was considered to be a significant improvement compared to previous methods used by other workers.