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Microbiology of beef carcasses before and after slaughterline automation

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SUMMARY

The bacterial status of beef carcasses at a commercial abattoir was monitored before and after slaughterline automation. Bacterial counts did not differ significantly overall ($P > 0{\cdot}05$) between the original manual line and the automated line for either morning or afternoon slaughter. On the manual line counts in the morning were lower than those from carcasses slaughtered in the afternoon, but on the automated line there was no difference between morning and afternoon counts. Due to highly significant line × sample site interaction for both morning and afternoon counts, overall differences among sample sites were not found by analysis of variance. However, principal components analysis revealed a significant shift in bacterial contamination among some sites due to slaughterline changes. The incidence of Enterobacteriaceae increased marginally following automation.

INTRODUCTION

Increasing automation and higher throughput on modern slaughterlines has generated considerable interest in their effects on carcass hygiene. This is especially so in cases where customers such as meat processors have microbiology laboratories to enforce their own bacteriological specifications on meat supplied.

Previous reports (Ingram & Roberts, 1976; Roberts, 1980) have suggested that modern developments in slaughter practices have had little or no effect on carcass hygiene and Roberts (1980) considered that bacteriological investigations are warranted prior to the introduction of large and costly 'improvements'. The capital expenditure on automated slaughter equipment is justified primarily on the grounds of increased efficiency rather than improved hygiene. Any improvement in bacteriological status of the carcass meat after such changes would generally be regarded as a bonus.

The difficulty of obtaining meaningful bacteriological data from red meat carcasses was described by Roberts, MacFie & Hudson (1980) and Ingram & Roberts (1976). Variations in bacterial count due to microbiological technique, by differences of sampling site on a carcass, between carcasses on the same day of slaughter, between the start (a.m.) and finish (p.m.) of the kill and in particular between daily batches of carcasses must all be taken into account.

Although Roberts, MacFie & Hudson (1980) ruled out counts of bacteria claimed to indicate faecal contamination of carcasses (viz. presumptive coliforms, Enterobacteriaceae and faecal streptococci) as a substitute for total viable counts in quality control sampling, they are sometimes used as a guide to slaughter and meat hygiene. Total viable counts at the end of the slaughterline are a useful guide to hygienic practice during slaughter and dressing, but there are few publications where adequate numbers of carcasses have been sampled (Nottingham, Penny & Harrison, 1974; Ingram & Roberts, 1976; Johanson *et al.* 1983).

The automation of a beef slaughterline at a modern commercial abattoir afforded a unique opportunity to establish any effect on the bacteriological status of the carcasses.

MATERIALS AND METHODS

Original manual slaughterline

Cattle were stunned by captive bolt, but no pithing was performed. Each animal, shackled with a chain around a hind leg, was then hoisted over an exsanguination trough and the major blood vessels in the neck severed. Following exsanguination the head was removed and the carcass lowered onto a cradle, the chain shackle removed, and partially flayed by slaughtermen using knives. The brisket was opened by a manually operated, electrically powered saw and the abdominal cavity opened by a slaughterman's knife. The carcass was then partially hoisted into the vertical position suspended by the hind legs from a 'beef tree', the neck remaining on the cradle while evisceration was completed. After flaying the carcass was raised to a fully vertical position and the cradle removed. The carcass was split with a manually operated, electrically powered chine saw, the beef sides trimmed free of loose fat, spray washed with hot water (impact temperature approx. 50 °C) and sent for weighing. Twelve men worked on the slaughterline with a throughput of about 25 animals per hour.

Automated slaughterline

The new beef slaughterline operated on an overhead powered rail system, carcasses hanging suspended by their hind legs moving along its rail system on an intermittent basis. Stunning and exsanguination were unchanged from the manual line, then slaughtermen standing on a raised metal platform flayed the hind legs and flank while others working at a lower level partially flayed the brisket and forelegs.

