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Microbiological aspects of polyphosphate injection in the processing and chill storage of poultry

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SUMMARY

During commercial processing of broiler chickens, injection of polyphosphate (Puron 604 or 6040) resulted in microorganisms being added to the deep breast muscle. The level of contamination was related to the microbiological condition of the injection solution.

Injection of polyphosphate had no effect on the shelf-life of fresh chilled carcasses held at 1° or 10 °C but changes were observed in the growth rate of microorganisms in the deep muscle and in the composition of the muscle microflora following storage.

Cross-contamination of carcasses and the transfer of organisms from the skin to the deep muscle during injection was demonstrated with a marker strain of *Clostridium perfringens*. However, both processes were influenced by the number of marker organisms applied initially to the skin.

The above findings are discussed in relation to the possible behaviour of any food poisoning bacteria present.

INTRODUCTION

Commercial polyphosphate preparations which have been developed for use in the food industry are mixtures of polymeric phosphates of varying chain length and ring size, usually with large proportions of pyrophosphate, tripolyphosphate and high molecular weight polyanions (Hargreaves, Wood & Jarvis, 1972). For many years such substances have been added to various meat products and it is claimed that they contribute to increased water retention, improvement in colour, flavour and texture of the product and reduced cooking losses (reviewed by Ellinger, 1972).

Polyphosphate treatment was first used commercially for poultry meat in the U.S.A. in 1963 (Mahon, Schlamb & Brotsky, 1971). At that time the only method available was one in which carcasses were immersed for lengthy periods in tanks of polyphosphate solution held under chill conditions. However, the uneconomical manner of using the polyphosphate and increasing use of mechanical immersion chillers led to the development of injection procedures in which the solution could be introduced directly into the deep muscle whilst the carcass remained on the processing line. The injection method was adopted only a few years ago by British processors but is frequently used now for adding polyphosphate to broiler chickens.

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Although most of the injected carcasses are chilled and then frozen, a small proportion are deep-chilled and sold fresh.

This paper describes the effect of polyphosphate injection on the microbial contamination of broiler chicken carcasses during processing and the subsequent behaviour of the organisms present during cold storage. It should be emphasized that the results obtained relate solely to polyphosphates and not to other injection processes used with poultry which have yet to be studied.

MATERIALS AND METHODS

Microbiological examination of injection solution and equipment

Samples of injection fluid were collected in sterile 1 oz screw-capped bottles from four different processing plants (A–D). Serial tenfold dilutions of each sample were prepared using 0·1% peptone diluent. Microorganisms were counted by means of the spread-plate method using duplicate plates of the following media.

Total colony count. This was obtained on Difco heart infusion agar (HI) incubated at 20 °C for 5 days.

Presumptive coliform bacteria. The medium used was Oxoid MacConkey No. 3 agar incubated at 37 °C for 24 h.

The equipment was sampled by swabbing at hourly intervals during the working day. Three successive cotton wool swabs (Exogen Ltd., Glasgow, Scotland) were used in sampling the entire surface of that part of the injector which is in contact with the carcasses being injected. The swabs were transferred to 10 ml of 0·1% peptone diluent and counts obtained as described above.

Collection of carcasses for storage tests

Broiler chickens were obtained from two commercial processing plants belonging to different companies. In both plants carcasses were being injected with polyphosphate solution after passing through the post-evisceration spray washer.

On each occasion both injected and non-injected carcasses were taken from a single batch of birds on the processing line. The average weight of the eviscerated carcasses selected for the experiment at each plant was *ca.* 1200 g. The polyphosphate dose used was 75 ml (Plant A) or 60 ml (Plant B) of a 5·0% (v/v) solution of Puron 6040*.

Individual carcasses were tagged to aid identification, allowed to pass through the water immersion chilling system and were collected in polythene bags after draining. The carcasses were packed in ice for transporting to the laboratory. On arrival, three carcasses from each batch were sampled immediately; the remainder were subdivided for storage in a cold room at 1° or 10 °C, each carcass being wrapped loosely.

* Puron 6040 is a 40% (w/v) aqueous solution of Puron 604, a food grade polyphosphate produced by Albright & Wilson Ltd., Oldbury Warley, Worcs.

Microbiological and sensory examination

A panel of four people examined the stored carcasses twice daily for changes in odour. For microbiological examination, three carcasses were taken at intervals from each experimental batch up to the time that the remaining carcasses were clearly spoiled.

