IDENTIFICATION OF BACTERIAL CRITICAL POINTS AND ANTIBACTERIAL EFFECT OF LACTIC ACID ON BEEF CARCASS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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Department of Veterinary Public Health
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1994

DECLARATION

I hereby declare that the thesis entitled **"Identification of Bacterial Critical Points and Antibacterial Effect of Lactic Acid on Beef Carcass"** is a bonafide record of research work and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Mannuthy,

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CERTIFICATE

Certified that the thesis entitled "**Identification of Bacterial Critical Points and Antibacterial Effect of Lactic Acid on Beef Carcass**" is a record of research work done independently by **Shri. K. Rajeev,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Introduction

INTRODUCTION

Food-borne infections and intoxications have assumed significance as a serious problem for mankind. In the hunt for animal protein, man is exposed to a formidable array of potential hazards in recent years. Even in developed countries, where advanced techniques and facilities are adopted, food-borne infections are frequently reported. Primarily the foods of animal origin were found to be the main sources of infection. At times, episodes of food-borne infections and intoxication are reported by Indian Press also. But the magnitude of the problem is not known.

During conventional slaughter and dressing processes, carcasses are contaminated with a variety of bacteria, both pathogenic and nonpathogenic. The presence of pathogenic bacteria is a potential health hazard to the consumers and nonpathogenic bacteria is responsible for spoilage of meat, resulting in economic loss to meat traders. Keeping quality of meat is highly dependent on initial microbial load and nature of organisms. Eventhough good manufacturing practices (GMP) are observed during slaughter, surfaces of carcasses become contaminated with various microorganisms. Under practical conditions of slaughtering, it is impossible to prevent microbial contaminations.

Even visually clean carcasses may carry relatively large number of bacteria on their surfaces. In India, it is widely reported that the hygienic conditions existing in slaughter houses is far from satisfactory; thus exposing the carcass to heavy bacterial load. The tropical location favours the microbial growth leading to rapid spoilage of meat.

The assessment of microbial contamination is necessary to evaluate the hygienic conditions prevailing in the production and processing of meat. During processing and subsequent handling, carcasses get exposed to surface bacterial contamination. The sources and extent to which contamination takes place depends on various factors. Assessment of these various factors have been given much importance to help in reducing the contamination level in the food chain. With this objective Hazard Analysis Critical Control Point (HACCP) system is being enforced in developed countries for producing wholesome meat. Among the various indices, the bacterial load on the carcasses and the variation between the locations on the carcasses have been found to help in exercising good manufacturing practices. These are the critical points (CP) which are to be identified and monitored. The CPs on carcasses have been reported to vary between

slaughter houses depending upon the local conditions and the management systems followed.

Evaluation of hygienic conditions as reflected in the CPs are primarily based on bacterial indices. Thus, bacterial examination forms an essential component in hygienic meat production. Bacteriological examination is normally time consuming, and hence identification of locations in the carcass - having the chances of maximum contamination, will help in quicker evaluation and taking appropriate remedial measures.

The basic approach for reducing bacterial load on carcass surface is to follow strict sanitary slaughter and fabrication practices (Ingram et al., 1956; Chandran et al., 1986). Even with best possible slaughter and dressing practices, the carcasses will still contain considerable microbial load on the surface. Consequently various physicochemical measures were practised to reduce bacterial load and/or their multiplication.

Various treatments like washing and spraying with or without sanitizers are being adopted for decontamination of carcasses. Recently the use of edible organic acids particularly lactic acid and acetic acid have been subjected to considerable investigation as a means of reducing bacterial

temperatures were adopted depending on the type of organisms and material tested. Incubation at 37°C was accepted for estimation of total viable count. Phosphate buffer or peptone water at different strength have been widely used to preserve bacteria before processing and incubation.

The present study was undertaken to examine six pre-determined locations of carcasses produced in two different systems of management to identify the critical bacterial points. The present study also cover the effects of application of lactic acid solutions at two different temperatures, one at ambient temperature and the other at 70°C, on carcass surface and compare their efficiency in controlling the bacterial load.

Review of Literature

REVIEW OF LITERATURE

Hazard Analysis Critical Control Point (HACCP) system was originally proposed and used in USA in 1965 (Sperber, 1991) as a quality control procedure to assess the safety in space food service system. Determination of critical control points is required to control any identified hazard. It is also necessary to establish procedure to monitor critical control points (Sperber, 1991). This concept has since been introduced in meat industry (Charlebois *et al.*, 1991).

The locations, practices, processing steps or procedures where control must and can be exerted are called critical control points (Baird-Parker, 1987).

Sampling sites are those locations to determine bacterial contamination of carcasses within an abattoir wherein the sites on the carcasses are constantly dirty (in a bacteriological sense) (Johansen *et al.*, 1983).

Sources of contamination on meat

Howe *et al.* (1976) determined the types of *E. coli* found on the surface of the carcass and in rectal contents of calves. In one third of the animals tested, *E. coli* strains found on the surface of a carcass belonged to the same

serotype as those found in the rectal sample of the same calf, indicating contamination during slaughter.

Mandokhot and Garg (1985) pointed out the various possible sources like chopping blocks, persons handling the meat equipments, hands etc. which might contaminate the meat during production and marketing. It was also suggested that enterococcus index and coliform index were best used in assessing the sanitary quality of foods including meat.

Whelehan et al. (1986) found that there was no significant difference between bacterial load of carcass produced in manual line and automated line.

Gustavasson and Karlsson (1989) conducted a bacteriological study to determine the critical process operations in abattoirs and found that the exposure of carcasses in the rapid and storage chillers together with cutting and deboning were the critical process operations.

Microbial contamination of carcasses arised from direct or indirect contact with animal's hide, legs or hooves, with gut contents or faecal material or contaminated equipment (Huis in't Veld et al., 1994).

Sites and areas for bacteriological evaluation on carcasses

Patterson (1968) suggested a suitable sampling

technique for freshly butchered cattle and sheep carcass by swabbing an area of 16 cm^2 on the rump, brisket and foreleg.

The highest microbial count in the brisket region of the carcass while assessing the microbial load on the surface at different sites (Patterson, 1971).

Wojtan and Kossakowska (1977) conducted bacteriological tests for the evaluation of sanitary quality of carcasses by taking swabs from abdomen and sternum of pig carcasses and from shoulder and buttock of beef carcasses.

Deshpande (1979) observed that mesophilic count in the neck region of beef carcass was comparatively less than the leg or skirt region.

Roberts et al. (1980) while investigating the bacterial load on carcasses at commercial abattoirs, selected neck, brisket, forerib, flank, sirloin, rump and round as different sites for sampling. They found the highest bacterial load in the forerib medial, followed by flank and the least in sirloin.

Fournand and Bertand (1981) evaluated microbial contamination of carcasses and of the air in eight abattoirs. Levels of contamination of the lean were considerably higher than those of fat parts of the carcasses. Contamination was

not homogenous for individual carcasses and the region of sternum was found to be highly contaminated than other parts.

Nortje and Naude (1981) after conducting a survey in local abattoirs, assessed the bacterial counts on the carcasses. Lower back and brisket region of carcasses showed higher counts, because they were most exposed to handling. The sites selected for the evaluation were hind limb, loin, groin, sternum, shoulder, forelimb and neck.

Johanson et al. (1983) while conducting a survey of hygienic quality of beef, selected neck, brisket, forerib, flank, round-lateral, round-medial, forerib-medial and flank groin as the sites for evaluation of bacterial load and found that the highest bacterial contamination was at brisket, round-medial and forerib-lateral and the least at flank-medial and forerib-medial.

Roberts et al. (1984) conducted bacteriological survey on beef carcasses in three abattoirs. Out of the different sites such as neck, brisket, forerib and round-medial, brisket was found to have highest level of contamination. They recommended that atleast 3 or 4 sites are to be sampled in future survey as single site will under estimate the contamination of carcasses.

Whelehan et al. (1986) studied the microbial load of beef carcasses before and after slaughter line automation. Sites on the carcasses selected for evaluation of bacterial load were neck, brisket, forerib, flank, hindlimb lateral and hindlimb medial.

Lasta and Fonrouge (1988) stated that bacterial count on carcass surface depended on size of the area sampled. Small sampling areas were not adequate to evaluate the hygienic quality of bovine carcasses.

Stolle (1988) observed that a consistently higher contamination was found on the lateral surface of beef carcasses and within this area, the most contaminated sites where on the forequarter. The biometrical analysis revealed a significant interaction between contamination and site, and an indicator function of the total viable count and the Enterobacteriaceae. For the study, he selected skin, midshoulder, flank, inner brisket, bed, silver side, top side and inner forerib for bacteriological evaluation of beef carcasses and found highest bacterial load on the skin and the minimum at silver side.

Nortje et al. (1989) conducted a microbiological survey of fresh meat in the super market with special attention to carcasses and contact surfaces. No consistency

was found in the contamination level of different parts of carcasses at different super markets, while, there was a tendency for fore quarters to be more contaminated than hind quarters. Sites selected for the evaluation were forelimb, forerib, hindlimb and silver side.

Tarwate et al. (1993) carried out investigations to analyse microbial load on fourteen different carcass sites in a buffalo slaughter line. The mean total viable count of carcass sites was 4.70 ± 0.40 log CFU/cm^2. The brisket, shank, neck, rib medial, plate medial and plate surfaces were the most contaminated sites.

Bacterial indices for hygienic quality

Miskimin et al. (1976) studied the relationship between indicator organisms and specific pathogens in potentially hazardous foods. Total aerobic plate count, coliform count and E. coli count were related to one another in both raw and ready to eat foods. Any of the three indicator tests was suitable to ensure the procedural integrity of food preparation activities. Total aerobic plate count was the most suitable method for the evaluation of microbiological quality of foods and the search for the specific pathogen was necessary to ensure the safety of foods.

Weisser (1979) isolated a total of 533 strains of streptococci from the organs of 413 of 3700 emergency slaughtered cattle (11.2%). From 5 per cent of another set of 800 emergency slaughtered calves he recovered S. dysagalactiae and S. uberis. These strains were associated with various conditions in slaughtered animals.

Kleeberger et al. (1980) conducted bacteriological examination of cattle carcasses, freshly slaughtered and those stored at 15°C for two days and at 7°C for four days. Most of the isolates were psychrotrophs. E. coli was the second most important organism.

Bachil (1983) studied the prevalence of E. coli in fresh meats. He reported that cent per cent samples of pork, mutton and chevon revealed E. coli contamination as compared to 91 per cent in buffaloe meat.

Tompkin (1983) suggested that indicator organisms were useful in meat and poultry products to assess three factors: microbiological safety, sanitation conditions during processing and keeping quality of product. Aerobic plate count, coliform and E. coli counts were the most commonly used indicators of sanitary quality for meat and meat products.

Lotfi et al. (1986) assessed the bacterial status of

emergency slaughtered cattle. Enterococci and coliforms were isolated from them.

Assessment of bacterial load on meat surface

Wojtan and Kossakowsk (1977) found correlation between aerobic and anaerobic bacterial counts with the counts of E. coli and enterococci on beef and pork carcasses.

Deshpande (1979) observed that the average total aerobic mesophilic count of beef carcass obtained from slaughter house was 12.5×10^8 whereas it was 30.3×10^7 and 41.03×10^7 in two different meat shops.

Firstenberg-Eden (1981) studied the mechanism of attachment of bacteria to meat surfaces and found that the number of attached bacteria as well as the attachment were dependent on the concentration of bacteria in the attachment suspension, the type of meat surface and on bacterial strain.

Maxcy (1981) stated that surface contamination in the form of discrete colony forming units was the main source of bacteria associated with meat spoilage.

Charlebois et al. (1991) evaluated the surface contamination of beef carcasses by faecal coliforms. Among

the different sites, the flank recorded the highest mean faecal coliform count per square centimetre.

Prieto et al. (1991) studied the distribution and evolution of bacteria on lamb carcasses during aerobic storage. Brisket and leg were the most contaminated areas.

Jericho et al. (1993) developed a repeatable automated method for estimating aerobic bacterial populations on surfaces of groups of beef carcasses. Some sample cluster sites were identified on beef carcasses. For the cluster sites, the lowest counts were found on thorax region and the highest on axilla region.

Procedure for bacteriological evaluation

Patterson (1971) examined the main methods in use in laboratories for the microbiological assessment of surfaces. He compared the microbial counts of different sites in cattle carcasses with alginate and cotton swabs and found that cotton swabs were generally more useful than alginate swabs in recovering bacteria from carcass surfaces.

Nottingham et al. (1975) reported that the spread plate counts were generally higher than pour plate counts for assessing bacterial load on carcasses and meat processing equipments. They also found incubation of plates at 25° and

30°C gave similar results, but 37°C counts were usually lower or more variable.