The hind hooves were removed and steel hooks inserted behind each main tendon. Each carcass then proceeded to a 'Nijhuis' downward-pulling hide stripper supported by two slaughtermen with hand held 'Flaymasters'. To prevent fracture of the backbone the carcass was electrically stimulated for 9 s during the latter part of the hide-stripping process. The head was removed and washed in a small cabinet with a water spray before being hooked to a separate powered rail parallel to the carcass rail. A slaughterman then opened the brisket with a small electrically powered saw and, with a knife, made a deep longitudinal cut in the back of the neck to assist later carcass splitting. The carcass was raised to a higher level by the powered rail and eviscerated. Stomach, intestines and offal, including

kidney knob fat, were dropped onto a stainless steel conveyor belt to a working level beneath the slaughterhall floor. The carcass was automatically positioned beneath a circular chine saw operated by hydraulic rams. During splitting each carcass was sprayed from fixed jets with water at *c.* 50 °C to remove bone dust and blood. Each side was then trimmed and spray washed with hot water from a hollow cone spray gun. A meat inspector finally examined the carcasses, trimmed and washed off any remaining loose fat or blood and then allowed it to be taken by the powered rail to the weighing area. After weighing and documentation the ears were removed and the carcasses were pushed by an operative wearing waterproof gloves to the chill rooms. At this stage sampling for bacteriological analysis took place on both the new and old slaughterline. The throughput of the new automated slaughterline was almost twice that of the old manual line using the same number of slaughtermen.

Bacteriological sampling

On each of 9 occasions 10 beef carcasses were taken at random from a morning's kill and on 3 of those days a further 10 carcasses were taken from the afternoon's kill. This was repeated 2 years later when the new automated slaughterline had been in use for about 9 months.

Carcasses were sampled using the template and wet and dry swab method described by Kitchell, Ingram & Hudson (1973), swabs being taken into 10 ml volumes of bacteriological diluent comprising 0·85% (w/v) NaCl+0·1% (w/v) peptone. Seven 100 cm^2 areas were sampled on each carcass from the following sites: neck, brisket, forerib, flank, flank/groin, round (hind leg) lateral surface and round medial surface (i.e. sites, 1, 2, 3, 4, 6, 8 and 9 in Roberts, MacFie & Hudson, 1980).

Transport of samples

Sample bottles were transported to the laboratory in an ice-cooled insulated container at 0 °C, stored at this temperature overnight and examined the next morning. Tests established that no measurable bacterial growth occurred in samples stored under these conditions.

Bacterial counts

Total viable counts (TVCs) were made on Standard Plate Count Agar (Oxoid CM463) incubated at 30 °C for 3 days using the loop/tile method described by Hudson, Roberts & Whelehan (1983). Counts of Enterobacteriaceae were made at 37 °C in Violet Red Bile Glucose Agar (Oxoid CM485) overpoured with the same medium. Dark red colonies 1–2 mm diameter surrounded by a reddish zone were counted.

Calculation of viable counts

Results were calculated using colony counts at two or more dilutions with weighting for dilution, as described by Farmiloe *et al.* (1954).

Statistical analysis

The bacterial counts were transformed to logarithms to the base 10 in order to

normalize them. Normality was monitored by plotting residuals from the analyses of variance described below.

A split-plot design analysis of variance was performed on the a.m. and p.m. data separately to examine the effects of slaughterline and site and their interaction. The sampling occasions (batches) for both slaughterlines formed the whole-plots and the ten carcasses examined within each batch the sub-plots. Thus to test for significant overall slaughterline differences the mean square due to slaughterline variation was compared with the whole-plot residual mean square (batch within slaughterline variation). Significant overall site differences were tested for by comparing the mean square due to site variation with the sub-plot residual mean square and the slaughterline × site interaction mean square where significant. Sampling occasions on which both a.m. and p.m. sampling was undertaken were used to examine the effects of sampling time in a third analysis of variance.

In addition the multivariate statistical technique of principal components analysis was performed (Pearson, 1901; Rao, 1964). The ten carcasses sampled on each occasion were averaged reducing the data set to 7 site counts, on each of 24 sampling occasions. The sites may be regarded as variables and thus the 24 sampling occasions may be represented in a 7-dimensional space. It is obviously impossible to visualize 7-dimensional graphs but principal components analysis reduces the effective dimensionality of the data by maximally explaining the variation amongst sampling occasions in some reduced dimensionality. It is not unusual for only 2 or 3 principal components to explain a vast majority of total variation due to intercorrelations among the variables (sites), and a plot of the samples relative to these principal components can reveal patterns in the data not previously suspected. The principal components are linear combinations of the sites and a study of the loadings or weightings of the sites on a particular component will indicate its importance on that component. Components are chosen so as to be uncorrelated with each other. All analyses were performed using the GENSTAT statistical package (Nelder, 1973).

