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The microbiological safety of duckweed fed chickens: a risk assessment of using duckweed reared on domestic wastewater as a protein source in broiler chickens

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Abstract

The possibility of transmission of pathogens from duckweed supplemented feed to chickens and consequently to the human consumer necessitated the microbiological testing of duckweed fed chickens. This assessment was thus done to determine whether there is transmission of pathogens from the duckweed supplemented feed to the chickens; determine whether such infection would be systemic or be confined to the gastro-intestinal tract of the birds; and to investigate the microbial load and distribution of the microbes with age. The study birds were sacrificed at 3, 6, 8 and 10 weeks of age and examined for the indicator organisms *Escherichia coli* and *Salmonella* spp. There was no discernible pattern in the microbial load of both the duckweed fed chickens and control birds with age although the control birds sampled clearly had a lower microbial load than the experimental flock. Some *Salmonella* and two enteropathogenic *E. coli* strains were isolated from control and experimental sub-samples at 3 weeks. There were no Salmonellae isolated in the subsequent batches of birds and feed although a number of *E. coli* were isolated. More isolates were obtained from the three weeks' sub-samples (collected during wet weather) than from all the other sub-samples. The use of duckweed at this inclusion rate under the processing conditions at Nemanwa was thus concluded to be microbiologically safe as long as due caution is exercised during the processing of the duckweed and handling of the birds. There are indications that the chickens may get contaminated especially during wet weather as evidenced by the isolation of *E. coli* and *Salmonella* spp from the first batch sub-samples. This was attributed to poor environmental sanitation at the plant particularly in view of the prevailing wet conditions at the time.
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1. Introduction

1.1. Excreta re-use in aquaculture

The floating macrophytes (duckweeds, water hyacinth and the ferns) have been shown to grow on wastewaters, accumulating the excess nutrients into their own biomass (Boyt et al., 1977). The macrophytes can later be processed into a variety of products such as poultry feed and fishmeal thereby bringing a cost recovery aspect into the waste management process (Hillman and Culley, 1978). The duckweeds (*Lemna* spp, *Spirodella* spp) have received the most attention because of their rapid growth to high biomass, ease of handling and a high protein content relative to the other macrophytes. Numerous studies have demonstrated the value of duckweed as a feed for poultry, fish and other animals (Skillicorn et al., 1993).

In Zimbabwe, duckweed was introduced into the facultative and the first of two maturation ponds at Nemanwa and Gutu Mupandawana growth points in Masvingo Province in June 1999. Poultry projects were started to incorporate a cost recovery aspect into the wastewater treatment process. The duckweed is harvested daily and dried. Kusina et al. (1999) reported that an inclusion rate of 10–20% w/w dry duckweed in broiler feed did not affect the growth performance or carcass composition of the birds while it offered a reduced cost of feeding them. Based on their recommendations duckweed was incorporated into the feed of broilers in the poultry projects. The birds have ad libitum access to the feed.

1.2. The microbiological risks in excreta re-use

The macrophytes grown on wastewaters have been shown to also accumulate microbial contaminants from

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the wastewaters (Boyt et al., 1977). When they are later processed for use the load can be reduced by drying. Earlier work has however shown that the length of time required for the total elimination of pathogens is highly variable thus there is a possibility of producing a contaminated duckweed product. Drying in itself has also been a major handicap to the acceptance of duckweed as commercial crop. No conventional technology has been able to produce a dried duckweed commodity without incurring a significant financial loss (Skillicorn et al., 1993).

Experimental evidence suggests that *Salmonella* infection of animals resulting from excreta use on fodder crops may be an unlikely event, requiring ingestion doses of organisms in excess of 10^4–10^5 (Hall and Jones, 1978). However, Blum and Feachem (1985) acknowledge that there is a possibility of human infection when people consume meat or milk from an animal that has consumed a crop (or fish) fertilised by excreta. The disease risks include infection with *Salmonella* spp and *Campylobacter jejuni*.

There is limited evidence to suggest that a small unknown percentage of human Salmonellosis outbreaks may be attributable to the consumption of animals reared on foodstuffs from excreta enriched ponds although no epidemiological studies have examined the risk of human disease from consuming meat or milk from these animals. Cattle Salmonellosis outbreaks and subsequent human outbreaks after consumption of milk from the cattle have been reported. The outbreaks were attributed to fertilisation or accidental contamination of pasture with sludge and or wastewater effluent (Burnett et al., 1980; Berderke et al., 1956). The most significant health hazard however, one that is generally overlooked, is the danger from handling and preparing contaminated products especially when the product can carry pathogens passively (Stuckey et al., 1986).