Yokoya and Zulzke (1975) described a new method for sampling meat surfaces by using a stainless steel plate with an oval hole in the centre and having bevelled inner edge to expose 8.24 sq cm.

Niskanen and Pohja (1977) carried out comparative studies on the sampling and investigation of microbial contamination of surfaces by the contact plate and swab methods. For flat firm surfaces the contact plate method was more suitable. Swabbing was better for flexible and uneven surfaces and also for heavily contaminated surfaces. In the investigation of bacterial numbers on cow carcasses, the results obtained by the swab method was on average 100 times greater than the contact plate method.

Lazarus et al. (1977) reported that secondary-tissue-removal-technique was better than moist swab contact method in assessing the microbial count of meat surfaces and that it provided a more representative value of the true microbial flora.

Olgaard (1977) described a new method for bacterial surface sampling of meat. Using a cotton wool swab stick, bacteria were transferred directly to the surface of a segment

of an agar plate. The results were regarded on relative levels rather than actual bacterial count.

Murthy (1983) compared different methods of plating and incubation temperature for recovery of contaminating bacteria in meat and found that spread plating resulted in higher rate of recovery of bacteria than pour plating. incubation temperature of 30 or 37°C may be used for screening retail market meat and 30°C for refrigerated meat.

Reuter (1984) assessed the suitability of non-destructive sampling methods for determining surface contamination of beef carcasses and found that the easiest method was the swab technique.

Anderson et al. (1987) evaluated swab and tissue excision methods for recovering micro-organisms from washed and sanitized beef carcasses. Excised tissues produced much higher counts than that recovered by swab method. Percentage recovered by swabbing appeared to be influenced by the characteristics of the area sampled on the carcass.

Fliss et al. (1991) compared surface sampling techniques for estimating total aerobic micro flora, total coliforms, faecal coliforms and E. coli on meat surfaces. Stomaching excised skin recovered the highest number of

bacteria than those by direct agar contact and double moist swab.

Media

Heartman (1960) stated that violet red bile agar was the commonest media used for enumeration of coliforms in frozen pot pies.

Oblinger (1975) compared the several media for the recovery of streptococci from a variety of foods. He reported that azide blood agar gave the highest recovery. K.F. streptococcus agar was also used and gave sufficient recovery.

Oblinger and Kennedy (1976) evaluated various diluents used for total counts, viz., Butterfields buffered phosphate solution, pure distilled water, solutions of 0.1 and 0.5 per cent peptone in distilled water and 0.85 per cent NaCl in distilled water. Of these, Butterfields diluent afforded the highest overall mean count, regardless of the incubation temperature. Although Butterfield's diluent, 0.1 per cent peptone and 0.5 per cent peptone solutions appeared to yield higher counts than the distilled water or 0.85 per cent NaCl solution, statistically the mean counts obtained for the five diluents were not significantly different from each other.

Ng and Stiles (1978) enumerated coliform bacteria and Enterobacteriaceae of samples of non frozen ground beef and frozen pork sausages obtained from different retail stores. The counts on violet red bile agar within 18-24 h incubation at 35°C gave reliable estimates of coliform bacteria and Enterobacteriaceae, with only 1.3 and 10.7 per cent false positives respectively.

Roberts et al. (1980) evaluated the effect of incubation temperature on assessment of bacterial load of several sites on commercial beef, pork and lamb carcasses at the end of slaughter line. Total viable count at 37°C was found to be the most useful bacteriological index.

Sanitizers

Kotula et al. (1974) reported that washing beef carcasses with chlorinated water caused a reduction in total aerobic bacteria count and also observed that washing under high pressure and temperature was more effective. The reduction in pH proportionately enhanced the effect.

Ockerman et al. (1974) reported that spraying lamb carcass with 12 per cent level of lactic acid was most effective for reducing bacterial level after seven days of storage when different levels 6, 12, 18 and 24 per cent lactic acid solutions were tried.

Mulder and Krol (1975) observed that bacterial growth on meat can be delayed by dipping into 2 or 3 per cent lactic acid solutions and an increase in the bacteriologically influenced keeping quality was also noticed. The treatment also produced a negative effect on the colour of the surfaces of beef.

Rubin (1978) reported lactic acid and acetic acids had synergistic inhibitory effect on Salmonella typhimurium which was 12 per cent more than the inhibition obtained by the acids independently.

Smith and Graham (1978) reported that treatment with hot water at 80°C for 10 seconds reduced coliform count from $100/cm^2$ to below the detection level i.e., one $cell/cm^2$ and counts of aerobic organisms decreased from 8500 to 3.0 $cells/cm^2$. The reduction rate of bacteria increased with increase in temperature. The treatment time had little effect on the bacterial count of treated sheep carcass.

Snijders et al (1979) reported that spraying fresh cattle carcasses with 0.5 per cent lactic acid solutions significantly reduced surface aerobic bacterial load. Use of 0.75 per cent lactic acid solutions resulted in a significant reduction and 1 per cent solution reduced all bacteria. The

effect of lactic acid persisted for three days under refrigerated condition.

Nelton and Mosson (1980) reported the effect of lactic acid treatment on the skin of freshly slaughtered pigs which were inoculated with Enterobacteriaceae. They observed that treatment with two per cent lactic acid solution produced the required lethality (2-3 log cycles reduction of counts) for Enterobacteriaceae within one minute.

Kelly et al. (1981) conducted experiment to determine the effect of temperature and chlorine content of water and duration of spraying in spray washing of lamb carcasses. Significant reduction in bacterial load was noticed. The rate of reduction was directly proportional to the temperature and the strength of chlorine. No significant difference in bacterial reduction was noticed with change in pressure of spray wash.

Restaino et al. (1982) reported that organic acids, specifically citric and lactic acid along with potassium sorbate had significant bacteriostatic effect on some food released microorganisms in culture media.

Sheridan (1982) stated three methods for cleaning lab carcasses: (i) Hot water (85-90°C) sprayed at high pressure (7 kg/cm^2), (ii) a pneumatic gun using water at 40-50°C and

7 kg/cm^2 pressure and (iii) scrubbing with a nylon or bristle brush. Bacteriologically the first method was marginally better, but it had no effect in enhancing normal shelf life.

Osthold et al. (1984) developed an acid spray (2% acetic acid; 1% lactic acid; 0.25% citric acid; 0.1% ascorbic acid; upto 100% water, as solvent) and tested on beef and sheep carcasses and observed that bacterial quality of acid treated carcasses were much better than those of controls. A selective inhibitory effect on Enterobacteriaceae and coliform bacteria was also noticed.

Woolthuis et al (1984) compared the effect of reduction of bacterial count by two treatments on fresh porcine liver, one by immersing it in 0.2 per cent lactic acid solution and the other in hot water at 65°C for 15 seconds. Though both treatments were effective, treatment with lactic acid was significantly more effective.

Smulders and Woolthuis (1985) determined the immediate and delayed microbiological effects of lactic acid decontamination of calf carcasses. As a result of 1.25 per cent (v/v) lactic acid treatment, aerobic colony counts were reduced by 0.8 $\log_{10}$ CFU/cm^2 as compared with initial counts of approximately 3.0 $\log_{10}$ CFU/cm^2 in controls. However reduction increased to 1.3 $\log_{10}$ CFU/cm^2 at 14 days postmortem

indicating some delayed effect of lactic acid. The percentage of samples positive for Enterobacteriaceae were reduced from 50 per cent to approximately 10 per cent.

Woolthuis and Smulder (1985) reported that lactic acid sprays on calf carcasses with concentrations upto 1.25 per cent (v/v) did not produce unacceptable discolourations and concentrations upto two per cent (v/v) were not significantly different from control in terms of flavour. Bactericidal properties of 1.25 per cent lactic acid sprays were quantified. Aerobic colony counts and enterobacteria showed marked reduction by this treatment.

Snijders et al. (1985) observed that lactic acid as a terminal decontaminant, in addition to good slaughter hygiene, produced both an immediate (bactericidal) and a delayed (bacteriostatic) effect which resulted in an extended shelf life of meat.

Acuff et al. (1987) conducted an experiment in which beef strip loins were decontaminated by spraying with various food grade acid solutions (1% lactic acid, 1% acetic acid and a mixture of 1% lactic acid, 2% acetic acid, 0.25% citric acid and 0.1% ascorbic acid). These were then vacuum packaged and stored at $4 \pm 1°C$ for a long duration. Mean aerobic plate counts of steaks fabricated from control and acid treated

loins, taken during different days of storage, were not significantly different.

Hamby et al. (1987) compared the effect of intermittent spray chilling and single spray treatment with one per cent acetic acid or one per cent lactic acid on the microbiological properties of beef cuts. They noticed significant reduction in aerobic plate count on the rib and close areas of carcass treated with acetic acid and reduction of aerobic plate count in all sampling areas sprayed with one per cent lactic acid in case of intermittent spray chilling. Single spray of lactic acid resulted in significant reduction in aerobic plate counts on strip loins and rib areas whereas treatment with one per cent acetic acid did not show significant effect.

Adam and Hall (1988) measured the inhibitory effect of lactic and acetic acids towards Salmonella enteritidis and E. coli. In weakly buffered media, an apparently synergistic interaction was observed between these two acids.

Marel et al. (1988) reported that decontamination with 1-2 per cent lactic acid solutions at pH two, when applied shortly before chilling, very significantly improved bacterial safety and increased the refrigerated shelf life of broiler carcasses.

Visser et al. (1988) assessed the effect of lactic acid decontamination on the microbiological condition and keeping qualities of veal calf tongues. Decontamination with two per cent (v/v) lactic acid, decreased the mesophilic aerobic count, from 5.6 to 2.7 $\log_{10}$CFU/cm^2. After 14 days storage the delayed effect of lactic acid was still observed.

Davey and Smith (1989) conducted an experiment in which E. coli inoculated beef sides were washed with water at different temperatures and with different exposure time. There was a significant linear relationship between log reductions in bacterial count and temperature of water which varied with exposure time.

Anderson and Marshall (1990a) reported that most effective treatment to decontaminate the lean beef muscle by sanitization was to dip in three per cent lactic acid at 70°C. As the temperature of the sanitizing agent is increased (from 25 to 70°C), concentration of sanitizing agent became an insignificant variable.

A mixture of acids at different temperatures was tried for bacterial count reduction of beef tissue by Anderson and Marshall (1990b) and observed that the effect was proportionate to temperature and concentration of acid

mixtures against S. typhimurium, Enterobacteriaceae and E. coli.

Izat et al. (1990) conducted a study to determine the effect of propylene glycol and lactic acid alone or in combination on levels of salmonellae on broiler carcasses. The two treatments were effective in completely eliminating salmonellae (0.25% lactic acid + 20% propylene glycol or 0.5% lactic acid + 20% propylene glycol). But both produced colour and odour problems.

Saoji et al. (1990) studied the preservative effect of one, two, three and four per cent of acetic and lactic acids on buffalo meat stored at refrigeration temperatures. The bacteriostatic or bactericidal effect increased with the increase in concentration of acids. Both acids showed pronounced effect on gram negative bacteria. Two per cent acetic acid and one per cent lactic acid were effective antimicrobial agents.

Smulders et al. (1990) reported that discolouration of meat surfaces did not occur at concentrations of approximately one per cent (v/v) lactic acid and upto two per cent did not cause off-flavours in meat. But significant reductions of bacterial flora was noted in these treatments.

Tomancova and Steinhowzer (1990) studied the effect of one per cent acetic acid, two per cent lactic acid and combination of one per cent solution of each of the acids on shelf life and sensory changes of vacuum packaged meat. They reported that the shelf life of sample treated with acetic acid, lactic acid and combination of acids increased by 15-17 days, 18-20 days and 20-24 days respectively.

Dixon et al. (1991) reported that spraying of steer carcasses with hot (55°C) one per cent lactic acid before evisceration and before entering the chiller produced lower mean aerobic plate counts.

Prasai et al. (1991) observed spraying with hot (55°C), dilute (1% v/v) lactic acid on beef carcass surfaces immediately after dehiding and after evisceration brought in a reduction in $\log_{10}$ aerobic plate count by more than 90 per cent. No further reduction was noted after 72 h of postmortem storage under chilling.

Anderson et al. (1992) designed a study on sanitizing beef surfaces to evaluate effects of mixtures of acetic, lactic, citric and ascorbic acids, changing the concentration and also acetic and lactic acids, individually at various temperatures. An increase in either acid concentration or treatment temperature decreased the number of residual viable

bacteria. Lactic acid was the most effective against S. typhimurium at 70°C.

Prasai et al. (1992) assessed microbiological effect of hot (55°C) 1% v/v lactic acid sprayed on the pork carcass surfaces immediately after dehairing, after evisceration or at both locations in slaughter house. Mean aerobic plate counts of all acid-treated carcass surfaces were numerically lower than those of control carcasses, but these reductions were not statistically significant.

