Control of the bacteriological condition of calve brain by improving hygiene

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Introduction

The bacteriological condition of edible slaughter byproducts and how this is affected by various handling procedures, packaging and storage conditions have been studied recently (Patterson and Gibbs 1979, Bijker 1981, Berry et al. 1981, Hanna et al. 1982a,1982b, Oblinger et al. 1982, Rothenberg et al. 1982). The byproducts studied included livers, hearts, tongues, gullets, udders, kidneys, skirts and tails.

Another byproduct viz. brain, which is economically important particularly in the veal calf slaughterline, has received very little attention. Calve brain is considered a delicatessen in Western European countries (Fisher 1977), usually as a fried dish but it is also used as a basic component of sausages or in soups (Ostertag and Schönberg 1955, Linke 1959). Calve brain contains approximately 9 % fat, 10 % protein, including all essential amino acids, and it is relatively rich in B vitamins and vitamin C as compared with lean veal (Soucis et al. 1969). It has been recommended as particularly fit for diabetes mellitus patients (Wetzel A.,cited by Ostertag and Schönberg 1955).

However, due to its particular tissue structure (i.e. a parenchymatous organ with very little mesenchymal interstitium) the conditions for bacterial colonization are optimal. The use of brain in culture media for fastidious microorganisms indicate this (Rosenow 1919). Important factors determining the keepability of calve brain are the extent of initial contamination as affected by manipulation and storage conditions from the moment of removal from the skull. The initial contamination is determined to a great extent by the stunning procedure. In essence three stunning methods are available for veal calves viz. concussive, electrical, and captive bolt stunning.The latter method is generally preferred as being most practical under the present conditions (Lambooy 1981). However, the captive bolt damages part of the brain, usually the cerebellum, durind which process skin and bone fragments are forced into the inner parts. Thus brain tissue is inoculated with a potentially hazardous microflora. Manual fixation of the head, splitting of the skull and removal of the brain imply yet other mechanisms of contamination.

Purpose of the present study was to investigate whether paying particular attention to hygienic measures during the removal of the brain from the skull would significantly improve the bacteriological condition of brains originating from veal calves stunned by means of a captive bolt.

Materials and Methods

Samples;
A total of 55 calves of the Dutch Friesian (FH-) breed were stunned mechanically by means of a captive bolt.

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Immediately after bleeding (approximately 5 minutes post mortem) carcasses were decapitated.Approximately 1 hour post mortem the heads were skinned and hot boned and the skulls were opened. The brain was removed either conventionally or "hygienically". Conventional practice in the plant implied manual fixation of the head, splitting of the occipital bone by means of an axe and removal of the brain; all these manipulations were carried out by one person. "Hygienic" practice relied on the same manipulations with exception of the removal of the brain from the skull, which was carried out by a second person wearing a fresh pair of surgical gloves during each removal in order to avoid cross contamination. Upon removal from the skull brains were placed in polythene trays and stored at 3 ± 1°C during 7 days. Samples were excised from two locations on the brain viz. i) the undamaged hemisphere and ii) the site of impact of the captive bolt, usually being the cerebellum.

Bacteriological examination;
Samples for bacteriological examination were excised immediately after collection and after 7 days of storage at 3 ± 1°C. After maceration of 10 g brain tissue in a Stomacher (Gerats and Snijders 1978) and resuscitation during 1¾-2 hours at ambient temperatures in 90 ml Buffered Peptone Water (van Leusden et al. 1982) numbers of colony forming units (cfu) of the following microorganisms were assessed:

- Total colony count; in poured plates of Tryptone Glucose Beef extract agar (Difco 0002-01); incubation 3 days at 30 °C and 14 days at 4 °C.
- *Enterobacteriaceae;* in poured plates of Violet Red Bile Glucose agar (Oxoid CM 485) with overlayer; incubation 20-24 hours at 37 °C (Mossel et al. 1962).
  In addition all samples showing plates containing 7 or more *Enterobacteriaceae* colonies (log cfu.$g^{-1} \geq 1.8$) were tested for *Salmonella* . For this purpose the remaining macerate (vide supra) was incubated at 37 °C during 24 hours whereupon 1.0 ml was inoculated in 10.0 ml Muller-Kauffman broth (Oxoid CM 343) and incubated at 43 °C for another 24 hours. After plating on Brilliant Green agar (Oxoid CM 329) and incubation during 24 hours at 37 °C, typical colonies were tested by agglutination and type of growth in Kligler Iron agar tubes (Difco 0086-01).

Mathematical analysis of data;
To determine significance of differences between counts these were analysed using Student t-tests.Samples with less than 7 colonies on the total colony count,- or *Enterobacteriaceae* plates of the first serial dilution (log cfu.g^{-1} < 1.8) were assigned counts of 1.8 log units.

Results and Discussion

Table 1 presents the total colony counts at 30 °C and at 4 °C as well as counts of *Enterobacteriaceae* expressed as log cgu.g^{-1} brain tissue excised from the undamaged hemisphere.

As a result of hygienic practices at day 1 in all counts the percentage of plates appropriate for enumeration have been drastically reduced. In the case of *Enterobacteriaceae* this occurred to such an extent that these bacteria remain "undetectable". Overall, the initial bacterial counts when hygienic practices are followed, are approximately 1.0 log unit lower than with conventional practice ($p<.001$).