Two types of sample were taken from each carcass, one comprising *ca.* 2 g of skin from under a wing plus *ca.* 3 g of skin and cut muscle tissue from the vent region (Barnes & Thornley, 1966). A second sample (*ca.* 5 g) was taken from the injected region of the deep breast muscle. This was obtained by dissecting each carcass on a sterile enamel tray using instruments sterilized just before use by dipping in ethanol and flaming. First, the breast skin and underlying layer of muscle were removed and discarded. Then fresh, sterile instruments were used to collect a sample of muscle in the region which had been penetrated by the injector needles.

Each sample was macerated for 2 min in 20 ml of 0·1 % peptone diluent using a Colworth Stomacher (Seward & Co. Ltd., Bury St Edmunds, Suffolk). Tenfold dilutions were prepared in 0·1 % peptone diluent and counts obtained as described above.

Identification of strains isolated. All the colonies present on HI plates prepared from the highest dilution of sample and incubated at 20 °C were taken. Each isolate was purified and examined for morphology, motility, Gram stain reaction, pigment production, oxidase reaction, arginine breakdown and mode of glucose utilization as described by Barnes & Thornley (1966). Where required, further tests were made as described by Cowan (1974).

Preparation and use of 'marker' organism

A non-haemolytic, heat-resistant, food poisoning strain of *Clostridium perfringens* was used. The organism, strain 25 LR, had been obtained from Dr Betty Hobbs, formerly of the Food Hygiene Laboratory, London, and preserved as a freeze-dried culture.

In order to obtain a readily identifiable marker strain, resistance of the organism to streptomycin was increased by streaking a cooked meat broth culture on 'gradient plates' (Szybalski & Bryson, 1952, cited by Braun, 1965) of 5 % (v/v) horse blood agar containing up to 1 mg/ml of streptomycin sulphate. Resultant colonies growing at the higher concentrations of antibiotic under anaerobic conditions were re-streaked on the same medium. In this way a strain of moderately high resistance was selected which grew well on anaerobic plates containing 500 μg/ml of the antibiotic. Eight other strains of *Cl. perfringens* isolated previously from poultry were found to grow on the basal medium with 100 but not 500 μg/ml of streptomycin sulphate.

The strain selected for use as a 'marker' organism was maintained in cooked meat medium.

Test method. Tests to investigate transfer of the 'marker' organism from the skin of an artificially contaminated carcass to the deep muscle of this and subsequent carcasses were carried out as follows. A stationary phase culture of the

test organism in cooked meat medium was diluted as required in 0·1 % peptone diluent. One ml of the diluted culture was applied with a sterile paint-brush to the surface of the breast skin of a plucked and freshly eviscerated carcass. In each case the inoculum comprised mainly vegetative cells at a concentration of (*a*) *ca.* 2/cm^2 or (*b*) *ca.* 2×10^4/cm^2 of breast skin. The contaminated carcass and subsequently five other carcasses of *ca.* 1600 g average eviscerated weight, were each injected with 90 ml of 5 % (w/v) Puron 604 using a pneumatically-operated hand injector unit (Autarky Machine Co. Ltd., Salfords, Surrey) of the type used in commercial processing plants. After injection the carcasses were transferred to plastic bags. For each level of inoculum, a total of four batches of six carcasses was treated as above. Two batches were held loosely wrapped at 25 °C for 24 hr before examination in order to allow multiplication of the 'marker' and facilitate detection. The remaining carcasses were examined immediately.

In each case, a 5 g sample of breast muscle was obtained following careful dissection and the use of aseptic precautions. The sample was macerated in 20 ml of 0·1 % peptone diluent as before. Tests for the 'marker' organisms were made by spreading 0·1 ml of the macerate over the surface of duplicate agar plates. The isolation medium used was 5 % horse blood agar containing 500 μg/ml of streptomycin sulphate and the plates were incubated under hydrogen in an anaerobic jar for 24 h at 37 °C. Typical non-haemolytic colonies were picked into cooked meat medium for confirmatory tests which included (*a*) inhibition of lecithinase by *Clostridium perfringens* type A anti-toxin, (*b*) fermentation of lactose and (*c*) liquefaction of gelatin as described by Willis (1964).

RESULTS

Contamination of injection solution and injector

In order to determine the extent to which polyphosphate solutions used in different processing plants were contaminated with microorganisms, random samples were taken from the injection reservoirs at plants A–D during the normal working period in each case. The solutions being used at all four establishments were prepared from either Puron 604 (powder) or 6040 (concentrated solution).