Materials and Methods

MATERIALS AND METHODS

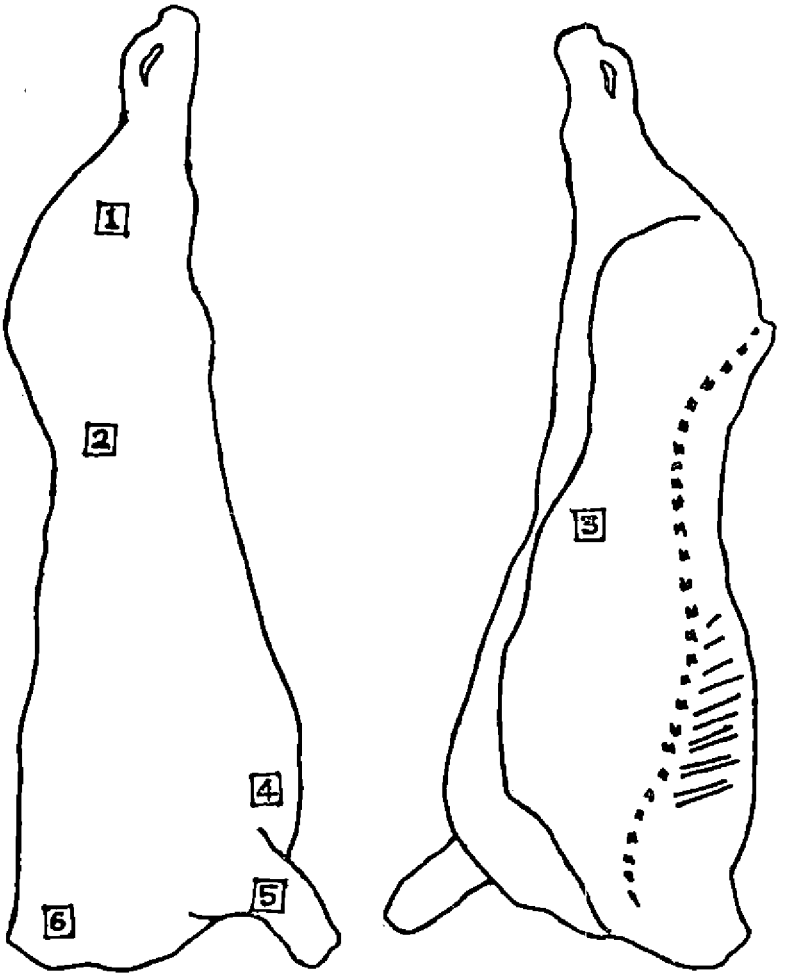
The carcasses for the study were obtained from the slaughter house attached to the College of Veterinary and Animal Sciences, Mannuthy and also from the Municipal slaughter house, Trichur. Beef carcasses from adult animals were subjected for the study. Ten carcasses from Municipal slaughter house were used exclusively for the determination of bacterial critical points. Ten carcasses from University slaughter house were used to study both bacterial critical points and sanitizing effect of lactic acid.

The methods of slaughter of animals were different in two slaughter houses. In the University slaughter house, animals were stunned with captive bolt pistol, followed by bleeding and flaying on the cradles. Thereafter, evisceration and splitting was done on rails. In Municipal slaughter house. stunning was done by a blow on the forehead with a metal hammer. Bleeding, flaying, evisceration and splitting were all done on the floor.

Bacterial critical point sites

The bacterial critical points were determined by assessing the total viable count (TVC) of six different

FIG.1 SAMPLING SITES ON BEEF CARCASS



LATERAL SURFACE *MEDIAL SURFACE*

1. *Hindlimb-lateral*
2. *Flank*
3. *Abdomen-medial*
4. *Forerib-lateral*
5. *Forelimb-lateral*
6. *Neck-lateral*

defined sites on each carcass. The following were the defined sites as indicated in the Fig.1.

(i) Hind limb-lateral

(ii) Flank

(iii) Abdomen-medial

(iv) Fore rib-lateral

(v) Fore limb-lateral and

(vi) Neck-lateral

Collection of samples

The samples were taken at the end of the slaughter line i.e. immediately after evisceration and splitting. Swabbing of specific area was done with the help of square aluminium template with 5x5 cm^2 internal measurement which can expose 25cm^2 area on the carcass surface.

The templates were sterilized in hot air oven before use. The swab was prepared using 1 cm wide aluminium plate by wrapping absorbent cotton to form about 1.5 cm wide swabbing surface. Absorbent cotton swabs were sterilized by autoclaving. The swabbing end was moistened with 0.1 per cent peptone water and allowed to drip. The exposed area on carcass surface was swabbed first from left to right with one side of swab, then top to bottom with the other side and finally from corner to corner with the tip of the swab. This

was then transferred into a test-tube containing 25 ml sterile 0.1 per cent peptone water as diluent. These samples were transported to the laboratory immediately after collection in a thermocol container. In the laboratory all samples were processed immediately.

The swabs in the peptone water were agitated thoroughly to emanate bacteria into the diluent. This formed the stock solution. The bacterial content of one ml of the stock solution will be the number of organisms per cm^2 of the carcass surface. From this stock solution 10 ml was transferred into a conical flask containing 90 ml of diluent, with a sterile pipette, to form one in ten dilution. Further 10 fold serial dilutions were made by transferring 1 ml of this solution into 9 ml of the diluent. Thus the dilutions were made upto 10^6.

Total viable count (TVC)

Total viable count (TVC) per cm^2 of the samples was estimated by pour-plate technique following the procedure recommended by American Public Health Association (APHA, 1976). The selected dilution of the sample was used for estimation of TVC. One ml each of the selected dilution was transferred into duplicate sterile petriplates with sterile pipette. To each of these petriplates, about 15-20 ml sterile

molten standard plate count agar (Hi-media, composition and preparation appended) maintained at 45°C in a water bath, was poured.

The contents in the petriplates were mixed by gentle clockwise and anticlockwise rotatory movements followed by forward and backward movements. The plates were left at room temperature to allow the medium to solidify. These plates were then incubated at 37°C for 24 hr, and examined at the end of incubation period. The plates having 50 to 300 colony forming units (CFU) were selected for colony counts. The colonies were counted with the help of a colony counter. The TVC of the surface area of carcass was estimated from the average number of CFUs in the plates, applying the dilution factor of the innoculum and expressed as $\log_{10}$CFU/cm^2.

Sampling for lactic acid treatments

The meat samples were collected from the neck region (the maximum bacterial load was observed during the bacterial critical point study) of carcasses with sterile precautions at the end of slaughter line. The size of the sample was about 300 cm^2 area, rectangular in shape, having a thickness of about 1.5 cm. Each sample was divided into three equal parts of about 100 cm^2 with the help of sterile stainless steel scissors and forceps. The three pieces were transferred

into separate sterile enamel trays (30 x 20 cm) and labelled as 'L' 'HL' and 'C' indicating lactic acid at ambient temperature, hot lactic acid and control respectively. The sample labelled 'L' was dipped completely in one per cent lactic acid solution in distilled water for 15 sec. Similarly the sample labelled 'HL' was completely dipped in one per cent lactic acid solution in distilled water at 70°C for 15 sec. Sample labelled C was kept as control. After acid treatment the samples were hung using sterile metal hooks and allowed to drain at ambient temperature for 1 h.

Estimation of bacterial load

Total viable count, coliform count and faecal streptococcal count on the surface of the control was estimated immediately after sampling. An area of 25 cm^2 was demarcated using sterile aluminium template. The swabbing of the area and preparation of diluent was done as mentioned in bacterial critical point study.

Total viable count was estimated as done for the determination of bacterial critical point study. The coliform count was made following the method described by Nordic Committee on Food Analysis (1966). Spread plate method of inoculation was done using Violet Red Bile agar (Hi-media; composition and preparation appended). One in hundred

dilution was selected as found suitable for coliform estimation from preliminary trials. One tenth of a millilitre (0.1 ml) of the innoculum was poured over the surface of media in duplicate petriplates with sterile pipette and spread evenly using a sterile 'L' shaped glass rod. The inoculated plates were incubated at 37°C for 24 h. After incubation, the purplish red colonies with a diameter of 0.5 mm or more, surrounded by a red precipitation zone and characteristic of coliforms, were counted. The number of CFU per cm^2 of the sample was estimated from the mean colony count applying dilution factor and was expressed as $\log_{10}CFU/cm^2$ of the sample surface.

Faecal streptococcal count was determined following spread plate technique described by Nordic Committee on Food Analysis (1968). K.F. streptococcal agar (Hi-media, composition and preparation appended) was used. One-in-ten dilution of the innoculum was selected, as found suitable for faecal streptococcal count estimation from preliminary trials. On the surface of medium in the petri plates, 0.1 ml of the innoculum was poured in duplicate plates with the help of sterile pipette and was spread evenly with sterile 'L' shaped glass rod. These plates were incubated at 37°C for 48 h. After incubation, colonies with pink to dark red colour surrounded by narrow white zone, characteristic of faecal

streptococci were counted. The number of CFU/cm^2 of the sample was estimated from the mean colony count, applying dilution factor and expressed as $\log_{10}$CFU/cm^2 of the sample surface.

The estimation of TVC, coliform count and faecal streptococcal count of all samples were also made one hour after the treatment.

Statistical analysis

The data were analysed statistically by using the method of analysis of variance and paired 'T' test as explained by Snedecor and Cochran (1967).

RESULTS

Total viable count (TVC) is an indication of the extent of bacterial contamination on carcasses. It is an indication of the level of hygiene at production points, influenced by the slaughter practices adopted and existing environment. Estimation of TVC from identified critical points of carcass is a sensitive method for evaluation of its hygienic standard. TVC assessed at six different sites on surface of ten carcasses each, examined from Municipal slaughter house, Trichur (MSH), and Kerala Agricultural University slaughter house (USH) are given in Table 1. The TVC expressed as the average log counts per cm^2.

The TVC at all points on carcasses from USH was significantly ($P < 0.01$) lower than the MSH samples. The highest TVC was found in the neck and the lowest in abdomen medial points. The TVC at selected sites of carcasses from USH and MSH are presented in Fig.2a and 2b respectively. The TVC at neck region was 5.44 CFU/cm^2 in samples collected from MSH and 4.39 CFU/cm^2 in USH samples. In forelimb, TVC was 5.33 in MSH samples whereas in USH samples it was 4.32. In the case of hindlimb TVC in MSH samples was 5.32 and 4.28 in USH samples. In forerib, TVC was 5.27 in MSH samples and 4.28 in USH samples. The flank has shown a TVC of 5.23 in MSH

samples and 4.26 in USH samples. The TVC on abdomen medial was 5.20 in MSH and 4.23 in USH samples. The comparative values of TVC at different sites of carcasses showed that the trend of bacterial contamination was almost similar on carcasses obtained from both slaughter houses.

The analysis of variance of TVC of six different sites on carcasses from MSH and USH is shown in Table 1a. Highly significant difference was noticed among the various sites of carcasses from both MSH and USH.

Analysis of logarithmic mean of TVC at different points of carcasses from the USH is given in Table 2. While testing the significance among the six sites, it was found that neck was significantly different from all the other sites, registering a maximum mean count. Next in order was the forelimb which was also significantly different from all the other sites. Among the remaining four sites, it was found that there was no significant difference between hindlimb forerib and flank. The abdomen medial showed the least count and was significantly different from hind limb and forerib.