RESULTS

It should be emphasized that all the carcasses examined were visually clean at the time of sampling. Since no visual differences were apparent, the bacteriological testing and statistical analyses were applied to assess 'hygiene'.

The analysis of variance of data from the morning slaughter sessions is shown in Table 1. There was no significant difference in numbers of bacteria on carcasses from manual and automated lines (Table 2). There were no overall sample site mean count differences due to a highly significant slaughterline × site interaction. The slaughterline × site mean counts are also shown in Table 2 and indicate that the rank order of site means was different between the two slaughterlines. The change from manual to automated line led to a mean increase in counts on the flank (site 4) from 3·18 to 3·49 and flank/groin (site 6) from 3·14 to 3·46 ($\log_{10}/cm^2$) and a corresponding decrease at the brisket (site 2) from 3·52 to 3·23 and round lateral surface (site 8) from 2·91 to 2·21.

The analysis of variance of data from the afternoon slaughter sessions is shown in Table 3. There was no significant difference in carcass bacterial mean count

Table 1. *Analysis of variance of data from carcasses sampled a.m.*

Source	Degrees of freedom	Mean square	*F* ratio
Whole-plots			
Line	1	0·54	< 1
Residual	16	11·06	—
Total	17	10·44	—
Sub-plots			
Site	6	19·60	61·89
Line × site	6	6·26	19·77
Residual	1230	0·32	—
Total	1242	0·44	—
Grand total	1259	0·57	—

Table 2. *Mean bacterial counts on beef carcasses from manual and automated slaughterlines during the morning slaughter at a commercial abattoir*

Overall mean count	Manual	Automated
$n = 630$	* 3·05	3·01
†S.E.D. = 0·19		

Slaughterline × carcass sample site interaction

Site	Manual	Automated
1	*2·66a	2·78b
2	3·52e	3·23c
3	3·02bc	3·17c
4	3·18d	3·49d
6	3·14cd	3·46d
8	2·91b	2·21a
9	2·90b	2·71b

Significance ($P < 0·05$, $n = 90$), different letters within a column.
Comparing sites S.E.D. = 0·08; comparing lines S.E.D. = 0·20.
*Mean $\log_{10}$ number per cm^2.
†S.E.D. = standard error of difference.

Table 3. *Analysis of variance of data from carcasses sampled p.m.*

Source	Degrees of freedom	Mean square	*F* ratio
Whole-plots			
Line	1	0·09	< 1
Residual	4	1·59	—
Total	5	1·29	—
Sub-plots			
Site	6	8·78	25·68
Line × site	6	2·09	6·12
Residual	402	0·34	—
Total	414	0·49	—
Grand total	419	0·50	—

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Table 4. *Mean bacterial counts on beef carcasses from manual and automated slaughterlines during the afternoon slaughter at a commercial abattoir*

Overall mean count	Manual	Automated
$n = 210$	*3·10	3·07
†S.E.D. = 0·12		

Slaughterline × carcass sample site interaction

Site	Manual	Automated
1	*2·77 a	2·58 b
2	3·61 d	3·37 cb
3	2·92 ab	3·24 c
4	3·21 c	3·55 d
6	3·30 c	3·37 cd
8	2·81 ab	2·11 a
9	3·07 bc	3·27 c

Significance ($P < 0·05$, $n = 30$), different letters within a column.
Comparing sites S.E.D. = 0·14; comparing lines S.E.D. = 0·18.
*Mean $\log_{10}$ number per cm^2.
†S.E.D. = standard error of difference.

Table 5. *Analysis of variance for combined a.m. and p.m. data*

Source	Degrees of freedom	Mean square	*F* ratio
Whole plots			
Line	1	5·21	1·58
Residual	4	3·30	—
Total	5	3·69	—
Sub-plots			
Time	1	12·09	31·59
Site	6	15·34	40·07
Line × Time	1	7·34	19·17
Line × Site	6	3·80	9·92
Time × Site	6	1·15	3·01
Line × Time × Site	6	0·29	< 1
Residual	808	0·38	—
Total	834	0·54	—
Grand total	839	0·56	—

between the two slaughterlines. Again there was no overall sample site difference in counts due to highly significant slaughterline × sample site interactions. The line × site means are shown in Table 4. The change from manual to automated line resulted in a mean increase in bacterial count on the forerib (site 3) from 2·92 to 3·24 and flank (site 4) from 3·21 to 3·55, and a corresponding decrease at the brisket (site 2) from 3·61 to 3·37 and the round lateral surface (site 8) from 2·81 to 2·11. The analyses of the a.m. and p.m. data both showed that a large source of variation was due to daily batches within slaughterline.

