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A Study of the Bacterial Population Fluctuations During Poultry Processing

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ABSTRAK

Penilaian kandungan bakteria di dalam sampel-sampel air cucian kulit 39 ekor ayam dan air basuhan daripada unit pemerosesan ayam telah menunjukkan bilangan bakteria yang semakin bertambah pada tiap-tiap peringkat pemerosesan. Bandingan bilangan bakteria pada peringkat awal dan peringkat akhir menunjukkan bahawa tahap E. coli *dan koliform telah bertambah sebanyak 231.1% dan 263.1% manakala kandungan* Salmonella *berkurang sebanyak 9%. Pemeriksaan efluen tangki simpanan ayam sebelum ia dibungkus, menunjukkan bahawa* E. coli *dan* Salmonella *dapat hidup di dalam cecair tersebut. Dalam masa eraman 4 jam, bilangan kedua-dua organisma telah menurun pada 30 min. yang pertama tetapi telah bertambah dengan cepat selepas waktu ini. Implikasi hasil kajian ini dibincangkan dengan tujuan untuk mengurangkan masalah jangkitan semasa pemerosesan.*

ABSTRACT

The microbial assessment of skin-washed water samples taken from 39 poultry carcasses and the wash water from a poultry processing unit revealed cumulative increases in the number of microflora at each stage of processing. A comparison of the bacterial population densities on the skin at the start and at the end of processing showed that the levels of E. coli *and coliforms increased by 231.1% and 263.1% respectively whereas the* Salmonella *concentrations decreased by about 9%. A positive correlation was obtained between the occurrence of bacteria and parameters such as wash water pH and temperature, and suspended solids concentration. An examination of the storage tank effluent which was used to store the poultry carcasses prior to packaging showed that* E. coli *and* Salmonella *were able to thrive in the liquid. During the 4 h incubation period, the levels of both organisms declined rapidly during the initial half hour, but quickly became adapted and multiplied in the subsequent hours. The implications of both, the field and laboratory studies are discussed with a view of reducing the level of contamination during processing.*

INTRODUCTION

With the increase in the price of fish, beef and other meats, more consumers have turned to poultry as an alternative source of protein. In contrast to the beef industry which is well regulated in terms of standards of slaughtering and processing, the poultry meat industry consists of numerous independent entities which manage their own slaughtering, cleaning and packaging activities, and which supply dressed chicken carcasses locally. It is likely that the demand for poultry meat will increase in the future since it is relatively cheaper than beef and other meat and this would result in the establishment of many poultry processing units or plants in the country.

One consequence of such an occurrence would be a lowering of quality control standards due to the volume of chickens being processed. Therefore some contamination will occur, the degree depending on the stage of processing, the sanitary conditions of the processing units, the temperature of the scald water, the equipment that they use and the personal hygiene practices of the personnel. In a fully automated processing plant or unit where manual contact is minimised, contamination will be low (Walker & Ayres, 1956) but the same may not be true in an ordinary or isolated processing unit.

This study was designed to examine the extent and source of contamination in a poultry processing unit situated in Selangor.