Table 3 shows the analysis of logarithmic mean of TVC at different points of carcasses from MSH. Test of significance of TVC at six different sites showed that the neck region having the maximum TVC was significantly different

Table 1. TVCs of different sites on carcasses collected from two slaughter houses

Sites	Mean log CFU/cm^2 ± standard error		t value
	MSH sample	USH sample	
Neck-lateral	5.44 ± 0.01	4.39 ± 0.01	84.67**
Forelimb-lateral	5.33 ± 0.01	4.32 ± 0.01	90.72**
Hind limb-lateral	5.32 ± 0.02	4.28 ± 0.02	49.10**
Forerib-lateral	5.27 ± 0.01	4.28 ± 0.01	66.10**
Flank	5.23 ± 0.02	4.26 ± 0.01	41.08**
Abdomen-medial	5.20 ± 0.03	4.23 ± 0.01	42.37**

* = P <0.05 ** = P <0.01

Table 1a. ANOVA of TVCs of different sites on carcasses from two slaughter houses

Variables	d.f.	Mean sum of squares	
		USH sample	MSH sample
Treatment	5	0.0285**	0.0720**
Error	54	0.0012	0.0032

* = P <0.05 ** = P <0.01

Table 2. Mean TVCs of different sites on carcasses from University slaughter house

Sites	Mean log CFU/cm^2
Hind limb-lateral	4.28^{ab}
Flank	4.26^{acd}
Abdomen-medial	4.23^{d}
Forerib-lateral	4.28^{abc}
Forelimb-lateral	4.32^{e}
Neck-lateral	4.39^{f}

Means having the same superscripts are not significantly different

Table 3. Mean TVCs of different sites on carcasses from Municipal slaughter house

Sites	Mean log CFU/cm^2
Hind limb-lateral	5.32^{a}
Flank	5.23^{bc}
Abdomen-medial	5.20^{b}
Forerib-lateral	5.27^{c}
Forelimb-lateral	5.33^{a}
Neck-lateral	5.44^{d}

Means having the same superscripts are not significantly different

FIG.2a TOTAL VIABLE COUNT AT CRITICAL POINTS OF CARCASSES FROM UNIVERSITY SLAUGHTER HOUSE

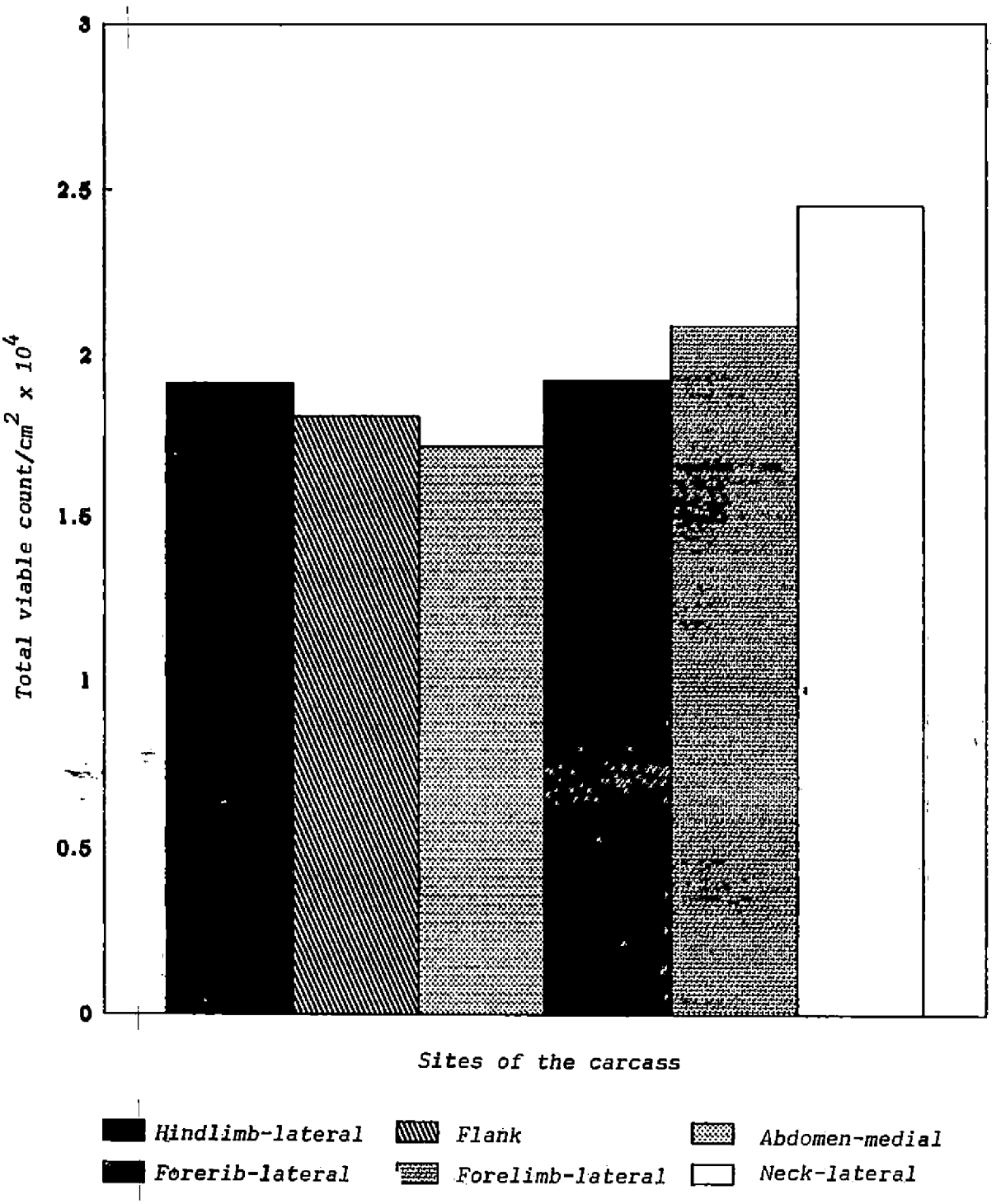


FIG.2b TOTAL VIABLE COUNT AT CRITICAL POINTS OF CARCASSES FROM MUNICIPAL SLAUGHTER HOUSE

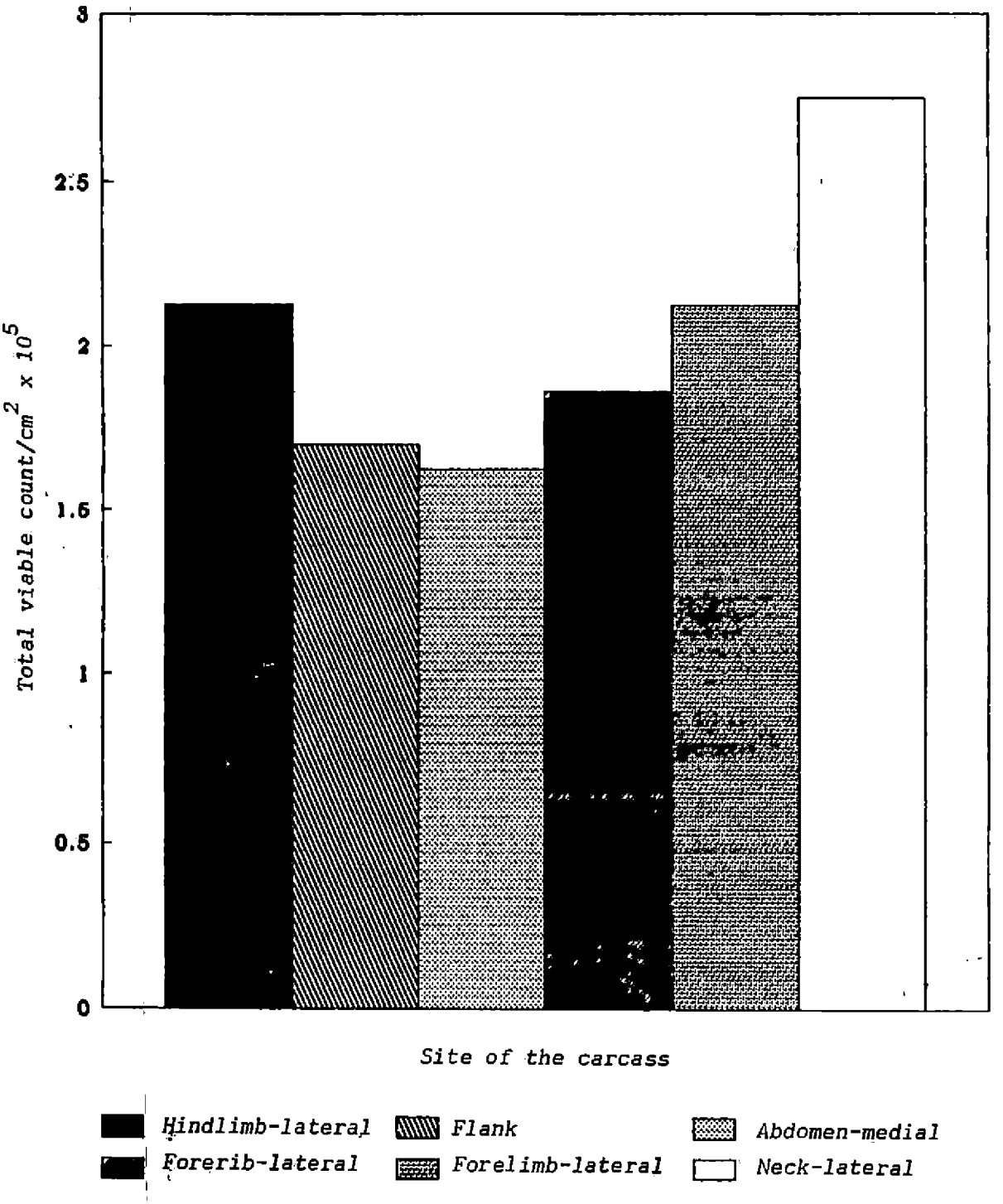
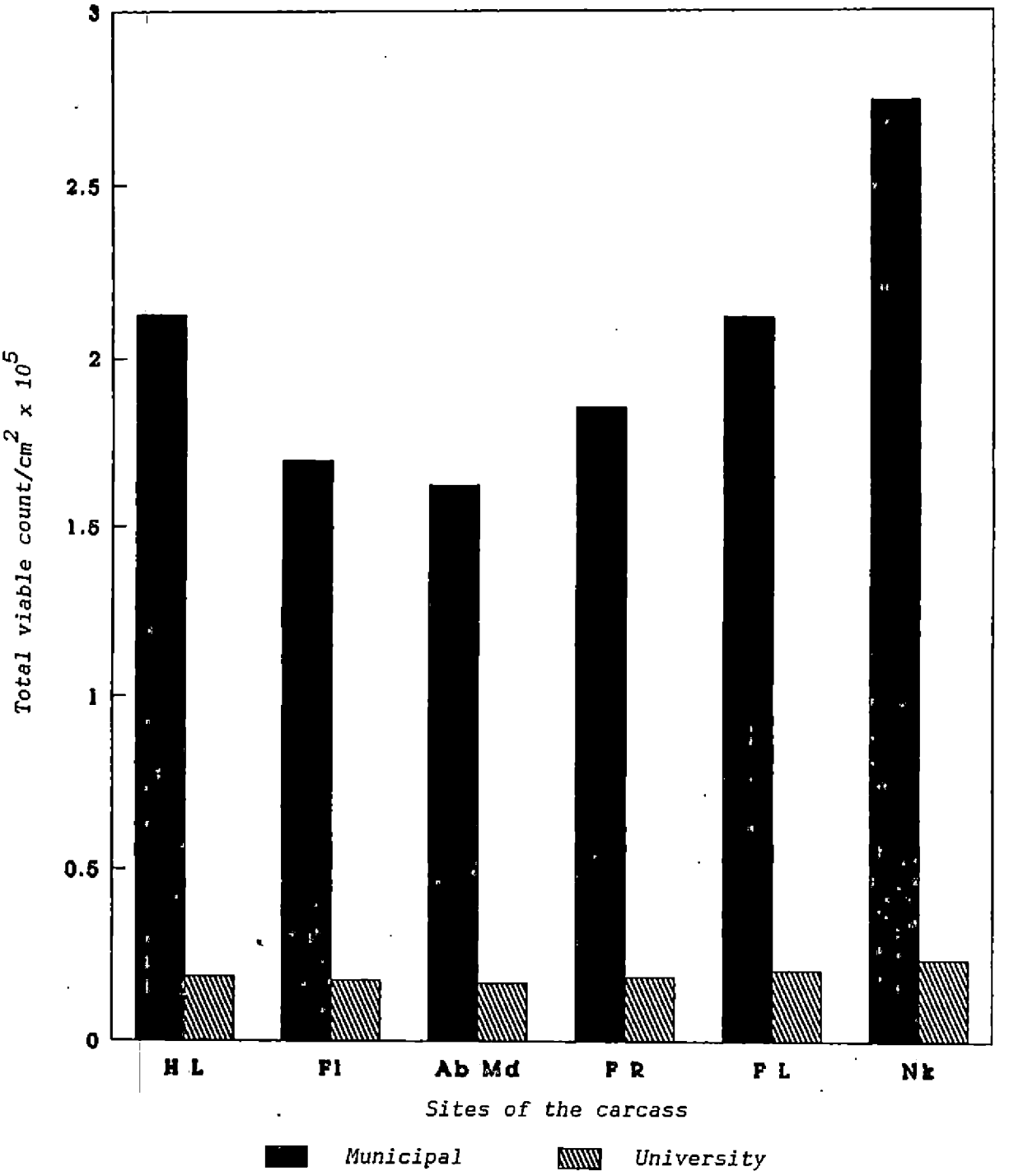


FIG.3 BACTERIAL LOAD AT CRITICAL POINTS OF CARCASSES FROM UNIVERSITY AND MUNICIPAL SLAUGHTER HOUSES



HL - Hindlimb-lateral *Fl - Flank* *Ab Md - Abdomen-medial*

FR - Forerib-lateral *FL - Forelimb-lateral* *NK - Neck-lateral*

from all other sites. The forelimb and hind limb were similar and were significantly different from all other sites. Among the remaining three sites, there was significant difference between forerib and abdomen medial and the latter showed the minimum TVC. Comparative bacterial counts at specified sites on carcasses obtained from MSH and USH are shown in Fig.3.