To compare carcass hygiene in the morning with that of afternoon slaughter 3 of the 9 visits were selected where carcasses were sampled during both a.m. and p.m. slaughter sessions on the same days. The analysis of variance is shown in Table 5. There were highly significant time × line and time × site interactions.

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Table 6. *Bacterial counts on beef carcasses from paired morning and afternoon slaughter sessions on manual and automated slaughterlines*

Time	Manual	Automated
am	*2·67a	3·02a
pm	3·10b	3·07a

Significance ($P < 0·05$; $n = 210$), different letters within a column.
Comparing times, S.E.D.† = 0·06.
*Mean $\log_{10}$ number per cm².
†S.E.D. = standard error of difference.

Table 7. *Combined (manual and automated slaughterline) bacterial counts on beef carcasses from paired morning and afternoon slaughter sessions*

	Site						
Time	1	2	3	4	6	8	9
am	*2·52a	3·14a	3·04a	3·18a	3·15a	2·35a	2·54a
pm	2·68a	3·49b	3·08a	3·38a	3·33a	2·46a	3·17b

Significance ($P < 0·05$, $n = 60$) = different letters within a column.
†S.E.D. = 0·11.
*Mean $\log_{10}$ number per cm².
†S.E.D. = standard error of difference.

Table 8. *Distribution of Enterobacteriaceae counts on beef carcasses from manual and automated slaughterlines at a commercial abattoir*

		Enterobacteriaceae/cm²		
Site		< 1	1–10	> 10
1	Manual	104	13	3
	Automated	78	37	5
2	Manual	67	48	5
	Automated	74	37	9
3	Manual	82	35	3
	Automated	44	52	24
4	Manual	81	37	2
	Automated	31	69	20
6	Manual	97	22	1
	Automated	55	54	11
8	Manual	93	23	4
	Automated	115	5	0
9	Manual	94	19	7
	Automated	107	13	0

Total number of counts per slaughterline = 840.

The line × time mean counts are shown in Table 6. The afternoon carcasses were significantly dirtier than the morning carcasses on the manual line but not on the automated line. The time × site mean counts are shown in Table 7, at all sites mean counts were higher in the afternoon but only significantly so on the brisket (site 2) and round medial surface (site 9).

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Table 9. *The 'weightings' of each beef carcass sampling site on each of the first two principal components together with the percentage of the original variation explained by each component*

	Component	
Site	1	2
1	−0·42	−0·01
2	−0·43	0·23
3	−0·28	−0·22
4	−0·31	−0·47
6	−0·27	−0·46
8	−0·40	0·68
9	−0·49	−0·07
% of original variation	61·9	19·0

Enterobacteriaceae

The minimum detection level was 0·2/cm². The overall incidence increased from 65·7 % on the manual line to 70·2 % on the automated line. The distribution of counts is shown in Table 8. On sites 1, 3, 4 and 6 the incidence increased significantly ($P < 0·001$) with automation, but decreased at sites 8 ($P < 0·001$) and 9 ($P < 0·05$) and was not significantly different at site 2. This pattern of change was very similar to that of TVCs.

Although the incidence of Enterobacteriaceae increased significantly with automation of the line, the numbers detected on both lines were low and of the same order as other surveys of fresh meat of good quality (Mossel, Dijkmann & Snijders, 1975, de Zutter & Van Hoof, 1982).

Principal components analysis

Principal components analysis of the reduced data revealed that 80·9 % of the total variation among the sampling occasions was accounted for by only two principal components, effectively reducing the data from seven dimensions to two. These principal components may be interpreted in terms of the variation among the sampling occasions they represent as a differential pattern of dirtiness of the seven sites. A plot of the two components will show the relative positions of the 24 sampling occasions.

Table 9 shows the loadings, or 'weightings', of each site on the first two principal components together with the percentage of total variation explained. When interpreting these loadings the magnitude and sign relative to one another are important.

The first principal component, accounting for 61·9 % of the total variation, had loadings of identical sign (negative) and similar magnitude for all the sites (Table 9). Therefore it represented a contrast between sampling occasions with high bacterial counts at all sites (low on the first principal component) and those with low bacterial counts at all sites (high on the first principal component).