MATERIALS AND METHODS

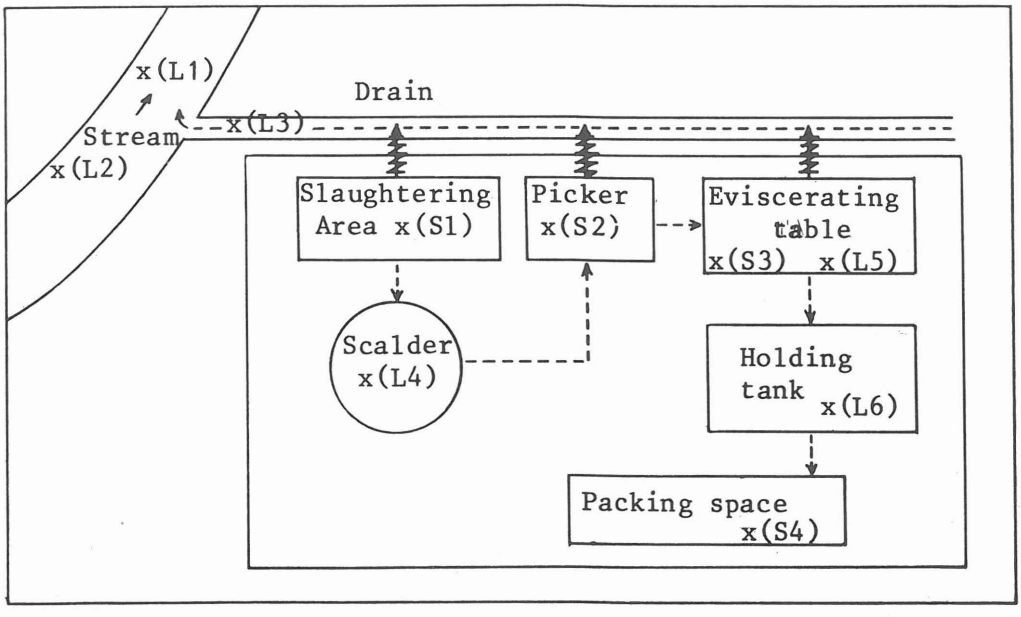
Poultry Processing Unit

Approximately 150 to 250 birds are slaughtered on each occasion three times a week for sale to clients. However, during the festive seasons, the number increases to 350 to 400 birds whence some spoilage occurs during storage of the processed chickens prior to their sale. Slaughtering usually starts at about 0730 h and by 1000h all of the chickens would have been processed and wrapped in plastic bags ready for sale. About 4 to 6 workers are employed in the unit depending on the work load.

Samples and Sampling Locations

Figure 1 shows a schematic layout of the processing unit. The sampling sites are marked X. Due to time and limited manpower, samples were taken once weekly between 0900 h and 1100 h for 13 weeks. At each sampling site, liquid effluent or skin washings were collected in triplicates.

The liquid effluent samples included water taken from the nearby stream, diluted blood and water from the drains surrounding the processing area, scald water from the scalder, water



