

Microbiological evaluation of dressing procedures for crocodile carcasses in Queensland

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SUMMARY: Microbiological testing of crocodiles during the dressing procedure has shown that sanitising the skin before skinning reduces the bacterial count on the skin and that dipping crocodile meat in 1.3% acetic acid solution effectively reduces bacterial levels. The total bacterial count on the processed meat sample was comparable with that obtained in the beef, pork and lamb industries. *Salmonellae* were isolated from 14 of the 72 carcasses. Most (65%) of these isolates were in *Salmonella* subspecies III, formerly classified as *Arizona*.

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Introduction

Until 1986, crocodiles in Queensland were farmed only for their skins. Since then the harvesting of crocodile meat has commenced and has steadily increased with demand. In 1988, the Veterinary Public Health Branch of the Queensland Department of Primary Industries, in close consultation with the Queensland Crocodile Industry, developed the first draft of *The Code of Practice for Hygienic Production of Crocodile Meat*. As crocodiles are known carriers of salmonellae (Manolis *et al* 1991) and the environment in which they are farmed is also considered to be infected, procedures were included in the Code of Practice to ensure that crocodile meat was safe for human consumption. The following two studies evaluated a number of existing or recently adopted dressing procedures in line with the Code of Practice.

Materials and Methods

Animals

We studied saltwater crocodiles (*Crocodylus porosus*) harvested from two farms in the Cairns area of Queensland. In study I (July 1991), 23 crocodiles were sampled from one farm (farm 1) over a two-day period. In study II (August 1992), 24 crocodiles were sampled from another farm (farm 2) on the first day and 25 crocodiles from farm 1 on the second day. The age of the crocodiles ranged from 3 to 5 years. All crocodiles harvested appeared healthy and free of disease. Crocodiles were denied access to feed for 2 to 3 days before slaughter. Selected crocodiles were humanely slaughtered in their pens by shooting in the head, immediately behind the cranial platform, using a .22 calibre rifle (Warwick 1990).

Carcass Handling

Once shot, the carcasses were immediately removed from the pens and sanitised. In study I, the bleeding site only was swabbed with 300 ppm chlorine solution. In study II, the entire carcass was scrubbed with a foaming, chlorine-based, alkaline sanitiser[§]. The necks were incised at the bleeding site and the animals left to bleed for about 15 min. Each carcass was rinsed with running water, suspended by the tail and stored in a refrigerated vehicle overnight. The storage temperature for study I was 4°C and for study II 2°C.

Skinning and Boning

After removal from the refrigerated vehicle the carcasses were again scrubbed with sanitiser and rinsed with running water. The cloacae were plugged with cotton wool swabs soaked with the sanitiser. The carcasses were then transferred to stainless steel skinning tables. To prevent any spillage of ingesta, the heads were placed in plastic bags, which were secured with masking tape.

During skinning, care was taken to try to prevent the meat from coming in contact with the external skin surfaces of the crocodile or the table surfaces. In study I stainless steel hooks were used to pin the skinned legs back onto the carcasses to prevent them from becoming contaminated. In study II the legs were wrapped in plastic to prevent contamination.

After skinning the carcasses were transferred to a boning table. In study I the carcasses were initially placed ventral side down and in study II dorsal side down. The back strap meat was removed when the carcass was on its ventral side and the tail and leg meat were removed when the carcass was dorsal side down. Leg meat was separated at the joints and other meat pieces separated into individual muscles. Only the external muscles were recovered for human consumption so evisceration was not performed.

The meat was treated with sodium metabisulphate[§] or acetic acid[#] before being drained and placed on polystyrene trays, over-wrapped with plastic film and stored in a freezer at -10°C for a minimum of 24 h. The surfaces of both the skinning and boning tables were washed clean with the sanitiser between processing each carcass.

Collection of Samples

Skin study I – After carcasses had been transferred from the refrigerated vehicle to the scrubbing platform three 24 cm² areas of skin on each crocodile (areas 1, 2 and 3 as shown in Figure 1) were swabbed using buffered peptone water^{**} (BPW) and microbiologically assessed as described below. After scrubbing the entire crocodile with the sanitiser and rinsing with running water, the 3 areas were swabbed again for microbiological assessment.

