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Full Length Research Paper

Impact of deep frying on furazolidone residues in chicken tissues

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This study investigated the effect of deep frying (210°C/15 min) on furazolidone residues in liver and muscle tissues of chicken. Furazolidone was administered (2 mg/kg body weight) orally to chicken daily for five days. The hens were then sacrificed at 1, 5, 24 168 and 264 h after treatment stopped and liver and muscle tissue samples obtained. The samples were deep fried, blended with distilled water and then centrifuged at 6000 rpm for five minutes. The supernatant was analyzed for the concentration of the drug using a using the Delvotest SP microbiological assay. A detection limit of 11.0 g/ml was obtained with spiked liver tissues contaminated with Furazolidone. Furazolidone residues were detected in fried liver and muscle tissues 264 h post treatment. It was concluded that furazolidone drug residues in chicken liver and muscle tissues were not destroyed by deep frying.

Key words: Furazolidone, depletion rate, delvotest test, poultry, deep frying.

INTRODUCTION

Various antibiotics, sulfonamides and coccidiostats are usually administered via feed or drinking water for the prevention and treatment of infectious diseases in laying hens. They also enhance feed efficiency, promote growth and improve productivity (Hermes, 2003; Gaudin et al., 2004; Bergwett, 2005). The uncontrolled and unlimited use of anticoccidial drugs may however lead to the accumulation of undesirable residues in the animals treated and their products. These residues may have adverse effects on both the animals and human beings (Lee et al., 2001; McCracken et al., 2005).

In high income countries, birds whose residues exceed tolerance levels are removed from the distribution line (Lee et al., 2001). However, in many low income countries such as Kenya, monitoring of drug residues is not done (Shitandi and Sternesjo, 2001). Hence, the risk consumers of poultry products are exposed to is not known.

Furazolidone is a nitrofuran that has been used for many years for treatment of bacterial and protozoan infections in poultry. It was banned in USA and European countries, but it's widely used in veterinary practice in low income countries like Kenya (Ali, 1999; Cooper et al., 2005). Furazolidone produces adverse reactions in both man and animals as it's carcinogenic and mutagenic (Hoogenboom et al., 2002). When administered, furazolidone metabolizes to its metabolite 3-amino-2-oxazolidinone (AOZ).

The Delvotest SP system is a broad spectrum screening test for the detection of antibiotic residues and sulphonamides in milk. This is a microbial inhibition assay that is based on the International Dairy Federation (IDF) reference method. It is the standard test method used for the detection of antibiotic residues in liquid milk at the pasteurizing plants (Fallon et al., 1995, 1996). The Delvotest SP system is dependent on rapid growth and acid production of *Bacillus stearothermophilus var. calidolactis*. Being a microbial assay, it can be used to test a broad spectrum of antibiotics (Food Safety / Authority of Ireland / 2002). In a previous study the potential of the *B. stearothermophilus var calidolactis* C953 as a test organism to detect a broad spectrum of drugs residue was demonstrated in unprocessed poultry meat (Shitandi et al., 2006). The fate of drug residues during heat processing is however unclear. This study thus investigated

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Table 1. Positive, doubtful and negative rates of detection for Furazolidone contaminated liver tissues.

Conc. (g/ml)	% Positive	% Positive/ Negative	% Negative
0.1	0	30	70
0.2	10	40	50
0.4	20	30	50
0.6	30	30	40
0.8	40	30	30
1.0	50	20	30
2.0	60	20	20
4.0	60	30	10
6.0	70	20	10
8.0	80	10	10
10.0	90	10	0
12.0	100	0	0
14.0	100	0	0
20.0	100	0	0

NB: The responses were determined from replicates of ten for each drug concentration.

the effect of heat treatment on furazolidone residues in liver and muscle tissues of chicken. It further evaluated the depletion rate of Furazolidone so as to establish appropriate withdrawal period for the drug using the Delvotest SP system

MATERIALS AND METHODS

Site of experiment

The experiment was carried out at the Taton farm and Microbiology Laboratory of Dairy and Food Science Technology Department, Egerton University. All experimental animals were acquired, retained, and used in compliance with the national laws and regulations of the research institution(s) of the authors. Experimental animals were properly housed and used in accordance with the research plan.

In vitro studies

Preparation of standards

100 mg of furazolidone (Cosmos, Kenya ltd) were weighed into a 100 ml flask and dissolved in 100 ml distilled water to produce a solution of 1000 g/ml (Stock solution A). Stock A was further diluted with distilled water (Stock B) producing a solution of 100 g/ml. Stock B (1 ml) was added to 4 ml of liver homogenate prepared at a dilution of 1:2 (tissue: distilled water) producing a solution of 20 g/ml. Standards of 14, 12 10, 8, 6, 4, 2, 1, 0.8, 0.6, 0.4, 0.2 and 0.1 g/ml were prepared from stock solution B using blank tissue solutions.

Determination of limits of detection

A sample of each standard homogenate was placed in a Delvotest SP tube and replicated ten times (a total of 10 tubes). Following the manufacturers instructions, the Delvotest SP tubes were incubated for thee hours in a water bath at a constant temperature of 64 ± 1^{o}C. The number of positive (Purple), doubtful (partly purple, partly yellow) and negative (yellow) colour changes were noted for each standard. Percentages positive, doubtful and negative were calculated for each standard tissue homogenate.