In microbiological terms the major source of variation among the 24 sampling occasions was 'overall' dirtiness, rather than contamination at a single site.

The second principal component had loadings of mixed sign and magnitude. Sites

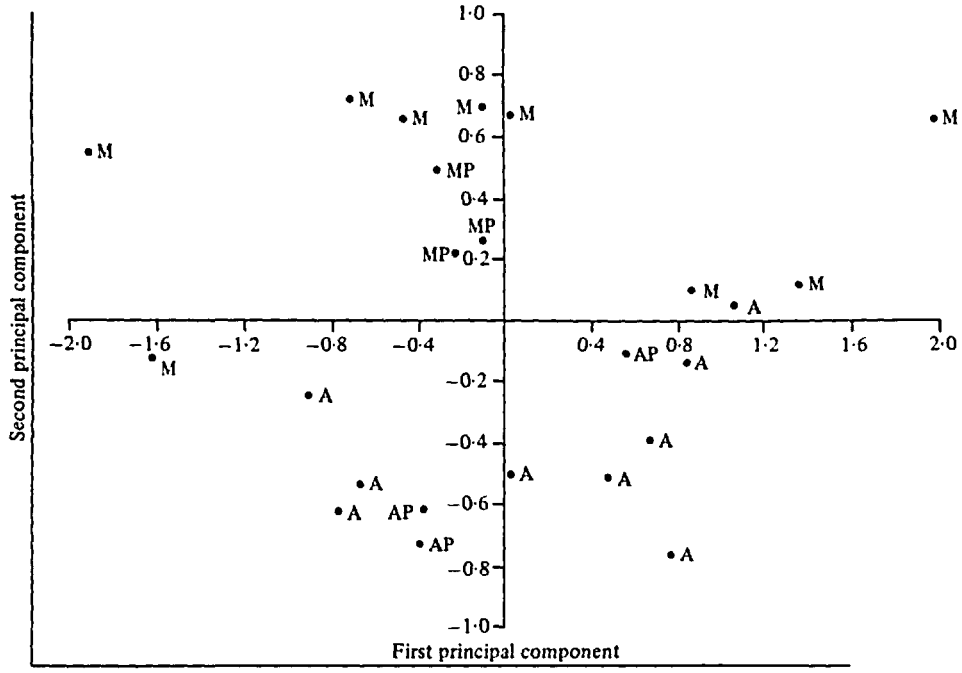

Fig. 1. Beef carcass sampling occasion scores plotted for the first and second principal components in relation to manual and automated slaughterlines. M, manual; A, automated; P, p.m.

1 and 9 had very small loadings on this component and may be ignored. The component represented a contrast between sampling occasions with low counts at sites 2 and 8 and high counts at sites 3, 4 and 6 (low on second principal component) and sampling occasions with high counts at sites 2 and 8 and low counts at sites 3, 4 and 6 (high on second principal component). This subtle contrast among the patterns of counts accounted for 19·0 % of the total variation.

The 24 sampling occasions are plotted relative to the first two principal components in Figure 1. The first principal component, which measured overall dirtiness, did not differentiate between the two slaughterlines. However, the second principal component clearly separated the sampling occasions on the old manual line (M) from those on the new automated line (A). The sampling occasions corresponding to the manual line tended to be high on this component implying high counts at sites 2 and 8 (positive weightings in Table 9, Component 2), coupled with low counts at sites 3, 4 and 6 (negative weightings, Table 9). The automated line sampling occasions tended to be low on this component implying the opposite pattern of counts. The change from manual to automated slaughterline has consequently been associated with a shift in bacteria from sites 2 and 8 to sites 3, 4 and 6 and in particular from site 8 to sites 4 and 6. The distribution of bacterial counts obtained is shown in Figure 2.

