Influence of Two Levels of Hygiene on the Microbiological Condition of Veal as a Product of Two Slaughtering/Processing Sequences

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ABSTRACT

In two experiments involving two groups of 20 calves each, the microbiological condition of veal produced in an alternative (Electrical Stimulation/Hot Boning) and a conventional (No Stimulation/Cold Boning) slaughtering/boning sequence was investigated. Two levels of hygiene were practiced, i.e. (a) "strictly hygienic" by using surgical gloves and disinfected knives, and (b) "hygienic" by using no gloves and only one (visually) clean knife at the start of incision. All hot-boned cuts were sprayed with a 1% v/v L-lactic acid solution, vacuum packed and immersed in ice water. Hot- and cold-boned cuts were stored at 2°C, as vacuum packs during 6 d and exposed to air for an additional week. Using a destructive method, samples for microbiological examination were taken from the 8-10th rib section of the dorsal carcass surface at the end of the slaughterline as well as before boning, and from the epimysium of longissimus cuts immediately after boning, 7 d post mortem (p.m.) upon opening vacuum packs and 14 d p.m. As compared with "hygienic" boning, "strictly hygienic" boning resulted in a significant decrease in aerobic colony count on longissimus cuts from 1.9 to 1.4 log/cm² and from 2.4 to 1.4 log/cm² for alternative and conventional procedures, respectively. An effect of lactic acid decontamination could not be demonstrated earlier than 7 d after opening of vacuum packs (14 d p.m.).

The keeping quality of meat is mainly determined by the nature and degree of the initial contamination of the carcass surfaces (13,16). Prevention of contamination during slaughtering and subsequent processing has therefore been identified as by far the most important factor in safeguarding the microbiological quality of meat (4,11). In addition to this, reduction of bacterial contamination of hot carcasses may be achieved by, amongst others, application of agents such as lactic acid (5,31). Treatment of cold boned meat with lactic acid appears to be far less effective (27).

Future slaughter and processing chains may include, amongst others, electrical stimulation, hot boning, packaging and chilling of high valued primal cuts. The microbiological consequences of some of these innovations have been reported recently (2,3,8,9,14,18,19,32). In one of our earlier studies (29), we examined some sensory meat quality characteristics of beef in such an alternative slaughter/processing chain (electrical stimulation - hot boning - vacuum packaging - chilling in ice water) in comparison with a conventional one (no stimulation - cold boning - vacuum packaging). A similar experiment with veal cuts providing data on the sensory meat quality traits was also carried out (30). Some microbiological data generated in the latter study are reported here.

This investigation evaluates the effect of two different levels of hygiene on the microbiological condition of cold-boned and hot-boned vacuum-packed veal cuts. The veal cuts were produced in alternative and conventional slaughtering/processing chains. Furthermore we investigated to what extent the microbiological condition is improved by lactic acid decontamination before vacuum packaging in association with the two levels of hygiene.

MATERIALS AND METHODS

Samples

Two experiments involving two groups of 20 calves each of the Friesian Holland (FH) breed of approximately 20 weeks old were carried out. Two groups of 10 calves each were stimulated electrically (ES) immediately after bleeding (approximately 2 min post mortem), using 300 V, 50 Hz, intermittent pulses of 2¼ s with 1½ s interval. Two groups of 10 calves each served as non stimulated controls (NS). All carcasses were subjected to a standard slaughtering procedure which included manual dehiding, evisceration/inspection, both followed by showering with tap water. Fifty five minutes post mortem carcasses were chilled in 3 "shock"-tunnels during 30 min each, at -14, -8, and -4°C, respectively, at an air velocity of 8 m/s and subsequently conveyed to a chilling room at 2°C and an air velocity of 0.2 m/s. Upon arrival in this chilling room (2½ h post mortem) longissimus samples of approximately 500 g with a mean temperature of 25°C were excised from two groups of 10 carcasses (hot-boned = HB). Twenty four hours post mortem two groups of 10 similar samples were taken from the cold carcass (cold-boned = CB).

Cutting from the hanging carcass was carried out under two different conditions, i.e. (a) "strictly hygienic", by using surgical gloves when handling longissimus samples and by disinfecting the knife with 70% ethanol and subsequent flaming after each excision (experiment 1), and (b) "hygienic", i.e. using a visually clean knife when starting the excision.
sion and no gloves (experiment 2). All hot-boned samples were sprayed with 1% v/v lactic acid (LA) solution (prepared from an 80% L-lactic acid stock solution; Chemie Combinatie, Amsterdam), vacuum packed and immersed in ice water for approximately 5 h. Both hot- and cold-boned samples were stored at 2°C during 6 d after which vacuum packs were opened. Parts of the longissimus samples were kept at 2°C for an additional 7 d for microbiological examination.