Lactic acid treatment

Beef collected from ten carcasses obtained from University slaughter house were subjected to treatment with one per cent lactic acid solution at two different temperatures, one at room temperature and the other at 70°C, to evaluate the sanitizing effect of lactic acid on meat. The initial TVC, coliform count and faecal streptococcal counts were estimated. It was 4.38, 2.58 and 2.50 log mean count/cm^2 respectively for TVC, coliform count and faecal streptococcal count. After 1 h, the bacterial count in respect of TVC, coliforms and faecal streptococci count were made on control, sample treated with one per cent lactic acid at ambient temperature (T_1) and sample treated with 1 per cent lactic acid at 70°C (T_2) (Table 4).

The bacterial counts in control, T_1 and T_2 at 1 h post treatment storage at ambient temperature were significantly different between control and T_1, control and T_2 and also

between T_1 and T_2. This phenomenon was noticed in TVC, coliform and faecal streptococcal counts.

After 1 h of storage at ambient temperature, in control, there was log mean increase to the extent of $0.04/cm_2$ for TVC, $0.05/cm^2$ for coliforms and $0.05/cm^2$ for faecal streptococcal counts in comparison to the counts at zero hour. In the case of T_1, reduction in bacterial count was noticed to the extent of 0.18, 0.22 and 0.26 mean log/cm^2 respectively for TVC, coliform count and faecal streptococcal count. In T_2, also there was reduction in TVC, coliform counts and faecal streptococcal counts to the extent of 0.51, 0.63 and 0.81 mean log count/cm^2, respectively.

The effect of lactic acid treatment on bacterial load on meat surface during storage for 1 h is shown in Table 4. The change in TVC on sample surface at 1 h on control, T_1 and T_2 in comparison to the initial load is represented in Fig.4. Similar change in coliform count and faecal streptococcal counts on samples at 1 h are shown in Fig.5 and 6 respectively. The treatment had highly significant effect in reduction of TVC, coliform count and faecal streptococcal count. TVC was significantly lower ($P < 0.01$) in T_1 and T_2 in comparison to the control at 1 h. TVC in T_2 was significantly lower than T_1 ($P < 0.01$). In the case of coliforms, the count was significantly lower ($P < 0.01$) in T_1 and T_2 in comparison

to the control at 1 h, T_2 having a significantly lower coliform count than T_1. Faecal streptococcal count was significantly lower in T_1 and T_2 than control at 1 h after the treatment, T_2 count being significantly lower ($P < 0.01$) than T_1.

Analysis of variance shows significant difference ($P<0.01$) between treatments with respect to TVC, coliform and faecal streptococcal counts (Table 5).

Log per cent change in bacterial count due to lactic acid treatment on meat surface during the period of 1 h storage is given in Table 6. This change is in relation to the initial count at zero hour. In the case of TVC, in control, there was an increase of 0.91 log per cent during 1 h of storage. In T_1 the log per cent reduction was 4.98. In T_2 also there was a reduction of 12.44 log per cent.

The coliform count increased by 1.93 log per cent at 1 h in control whereas there was reduction of 10.26 and 25.85 log per cent in T_1 and T_2 respectively. Faecal streptococcal count had shown an increase of 2.00 log per cent at 1 h in control. In T_1, there was a reduction of 12.15 log per cent and in T_2, the reduction was 33.72 log per cent.

Table 4. The effect of lactic acid treatment on bacterial load on meat surfaces (mean log CFU/cm^2 ± standard error)

Type of count	Initial load at 0 h	Count at 1 h		
		Control (untreated)	Lactic acid at ambient temperature (T_1)	Lactic acid at 70°C (T_2)
TVC	4.38^a ± 0.01	4.42^a ± 0.01	4.20^b ± 0.01	3.87^a ± 0.02
Coliform count	2.58^a ± 0.03	2.63^a ± 0.03	2.36^b ± 0.02	1.95^c ± 0.03
Faecal streptococcal count	2.50^a ± 0.03	2.55^a ± 0.02	2.24^b ± 0.03	1.69^a ± 0.06

Means having the same superscripts are not significantly different. Comparisons were made row-wise

Table 5. ANOVA of different bacterial counts after lactic acid treatment

Source	d.f.	Mean sum of squares		
		TVC	Coliform count	Faecal streptococcal count
Treatment	3	0.6001**	0.9468**	1.5819**
Error	36	0.0026	0.0084	0.0189

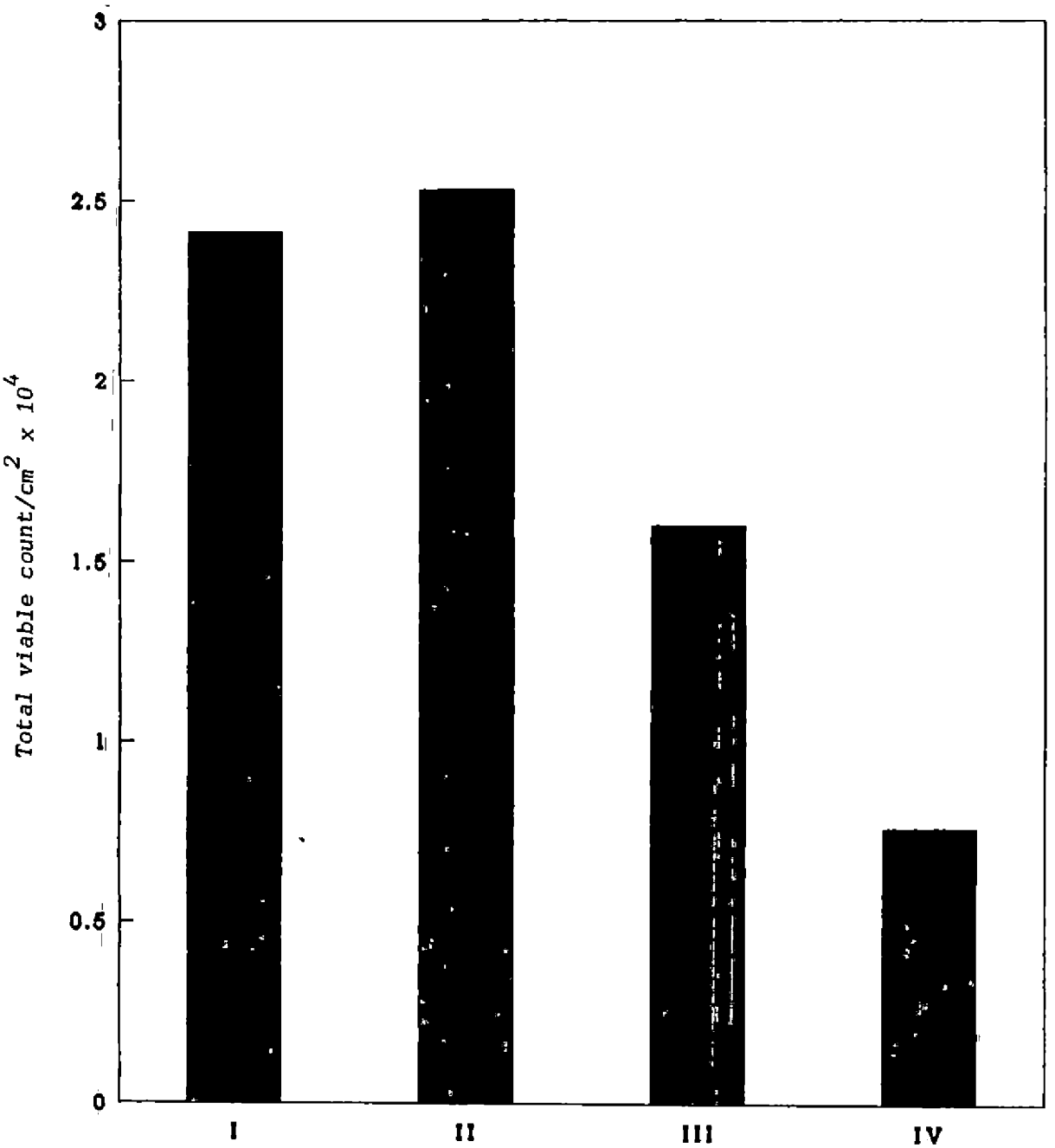
* P <0.05 ** P <0.01

Table 6. Effect of lactic acid treatment on bacterial load on meat surface at 1 h of storage

Type of count	Log per cent change in bacterial count		
	Control	Treatment I (Lactic acid)	Treatment II (Hot lactic acid)
TVC	(+) 0.91	(-) 4.98	(-) 12.44
Coliform count	(+) 1.93	(-)10.26	(-) 25.85
Faecal strepto-coccal count	(+) 2.00	(-)12.15	(-) 33.72