Legend: L — liquid samples
S — skin/tissue wash samples
———→ — flow of poultry processing
~~~~→ — flow of wastewater

Fig. 1. Schematic diagram of the layout of the study poultry processing unit (see legend).
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samples from the eviscerating table, and water from the storage tank where the dressed chickens were immersed prior to packaging.

Skin washings were carried out immediately after slaughtering but before processing (a patch was cleared of feathers), after defeathering from the picking machine, and immediately before packaging. Some liver and kidney tissue washings were also taken during the evisceration of the carcasses.

All liquid samples were collected in sterile 200 ml reagent bottles.

Skin and Tissue Sampling

The method described by Williams (1967) was used to evaluate the skin and tissue surface associated bacteria since it was shown to be more efficient compared to the conventional swab technique (Kotula, 1966). A sterile metal cylinder of approximately 21 cm diameter and opened at both ends was pressed onto the surface of the carcass near the thigh or breast region so that a fixed area was circled. The skin surface was then gently scraped using a sterile spatula with 15 ml of sterile quarter strength Ringer's solution. The solution was then pipetted into a sterile universal bottle and brought back to the laboratory for analysis. Similarly, the liver and kidney tissues were shaken vigourously by hand, in Ringer's solution and analysed.

Parameters Examined

pH and Temperature. The pH of the water samples was measured using a Beckman digital pH meter. The small volume of sample used for the pH measurement was discarded after the measurement.

The temperature of the water samples was measured *in situ* using an ordinary mercury thermometer.

Suspended Solids. For this purpose, 50 ml of the liquid sample were filtered through a Whatman GF/C glass fibre filter which had been dried to constant weight before. They were then dried in an oven at 44°C for 24 – 48 hours. The suspended solids concentration was calculated from the difference in weight of the glass fibre filter before and after filtration of the sample.

Enumeration of Salmonellae. The number of salmonellae in each liquid sample was determined using the Most Probable Number (MPN) method (Swaroop, 1951). Ten, 1.0 and 0.1 ml aliquots of each sample were enriched in equivalent volumes of double strength Rappaport's broth in sterile universal bottles. Each bottle was thoroughly shaken and incubated at 39°C in a thermostatically controlled water bath for 24 h. Using sterile techniques, a loopful of sample from each bottle was streaked onto Xylose Lysine Deoxycholate (XLD) agar and incubated for 18 – 24 h at 37°C. Presumptive salmonellae are shown by the growth of black centred colonies on the XLD agar. These were then confirmed by biochemical tests.

For the skin washing samples taken just after slaughtering and before packing, the direct spread plate technique was used to obtain more precise results. For this purpose, 0.1 ml of the sample was spread onto XLD agar and incubated at 37°C. After 18 – 24 h, the number of black-centred colonies were counted.

*Enumeration of **E. coli** and Coliforms.* Each sample was serially diluted and 0.1 ml of appropriate dilutions was spread onto Violet Red Bile Dextrose (VRBD) agar. On this medium, both *E. coli* and coliforms apeared as reddish purple and pinkish colonies respectively. Plates incubated at 44°C for 24 h yielded. *E. coli* only as the coliforms are suppressed at this higher temperature. In contrast, incubation at 37°C yields both the coliforms and *E. coli.*

*Growth of **S. typhimurium** and **E. coli** in the Storage Tank Effluent.* For this purpose, about 600 ml of effluent was obtained from the holding tank when it was half full with eviscerated chicken carcasses. The effluent was sterilized by filtration through a Whatman filter followed by a Glass Fibre filter and finally using aseptic techniques, through a Gellman 0.45 filter. Pure isolates of *S. typhimurium* and *E. coli* which had

TABLE 1
Composition of the various liquid samples taken at the different sites

Parameter	samples L1	L2	L3	L4	L5	L6
pH	6.44	6.40	7.05	6.71	6.91	
Suspended solids (mg/l)	53.7	47.4	261.4	1410	386	74.3
Temperature (°C)	27.4	27.3	28.7	30.1	28.9	
*Salmonella**	1.57	1.26	2.52	0	1.99	1.66
E. coli	1.57	1.26	2.52	0	3.57	3.48
Coliforms	3.02	2.89	4.08	0	3.74	3.64

*All bacterial concentrations are expressed as Log_{10} nos./1.

been isolated previously from the effluent was used to prepare a suspension in Ringer's solution containing $10^6 - 10^7$ cells/ml.

Two hundred ml aliquots of the filter-sterilized effluents were placed in sterile conical flasks capped with aluminium foil and incubated in a water bath set at 29°C. A ml of each bacterial suspension was inoculated into each flask and gently swirled. One ml samples were withdrawn from the flasks at intervals of 0, 0.5, 1, 1.5, 2, 3 and 4 h. Samples were spread-plated on nutrient agar and incubated at 37°C for 24 h before counting.

RESULTS AND DISCUSSION

Table 1 shows the composition of the liquid samples taken at the various sites indicated in *Figure 1*. It was found that the *Salmonella* and coliform levels were relatively higher downstream at the point of discharge as compared to their levels upstream. This could be attributed to contamination from the poultry processing unit since these bacterial species do not normally constitute the soil bacterial flora.