Skin study II – After each crocodile carcass was transferred from the refrigerated vehicle to the scrubbing platform, one 24 cm² area of skin (area 1 in Figure 1) was swabbed. After scrubbing the entire crocodile with the sanitiser and rinsing with running water, 24 cm² area of skin, on the thoracic side of area 1 (area 4 in Figure 1) was swabbed.

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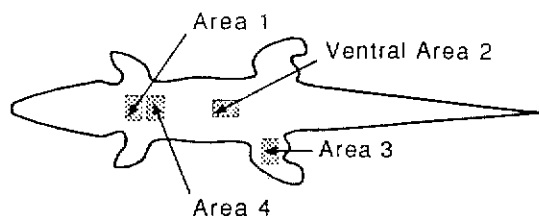


Figure 1. Dorsal view of crocodile showing swabbing sites

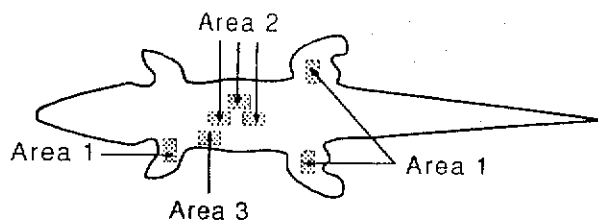


Figure 2. Dorsal view of skinned crocodile before boning showing swabbing sites on the meat

Meat study I – Back strap and leg meats were selected for testing as these cuts were more prone to handling contamination. After partial boning, swabs were taken of three 24 cm² areas at random from the back strap and leg meat areas (areas 1 and 2 in Figure 2) for microbiological testing. The meat was then dipped in an aqueous solution of sodium metabisulphite (30 mL/L) for 3 min. However, this procedure was later replaced by dipping the meat in a 15 mL/L aqueous solution of 100% food grade glacial acetic acid at 23°C for 10 s. After dipping, the meat sections were allowed to drain before being swabbed again for further assessment.

Meat study II – As more carcasses were sampled in this study only the blade meat was selected for sampling. After partial boning and separation into meat cuts, a swab was taken from the blade area (area 3 in Figure 2) for testing. On the first day of study II the meat was dipped in a solution containing 13.3 mL/L acetic acid at 23°C for 10 s. However, during the second day the concentration of the acetic acid bath was varied from 10 to 30 mL/L and the immersion time from 10 to 20 s. The blade meat was swabbed again after the meat had been dipped in the acetic acid bath and drained.

Antibacterial bath – A sterile pipette was used to collect a 1 mL sample from the antibacterial bath after a lot of carcass meat had been dipped. This procedure was the same for both studies.

Microbiological Methods

Swabbing procedure – A stainless steel template (8 cm × 3 cm) with an elongated angled handle was used to collect the skin and meat samples. The template was dipped in alcohol and flamed, cooled and then placed over the area to be swabbed. Cotton bud swabs were moistened thoroughly in BPW, drained up the side to remove excess moisture and then swabbed one way along the template area with continued rotation of the cotton to allow maximum contact with the area before swabbing at a 90° rotation along the other direction of the template. The template was dipped in alcohol and flamed between uses.

Salmonella isolation – After each site was swabbed in BPW, the swab was placed immediately in 10 mL of BPW of which 0.1 mL was plated onto Bismuth Sulphite Agar^{††} (BSA) before incubating both media at 37°C over-night. After 24 hours 0.1 mL of the BPW was transferred to 10 mL of Rappaport-Vassiliadis enrichment broth[‡] and 1 mL of BPW was transferred to 10 mL tetrathionate broth[‡]. The former was incubated at 37°C and the latter at 43°C for 24 hours before a loop of the broth was plated onto BSA. The 1 mL dip sample was added to 9 mL BPW and processed as above. Colonies with the appearance of salmonellae were removed for biochemical identification. Suspect *Salmonella* were sent to the Australian Salmonella Reference Centre in Adelaide for serotyping.

Total aerobic count – Volumes of 1 mL of the inoculated BPW were diluted serially in 9 mL amounts of sterile saline. Some 0.1 mL amounts of each dilution were spread over Plate Count Agar[‡] (PCA) so that dilution factors of 10⁻⁶ were achieved. Colony numbers were calculated after 2 days at 37°C.

