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F10 PRODUCTS

Determination of disinfectant residues in tissue after oral supplementation of drinking water with F10SC disinfectant

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Summary

F10 Super Concentrate disinfectant (F10SC) was administered to poultry continuously in the drinking water at two dilution rates (1:1000 and 1:250). Tissue samples were examined for disinfectant residues using a biological disc inhibition assay at day 35 of the study. No significant residues (P>.0.05) were detected in liver, muscle or kidney tissues at either dilution rate compared with a control group. There was no significant increase in mortality (P>0.05) in the F10SC disinfectant treatment groups over the control group. It is concluded that F10SC disinfectant can be added to the water supplies of poultry and other avian species or by nebulising in an attempt to reduce disease or improve water quality without the risk of producing tissue residues.

Introduction

F10 Super Concentrate disinfectant (F10SC) is a novel quaternary ammonia and biguanide compound based disinfectant which by independent tests and trials has been shown to be effective against gram negative and gram-positive bacteria, enveloped and non-enveloped viruses in addition to fungal spores. Similarly the disinfectant has been shown to be non-toxic, non-irritant, non-corrosive and biodegradable . Due to both its safety and efficacy F10SC disinfectant has been increasingly used in applications where the diluted solution is inhaled through nebulisation or as an aerosol over-spray (fogging) rather than just as a surface disinfectant. It has been administered through the drinking water to improve the water quality in automatic drinking systems in both broiler and layer operations thereby reducing bacterial challenges during production. It has been used as therapy during outbreaks against infective agents such as *Avian Influenza Virus, Newcastle Disease Virus* and *Infectious Bursal Disease Virus.*

Fogging the enclosed air space in commercial poultry houses as well as in setters and hatchers regularly with F10SC disinfectant has been demonstrated to significantly reduce environmental contamination with *Aspergillus fumigatus* spores. F10SC disinfectant has also been utilized to treat respiratory infections in both captive exotic birds, birds of prey, and reptiles by nebulisation. It has been used in the reduction of Psittacine Beak and Feather Disease infection in African Grey parrots by reducing contamination of the environment with *Circo Virus spp*. In addition it has been used in the treatment of circovirus infection in combination with avian gamma interferons. Due to the increasing alternative uses of F10SC in the prevention and treatment of disease there is a need to rule out the possibility of tissue residues from continuous oral treatment. The aim of this study therefore was to investigate whether F10SC disinfectant could be safely administered orally to birds over a period of time without side effects or detectable tissue residues.

Fogger used to overspray in aviaries and poultry houses in the presence of the birds.

Material and Methods

The trial was conducted under the auspices of the Republic of South Africa's Agricultural Research Council (ARC) at their Animal Nutrition and Animal Products Institute facility at Irene, Gauteng Province. Subsequent sensitivity and tissue analysis testing was carried out at the ARC's Onderstepoort Veterinary Institute's (OVI) Residue Laboratory (South African National Accreditation Service (SANAS) accredited laboratory).

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The trial was conducted using 153 as hatched Ross 788 day-old broilers obtained commercially. The birds were placed randomly into a small broiler experimental house. The facility consisted of 9 pens with each pen containing 17 chickens. This study was run in the form of a 3 x 1 block design with each treatment replicated 3 times.

During the period no additives, growth stimulants, coccidiostats or medicines were included in the feed.

Upon arrival at the research site, the chickens were examined and any obviously sick or dehydrated birds were culled. Standard management techniques were followed as described by the suppliers of the Ross chickens (Ross Broiler Management Manual, 2002) and the same care and management was provided to all the birds used in the study. All birds were housed in wire pens with the floor covered with approximately 5cm wood shavings. Each pen was equipped with a 10-liter fountain drinker and a plastic tube feeder. The house temperature at the start of the study was kept as close as possible to 32 °C whereafter it was decreased with a gradient as the chickens grew older. All the broilers received broiler starter and finisher diets formulated to commercial specifications that contained no additional medication or supplementation. The feed was formulated and mixed at the ARC Poultry Nutrition facilities at Irene. The birds received no vaccines.

The experimental house was divided into two areas. The control group of chickens was placed and reared in the one side of the house. The two treatment groups that were supplemented with F10SC via the drinking water with respectively 1:1000 and 1:250 concentrations (10ml and 40ml/ 10-l drinking water) were placed opposite each other on the other side of the house. Footbaths containing F10SC in the water were placed on both sides of the control and treatment pens respectively for the personnel to walk through in the broiler house. The F10SC was prepared fresh on a daily basis and placed into the footbaths and drinkers. Chickens were weighed weekly, and their feed intake recorded. The water consumption was recorded throughout the study.