Immediately after slaughtering, the carcasses were immersed for 3 – 4 mins in the scalder to soften the skin and hence facilitate defeathering. As shown in Table 1, no bacteria were detected in the scald water. This was because the high water temperature would have killed all of the free bacteria in the water including those attached to minute pieces of floating debris and suspended solids. Nevertheless, some bacteria might have survived on the skin of the carcasses since the feathers were still intact and thus acted as a buffer against the high water temperature. This was confirmed when after defeathering, significant numbers of bacteria were detected on the skin of the carcasses (Table 2). The bacterial concentration on the skin for *Salmonella, E. coli,* and coliforms were found to be higher at sampling site S2 compared to S1. This slight increase could be due to survival of the bacteria in the follicles of the unplucked birds during scalding or as a result of contamination from the picker (defeathering) machine. The picker might be clean initially but after defeathering of several carcasses, it could have accumulated some bacteria and transmitted them on to incoming carcasses.

Further contamination could have occurred during evisceration when the poultry carcasses were silt open manually and the viscera removed. Owing to the large number of birds to be processed, it was observed that some intenstines were ruptured and this released the intenstinal flora. The results in Table 1 indicated that this was a significant source of contamination whereby high concentrations of salmonellae, *E. coli,* and coliforms were detected in the liquid samples which drained off from the eviscerating table.

TABLE 2
Bacterial composition of the various skin and tissue washings

Parameter	Sample				
	S1	S2	S3a*	S3b**	S4
pH	6.65	6.67	—	—	6.72
Salmonella +	1.08	1.34	2.06	2.09	1.04
E. coli	1.75	1.89	3.45	3.38	2.27
Coliforms	2.18	2.27	4.20	4.36	2.74

* S3a = liver) Bacterial concentrations expressed as Log_{10} nos./gm.
** S3b = kidney) wet weight.
\+ All skin-washed bacterial concentrations expressed as Log_{10} nos./cm^2.

TABLE 3
A comparison of the bacterial densities on the skin of the poultry carcasses at the start and end of processing

Organism	Bacterial conc. (Log_{10} nos./cm^2)			
	After slaughtering	Before packing	% Increase	% Decrease
Salmonella	1.08	1.04	—	8.8
E. coli	1.75	2.27	231.1	—
Coliforms	2.18	2.74	263.1	—

The liver and kidney samples also showed very high counts of salmonellae, *E. coli,* and coliforms with log concentrations of 2.06, 3.38 and 4.20 respectively. The results indicated a fractionally higher contamination for the kidney tissues compared to the liver.

After evisceration and decapitation, the carcasses were briefly washed under running tap water near the evisceration table and then placed into a large concrete storage tank (1 m × 1.5 m) which was three-quarter full of tap water. The carcasses were usually left in the tank for about 2 h until all the processing was completed before being briefly rinsed with tap water and packed.

Examination of the water in the holding tank showed relatively lower bacterial concentrations compared to the water from the evisceration table. The log concentrations for salmonellae, *E. coli,* and coliforms were 1.66, 3.48 and 1.66 organisms per litre respectively, indicating reductions of 53%, 19% and 12% respectively compared to sampling site L5.

Skin samplings just prior to packing demonstrated an accumulation of bacteria. This increase in bacterial concentration on the skin could be attributed to bacterial accumulation at each stage of the processing after scalding. With the exception of the salmonellae, the other bacterial counts showed an increase on the skin surface. The data in Table 3 showed that the *E. coli* and coliforms concentrations had increased 2.5 fold after processing but the salmonellae concentration decreased by about 9 per cent.

Overall, the results showed that the final product leaving the processing unit still contained significant numbers of bacteria. The liquid effluents (blood and water) which were discharg-

ed into the drains and finally into the receiving stream alone may harbour up to 10^4 organisms/ml. The consequences of the release of large numbers of bacteria into the environment need to be evaluated in a more detailed study, but the sanitary significance of the presence of potentially pathogenic organisms in the effluents from the unit is obvious especially for grazing livestock which drink water from the contaminated stream. It can be assumed that these bacteria will persist after being discharged into the environment since the pH of the stream water samples is not restrictive to bacterial growth (Hendricks, 1972). In addition, the stream water temperature of about 27°C was also conducive to microbiological growth especially in the absence or minimal presence of predator species. As such, the effluents from the processing unit should be treated prior to its release.

With regards to the levels of bacteria on the skin of the carcasses, it was shown that the present method of processing did little to reduce the numbers originally present. Scalding had little effect since it was shown that the bacterial concentration before and after scalding did not change much. Nonetheless, since the effect of high temperature on bacterial survival has been established, it can be inferred that the brief immersion of the slaughtered birds (feathered) in the hot water was not sufficient to kill the bacterial flora on the skin. Furthermore, the rapid and continuous immersion of the carcasses had reduced the temperature of the initially boiling water. Thus, salmonellae, *E. coli,* and coliforms were still detected on the skin of the birds. Although the number of pathogenic salmonellae had decreased slightly at the end of processing, their presence was alarming.