\+ increase - reduction

FIG.4 EFFECT OF LACTIC ACID TREATMENTS OF BEEF ON TOTAL VIABLE COUNT AT ONE HOUR



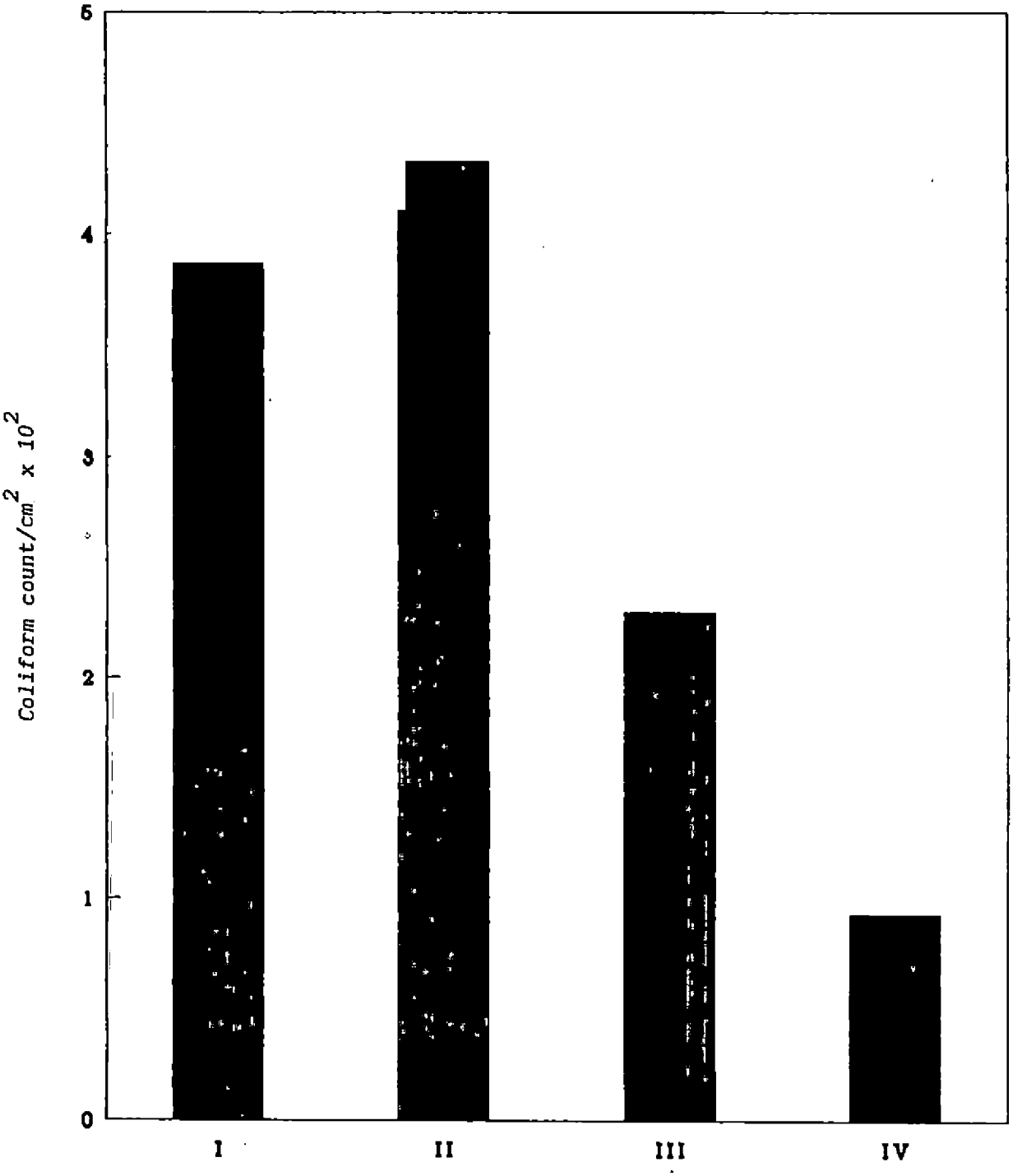
I Control at 0 h

II Control at 1 h

III 1% lactic acid of ambient temperature

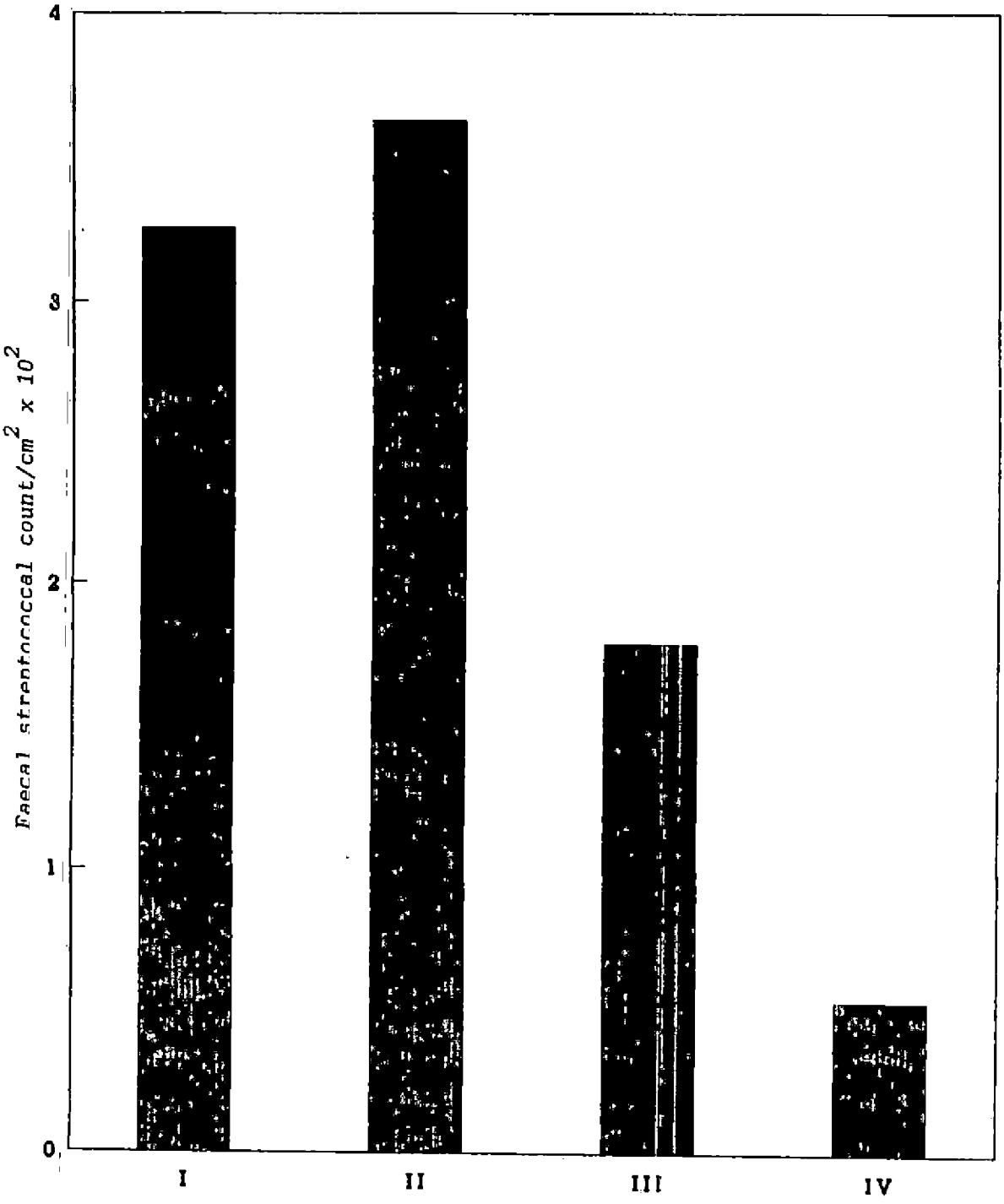
IV 1% lactic acid at 70°C

FIG.5 EFFECT OF LACTIC ACID TREATMENT OF BEEF ON COLIFORM COUNT AT ONE HOUR



I Control 0 h
II Control at 1 h
III 1% lactic acid of ambient temperature
IV 1% lactic acid at 70°C

FIG.6 EFFECT OF LACTIC ACID TREATMENT OF BEEF ON FAECAL STREPTOCOCCAL COUNT AT ONE HOUR



I Control at 0 h

II Control at 1 h

III 1% lactic acid of ambient temperature

IV 1% lactic acid at 70°C

Discussion

DISCUSSION

Theoretically, meat derived from healthy animal should be sterile. But during conventional slaughter procedures and further processing, the carcasses get contaminated with microorganisms which will affect its keeping quality and wholesomeness. Thus estimation of microorganisms has been universally recommended to ensure good manufacturing practices. Contamination of carcass is principally a surface phenomenon and evaluation of bacterial load on the entire carcass surface is impracticable. Hence application of Hazard Analysis Critical Control Points (HACCP) system has been recommended in the production, processing and marketing of meat. Implementation of this system involves identifying critical points on the carcass. Critical points, in a bacteriological sense, are those which are more prone for contamination. Identification of these anatomical sites will help in further monitoring the hygienic quality of meat and measures can be taken in reducing the contamination level.

Six anatomical sites in the carcass were identified in the present study as critical points and the total bacterial load on those locations were evaluated under two different systems of slaughter practices.

Ten carcasses each from University slaughter house (USH) and Municipal slaughter house (MSH) were examined for bacterial load at six critical points. The maximum bacterial load was found at the neck-lateral followed by forelimb-lateral, hindlimb-lateral, forerib-lateral, flank and the minimum at abdomen-medial region in that order. This phenomenon was noticed in carcasses from both slaughter houses. Sources of bacterial contamination were the skin, intestinal contents, utensils and personnels at the time of slaughter. The highest bacterial load in carcasses from both houses, was noted in the neck-lateral and this may be due to various factors. Bleeding of the animals were effected by bilateral severance of the carotid arteries and jugular veins by a transverse incision across the throat region caudal to the larynx. During this process microorganisms from the skin, knife, oesophagus and trachea get disseminated in the region. The contamination in this area is likely to be further aggravated during flaying and evisceration, when these operations are done with little precaution. The abdomen medial area was found to have the minimum bacterial load. This may be due to the minimum handling compared to other sites during operation and other environmental contaminating sources. Only during evisceration this part come in contact with the handlers and that too, the minimum, as the abdominal viscera is removed without opening them.

In the order of contamination forelimb-lateral, hindlimb-lateral, forerib-lateral and flank were found next to neck. Forelimb-lateral was also subjected to frequent handling during various process of slaughter.

Generally there is a tendency for higher level of contamination in forequarters than the hind quarters as reported by Nortje et al. (1989). When the carcass is suspended by the hindlimb, there is chance of accumulation of bacteria at the lower portion of the hanging carcass. A higher bacterial load in the forelimb-lateraland neck-lateral observed during the present study may be attributed to this.

For evaluation of the bacterial load of the carcass, it is not sufficient to test a single site. Roberts et al. (1984) suggested to estimate bacterial load at least from three or four sites, as single site would under-estimate the contamination of carcasses. The results obtained in the present study also substantiate the above contention and it is suggested that more than one site are to be examined to assess the bacterial load on the carcass.

The analysis of the data showed that there was highly significant difference in bacterial load between the sites. This indicate that the association of the sites with the factors responsible for bacterial contamination is not

uniform. Though the general pattern of bacterial load of carcass obtained from the two slaughter houses was similar, quantitatively they were significantly different. The bacterial load was higher on carcasses from MSH than that of USH. The infrastructural facilities of these two slaughter houses are widely different. The USH is built with facilities for hygienic production of meat with adequate construction, environmental protection, overhead rails and professional supervision. These facilities are not existing in the MSH. Hence the highly significant difference in bacterial load is a reflection on the facilities available and practices followed.

Lactic acid treatment

The basic approach for reducing initial microbial load on carcass surface is to follow strict sanitary slaughter and fabrication process (Chandran et al., 1986). Even with the best possible slaughtering and dressing practices, carcass will still contain considerable microbial load. In order to control bacterial load, various treatments like washing or spraying the carcass, using hot or cold water, with and without sanitizers have been tried and evaluated. Use of edible organic acids, particularly lactic acid and acetic acid have been subjected to considerable investigations as a means of reducing bacterial contamination on fresh meat (Smulders and Woolthuis, 1985; Snijders et al., 1985).

In the present study, for sanitization of meat, one per cent lactic acid at two different temperatures were used to evaluate their sanitizing effect on beef. For the purpose of evaluation of sanitizing effect, total viable count (TVC), coliform count and faecal streptococcal count were estimated. The above organisms are indicators of sanitary standards of the product. The initial load of TVC was 4.38, coliforms 2.58 and faecal streptococci 2.50 log CFU/cm^2, before the lactic acid treatment was done. Evaluation of the bacterial load on samples after 1 h, indicated appreciable reduction in treated samples in comparison to the control.

These changes were highly significant. The bactericidal and bacteriostatic effect of lactic acid is well documented. Lactic acid is listed as 'generally recognised as safe' (GRAS) in United States (Food and Drug Administration, 1981). Similarly in Europe it is considered as harmless constituent of foods. Lactic acid exerts both an immediate (bactericidal) and a delayed (bacteriostatic) effect, that results in extended shelf life of meat (Snijders et al., 1985). Short chain fatty acids such as acetic and lactic acids are the most widely used organic acids for the preservation of fresh red meat. Antibacterial effects of these acids is due to both the depression of pH below range and the inhibition by undissociated acid molecules

(Ingram et al., 1956; Adams and Hall, 1988). Lactic acid at one per cent (pH 2.4) has got bactericidal property without adversely affecting the sensory attributes of food (Smulders et al., 1986).

The effect of acid treatment on TVC observed at 1 h post treatment indicated, in comparison to control that the one per cent acid solution at room temperature (T_1) had brought about a reduction of 4.98 per cent, while the lactic acid at 70°C (T_2) had effected a reduction of 12.44 per cent. The difference in TVC between the treatments was 7.46 per cent which could be attributed to the effect of higher temperature.

Better sanitizing effect as evidenced by a reduction in the total count in T_2 can be attributed to the higher temperature of lactic acid solution. Bacteria are susceptible to destruction by higher temperature. Lactic acid exerted stronger microbicidal effect at 35°C than at chill room temperature (Park and Martin, 1972). It also reduced bacterial count when used at 70°C than at 20°C (Anderson et al., 1992). The present observation is in agreement with the above reports and there appears to be a synergistic action between the acid and temperature in its sanitizing effect.

The effect of acid treatment of beef on coliform count showed that there was a reduction of 10.26 per cent in sample

treated with one per cent lactic acid at room temperature in comparison to the control. In the case of samples treated with lactic acid solution at 70°C, the reduction was 25.85 per cent as compared to the control. Between the treatments the samples treated with hot acid solution had shown a beneficial reduction of 15.59 per cent. Pronounced antibacterial effect of one per cent lactic acid on gram negative bacteria was reported by Sherikar *et al*. The present observation also substantiate this report. The enhanced antibacterial effect of lactic acid was achieved by increasing the temperature of sanitizing solution. When lactic acid was applied on calf carcass, Enterobacteriaceae count was markedly reduced (Woolthuis and Smulders, 1985). Lactic acid in meat appears to exert selective effect on gram negative flora mainly by reducing the pH (Gill and Newton, 1982). The trend in reduction of *E. coli* count was observed as the concentration of the acid was increased (Anderson *et al*., 1990).

Percentage of faecal streptococci in the meat samples indicate poor hygienic status of production. The study indicate treatment with lactic acid can reduce the contamination.

The effect of acid treatments of samples with respect to the load of faecal streptococci indicated that marked reduction was achieved. The difference in counts between

control and T_1 was 12.15 per cent and that between control and T_2 was 33.72 per cent. Thus, between T_1 and T_2, the difference was 21.57 per cent, the count being lower in T_2. This effect may be due to the elevated temperature of acid solution.

Different members of bacterial population responded in different ways to acid treatments with varying concentration and temperature (Anderson et al., 1992). Reports on effect of lactic acid treatment, specifically on faecal streptococci on meat surface, is not seen available. However acid treatment, especially at higher temperature, is generally effective in markedly reducing bacterial load (Anderson and Marshall, 1990a and b). This may be applicable to faecal streptococci also.

The result of the present study indicate that the treatment of beef immediately after production, with one per cent lactic acid solution, brings about significant reduction in bacterial load and thus improve the hygienic quality of meat. This effect is enhanced by using hot lactic acid solution, without affecting the wholesomeness of meat. Since the initial bacterial load has a bearing on the shelf life of meat, sanitization with 1 per cent lactic acid solution will help in reducing the initial microbial load and thus prolong the storage period. In view of the tropical climate, existing

poor hygienic status at production sites and marketing system it is recommended that sanitization of carcass at production point with one per cent lactic acid preferably at 70°C will help to improve bacterial quality of meat in retail market.