It has been suggested that the public health hazards due to excreta re-use be minimised by lengthening the food chain (Stuckey et al., 1986). Implicit in this suggestion is the assumption that the microbial load will decrease through the food chain. There has however been no purpose designed study to ascertain this.

1.3. The pathogens of interest in excreta re-use and poultry

The members of the family Enterobacteriaceae are inhabitants of the human intestinal tract in health and disease. They are among the most important bacteria medically as a number of them are human intestinal pathogens. The organisms *Salmonella* spp and *E. coli* are frequently used as indicators of faecal pollution because of their consistent occurrence in the faeces of warm blooded animals.

1.3.1. Escherichia coli

E. coli is often used as an indicator of pollution by mammalian faeces. Its presence is taken to indicate the presence of other more dangerous enteric bacteria and other types of micro-organism although the organism itself is not normally pathogenic. A few serovars play an important role in intestinal and extra-intestinal disease although the organism remains primarily an opportunistic pathogen. In Zimbabwe the more important pathogens associated with poultry are the intestinal pathogen groups: enteroheamorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC).

EHEC are represented by a single strain (serotype O157:H7), which causes a diarrhoeal syndrome in which there is copious bloody discharge accompanied by abdominal cramps but with no fever. Doyle and Schoeni (1987) found the organism in 1.5% of chicken and turkey samples in a supermarket. ETEC are an important cause of diarrhoea in infants and travellers in underdeveloped countries or regions of poor sanitation. They are acquired by ingestion of contaminated food and/or water. EPEC induce a watery diarrhoea similar to ETEC. EIEC closely resemble *Shigella* in their pathogenic mechanisms and the kind of clinical illness they produce, that is a dysentery like diarrhoea with fever.

1.3.2. Salmonella spp

The Salmonellae are rod shaped bacteria found in animals especially poultry and pigs. Salmonellosis, the clinical infection caused by Salmonella infection is the most common food borne illness. *Salmonella typhi* and the paratyphoid bacteria normally produce typhoid or typhoid like fever in humans. The other forms of Salmonellosis generally produce milder symptoms. All age groups are susceptible to Salmonellosis but the symptoms are most severe in the elderly, infants, and the infirm patients. In Zimbabwe in the context of poultry the more important serogroups are B, C3, D and 53. Within these the divisions 1, 4, 5 and 12 are of particular interest (Gwanzura, personal communication).

1.4. Microbiological testing

In 1995 the World Health Organisation (WHO) and the Food and Agricultural Organisation (FAO) in a joint report defined risk assessment as "the scientific evaluation of known or potential adverse health effects resulting from human exposure to food borne hazards" (Notermans and Jouve, 1995). Microbiological testing forms an integral basis of risk assessment in food production systems.

The microbiological safety of a finished food product is of prime concern to both the producer and the consumer. It assures the consumer of a disease free food and the producer a continued stake in the market more so in

this present day when consumer awareness is greater than ever before. The microbiologically safe food is not sterile, rather it has tolerable levels of micro-organisms that are not a threat to the consumer. It is also free of those that are fatal even at low doses or their toxins.

The micro-organisms in complex food systems do not always behave as predicted by laboratory studies of pure cultures. One of the objectives of microbiological testing is to determine the potential for micro-organisms to survive or multiply during production, processing, distribution, storage and preparation for consumption of the food (Silliker, 1995). The exercise is therefore done bearing in mind those factors that would influence the potential contamination of the food with pathogens as well as their growth and survival in it.

1.5. Objectives

The possibility of transmission of pathogens from the duckweed supplemented feed to the birds and consequently to the human consumer necessitates the microbiological testing of the chickens. This microbiological assessment was thus done:

(a) To determine whether there is a transmission of pathogens from the duckweed supplemented feed to the chickens.
(b) To determine whether the infection, if it occurs, becomes systemic or it is confined to the gastro-intestinal tract of the birds.
(c) To ascertain the patterns if any, in the microbial load and distribution of the microbes in selected tissues with age.

2. Materials and methods

2.1. Rearing of the birds

A flock of 50 birds (Crest Breeders day old chicks) was used. The birds were on the conventional duckweed free diet for 21 days. At 3 weeks of age the experimental flock (25 birds) was put on a duckweed supplemented diet while the control flock continued on the conventional diet. The birds had ad libitum access to the feed throughout the study period. The chickens were sacrificed at various stages of their growth specifically at 3, 6, 8 and at 10 weeks of age. At each stage the birds were aseptically dissected to obtain the required subsamples.