Microbiological examination

Samples for microbiological examination were taken at 7 stages in the slaughtering/processing sequence, i.e. (a) 45 min post mortem; sampling from all carcass surfaces at a site adjacent to the 8-10th thoracic vertebrae, (b) 3-3.5 h post mortem; similar sampling from the surfaces of two groups of 10 carcasses, before hot boning, (c) 3-3.5 h post mortem; sampling from two groups of 10 hot-boned longissimus cuts before lactic acid decontamination and vacuum packaging, (d) 24 h post mortem; sampling from the surfaces of two groups of 10 carcasses, before cold boning, (e) 24 h post mortem; sampling from two groups of 10 longissimus cuts, before vacuum packaging; (f) 7 d post mortem; sampling of all longissimus cuts, and (g) 14 d post mortem; sampling of all longissimus cuts.

Sampling was carried out by using a destructive method (31). With sterile cork borers two tissue discs (approximately 2-3 mm thick), 4.5 cm² each, were punched out. After maceration of two punched out specimens in 27 ml of Tryptone Soy Broth (Oxoid CM 129) in a Stomacher (10), resuscitation during 1½-2 h was carried out at ambient temperature (21,24). Numbers of colony forming units (cfu) of the following microorganisms were ultimately assessed in a mackerel, of which 1 ml corresponded to a sample of 0.33 cm²: (a) aerobic colony count: in poured plates of Tryptone Glycose Beef Extract agar (Difco 0002-001); incubation 72 h at 30°C, (b) Enterobacteriaceae: in poured plates of Violet Red Bile Glucose agar (Oxoid CM 485) with overlay; incubation 20 h at 37°C (21), in addition all samples leading to plates containing 7 or more Enterobacteriaceae colonies (log cfu/cm²≥1.3) were tested for the presence of Salmonella. For this purpose the samples were resuscitated in Tryptone Soy Broth (Oxoid CM 129) during 24 h at 37°C after which 1.0 ml was inoculated in 10 ml of Muller-Kauffmann medium (Oxoid CM 343) and subsequently incubated at 43°C for 24 h. After plating on Brilliant Green agar (Oxoid CM 329) and incubating for 24 h at 37°C, typical colonies were tested for agglutination by polyvalent and subsequently by group sera and type of growth in Kliger Iron agar tubes, (c) yeasts and molds: on spread plates of Oxystreptacyclin Gentamicin Dextrose Yeast extract agar (6,7,22); incubation 5 d at 20°C.

Mathematical analysis of data

Differences between cfu counts were assessed using Student paired t-tests. To determine significance of differences in aerobic colony counts, samples with less than 7 colonies (log cfu <1.3) in the first decimal dilution plate, and therefore inappropriate for colony assessment (20) were assigned a count of 1.3 log units. Since all selective media, i.e. those for Enterobacteriaceae and yeasts and molds showed hardly any colonies, differences for these microorganisms were not evaluated statistically.

RESULTS AND DISCUSSION

Tables 1 and 2 present aerobic colony counts, counts of Enterobacteriaceae and of yeasts and molds expressed as log N cfu/cm² carcass, or longissimus surface. At 45 min post mortem relatively low initial counts were obtained consistently under all experimental conditions. Careful manual dehiding and subsequent showering may have contributed to this. Earlier, unpublished experimental work on the same slaughter line revealed a high standard of hygiene resulting in mean aerobic colony counts (3 d, 30°C) not exceeding 3.7 log units on the generally highly contaminated regions, i.e. breast and perineal areas. Contamination or transmission of bacteria on longissimus cuts have, particularly for "strict hygiene", been severely restricted. Furthermore, even the practice referred to as "hygiene" implied cutting from a hanging carcass and consequently little cross contamination occurred here as well (1).