Summary

SUMMARY

During the conversion of animals into carcasses, either in the conventional or modern slaughter and dressing processes, beef carcasses get contaminated with a variety of bacteria. The surface bacterial load on the carcass at the end of the slaughter line is an indication of the hygienic status of production and has an important bearing on the storage life.

The methods for obtaining a meaningful information on the bacterial status of the carcass is limited by the constraints of time and cost. Even within a carcass, there may be bacteriologically dirty areas, that will have comparatively higher level of contamination. Hence identifying locations on the carcass having the chances of maximum contamination, i.e., bacterial critical points, will help in quicker evaluation of bacterial contamination and in taking appropriate control measures. Even with the best possible slaughter and dressing practices, the carcasses will still contain considerable bacterial load. Among the various treatments, washing the carcass with edible organic acid solutions like lactic acid and acetic acid are widely used to reduce the surface bacterial load.

The present study is to identify the bacterial critical points on beef carcass surfaces and also to assess and compare the sanitizing effect of lactic acid solutions at two different temperatures, one at room temperature and the other at 70°C.

Ten carcasses each from University slaughter house (USH) and Municipal slaughter house (MSH) were subjected to identification of bacterial critical points. The evaluation of total viable count (TVC) was made following the method described by the American Public Health Association (1976). Six different sites on each carcass viz., hindlimb-lateral, flank, abdomen-medial, forerib-lateral, forelimb-lateral and neck-lateral, were chosen at the end of the slaughter line. The estimated TVCs were expressed as log CFU/cm^2.

The TVCs at all points on carcasses from USH were found to be lower than the MSH samples and this was highly significant. Highly significant difference was noticed between the various sites on carcasses from both USH and MSH. Comparatively lower level of contamination on the carcasses from USH is likely to be due to the improved infrastructural facilities and practices.

The highest count was found in the neck-lateral followed by forelimb-lateral, hindlimb-lateral, forerib-

lateral, flank and abdomen-medial, in that order, for both USH and MSH samples.

The highest count at the neck-lateral may be due to various factors like the cutting of the neck, trachea and oesophagus during bleeding. Frequent handling of the exposed areas by the operators and contamination from the blade of the knife could also have contributed to the higher bacterial load.

The results of the study indicate that, for identification of bacterial critical points to evaluate the sanitary standard of beef carcasses, neck-lateral, forelimb-lateral, hindlimb-lateral and forerib-lateral may be examined as they are found to be highly contaminated sites.

For lactic acid treatment, ten beef carcasses from USH were selected. Samples were collected from the neck region of carcasses and initial load of bacteria such as TVC, coliform count and faecal streptococcal count were estimated. The coliform and faecal streptococcal count were made following the method described by Nordic Committee on food analysis (1966 and 1968). Approximately 100 cm^2 area of each sample was treated individually with one per cent lactic acid solution at ambient temperature and at 70°C for 15 sec. A control sample was also kept. The estimation of TVC, coliform

count and faecal streptococcal count of samples were made before and one hour after treatment.

The TVC in samples treated with lactic acid solution at ambient temperature has shown a mean reduction of 4.98 log per cent whereas, treatment with hot lactic acid solution brought a mean reduction of 12.44 log per cent in comparison to the control. The mean reduction of coliform count was 10.26 log per cent on samples treated with lactic acid solution at ambient temperature and the reduction was 25.85 log per cent on samples treated with hot lactic acid solution. In case of faecal streptococcal count the corresponding reductions were 12.15 log per cent and 33.72 log per cent, respectively. The higher reduction in bacterial counts on samples treated with hot lactic acid solution, in comparison to that at ambient temperature indicate that the temperature has got added sanitizing effects.

Use of lactic acid solution at one per cent level as a sanitizer for beef was found to produce highly significant effect in bacterial reduction. When the temperature of the lactic acid solution was elevated to 70°C, added sanitizing effect was observed. It is suggested that washing beef carcasses with one per cent lactic acid solution, preferably at 70°C, will help in reducing initial bacterial load and thus extend the period of storage in retail market meat.

References

REFERENCES

Acuff, G.R., Vanderzant, C., Savell, J.W., Jones, D.K., Griffin, D.B. and Ehlers, J.G. (1987). Effect of acid decontamination of beef subprimal cuts on the microbiological and sensory characteristics of steaks. Meat Sci. 19 (3): 217-226.

Adams, M.R. and Hall, C.J. (1988). Growth inhibition of food-borne pathogens by lactic and acetic acids and their mixtures. Int. J. Fd. Sci. Technol. 23: 287-292.

American Public Health Association. (1976). Compendium of methods for the microbial examination of foods. Ed. Speck, M.L. APHA, Washington DC.

Anderson, M.E., Huff, H.E., Naumann, H.D., Marshall, R.T., Damare, J.M., Pratt, M. and Johnston, R. (1987). Evaluation of an automated beef carcass washing and sanitizing system under production conditions. J. Fd. Prot. 50 (7): 562-566.

Anderson, M.E. and Marshall, R.T. (1990a). Reducing microbial populations on beef tissues: Concentration and temperature of an acid mixture. J. Fd. Sci. 55 (4): 903-905.

Anderson, M.E. and Marshall, R.T. (1990b). Reducing microbial populations on beef tissues: Concentration and temperature of lactic acid. J. Fd. Saf. 10: 181-190.

Anderson, M.E., Marshall, R.T. and Dickson, J.S. (1992). Efficacies of acetic, lactic and two mixed acids in reducing numbers of bacteria on surfaces of lean meat. J. Fd. Saf. 12: 139-147.

Bachil, V.N. (1983). Prevalence of E. coli and Enteropathogenic E. coli in fresh meats. Indian J. Microbiol. 23 (4): 223-227.

*Baird-Parker, A.C. (1987). The application of preventive quality assurance. In: Elimination of pathogenic organisms from Meat and Poultry, Smulders, F.J.M. (Ed.). Elsevier Science Publishers, Amsterdam. pp. 149-161.

Chandran, S.K., Savell, J.W., Griffin, D.B. and Vanderzant, C. (1986). Effect of slaughter dressing, fabrication and storage conditions on the microbiological and sensory characteristics of vaccum packaged beef steaks. J. Fd. Sci. 51: 37-39.

Charlebois, R., Trudel, R. and Messier, S. (1991). Surface contamination of beef carcasses by fecal coliforms. J. Fd. Prot. 54 (12): 950-956.

Davey, K.R. and Smith, M.G. (1989). A laboratory evaluation of a novel hot water cabinet for the decontamination of sides of beef. Int. J. Fd. Sci. Technol. 24: 305-316.

Deshpande, P.D. (1979). Studies on the sanitation and bacteriological aerobic examination of meat. M.V.Sc. Thesis submitted to All India Institute of hygiene and public health, Calcutta.

Dixon, Z.R., Acuff, G.R., Lucia, L.M., Vanderzant, C., Morgan, J.B., May, S.G. and Savell, J.W. (1991). Effect of degree of sanitation from slaughter through fabrication on the microbiological and sensory characteristics of beef. J. Fd. Prot. 54 (3): 200-207.

Firstenberg-Eden, R. (1981). Attachment of bacteria to meat surfaces: A review. J. Fd. Prot. 44 (8): 602-607.

Fliss, I., Simard, R.E. and Ettriki, A. (1991). Comparison of three sampling techniques for microbiological analysis of meat surfaces. J. Fd. Sci. 56 (1): 249-252.

Food and Drug Administration (1981). Substances generally regarded as safe. In: Code of Fedaral regulations, Title 21, Sec. 182. 1061. U.S. Government printing office, Washington, DC.

*Fournaud, J. and Bertaud. (1981). Contamination of air in abattoirs and bacteriological quality of beef carcasses. Proceedings of the European meeting of meat research workers. 1 (27): 188-191.

Gill, C.O. and Newton, K.G. (1982). Effect of lactic concentration on growth on meat of gram-negative Psychrotrophs from a meat works. Appl. Environ. Microbiol. 43 (2): 284-288.

Gustavsson, P. and Karlsson, R. (1989). Determination of critical process operations for spoilage bacteria in beef production: Proceedings of World Association of Veterinary Food hygienists Xth International symposium in stockholm. pp. 147-154.

Hamby, P.L., Savell, J.W., Acuff, G.R., Vanderzant, C. and Cross, H.R. (1987). Spray-chilling and carcass decontamination systems using lactic and acetic acid. Meat Sci. 21 (1): 1-14.

Hartman, P.A. (1960). Further studies on the selectivity of violet red bile agar. J. Milk Fd. Technol. 23 (1): 45-48.

Howe, K., Linton, A.H. and Osborne, A.D. (1976). An investigation of calf carcass contamination by Escherichia coli from the gut contents at slaughter. J. Appl. Bacteriol. 41 (1): 37-45.

Huis in't Veld, J.H.J., Mulder, R.W.A.W. and Snijders, J.M.A. (1994). Impact of Animal Husbandry and slaughter technologies on microbial contamination of Meat: Monitoring and control. Meat Sci. 36 (1&2): 123-154.

Ingram, M., Ottoway, F.J.H. and Coppock, J.B.M. (1956). The preservative action of acid substances in food. Chem. Ind. 42: 1154-1163.

Izat, A.L., Hierholzer, R.E., Kopek, J.M., Adams, M.J., Mcginnis, J.P. and Reiber, M.A. (1990). The use of propylene glycol and/or lactic acid in chill water for reducing salmonellae on broilers. J. Fd. Proc. Preserv. 14: 369-374.

Jericho, K.W.F., Bradley, J.A., Gannon, V.P.J. and Kozub, G.C. (1993). Visual demerit and microbiological evaluation of beef carcasses: Methodology. J. Fd. Prot. **56** (2): 114-119.

Johanson, L., Underdal, B., Grosland, K., Whelehan, O.P. and Roberts, T.A. (1983). A survey of the hygienic quality of beef and pork carcasses in Norway. Acta. vet. scand. **24** (1): 1-13.

Kelly, C.A., Dempster, J.F. and Mcloughlin, A.J. (1981). The effect of temperature, pressure and chlorine concentration of spray washing water on numbers of bacteria on lamb carcasses. J. Appl. Bacteriol. **51**: 415-424.

*Kleeberger, A., Schafer, K. and Busse, M. (1980). Ecology of enterobacteria on slaughter house meat. Fleischwirtschaft. **60** (8): 1529-1531.

Kotula, A.W., Lusby, W.R., Crouse, J.D. and Vries, B.de. (1974). Beef carcass washing to reduce bacterial contamination. J. Anim. Sci. **39** (4): 674-679.

Lasta, J. and Fonrouge, R. (1988). Significance of samples taken for bacterial counts from reduced areas of bovine carcasses. J. Fd. Prot. 51 (3): 214-217.

Lazarus, C.R., Abu-bakar, A., West, R.L. and Oblinger, J.L. (1977). Comparison of microbial counts on beef carcasses by using the Moist-swab contact method and secondary tissue removal technique. Appl. Environ. Microbiol. 33 (1): 217-218.

*Lotfi, A.Y., Nasr, S., Hefnawy, Y., Youssef, H., Abdel-Rhman, H. and El-Kassem, A.H. (1986). Bacterial status of emergency slaughtered cattle in Assiut. Fleischwirtsch. 66 (4): 577-578, 579.

Marel, G.M. Vander, Vries, A.W.De., Logtestiju, J.G. Van and Mossel, D.A.A. (1989). Effects of lactic acid treatment during processing on the sensory quality and lactic acid content of fresh broiler chickens. Int. J. Fd. Sci. Technol. 24: 11-16.

Mandokhot, V.U. and Garg, S.R. (1985). Microbiological quality of fresh and processed meats and their quality control. Indian Fd. Packer. 39 (6): 45-49.

Maxcy, R.B. (1981). Surface micro envoironment and penetration of bacteria into meat. J. Fd. Prot. 44 (7): 550-552.

Miskimin, D.K., Berkowitz, K.A., Riha Jr. W.E., Franke, W.C., Buchanan, R.L. and U'leary, V. (1976). Relationships between indicator organisms and specific pathogents in potentially hazardons foods. J. Fd. Sci. 41 (5): 1001-1006.

Mulder, S.J. and Kroll, I.R.B. (1975). The effect of lactic acid on the bacterial flora and the colour of fresh meat. Fleischwirtsch 55 (9): 1255-1258.

Murthy, T.R.K. (1983). Aerobic mesophilic count of fresh and refrigerated ground mutton: effect of plating and incubation temperature. J. Fd. Sci. Technol. 20 (2): 83-84.

*Nelton, P.V. and Mossel, D.A.A. (1980). The ecological consequences of decontaminating rain meat surfaces with lactic acid. Archiv fur Lebensmittel hygiene. 31 (6): 190-91.

Ng, L.K. and Stiles, M.E. (1978). Enterobacteriaceae in ground meats. Can. J. Microbiol. 24: 1574-1582.