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TABLE 1
Bacterial contamination (geometric means) on the skin of *Crocodylus porosus* before and after treatment with a sanitiser

Treatment	Study I		Study II		CV(%)*
	Day 1 (Farm 1)	Day 2 (Farm 1)	Day 1 (Farm 2)	Day 2 (Farm 1)	
Number of crocodiles	14	9	24	25	NA
Before treatment	149 ^{†ab‡}	83 ^a	271 ^b	13 ^c	14
After treatment	25 ^a	8 ^c	213 ^b	10 ^c	13
Reduction (%)	81 ^{2a}	88 ^{2a}	22 ^b	24 ^b	192

* Coefficient of variation from analysis of variance (in log units)

† Number of organisms per cm² × 10²

‡ In the same row, means with the same letter are not significantly different (P > 0.05). The superscript² indicates that the percentage value is significantly different from 0 (P < 0.05)

NA Not applicable

Statistical Analysis

Comparisons between 'treatment' groups (days, farms) were carried out using a one-way analysis, using individual carcass/dip sample variation as the error term. Where there were more than two treatments, pairwise comparisons were made using a protected LSD procedure. Bacterial counts were subjected to a log transformation before analysis, with geometric means generated. For percentage reduction in bacterial levels, analyses were performed on the differences in log (counts) and the means back-transformed to yield percentage figures. Coefficients of variation for each analysis were calculated to indicate the relative precision of each analysis. The percentage reduction estimates for each treatment were also compared against 0 (using a 1-tailed *t*-test on the transformed units) to test whether the reductions were in fact significant in themselves. Regression analysis was used to test for changes in bacterial concentrations in the dip solution as more carcasses were processed.

Results

Total Counts

Skin studies I and II – The results (Table 1) showed that there was a marked reduction in counts on skin after scrubbing with sanitiser. However, there was a difference in percentage reduction between studies I and II. This difference was due to animals from farm 2 having higher levels of bacteria both before and after foam scrubbing of the skin (Table 1).

Meat study I – The bacterial results of the meat samples demonstrated that there were lower numbers of bacteria on the pre-dipped back strap and leg meat on day 2 (Table 2). The bacterial results for the meat samples in study I demonstrated that there was a greater

TABLE 2
Bacterial contamination (geometric means) on the meat
of *Crocodylus porosus*

Site	Study I		Study II		CV(%) [*]
	Day 1 (Farm 1)	Day 2 (Farm 1)	Day 1 (Farm 2)	Day 2 (Farm 1)	
Number of crocodiles	11 [†]	6 [†]	24	25	
Back strap meat before dip	209 ^{‡a§}	64 ^a	‡	-	23
Back strap meat after dip [#]	117 ^a	8 ^b	-	-	23
Reduction (%)	51 ^{2a}	92 ^{2b}	-	-	46
Leg meat before dip	618 ^a	201 ^b	-	-	9
Leg meat after dip [#]	302 ^a	69 ^b	-	-	15
Reduction (%)	45 ^{2a}	66 ^{2a}	-	-	117
Blade meat before dip	-	-	184 ^a	98 ^b	19
Blade meat after dip [#]	-	-	47 ^a	30 ^a	31
Reduction (%)	-	-	72 ^{2a}	67 ^{2a}	110

* Coefficient of variation from analysis of variance (in log units)

† Two animals used as controls and one sample, which was contaminated, are not included

‡ Number of organisms per cm²

§ In the same row, means followed by the same letter are not significantly different ($P > 0.05$). The superscript ² indicates that the percentage is significantly different from 0 ($P < 0.05$)

¶ No data collected

For study I, day 1, the antibacterial dip was metabisulphite; acetic acid was used for the remainder of the studies (see text for details)

TABLE 3
Salmonellae isolated from crocodile skin and meat examined
in studies I and II