Grey parrot in a nebulisation chamber. The compressor is outside the chamber while the pot of nebulisers drug is inside with the bird. Such a chamber is also suitable for reptiles and small mammals.

The water treatments were administered to each group as follows:

- Control (Pens 1,2,3): Broilers that received only clean drinking water containing no F10SC disinfectant.
- Dilution (Pens 4,5,6): Broilers that received drinking water containing 1:1000 F10SC
- Dilution (Pens 7,8,9): Broilers that received drinking water containing 1:250 F10SC .

Broilers were selected randomly from each group (16 broiler chickens from the control treatment and 30 broiler chickens each from both the F10SC treatments). After the birds had been killed humanely tissue samples (158) where initial taken from across the 3 groups consisting of breast meat and liver and a further (14) confirmation samples of thigh meat and kidney. Feed and water samples were also submitted to the ARC-OVI Laboratories for residue detection. A biological assay based on disc inhibition zones in cultures of *Bacilis subtilis* were used to detect disinfectant residues. A sensitivity evaluation test determined that F10SC disinfectant would be detected at concentrations of 1:9000 and greater using the indicator microorganisms (*Bacilis subtilis*)

Results

Statistical analysis of the performance data utilised a procedure test with significance reported with 95% confidence limits.

Detection of F10SC residues in tissue residues at day 35

A summary of the feed and water samples and the tissue sample residue tests are given in Table 1 below.

Table 1

Sample	Negative	Positive
Feed negative control	2	0
Water untreated negative control	1	0
Water treated with 1:1000 F10SC positive control	0	2
Water treated with 1:250 F10SC positive control	0	2

Breast muscle meat (control)	17	0
Breast muscle meat - (1:1000 F10SC)	31	0
Breast muscle meat - (1:250 F10SC)	31	0
Liver - (control)	15	2
Liver - (1:1000 F10SC)	25	6
Liver - (1:250 F10SC)	31	0
Kidney - (control)	2	0
Kidney (1:1000 F10SC)	4	0
Thigh muscle meat - (control)	2	0
Thigh muscle meat (1:1000 F10SC)	6	0
	164	8

Liver samples (Table 1) from individuals in the control group and 1:1000 F10SC dilution group indicated evidence of F10SC disinfectant residue despite the muscle samples producing negative results. Accumulation of chemical substances in the liver would most probably be expected to also occur in kidney tissue but this was not detected. Further tests on these 6 chickens on further muscle and kidney samples were negative for residues of F10SC. The disc diffusion based assay is regarded as highly sensitive but with lower specificity. The results on the liver samples were therefore considered to be “false positives” due to imperfect specificity of the disc diffusion assay. It was therefore concluded there was no accumulation of F10SC residues in the tissue samples.

Effect of F10SC disinfectant supplementation on mortality

Mortalities encountered over the 35 day test period are shown in Table 2.

Table 2.

Date	Pen No.	Mortalities	Treatment	Diagnoses
01/02/04	8	1	1:250 F10SC	Septicemia
08/02/04	2	1	Control	Septicemia
09/02/04	3	1	Control	No macroscopic lesion
19/02/04/	1	1	Control	Hepatoses
19/02/04	7	1	1:250 F10SC	No macroscopic lesion

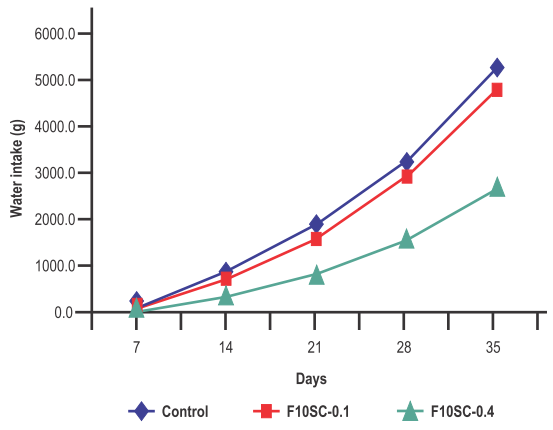
The incidence of 3% mortality was low. There was no significant difference (P>.0.05) in mortality or incidence of disease between the control group and F10SC treatment groups.