After 7 days of storage all samples of the conventionally collected brains lead to plates appropriate for

Total colony count (3d,30°C)	100 %	5.0 ±0.9$^{b\nabla}$	100 %	3.9 ±0.9^{a}	100 %	6.9 ±0.7^{d}	100 %	6.4 ±0.8^{c}
Total colony count (10d,4°C)	100 %	3.8 ±0.7^{b}	64 %	2.5 ±0.5^{a}	100 %	7.0 ±0.7^{d}	100 %	6.5 ±0.9^{c}
Enterobacteriaceae (1d,37°C)	87 %	3.5 ±1.2^{b}	18 %	2.2 ±0.2^{a}	100 %	5.1 ±0.8^{d}	91 %	4.2 ±0.8^{c}

$^{\nabla}$In horizontal rows figures with different superscripts differ significantly ($p<.025$); a is preferable to b, b to c and c to d

These results show that with respect to damaged brain areas hygiene results in a less marked reduction of the percentage of plates appropriate for enumeration as compared with intact hemispheres: only total colony counts at 4 °C and *Enterobacteriaceae* counts show smaller percentages than controls at day 1. Nonetheless all mean bacterial colony counts at that moment are more tha 1 log unit lower. At day 8 only *Enterobacteriaceae* count shows a slightly reduced percentage of appropriate plates ($p>.05$) whilst mean values for total colony counts at 30 an 4 °C and *Enterobacteriaceae* are reduced 0.5 0.5 and 0.9 log units respectively ($p<.025$). All samples positive for *Enterobacteriaceae* were examined for salmonellae but none were found to be positive.

These results show that in spite of the inevitable contamination of calve brain with skin fragments that have been punched out in the course of the stunning process, hygienic practices may yet improve the bacteriological condition significantly.

It seemed worthwile to investigate whether alternative stunning procedures not involving mechanical damaging of brain tissue would produce calve brain of superior bacteriological quality. For that purpose a series of experiments was initiated in which "kosher" slaughtering)i.e. slaughtering according to the Jewish rite implying sticking without previous stunning) was used as a model for such alternatives. The first data from these experiments show that at day 1 total colony counts at 30 and 4 °C and *Enterobacteriaceae* colony counts all remain under their respective detection limits. At day 8 this is still the case for *Enterobacteriaceae* colony counts. However total colony counts at 30 and 4 °C at that moment amount to values almost as high as the undamaged brains of mechanically stunned calves. These data illustrate once more the need to seggregate enteric contamination of foods of animal origin from subsequent proliferation of bacteria of environmental origin (Mossel 1982). These results also substantiate that brain tissue is extremely susceptible tp bacterial spoilage under the conditions of chilled storage which are conventionally applied.

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Table 1 The bacteriological condition of undamaged hemisheres of calve brain after conventional (n=30) and hygienic (n=22) collection as measured initially (day 1) and after 7 days of storage at 3±1 °C (day 8). Plates appropriate for enumeration (%), mean bacterial colony counts and standard deviations(log cfu.g^{-1})

	day 1				day 8			
	conventional		hygienic		conventional		hygienic	
Total colony count (3d,30°C)	100 %	3.8 ±0.6$^{b\nabla}$	66 %	2.5 ±0.6^{a}	100 %	6.2 ±0.7^{d}	92 %	4.9 ±0.6^{c}
Total colony count (14d,4°C)	97 %	3.0 ±0.5^{b}	9 %	2.1 ±0.1^{a}	100 %	6.4 ±0.8^{d}	86 %	5.2 ±0.6^{c}
Enterobacteriaceae (1d,37°C)	30 %	2.6 ±0.4^{b}	-	< 1.8^{a}	100 %	4.8 ±0.6^{d}	27 %	2.6 ±0.4^{c}

▽ In horizontal rows figures with different superscripts differ significantly ($p<.001$); a is preferable to b,b to c and c to d.

enumeration on all media. Hygienically collected samples, however, still showed a small percentage of "negative" total colony counts at 30 and 4 °C whereas the percentage of *Enterobacteriacea* "positive" samples had not exceeded 27 %.

Both at day 1 and day 8 all samples showing more than 7 colonies of *Enterobacteriaceae* on the first serial dilution were examined for salmonellae. Substantiating earlier studies on the bacteriological condition of veal in the same plant (Smulders et al 1982), in the present study once again no samples were found to be positive. This is indicative for a relatively high standard of plant hygiene (Hobbs 1964).

Table 2 presents total colony counts at 30 and 4 °C as well as counts of *Enterobacteriaceae* expressed as cfu.g^{-1} brain tissue excised from the site of impact of the captive bolt.

Table 2 The bacteriological condition of calve brain at the site of impact of the captive bolt after conventional (n=30) and hygienic (n=22) collection as measured initially (day 1) and after 7 days of storage at 3 ±1°C (day 8). Plates appropriate for enumeration (%), mean bacterial colony counts and standard deviations (log cfu.g^{-1})

day 1		day 8	
conventional	hygienic	conventional	hygienic