Anti-bacterial agent	Treatment stage	Sample site	Isolate	
Metabisulphite	Pre-wash	Skin	S IIlb ser 61:z52:z53	
		Pre-dip	Back meat	S IIIb ser 61:z52:z53
			Leg meat	S IIlb ser 38:1,v:z53:{z54} S IIIb ser 48:k:1,5,(7) S Bahrenfeld S Typhimurium phage type 1
	Post-dip	Back meat	S IIlb ser 38:1,v:z53:{z54}	
			S IIIb ser 61:z52:z53 S Bahrenfeld	
		Leg meat	S IIIb ser 38:1,v:z53:{z54} S IIlb ser 38:1,v:z53:{z54} S IIlb ser 48:k:1,5,(7)	
Acetic acid	Pre-wash	Skin	S IIlb ser 50:k:z * S IIIb ser 50:r:z35 S Typhimurium phage type 1	
		Foot abscess	S Typhimurium phage type 1*	
		Back meat	S IIIb ser 50:r:z35	
	Pre-dip	Leg meat	S Poona	
		Blade meat	S IIlb ser 50:r:z35*	
	Post-dip	Leg meat	S Adelaide	

* Study II

Note: In this table for reasons of space *Salmonella* subspecies IIb serovar is written S IIlb ser followed by the antigenic formula.

reduction in total bacterial counts when acetic acid was used as the dip (day 2) compared with metabisulphite (day 1). This occurred with both back strap and leg meat, though the latter difference was not statistically significant.

Meat study II - The blade meat sampled showed that pre-dip levels for farm 1 were lower than those for farm 2. The reductions in contamination on both farms were of a similar order (67%, 72%) (Table 2) and comparable with the reduction recorded on day 2 in study I.

Salmonellae

Eleven of the 23 crocodiles (48%) examined in the first study carried salmonellae. These included S Typhimurium phage type 1, S Adelaide, S Poona, S Bahrenfeld, S subsp IIIb ser 38:1,v:z53:{z54}, S subsp IIIb ser 48:k:1,5,(7), S subsp IIIb ser 50:r:z35, S subsp IIIb ser 61:z52:z53. In the second study, only 3 of the 49 crocodiles (6%) sampled carried salmonellae. These were S subsp IIIb ser 50:k:z, S subsp IIIb ser 50:r:z35 and S Typhimurium phage type I.

The salmonellae and their distribution on the skin and in the tissues, together with treatment applied, are shown in Table 3.

Dip

Regressing bacterial levels (log transformed) against number of carcasses immersed (up to 6 for metabisulphite, 9 for acetic acid) resulted in no significant trends for the 3 metabisulphite dips either individually or collectively. There were average counts of 18×10^2 bacteria per mL of metabisulphite dip irrespective of how many carcasses (up to 6) had been dipped. For the acetic acid dip, bacteria counts remained lower than 100 per mL even after 9 carcasses had been dipped.

Discussion

Dirt, dust and faecal matter on the hides of animals contribute to the microbial contamination of carcasses at slaughter houses (Dickson and Anderson 1992; Selgas *et al* 1993). Such material is common on the animal but increases during the transport to the abattoir. Wray *et al* (1991) showed in their survey that 20.6% of vehicles transporting calves to market were contaminated with salmonellae. Meat and meat products are also exposed to further contamination during the handling and processing stages of the procedures (Hechelmann and Kasproviak 1992). Quality control can minimise the potential risk to consumers and one of the first procedures available is sanitising the carcass to reduce the initial concentrations of bacteria (Dickson and Anderson 1992).

In our survey, farm 1 undertook a number of procedural changes between studies I and II. They introduced a preliminary scrub with sanitiser immediately after slaughter and before suspending the carcasses in the refrigerated vehicle overnight. The water supply to the crocodile pens was also chlorinated and the pens were converted from dirt to cement floor. The result was a pre-scrub skin contamination at the abattoir of 13×10^2 bacteria per cm² in study II. While there was also a difference in the percentage reduction of total count due to foaming between the two studies (81 and 88% - study I, 24% - study II), this was more likely due to the change in sampling pattern of the skin after scrubbing. In the first study, samples were taken from the same sites before and after scrubbing. Thus, the numbers were already depleted before the foam cleaning. In the second study, the post-scrubbing samples were taken from a site adjoining the pre-scrubbing site.

The hygienic practices undertaken at farm 2 were not as meticulous as at farm 1 and this is reflected in the higher skin total bacterial count of 271×10^2 per cm². Scrubbing with sanitiser at the abattoir caused a percentage reduction in bacterial counts similar to farm 1. Therefore, scrubbing the skins with sanitiser at the abattoir before skinning reduced bacterial numbers and thus reduced the risk of excessive

contamination of the meat during the skinning process. There was also value noticed in scrubbing with sanitiser at the farm before the animals were hung overnight in the refrigerated transport vehicles. To further minimise the numbers of bacteria on the meat after handling (skinning, deboning), an antibacterial agent was used as a dip before packaging.