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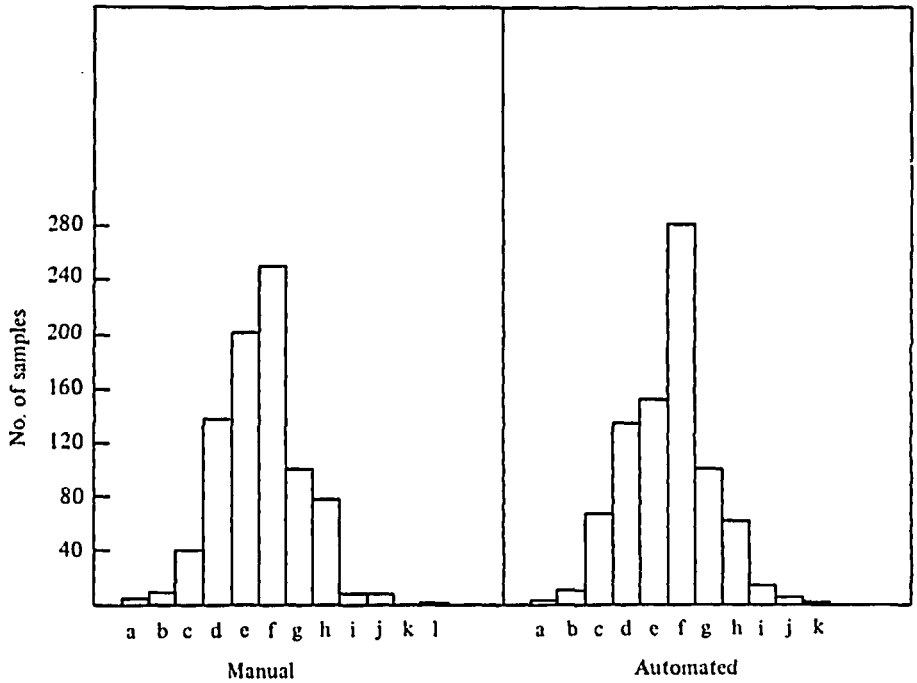

Fig. 2. Distribution of bacterial counts, on beef carcasses from manual and automated slaughterlines. Log_{10} bacteria/cm^2: a, 0·5–1·0; b, 1·0–1·5; c, 1·5–2·0; d, 2·0–2·5; e, 2·5–3·0; f, 3·0–3·5; g, 3·5–4·0; h, 4·0–4·5; i, 4·5–5·0; j, 5·0–5·5; k, 5·5–6·0; l, 6·0–6·5.

DISCUSSION

The overall level of bacterial contamination of the carcasses (3·05 and 3·01, morning slaughter; 3·10 and 3·07 afternoon slaughter, for manual and automated lines respectively) was of the same order as has been reported in England (1·9 to 3·7); Sweden (2·2 to 3·4) and New Zealand (1·3 to 4·3) (all from Ingram & Roberts, 1976; their Table V) and Norway (1·26 to 3·85, Johanson *et al.* 1983). Two more recent surveys in seven member-states of the European Communities yielded the following mean bacterial counts on freshly slaughtered beef carcasses: – Survey I: 3·85, 2·77, 2·29, 3·14, 2·45, 2·75 and 3·23; Survey II: 3·78, 3·15, 2·35, 3·50, 2·48, 3·11 and 3·33 (Roberts *et al.* 1984).

Nottingham, Penny & Harrison (1974) found little difference between the rail (vertical) and cradle (horizontal) flaying systems in the production of beef carcasses of high microbiological quality, but found that much of the contamination occurred during flaying as a high proportion of the isolates appeared to be of soil origin. In this survey the hides of animals were of average appearance with no exceptionally heavy soiling.

The change from a manual to an automated beef slaughterline at this abattoir did not greatly affect the overall bacteriological status of the carcasses. Small, but statistically significant, differences between levels of contamination on morning and afternoon manually-slaughtered beef carcasses became insignificant with the introduction of the automated line. There was however a shift in bacterial numbers

from the lateral surface of the round to the groin/flank region presumably due to different methods of handling the carcasses.

Principal components analysis is now a widely used technique, even being available on microcomputers. Analysis of variance and principal components analysis both attempt to identify the same sources of variation. The former provides established tests of significance while the attraction of principal components analysis is its ability to reduce a set of data to a few meaningful dimensions. In the data presented here the first two components were found to have meaning and together accounted for over 80% of the total variation and probably all the systematic variation. The first principal component measured overall dirtiness but, of more interest, the second principal component represented a shift in bacterial contamination from some sites to others as a result of automation of the slaughterline. The plot of the data relative to these components clearly differentiated the pre- and post-automation sampling occasions. Principal components analysis will be particularly useful in other studies of this nature where subtle, rather than large, differences occur among the experimental factors.

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