Table 1 shows that all solutions were contaminated. The higher counts obtained at plants A, C and D are consistent with the observation that no particular precautions were taken by plant personnel to exclude contaminants during preparation of the solutions. The lowest counts were obtained from plant B where the reservoir was suitably covered, fitted with a piped water supply and sited well away from the eviscerating area in which the carcasses were being injected. Coliform bacteria were not detected in the plant B sample and the total count at 20 °C was only 60/ml. Of 12 isolates obtained from this sample, 6 were identified as *Pseudomonas* spp., 4 as strains of the *Acinetobacter* group and 1 each as *Aeromonas* and *Alcaligenes* spp. These organisms may have originated in the factory water supply.

As expected, injection of the first carcass resulted in immediate contamination of the injector needles and guide plate. The contaminating organisms could have

Table 1. *Microbial contamination of commercial polyphosphate injection solutions*

Processing plant	Orgs./ml	
	Total count, HI at 20 °C	Coliform bacteria
A (day 1)	$8{\cdot}0 \times 10^3$	$1{\cdot}2 \times 10^3$
(day 2)	$2{\cdot}5 \times 10^4$	$4{\cdot}0 \times 10^2$
B	60	< 5
C	$3{\cdot}0 \times 10^2$	NT
D	$3{\cdot}0 \times 10^3$	$3{\cdot}8 \times 10^2$

NT = not tested.

been derived from both the carcass skin and the injection solution. Swab samples taken from the injector at seven 1 h intervals during processing at plant C gave total counts at 20 °C of $2{\cdot}3 \times 10^5$–$2{\cdot}3 \times 10^6$/sample together with $1{\cdot}0 \times 10^3$–$1{\cdot}5 \times 10^5$ coliforms as an indication of faecal contamination. Although the counts showed relatively slight variation during the injection of many thousands of carcasses, contamination of deep muscle in each carcass and cross-contamination between carcasses would appear inevitable.

Behaviour of microorganisms in deep muscle during cold storage

In attempting to relate the microbiological condition of the deep muscle to that of the solution being injected, the carcasses used in these experiments were collected from the processing line immediately after taking a sample of polyphosphate solution from the injection reservoir.

Changes in total counts obtained from the breast muscle of injected and non-injected carcasses (plant A) during storage at 1° and 10 °C are shown in Fig. 1(*a*) and (*b*). Tests were carried out at 10 °C because the growth of faecal coliform bacteria would be theoretically possible at this temperature (Michener & Elliott, 1964). Before storage, the contamination of injected muscle was found to be > 100-fold greater than in non-injected controls and corresponded with the high counts obtained from the solution on day 2 at plant A (Table 1). During storage at 1 °C, however, the organisms present in the injected muscle showed a pronounced lag period before growth occurred and, when carcasses spoiled in the usual way due to surface growth of bacteria, there was little difference between injected and non-injected birds in the total counts obtained from the muscle. The effect of polyphosphate injection in retarding microbial growth in deep muscle was less in birds held at 10 °C and at this temperature the final counts remained higher in muscle from the injected carcasses (Fig. 1*b*).

Since the polyphosphate solution injected into the breast muscle is unlikely to penetrate all parts of the carcass, it was not surprising that injection had no effect on the shelf-life of carcasses stored at either 1° or 10 °C. At spoilage, total counts obtained from skin samples were all in the region of 10^8/g, regardless of treatment or storage temperature.