2.2. Preparation of the duckweed

The duckweed was harvested from the wastewater treatment ponds (secondary ponds) at Nemanwa using a small boat and a scoop net. It was dried in the air for four days followed by six days of sun-drying. The dried duckweed was ground and incorporated into the poultry feed at a rate of 20% w/w.

2.3. Isolation of the indicator organisms

The birds were checked for the faecal contamination indicators *E. coli* and *Salmonella* spp.

2.3.1. Escherichia coli

(a) *Selective and differential culture:* The liver, heart with the lungs and cloaca with the intestine were used. The feed (both conventional and duckweed supplemented) was also analysed. The tissues were macerated lightly using sterile surgical blades and then suspended in sterile quarter strength Ringer solution for half an hour. The suspensions were cultured on Mackonkey medium, a differential medium for the isolation of coliforms and intestinal pathogens. The suspensions were also cultured on Sorbitol Mackonkey agar for the selective and differential culture of *E. coli* strain O157: H7. Suspected *E. coli* colonies were purified on Nutrient agar.

(b) *Screening of isolates:* Pure *E. coli* cultures on Nutrient agar were screened by means of a Gram stain. *E. coli* are short Gram negative rods. The isolates were confirmed biochemically using the iMVPC and Eosin Methylene Blue tests.

2.3.2. Salmonella spp

(a) *Enrichment and selective culture:* The liver, heart with the lungs, cloaca with the intestine, and skeletal muscle were used in the analyses. The feed (conventional and duckweed supplemented) was also analysed. Lightly macerated tissues were pre-enriched in buffered peptone water and enriched in Single Strength Selenite Brilliant Green Broth for Salmonellae. Positive enriched cultures were cultured on Xylose Lysine Desoxycholate agar (XLD) a selective and differential medium for the Salmonellae. Suspected Salmonellae colonies were further purified on XLD. The pure cultures were confirmed using Triple Sugar Iron, Lysine Iron agar and Urea Utilisation tests.

(b) *Serotyping:* The isolates were serotyped using the slide agglutination tests. Two drops of sterile saline were placed on a slide and each isolate emulsified to form a smooth suspension. Undiluted antisera was then added to one suspension and a further drop of saline added to the other suspension

(c) *E. coli:* The confirmed isolates were serotyped using EPEC agglutinating antisera using slide agglutination tests.

(d) *Salmonella spp:* The confirmed isolates were serotyped using Polyvalent 'O' antisera. Polyvalent 'O' agglutinates groups A to G.

3. Results

3.1. Escherichia coli

(a) *Bacterial load:* There was no notable change in the bacterial load of the feed throughout the study period although the supplemented feed had a higher load than the control feed. The cloaca and intestine sub-sample had the largest diversity of isolates at 3 weeks of age. By 6 weeks through to slaughter age however, only one type of isolate was being obtained from the control sub-sample. Three apparently different isolates were present in the experimental sub-samples. The heart and lungs sub-sample also consistently had the largest diversity of potential *E. coli* isolates. By slaughter age however this had also decreased appreciably although it still remained the highest in comparison with the other sub-samples. The liver had the least load. Only a few isolated colonies were obtained throughout the study period. Table 1 below summarises the results of the selective culture for *E. coli* and Table 2 shows the total numbers of confirmed isolates.

(b) *Serotyping:* Two of the *E. coli* strains from the 3 weeks sub-samples were enteropathogenic. At slaughter age, three EPEC strains were isolated from the liver sub-sample of the experimental flock.

3.2. Salmonella

(a) *Bacterial load:* During enrichment there was growth in the enrichment medium in a number of sub-samples except the liver although these later proved not to be *Salmonella*. Yellow colonies on XLD agar that were possibly *E. coli*, *Enterobacter*, *Klebisella*, *Citrobacter*, *Proteus* and *Serratia* were obtained. Table 3 summarises the results of the enrichment procedures.

(b) *Serotyping:* Poly O positive Salmonella was detected in the feed samples at 3 weeks of age. The same serogroup was also isolated from the cloaca and intestine sub-sample. No further Salmonellae were isolated during the study period.

4. Discussion

The results of the selective culture indicate that the birds did not have any systemic microbial load. This is indicated by the absence of *E. coli* and *Salmonella* spp in the liver sub-samples (Chamanza, personal communication). *Salmonella* is important as a pathogen in raw poultry and also as an indicator of faecal contamination. The fact that none of it was isolated in the heart, lungs, liver and other sub-samples confirms the microbiological safety of the birds. The *E. coli* that was found in the liver sub-sample probably migrated there after slaughter as it is a common phenomenon for microbial inhabitants of the gut to migrate to the liver after a bird has been killed.