The effect of "strictly hygienic" practices in both alternative and conventional slaughtering/processing procedure is evident: samplings immediately after hot boning (3½ h post mortem) and cold boning 24 h post mortem) revealed counts 0.5 and 1.0 log units lower (p<.025 and p<.001), respectively, than with "hygienic" practices. Lactic acid did not appear to affect counts at 7 d post mortem in comparison to counts resulting from "hygienic" practices only. This agrees with data of Snijders et al. (31), who found less marked effects of decontamination with decreasing initial aerobic colony counts. However, at 14 d post mortem the decontaminating effect of lactic acid became manifest. There is no reason to expect any delayed microbiological effect of hot boning or cooling in ice. Consequently, the only variable that could have caused the difference in bacterial counts at 14 d post mortem is lactic acid decontamination. This is substantiated by data from the literature, particularly those reporting the extension of the lag phase of psychrotrophic microorganisms (12,26) and of a mesophilic one like Escherichia coli (28) as a result of acid treatment.

TABLE 1. The effect of two levels of hygiene on the microbiological condition of veal produced in a conventional slaughtering/processing sequence; mean bacterial counts and standard deviations (log N cfu/cm²) for ten specimens per subgroup.

<table>
<thead>
<tr>
<th>Test</th>
<th>Carcass surface</th>
<th>Cold boning</th>
<th>Epimysium of longissimus cuts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 min p.m.</td>
<td>24 h p.m.</td>
<td>24 h p.m.</td>
</tr>
<tr>
<td>Aerobic colony count</td>
<td>3.0±0.3</td>
<td>3.2±0.3</td>
<td>Strictly</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>1.6±0.4</td>
<td>n.e.</td>
<td>Hygienic</td>
</tr>
<tr>
<td>Yeasts (and molds)</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
<tr>
<td>Aerobic colony count</td>
<td>3.2±0.7</td>
<td>2.6±0.4</td>
<td>2.4±0.4</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>1.7±0.4</td>
<td>1.4±0.1</td>
<td>Hygienic</td>
</tr>
<tr>
<td>Yeasts (and molds)</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
</tr>
</tbody>
</table>

Δ = In the vertical rows superscripts not containing a common character differ significantly (p<.05).
V = Subscripts in counts of Enterobacteriaceae and yeasts and molds represent the number of plates appropriate for colony assessment from which means have been calculated.
n.e. = not examined.
- = log cfu Enterobacteriaceae/cm² <1.3; log cfu yeasts and molds/cm² <2.3.
Enterobacteriaceae cfu counts were extremely low on all carcass surfaces. Most poured plates remained sterile or showed less than 7 colonies in the first decimal dilution. All other samples were examined for salmonellae but none were found to be positive. This may seem surprising. However, as early as 1964 Hobbs (15) indicated that marked improvement of the hygiene practices at calf slaughter lines may result in a considerable reduction of veal samples being contaminated with salmonellae. As can be expected from the low aerobic colony counts (17), extremely low counts of yeasts and molds were found.

Table 2. The effect of two levels of hygiene on the microbiological condition of veal produced in an alternative slaughtering/processing sequence; mean bacterial counts and standard deviations (log N cfu/cm²) for ten specimens per subgroup.

<table>
<thead>
<tr>
<th>Test</th>
<th>Electrical stimulation</th>
<th>Carcass surface</th>
<th>Hot boning</th>
<th>Lactic acid/vacuum packaging/cooling in icewater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>45 min p.m.</td>
<td>3½ h p.m.</td>
<td>7 h p.m.</td>
</tr>
<tr>
<td>Aerobic colony count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td>2.8 ± 0.8</td>
<td>3.2 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Yeasts (and molds)</td>
<td></td>
<td>n.e.</td>
<td>n.e.</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td>1.4 ± 0.12</td>
<td>2.3 ± 0.62</td>
<td></td>
</tr>
<tr>
<td>Yeasts (and molds)</td>
<td></td>
<td>n.e.</td>
<td>n.e.</td>
<td></td>
</tr>
<tr>
<td>Aerobic colony count</td>
<td></td>
<td>2.8 ± 0.7</td>
<td>3.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Yeasts (and molds)</td>
<td></td>
<td>n.e.</td>
<td>n.e.</td>
<td></td>
</tr>
</tbody>
</table>

\[ \Delta = \text{In the vertical rows superscripts not containing a common character differ significantly (p< .05).} \]

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n.e. = not examined.

— = log cfu Enterobacteriaceae/cm² <1.3; log cfu yeasts and molds/cm² <2.3.

**Table 2.** The effect of two levels of hygiene on the microbiological condition of veal produced in an alternative slaughtering/processing sequence; mean bacterial counts and standard deviations (log N cfu/cm²) for ten specimens per subgroup.

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REFERENCES