Niskanen, A. and Pohja, M.S. (1977). Comparative studies on the sampling and investigation of microbial contamination of surfaces by the contact-plate and swab methods. J. Appl. Bacteriol. 42 (1): 53-63.

Nordic committee on food analysis (1966). Determination of the content of thermo tolerant coliform bacteria in foods. (62) UDC 576. 861. 48.

Nordic committee on food analysis (1968). Determination of faecal streptococci in foods (68) UDC 576. 851. 21.

Nortje, G.L., Nel, L., Jordaan, E., Naude, R.T., Holzapfel, W.H. and grimbeek, R.J. (1989). A microbiological survey of fresh meat in the supermarket trade, Part 1: Carcasses and contact surfaces. Meat Sci. 25 (1): 81-97.

Nortje, G.L. and Naude, R.T. (1981). Microbiology of beef carcass surfaces. J. Fd. Prot. 44 (5): 355-358.

Nottingham, P.M., Rushbrook, A.J. and Jury, K.E. (1975). The effect of plating technique and incubation temperature on bacterial counts. J. Fd. Technol. 10: 273-279.

Oblinger, J.L. (1975). Recovery of streptococci from a variety of foods: A comparison of several media. J. Milk. Fd. Technol. 38 (6): 323-326.

Oblinger, J.L. and Kennedy, Jr. J.E. (1976). Evaluation of diluents used for total counts. J. Milk Fd. Technol. 39 (2): 114-116.

Ockerman, H.W., Borton, R.J., Cahill, V.R., Parrett, N.A. and Hoffman, H.D. (1974). Use of acetic and lactic acid to control the quantity of micro organisms on lamb carcasses. J. Milk Fd. Technol. 37 (4): 203-204.

*Olgaard, K. (1977). Determination of relative bacterial levels on carcasses and meats - a new quick method. J. Appl. Bacteriol. 42 (3): 321-329.

Osthold, W., Shin, H.K., Dresel, J. and Leistner, L. (1984). Improving the storage life of carcasses by treating their surfaces with an acid spray. Fleischwirtsch. 64 (7): 828-830.

Park, H.S. and Martin, E.H. (1972). Inactivation of Salmonella typhimurium by sorbic acid. J. Milk Fd. Technol. 35: 532-539.

Patterson, J.T. (1968). Hygiene in meat processing plants: Methods of assessing carcass contamination & methods of reducing carcass contamination. Rec. Agric. Res. 17: 1-5, 7-12.

Patterson, J.T. (1971). Microbiological assessment of surfaces. J. Fd. Technol. 6: 63-72.

Prasai, R.K., Acuff, G.R., Lucia, L.M., Hale, D.S., Savell, J.W. and Morgan, J.B. (1991). Microbial effects of acid decontamination on beef carcasses at various locations in processing. J. Fd. Prot. 54 (11): 868-872.

Prasai, R.K., Acuff, G.R., Lucia, L.M., Morgan, J.B., May, S.G. and Savell, J.W. (1992). Microbiological effects of acid decontamination of pork carcasses at various locations in processing. Meat Sci. 32: 413-423.

Prieto, M., Garcia, M.L., garcia, M.R., Otero, A. and Moreno, B. (1991). Dinstribution and evolution of bacteria on lamb carcasses during aerobic storage. J. Fd. Prot. 54 (12): 954-949.

Restaino, L., Komatsu, K.K. and Syracuse, M.J. (1982). Effects of acids on potassium sorbate inhibition of food related micro organisms in culture media. J. Fd. Sci. 42 (1): 134-138.

Reuter, G. (1984). Suitability of non-destructive sampling methods for determining the surface microbial content of beef carcasses. Fleischwirtsch. 64 (10): 1247-1252.

Roberts, T.A., Hudson, W.R., Whelehan, O.P., Simonsen, D., Olgaard, K., Labots, H., Snijders, J.M.A., Vanhoof, J., Debevere, J., Dempster, J.F., Devereux, J., Leistner, L., Gehra, H., Gledel, J. and Fournaud, J. (1984). Number and distribution of bacteria on some beef carcasses at selected abattoirs in some member states of the European communities. Meat Sci. 11 (3): 191-205.

Roberts, T.A., MacFie, H.J.H. and Hudson, W.R. (1980). The effect of incubation temperature and site of sampling on assessment of the numbers of bacteria on red meat carcasses at commercial abattoirs. J. Hyg. Camb. 85: 371-380.

Rubin, H.E. (1978). Toxicological model for a Two-Acid system. Appl. Environ. Microbiol. 36 (4): 623-624.

Saoji, S.S., Sherikar, A.T., Bhilegaonkar, K.N. and Karkare, U.D. (1990). Preservative effect of acetic and lactic acids on buffalo meat stored at refrigeration temperature. J. Bomba6 Vet. Coll. 2 (1): 37-45.

Sheridan, J.J. (1982). Problems associated with commercial lamb washing in Ireland Meat Sci. 6: 211-219.

Sherikar, A.J., Saoji, S.S. and Bhogwat, S.S. Effect of Acid Decontamination on Baffalo meat. Personnel communication.

Smith, M.G. and Graham, A. (1978). Distruction of Escherichia coli and salmonellae on mutton carcass by treatment with hot water. Meat Sci. 2 (2): 119-128.

Smulders, F.J.M., Barendsen, P., Van Logtestijn, J.G., Mossel, D.A.A. and Van Der Marel, G.M. (1986). Review: Lactic acid: considerations in favour of its acceptance as a meat decontaminant. J. Fd. Technol. 21: 419-436.

Smulders, F.J.M. and Woolthuis, C.H.J. (1985). Immediate and delayed microbiological effects of lactic acid decontamination of calf carcasses-influence on conventionally boned versus Hot - boned and vacuum - packaged cuts. J. Fd. Prot. 48 (10): 838-847.

Smulders, F.J.M., Barendsen, P., Logtestiju, J.G.Van., Mossel, D.A.A. and Marel, G.M. Vander. (1986). Review: Lactic acid: considerations in favour of its acceptance as a meat decontaminant. J. Fd. Technol. 21: 419-436.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical methods. 6th ed. Oxford and IBH Publishing Co., Hew Delhi. pp. 59-62, 258-298.

Snijders, J.M.A., Schoenmakers, M.J.G., Gerats, G.E. and Pijper, F.W. (1979). Decontamination of hot beef carcasses with organic acids. Fleischwirtsch. 59 (5): 656-663.

Snijders, J.M.A., Van Logtestijn, J.G., Mossel, D.A.A. and Smulders, F.J.M. (1985). Lactic acid as a decontaminant in slaughter and processing procedures. Vet. Q. 7 (4): 277-283.

Sperber, W.H. (1991). The modern HACCP system. Fd. Technol. 45 (6): 116-118, 120.

Stolle, F.A. (1988). Establishing microbiological surveillance programmes at slaughter lines - A new concept of meat hygiene. Meat Sci. 22 (3): 203-211.

Tarwate, B.G., Sherikar, A.T., Murugkar, H.V. and Paturkar, A.M. (1993). Studies on the levels of microbial contamination of carcass sites in a buffalo slaughter line. Indian Vet. Med. J. 17: 33-35.

*Tomancova, I. and Steinhauser, L. (1990). Influence of organic acids on the shelf-life of packaged meat. Prymysl potravin. 41 (8): 405-408. Cited in Fd. Sci. Technol. Abst. 1991. 23 (9): 939.

Tompkin, R.B. (1983). Indicator organisms in meat and poultry products. Fd. Technol. 37 (6): 107-110.

Visser, I.J.R., Koolmees, P.A. and Bijker, P.G.H. (1988). Microbiological conditions and keeping quality of veal tongues as affected by lactic acid decontamination and vacuum packaging. J. Fd. Prot. 51 (3): 208-213.

*Weisser, W. (1979). Isolation of streptococci from organs of emergency slaughtered cattle and calves at bacteriological meat inspection. Archiv fur Lebensmittel hygiene 30 (4): 137-142.

Whelehan, O.P., Hudson, W.R. and Roberts, T.A. (1986). Microbiology of beef carcasses before and after slaughter line automation. J. Hyg. Camb. 96: 205-216.

*Wojton, B. and Kossakowska, A. (1977). The use of different bacteriological tests for the evaluation of sanitary quality of carcasses. Bull. Vet. Inst. Pulawy. 21 (3/4): 69-74 Cited in Vet. Bull. 1979. 49 (3): 1759.

Woolthuis, C.H.J., Mossel, D.A.A., Van Log testijn, J.G., De Kruijf, J.M. and Smulders, F.J.M. (1984). Microbial decontamination of porcine liver with lactic acid and hot water. J. Fd. Prot. 47 (3): 220-226.

Woolthuis, C.H.J. and Smulders, F.J.M. (1985). Microbial decontamination of calf carcasses by lactic acid sprays. J. Fd. Prot. 48 (10): 832-837.

Yokoya, F. and Zulzke, M.L. (1975). Method for sampling meat surfaces. Appl. Microbiol. 29 (4): 551-552.

* Originals not consulted

Appendices

Appendix - I

Plate Count Agar

Tryptone	-	5.0 g
Yeast extract	-	2.5 g
Dextrose	-	1.0 g
Agar	-	15.0 g
Aq. dist.	-	1000 ml

Dissolved the ingredients in distilled water and adjusted the pH to 7 $\pm$ 0.2 with 0.1 N sodium hydroxide solution sterilized by autoclaving at 15 lbs for 15 minutes.

Appendix - II

Violet Red Bile Agar

Peptone	-	7.0 g
Yeast extract	-	3.0 g
Bile salt mixture	-	1.5 g
Lactose	-	10.0 g
Sodium chloride	-	5.0 g
Agar	-	15.0 g
Neutral red	-	0.03 g
Crystal violet	-	0.002 g
Aq. dist.	-	1000 ml

Dissolved the peptone, yeast extract, bile salt mixture, agar and sodium chloride in distilled water by steaming. Then cooled to 50°C and adjusted the pH to 7.4 $\pm$ 0.02 with 0.1 N sodium hydroxide solution. Lactose, neutral red and crystal violet were added and autoclaved at 15 lbs for 15 minutes. Hot medium was poured into sterile petridishes and allowed to solidify.

Appendix - III

K.F. Streptococcal agar

Proteose peptone	- 10.0 g
Yeast extract	- 10.0 g
Sodium chloride	- 5.0 g
Sodium glycero phosphate	- 10.0 g
Maltose	- 20.0 g
Lactose	- 1.0 g
Sodium azide	- 0.4 g
Agar	- 20.0 g
Aq. dist.	- 1000 ml

Boiled to dissolve the ingredients completely and adjusted the pH to 7.2 $\pm$ 0.2 with 0.1 N sodium hydroxide solution. Autoclaved at 15 lbs at 10 minutes. Cooled to 60°C and 1 ml of one per cent TTC (Tryphenyl Tetrazolium Chloride) was added asceptically into each 100 ml of the sterile medium. Mixed thoroughly to obtain uniform distribution of TTC in the medium and poured into sterile petridishes.

IDENTIFICATION OF BACTERIAL CRITICAL POINTS AND ANTIBACTERIAL EFFECT OF LACTIC ACID ON BEEF CARCASS

By

K. RAJEEV

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

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ABSTRACT

During the process of slaughter and subsequent processing, the beef carcass is exposed to bacterial contamination. Bacterial load is one of the parameters for assessment of the sanitary conditions in slaughter operations. It is tedious and time consuming to evaluate bacterial load of carcass surface as a whole. Therefore assessment of bacterial load on certain points (critical points) in the carcass which are more frequently exposed to contaminants will help in quick assessment of sanitary standard. In the present study six critical points were selected on beef carcasses to evaluate the bacterial contamination. Carcasses from two slaughter houses differing in infrastructural facilities were used for this assessment and comparison. There was significant difference in the level of bacterial contamination on critical points of carcasses obtained from the two sources. Significant difference was noticed between points as well. Among the critical points, neck-lateral has shown highest level of contamination. This may be due to chances of exposure to contaminants during bleeding and flaying. The abdomen-medial was comparatively less contaminated.

In spite of conscious precautions, carcasses invariably get contaminated. In order to minimise the

bacterial load on carcass at the end of slaughter line, washing carcass with sanitizers is one of the methods adopted in meat trade.

Lactic acid one per cent solution, when used as sanitizer for washing beef carcasses immediately after slaughter, has shown significant reduction in total viable count, coliform count and faecal streptococcal count estimated 1 h after treatment. When hot lactic acid solution at 70°C was used for washing, significant reduction in the above counts in comparison to the first treatment was observed. This added benefit can be attributed to the enhanced temperature of the solution. It is concluded that one per cent lactic acid solution, preferably at 70°C, can be effectively used as a sanitizer on beef carcass surface for reduction of initial bacterial load and this helps in prolonging the storage life under the retail marketing condition.