The safety of the birds is further reinforced by the cloaca with intestine isolates. Only one isolate was obtained from the control sub-samples after 3 weeks of age and a consistent 3 isolates every time from the experimental batches. These results indicate that the use of duckweed does alter the microbial flora of the birds, at

Table 1
A summary of the selective culture of *E. coli*

Age of birds (weeks)	Liver		Heart and lungs		Cloaca and intestine		Feed	
	Trt 1	Trt 2	Trt 1	Trt 2	Trt 1	Trt 2	Trt 1	Trt 2
3	–	–	–	–	+	+	+	+
6	–	–	+	–	+	+	+	+
8	–	–	+	–	+	+	+	+
10	–	–	–	–	+	+	+	+

Trt 1 refers to the sub-samples from the birds that were on a duckweed supplemented diet and Trt 2 refers to the birds that were on the conventional duckweed free diet.
(–) denotes less than 10 colonies on the plate and (+) greater than 10 colonies per plate.

Table 2
The total numbers of confirmed *E. coli* isolates

Age of birds (weeks)	Liver		Heart and lungs		Cloaca and intestine		Feed	
	Trt 1	Trt 2	Trt 1	Trt 2	Trt 1	Trt 2	Trt 1	Trt 2
3	0	0	0	0	4	4	2	2
6	1	0	3	0	3	1	0	1
8	0	1	5	3	3	1	5	2
10	2	*	4	*	3	*	2	*

Trt 1 refers to the sub-samples from the birds that were on a duckweed supplemented diet and Trt 2 refers to the birds that were on the conventional duckweed free diet.
* denotes that no samples were available.

Table 3
A summary of the enrichment results for *Salmonella*

Age of birds (weeks)	Liver/skeletal muscle		Heart and lungs		Cloaca and intestine		Feed	
	Trt 1	Trt 2	Trt 1	Trt 2	Trt 1	Trt 2	Trt 1	Trt 2
3	−	−	−	−	+	+	+	+
6	−	−	−	−	−	−	+	+
8	−	−	+	+	+	+	+	+
10	−	*	+	*	+	*	+	*

Trt 1 refers to the sub-samples from the birds that were on a duckweed supplemented diet and Trt 2 refers to the birds that were on the conventional duckweed free diet.
(+) denotes a change from green to a brick red of the enrichment medium that indicates growth. (−) denotes no change in colour.
* indicates that no samples were available.

least relative to the control that was reared on a duckweed free diet. This can also be seen in the results from the feed analysis. The control feed consistently had a lower microbial load that the duckweed supplemented feed.

The same serotypes of *E. coli* and *Salmonella* were isolated from the experimental feed sub-sample and chicken tissues. The birds probably got contaminated due to poor environmental sanitation in the plant rather than due to contaminated feed. The feed itself was also contaminated possibly due to insufficient drying or again due to poor environmental sanitation. The situation was likely brought about and aggravated by the wet weather at the time of sampling which could have interfered with the drying processes and increased the chances of contamination by the workers of the duckweed and the birds.

The absence of *E. coli* and *Salmonella* are generally considered as indicators of proper slaughter and handling. In the case of this study their presence indicated improper handling. While the overall results indicate that there is no systemic infection in the birds that may be attributed to the use of duckweed in feed, they also point out that there is potential for it. There is clearly a transmission of microbes from the weed to the birds which necessitates a thorough drying of the weed to kill off all pathogens at this point, before the weed is incorporated into feed.

Salmonella was only isolated from the three week sub-samples before the birds were put on a duckweed supplemented diet and there was none detected in the subsequent batches (after the introduction of the duckweed treatment). Although *Salmonella* is important as a contaminant of unprocessed poultry products and is frequently included as an enteric pathogen to detect faecal contamination, its numbers in sewage are usually low. It is therefore unlikely in cases of minimal to moderate contamination that the *Salmonella* will be isolated and this should be borne in mind.

This study served as a hazard analysis of the chicken project. It led to the identification of a potential hazard. It is therefore recommended that a quality assurance system based on HACCP principles be put in place to ensure the microbiological safety of the birds. The rationale behind HACCP is to avoid the contamination of foodstuffs (in this case poultry) whenever possible and this includes the inadvertent contamination of the animal, employees and the environment. Contamination can be minimised or eliminated altogether by following appropriate environmental sanitation procedures including those of employee hygiene.

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