Effect of F10SC disinfectant supplementation on water and feed intake.

Although the sole purpose of the study was to determine F10SC residues in the tissue samples taken a note was nevertheless made of water and feed intake over the 35 day period. It should be noted that the manufacturers recommended concentrations of F10SC for **continuous supplementation of drinking water** to improve water quality are from 1:20,000 to 1:40,000 and from 1:2500 to 1:10,000 when used to reduce bacterial infections in poultry dependant upon the type of micro-organism contamination.

Cumulative water intake (g) up to 35 days of age.

Figure 1: Broiler cumulative water intake over 35 days of age

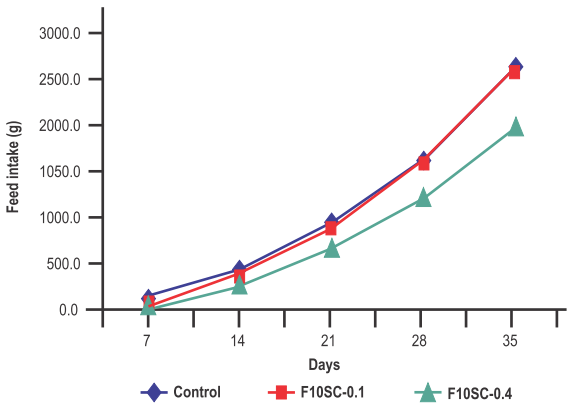


In general, the cumulative water consumption values were respectively 88% and 56% lower for the 1:1000 F10SC and 1:250 F10SC groups compared to the control over the 5-weeks rearing period.

At higher concentrations drinking water treated with F10SC has an increasingly bitter taste. Taste tests have shown that at concentrations of 1:2000 and above no chemical taste is observed.

Cumulative feed intake (g) up to 35 days of age.

Figure 2: Broiler cumulative feed intake over 35 days of age



Compare to the control the average magnitude over 35 days for cumulative feed intake differed with 98% and 68% for 1:1000 F10SC and 1:250 F10SC respectively.

Permanent misting/fogging system in poultry house to reduce airborne contamination

Open misting/fogging system used in a nebulising therapy to treat airsaccullitis in adult ostriches

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Conclusions

This study was not designed to evaluate the disinfectant F10SC in an application in a commercial broiler production unit as other studies have demonstrated potential uses and effectiveness of the disinfectant in disease control. The sole purpose of this study was to prepare chickens over a rearing period of 35 days to determine whether F10SC has any residual effect in broiler tissue that could be detected in an accredited inhibitory substances laboratory test.

Although the concentrations used in the study of 1:1000 and 1:250 of F10SC far exceed the manufacturer's recommendations for continuous long-term use in drinking water the results showed there was no indication of residues in chicken meat (breast and thigh) or organs (liver and kidneys). The results suggest that the manufacturer's recommended concentrations of F10SC for improving water quality to reduce microbiological contamination of 1:20,000 to 1:40,000 and from 1:2500 to 1:10,000 when used to reduce bacterial infections will not result in any inhibitory substances residues.

Alternatively the addition of F10SC to the drinking water of poultry to reduce the occurrence of bacterial, *Salmonella or E.coli,* infections and mycotic disease in poultry would be potentially very useful.

Similarly the use of F10SC as a long-term treatment in exotic birds might also be considered to be a safe option in refractory cases of aspergillosis where F10SC disinfectant is used by nebulisation as therapy for the treatment of *Aspergillus fumigatus* in exotic avian species or by over-spraying (fogging) in the presence of birds to reduce surface and airborne microbiological contamination at the higher recommended concentrations of 1:250; F10SC is unlikely to result in a build-up of chemical residues or side effects sometimes associated with systemic treatments.

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Related Tests

Airspace Decontamination using F10SC Disinfectant

Tests were conducted by The SABS Microbiology Dept to determine the effectiveness of F10SC Disinfectant to eliminate airborne micro-organisms.

The ambient air was sampled after introduction of a $\log^5$ *Staphylococcus epidermidis* suspension and again after release of the F10 aerosol spray with the SAS Air Sampler at a rate of 360 litres for 2 minutes. Viable micro-organisms were recovered on the surface of Rodac plates (nutient agar) used in the sampler at 10, 15, 20 minutes. No survivors were recovered after the release of the F10SC aerosol disinfectant.

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