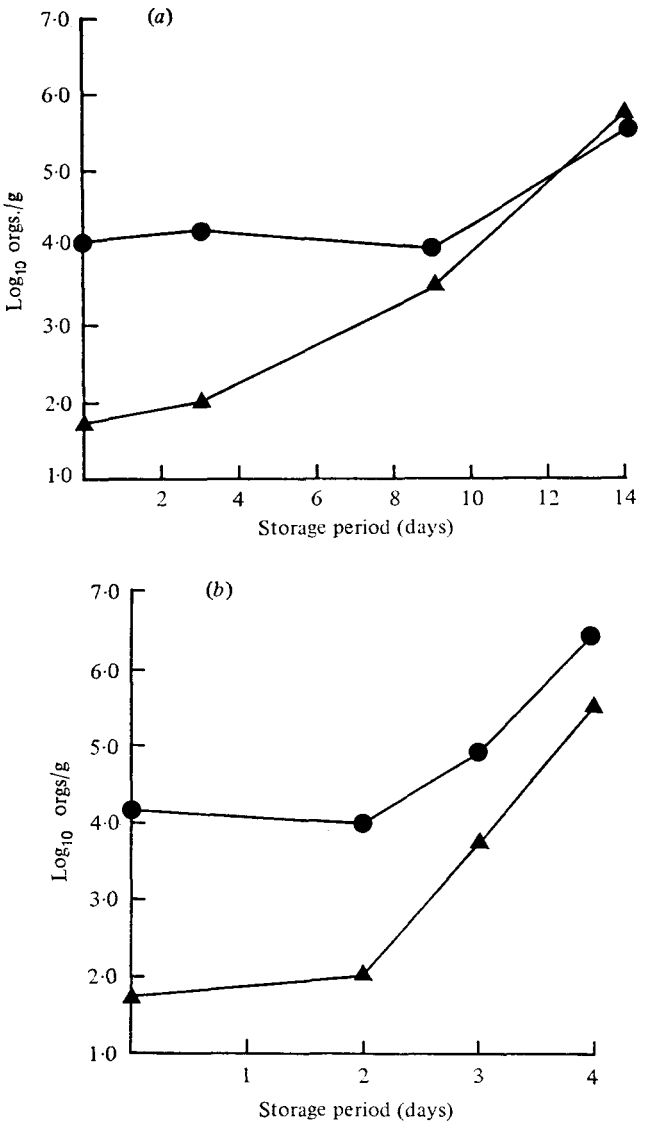

Fig. 1. Total counts at 20 °C from deep breast muscle of polyphosphate-injected (●) and non-injected (▲) carcasses stored at (*a*) 1 °C, (*b*) 10 °C. Geometric mean of results from three birds in each case.

In plant B, where the injection solution contained relatively few micro-organisms, the contamination of muscle in the injected birds was initially below the level of detection and there was no evidence of microbial growth during storage of carcasses at 1 °C. At 10 °C, however, the total counts increased considerably in the muscle, reaching $5{\cdot}0 \times 10^5$/g in non-injected controls and $1{\cdot}1 \times 10^6$/g in the injected birds.

Table 2 shows the incidence of coliform bacteria in the muscle, both before and after storage of carcasses obtained from plants A and B. With the exception of

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Table 2. *Influence of polyphosphate (Puron) on the growth of coliform bacteria in the deep breast muscle of carcasses stored at 1 ° and 10 °C*

Processing plant	Bird group	Orgs./g Before storage	After storage at 1 °C	After storage at 10 °C
A	Control	< 60	< 60	$2{\cdot}2 \times 10^3$
	Injected	63	$2{\cdot}4 \times 10^4$	$1{\cdot}0 \times 10^6$
B	Control	< 60	< 60	$2{\cdot}2 \times 10^2$
	Injected	< 60	< 60	$7{\cdot}0 \times 10^3$

Carcasses stored at 1 °C for 14 days or 10 °C for 4 days (plant A) or 5 days (plant B).

Table 3. *Influence of polyphosphate (Puron) on the bacterial flora of deep breast muscle in carcasses held at 1° or 10 °C until spoiled*

	Percentage distribution of genera or types			
Bird group ...	Control		Injected	
Storage temp (°C) ...	1	10	1	10
Acinetobacter gp.	3	38	6	7
Aeromonas	0	0	2	29
Enterobacteriaceae	0	0	15	64
Gram-positive spp.	27	21	2	0
Pseudomonas (pigmented)	0	10	40	0
Pseudomonas (non-pigmented)	70	29	29	0
Unclassified strains	0	2	6	0
Total strains	30	42	52	44

birds from plant B stored at 1 °C, there was evidence that cold-tolerant coliforms had multiplied during storage and the final counts from injected carcasses were always higher than the controls. Results obtained from plant A carcasses stored at 1 °C indicate the presence of strains capable of growth at both 1° and 37 °C.

The influence of polyphosphate on the composition of the bacterial flora developing in the deep muscle is indicated in Table 3. With carcasses stored at 1 °C, the main change was in the relative proportions of pigmented and non-pigmented pseudomonads present but at 10 °C a greater effect was observed. Whilst *Acinetobacter* and *Pseudomonas* spp. predominated in the muscle of control birds, injected carcasses showed a predominance of Enterobacteriaceae and *Aeromonas* spp. and pseudomonads were not found.

Seven strains of Enterobacteriaceae obtained from injected birds held at 10 °C were subjected to further tests. None produced indol or fermented lactose at 44 °C and hence they were not *Escherichia coli* type I. All strains grew well at 37 °C and were negative in the Voges–Proskauer test but varied in their ability to ferment lactose and citrate, liquefy gelatin or give a positive reaction in the Methyl Red test; their identity has yet to be established.