Several means are available to physically remove contaminating bacteria from a meat surface. These include water rinsing, chlorine, organic acids and gamma irradiation (Dickson and Anderson 1992). In study I sodium metabisulphite solution was used as a dip. This chemical was originally used in the prawn industry as a preservative but the results showed that the metabisulphite solution became contaminated after successive dipping of the processed meat.

Acetic and lactic acids have been used in the sanitisation of beef (Quarrey-Papafio *et al* 1980) and lamb (Ockerman *et al* 1974) carcasses because they have good bactericidal activity (Acuff *et al* 1987) and are regarded as safe additives (Food and Drug Administration 1982). The organic acids are also effective in reducing *Salmonella* contamination on meat (Ockerman *et al* 1974; Dickson and Anderson 1991; Ostling and Lindgren 1993).

The use of acetic acid as a successful antibacterial treatment procedure for pork was developed by the CSIRO Division of Food Processing, Meat Research Laboratory, Cannon Hill, Queensland, and is reported in *Guidelines for Acetic Acid Treatment of Chilled Pork before Vacuum Packaging*. This involved the dipping of pork meat in a 1.5% solution of acetic acid held at 55°C for 10 s. This procedure was modified for the processing of crocodiles in Cairns where meat was dipped in 1.3% acetic acid at 23° for 10 s. Our results indicated that the bacterial count in the acetic acid bath did not reach high concentrations even after meat from 9 crocodiles had been dipped. Meat dipped for longer periods of time (15 to 20 s) or dipped in higher concentrations of acetic acid (1.5 to 2%) showed discolouration that looked unappealing as well as leaving behind the distinct odour of the acid. Discolouration also occurred if pools of solution were allowed to remain on the meat (without proper draining). There was a greater percentage reduction in bacterial numbers (66 to 92%) on the meat dipped in acetic acid than on meat dipped in metabisulphite solution (45 to 51%).

The results indicated that before dipping, the leg meat samples contained more bacteria than either the back strap or blade meat samples (Table 2). This would be due to increased handling of the legs during the processing procedure and to accidental cross-contamination from the outer skin surface onto the exposed meat during skinning, as this operation is awkward and prone to skin fold-back. A similar result was seen after dipping. During the first study, legs, which had not been boned, were not submerged in the antibacterial solution because the baths were shallow. Therefore, these figures may not be a true indication of the effectiveness of the antibacterial solution. Leg meat was not sampled during the second survey.

The techniques used to determine total counts and presence of salmonellae were dictated by the necessity to cause as little disruption to the flow of the slaughtering process and the limited availability of bench space and incubation apparatus. To this end, a wet swab technique was substituted for the recommended removal of numerous cubes of meat. The crocodile meat industry is quite lucrative and the swab method was not destructive to the product. As well as this, the swab method was much faster than the cutting technique and thus did not interfere with the normal 'line' operation.

Swabbing of the meat and calculation of the number of organisms on a per cm² basis still allowed a comparison with results obtained from the meat industry.

Total aerobic counts were calculated from agar plates incubated at 37°C for 2 days. Usually plate counts are determined after 2 to 4 days at 25°C so that psychrophiles will have a better chance to grow. Because of the restricted availability of incubators on the field trip it

was decided to incubate all plates at 37°C. This also complied with the initial decision to follow procedure set out in the seafood industry, which often uses 35 to 37°C for incubation of PCA (Singh *et al* 1987; Ingham and Moody 1990; Wibowo *et al* 1992). As well as this, our concern was more for organisms of potential human health risk than food spoilage organisms.

The use of tetrathionate and Rappaport-Vassiliadis broths in conjunction with BSA was used for *Salmonella* isolation as recommended by Harvey and Price (1983) where a variety of media is required to cope with the different serotypes. Routinely, Brilliant Green Agar is used in our laboratory for *Salmonella* isolation as well. However, space for and transport of extra media were not possible for this survey.