A positive correlation was observed between the levels of bacteria and the concentration of suspended solids in the effluents. Within the range of 38.6 – 47.4 mg/l, the correlation between suspended solids and salmonellae, and coliforms were 0.73 and 0.72 respectively. As the concentration of suspended solids decreased at sites L1 to L2 and L5 to L6, there was also a corresponding drop in the concentration of salmonellae, coliforms and *E. coli.* Although the scald water had the highest concentration of suspended solids, no bacteria were detected due to the high water temperature. This suggests that the majority of bacteria were attached to the suspended solids in the effluents.

The detection of pathogens in the water in the holding tank indicated a necessity to determine whether the effluent was conducive for the survival and multiplication of salmonellae and coliforms and hence as a source of contamination to the poultry carcasses prior to packing. Results obtained *(Fig. 2)* indicated that both organisms were able to multiply in the effluent. After an initial drop during the first half hour, the numbers of salmonellae and *E. coli* increased in the next 3 hours. The effluent temperature was fixed at 29°C since this was the temperature of the water in the holding tank. It appeared that the water in the holding tank was able to support the growth of bacteria, including pathogens, even in the absence of any external energy source such as organic debris. Thus, this might cause carcasses introduced into the holding tank prior to packing to become contaminated.

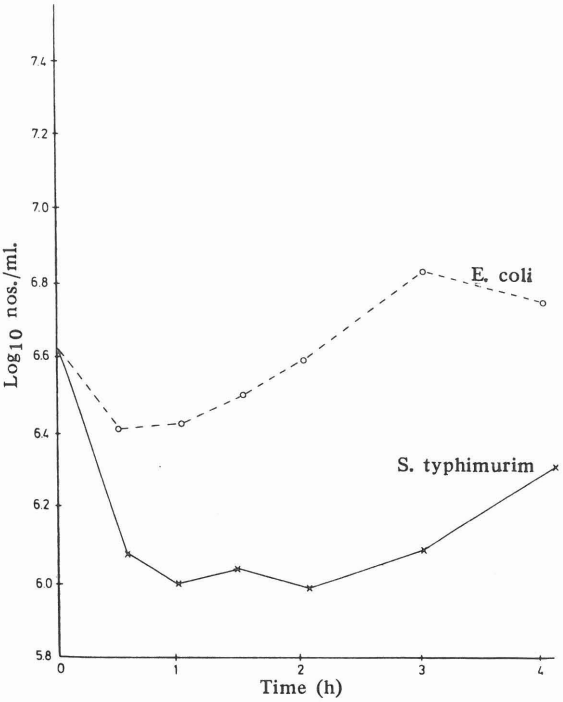

*Fig. 2. Growth of **E. coli** and **S. typhimurium** in the storage tank effluent during 4 h incubation at 29°C.*

CONCLUSION AND RECOMMENDATIONS

The results indicated that there was a significant net build-up of bacterial contamination on the skins of the poultry being processed. Of these, *E. coli* and coliforms increased by 231.1 and 263.1 per cent respectively whereas the *Salmonella* concentrations decreased by 8.8 per cent after processing. An important consideration is that these bacteria are able to thrive on the skins of the processed chickens and thus increase the possibility of spoilage. Several steps may be taken to minimize contamination during processing to reduce spoilage. These include the following:

1. The floor and the benchtops of the processing unit especially the slaughter and evisceration areas should be washed before and after processing with some disinfectant e.g. chlorine. This will reduce contamination of the chicken carcasses by the indigenous bacteria/protozoa populations which may be present at such places.

2. The top of the evisceration table which is slightly V-shaped was found to hold water. The poultry to be eviscerated are usually piled on the table and they cover the single hole at the bottom of the table near the edge, thus blocking the drainage. It is recommended that 5 holes be made on the evisceration table, one hole at each corner and another hole in the centre. In addition, plastic or rubberhoses should be attached to these holes so that the effluent can be channeled into the drain and not splash onto the floor.

3. The poultry carcasses should not be left immersed in the holding tank for longer than one hour. This is because *E. coli* and *Salmonella* are able to multiply in the water after a short incubation period of 1 hour. Therefore, it is desirable to wash and pack the processed poultry immediately after evisceration and washing. However, in circumstances where labour is short, storage may be permissible but running water should be allowed to flow through the holding tank and thus reduce the concentration of bacteria present in the water, or chilled water be used in the holding tank to control bacterial activity.

4. To reduce the skin associated bacterial flora, it may be advantageous to wash each poultry carcass with a mild disinfectant such as chlorine solution. However, to avoid the creation of possible hazards from the formation of organochloride compounds, concentrated brine solutions (4%) may be used instead.

5. Environmental sampling of the stream water showed gross contamination with salmonellae and coliforms. These probably arise from the processing unit itself. In order to avoid the possible risk of contamination to cattle and other livestock which graze near the lower reaches of the stream, it is recommended that the effluent from the unit be channeled into a proper oxidation pond with a retention time of about 2 – 3 weeks for treatment. Nonetheless, this would be a futile effort if the areas surrounding the chicken coops adjacent to the processing unit are not drained properly and become a major source of contamination for the stream due to surface run-off of rain water.

6. Another alternative to reduce contamination is to modify the approach in the processing method. After defeathering, the birds should be eviscerated dry without any water flowing on the table. Each bird is then washed briefly in a tank of concentrated brine solution in a holding tank and then under the tap for a few minutes to remove the brine solution before packing. In this way, the bacterial flora will not be widely dispersed on the evisceration table and also during subsequent processing. In addition, the volume of water used will be reduced significantly.

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