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Table 4. *Role of polyphosphate injection in transferring a marker strain of* Clostridium perfringens *from the skin of one artificially contaminated carcass to the deep muscle of this and other previously uncontaminated carcasses*

Approx. no. of orgs./cm² on skin of first carcass	Carcass no.	Treatment	Presence (+) or absence (−) of marker in deep muscle	
			Expt. I	Expt. II
2×10^4	1	No storage	+	+
	2		−	−
	3		−	−
	4		−	−
	5		−	−
	6		−	−
2×10^4	1	Stored*	+	+
	2		+	+
	3		+	−
	4		+	−
	5		+	+
	6		−	−
2	1	No storage	−	−
	2		−	−
	3		−	−
	4		−	−
	5		−	−
	6		−	−
2	1	Stored*	+	−
	2		−	−
	3		−	−
	4		−	−
	5		−	−
	6		−	−

* Held at 25 °C for 24 h.

Transfer of organisms from skin surface to deep muscle during injection

Experiments were carried out in the laboratory to determine the extent to which organisms on the skin could contaminate deep muscle tissue during the injection process.

Using the marker strain of *Clostridium perfringens* 25 LR, we found that injection resulted in both contamination of deep muscle and cross-contamination between carcasses when the breast skin of one carcass contained *ca.* 2×10^4 orgs./cm² before injection (Table 4). Since deep muscle contamination was detected only after incubation for 24 h at 25 °C, the number of organisms transferred from the skin is likely to be small, but following injection 5 out of 6 carcasses were found to be carrying the marker in the deep muscle in one experiment and 3 out of 6 in another.

As expected, the chances of transferring the marker to the deep muscle were reduced considerably when a small inoculum was used (*ca.* 2 orgs./cm²) and only one positive sample was obtained from all 12 carcasses used in the two experiments

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following injection and storage. Once in the deep muscle, however, the anaerobe was able to multiply readily during storage at 25 °C and each of the positive samples obtained from the high and low dose skin inocula yielded $> 1{\cdot}2 \times 10^4$ orgs./g. Hence, there was no indication that the Puron solution had markedly inhibited growth of the test organism at 25 °C in the muscle tissue.

DISCUSSION

The deep breast muscle of a processed carcass usually contains few micro-organisms (Barnes & Impey, 1975; Fig. 1*a*, *b*) though their numbers increase during storage under chill conditions. This study has demonstrated that poly-phosphate solutions used commercially are contaminated with microorganisms and on injection may increase the numbers present in deep muscle by >100-fold (plant A). However, during storage at 1 °C, the final counts obtained from injected birds were no higher than those from non-injected controls, thus indicating an inhibitory effect of Puron on the muscle flora. Elliott, Straka & Garibaldi (1964) found a similar effect on the spoilage pseudomonads when carcasses were immersed in a 3% solution of polyphosphates and held at 1 °C for 20–24 h. In the present study the inhibitory effect on the muscle flora was less following storage at 10 °C and a tenfold difference remained between injected and non-injected carcasses with internal counts reaching several millions/g. Nonetheless, there was no detect-able effect of deep muscle contamination on the time to spoilage of carcasses, whether injected or not, which occurs at population counts of *ca.* 10^8/g.

Although coliform bacteria were detected and some grew during storage at 10 °C, none were identified as *Escherichia coli*, type I which is indicative of faecal contamination. Cold-tolerant coliforms are commonly found on various animal products (Eddy & Kitchell, 1959).

Although it is impossible to determine from existing data whether polyphosphate injection is in any way associated with the increasing incidence of food-poisoning due to poultry, especially that caused by *Clostridium perfringens*, laboratory tests with artificially inoculated birds, using a higher degree of contamination than that found in normal practice (Mead & Impey, 1970; Lillard, 1971), established that the process of injection is capable of both carrying bacteria on the skin into deep muscle tissue and causing cross-contamination of carcasses. The potential hazard is related to the introduction of organisms into a site where they may survive and grow, particularly during any period of holding at ambient or higher temperatures. If cooking is inadequate, the growth rate of surviving *Cl. perfringens* in the cooling meat can be very rapid (Mead, 1969).

It is clear, therefore, that injecting carcasses with polyphosphates presents a potential hazard as anticipated by Marshall (1975) and Hobbs & Roberts (1976). For this reason it seems advisable that the Industry should develop a code of practice for the use of injection procedures which will ensure that the solutions used are as nearly sterile as is possible commercially, that the injection equipment is handled and maintained in a hygienic manner and that each carcass is washed thoroughly before injection in accordance with the best conditions of processing practice.

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