Of the samples taken from the skin and meat during the two studies, 11 (48%) of the 23 crocodiles sampled during the first study and three (6%) of the 49 crocodiles sampled during the second study recorded at least one *Salmonella*. In the first study, seven *Salmonella* were obtained from meat products (six after dipping in metabisulphite solution and one after dipping in acetic acid) destined for human consumption, while, in contrast, no *Salmonella* were detected on final meat products during the second study.

Apart from the skinning process, the most likely source of meat contamination with salmonellae during the abattoir procedure is from the cloaca. This is a known site for isolation of *Salmonella* (Manolis *et al* 1991). In pigs, 66% of carcass contamination is faecal in origin (Dickson and Anderson 1992). During the first study, it was noted that after skinning, the carcasses were transferred to a boning table where they were placed with their ventral side on the table surface. Carcasses were then turned over onto their dorsa during the boning operation. As the cloaca is only plugged and not sealed, any contamination from this opening could contaminate the table surface and subsequently the carcass. During the second study, the abattoir workers had introduced routine extra cleaning of the benches, use of surgical gloves, less handling of the carcass during skinning and, with more experience, had reduced the skinning time. As well as this, the carcasses were placed dorsa down on the boning table thus reducing the risk of cloacal contamination.

Salmonella serovars, especially *Salmonella* subsp III, are frequently isolated from healthy reptiles, including crocodiles and are part of the normal flora of the intestine of these reptiles (Chiodini and Sundberg 1981; Harvey and Price 1983; Debyser and Zwart 1991; Obwolo and Zwart 1993). These serotypes have rarely been incriminated as the cause of gastro-enteritis in man (Chiodini and Sundberg 1981; Ladds and Donovan 1989). Their zoonotic danger to man is doubtful (Harvey and Price 1983; Debyser and Zwart 1991) and *Salmonella* subspecies IIIb are regarded as opportunistic pathogens in patients with serious, underlying diseases who are immunocompromised by use of suppressive drugs (Kraus *et al* 1991). Of the 1545 and 1656 recorded observations of *Salmonella* recorded from humans in Queensland during 1991 and 1992 (National Salmonella Surveillance Scheme, University of Melbourne Annual Reports of 1991 and 1992), only 11 (0.7%) and 23 (1.1%), respectively, were attributable to *Salmonella* subspecies IIIb. The major proportion of *Salmonella* isolated during our work was *Salmonella* subspecies IIIb (65%). This is not dissimilar to other workers. Debyser and Zwart (1991) recorded 51.2% of their *Salmonella* isolates from reptiles as *Salmonella* subspecies IIIb, while Obwolo and Zwart (1993) identified 87.5% of their *Salmonella* isolated from crocodiles as *Salmonella* subspecies IIIb.

The average salmonellae occurrence over the two studies (19.4%) was comparable with results from other crocodile studies where the prevalence of salmonellae on flesh for human consumption has been recorded as 19.2% for *C porosus* (Manolis *et al* 1991) and 33% for frozen *Crocodylus niloticus* meat (Madsen 1993). The range (6 to 48%) of salmonellae in our studies was also comparable with other meat industries including beef [3.3% (0.2 to 21%)]; kangaroo [11.1%

(7.8 to 15%); domestic pigs [16% (0.4 to 76%)]; poultry [33.4% (5.0 to 79%)] and feral pigs [34.4% (5.6 to 68%)] (D'Aoust 1989; Bensink *et al* 1991).

The average total bacterial count of 39 per cm² from samples of final product during the second study compared very favourably with results from the beef industry (total counts of 10³ to 10⁴ per cm², Ingram and Roberts 1976), the pig industry (total counts of 10³ per cm², Gill and Bryant 1992) and the camel industry (total counts of 7.2 × 10³ per cm², Hamdy 1991). A total count of < 5 × 10⁶ per cm² has been suggested as a criterion for carcasses and fresh meat at processing plants (Hechelmann and Kasprowiak 1992).

Adherence to a strict set of guidelines, including scrubbing of carcasses at the farm as well as before skinning, cleansing of preparation and boning tables, proper handling of carcasses and use of an appropriate antibacterial dip such as acetic acid, ensures that crocodile meat products available for the retail market compare favourably with other meat commodities both with regard to salmonellae and total bacterial count. Crocodile meat is fit for human consumption. However, as in the case of all meat products, it is ultimately up to the consumer to handle and cook the product correctly.

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