Percent positive responses were plotted against tissue homogenate drug concentration to determine the detection limit of the Delvotest SP. The limit of detection was defined as the concentration where 95% of the test results were positive. Liver and muscle tissues that were free of antimicrobial drugs were used as the negative control. Liver and muscle tissue spiked at 50 g/ml were used as positive controls.

In vivo studies

Eighteen broiler chicken (Ross Breed) one day old were purchased from Kims Poultry farm (Nakuru, Kenya) and reared for six weeks at the poultry section of Animal Science Department, Egerton University. The birds had access to water and antibiotic free broiler feed *ad libitum* (the feed was obtained from Unga Limited, Nakuru). The birds were gavaged with a commercial oral suspension of Furazolidone once daily for five days at a dosage of 2 mg/kg of body weight. After the last treatment, the birds were sacrificed by decapitation in groups of thee at intervals of 1, 5, 24, 168 and 264 h. The liver and breast muscle samples were collected and frozen at -20^{o}C until analyzed for furazolidone residues. Thee birds served as controls and were killed before the treatment began. The liver and muscle tissues were deep fried at 210^{o}C for 15 min. The tissues were homogenized with distilled water at a ratio of 1:2 (tissue: distilled water). The homogenates were centrifuged for five minutes at 6000 rpm to eliminate tissue debris which appeared to inhibit diffusion of the drug into the medium. Supernatant from the homogenates were analyzed for residues using the Delvotest SP system as earlier described. The percentages of positive, doubtful and negative responses were calculated for the tissues at the different concentrations and plots of concentration versus percentage positive made. Semi quantification of furazolidone residues was done by comparing the obtained growth inhibition of samples and that of standards.

Data analysis

Data was analyzed using Chi Square test at 95% confidence interval. The test was used to compare depletion rates of the drug in liver and muscle tissues.

RESULTS

In vitro studies

The results of Furazolidone contaminated liver tissues are shown in Table 1. A detection limit of 11.0 g/ml (Figure 1) was obtained with spiked liver tissues contaminated with Furazolidone. 100% positive Delvotest response was achieved for concentrations greater than or equal to 11 g/ml.

In vivo studies

The Delvotest SP showed a 30% positive response at 1 h

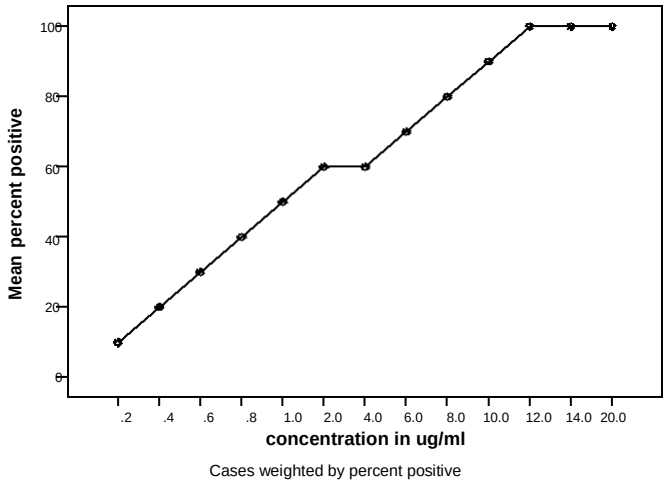

Figure 1. Limit of detection of Furazolidone in liver tissues by Delvotest SP.

Table 2. In vivo results for liver tissues in Delvo test

Withdrawal Hours	% Positive	% Negative	% Doubtful	Conc (g/ml)
1	30	30	40	0.6
5	20	50	30	0.4
24	20	40	40	0.4
168	10	20	70	0.2
264	10	10	80	0.2

NB: The responses were determined from replicates of ten for each sample.

post treatment and 10% at 264 h post treatment. The 30% positive response did correspond to a Furazolidone concentration of 0.6 ug/ml in deep fried liver tissues at 1 h after treatment. The concentration of furazolidone in liver tissues declined steadily between 1h and 264 h post treatment. More doubtful samples appeared 5 h post treatment at 50%.The least concentration determined in liver was 0.2 g/ml 264 h post treatment (Table 2). The results indicate the presence of residues 11 days post treatment. The results were also presented graphically in figure 1

The Delvotest SP detected a maximum furazolidone concentration of 0.8 g/ml in deep fried muscle tissues at 1h after treatment. The concentration of furazolidone in muscle tissues declined rapidly between 1h and 24 h post treatment. Afterwards the concentration remained relatively constant between 24 and 168 h post treatment. This was followed by a decline to 0.2 g/ml at 264 h post treatment (Table 3) . Like for liver tissues, the muscles also had residues 11 days post treatment.

The depletion rate of the drug in liver and muscle tissues were compared using chi – Square test which gave a p value of 0.227 (Table 4). The findings suggest that

Table 3. In vivo results for muscle tissues in Delvo test

Withdrawal Hours	% Positive	% Negative	% Doubtful	Conc (g/ml)
1	40	30	30	0.8
5	30	50	20	0.6
24	10	80	10	0.3
168	10	90	0	0.3
264	10	30	60	0.2

NB: The responses were determined from replicates of ten for each sample.

Table 4. Chi – square test.

	Value	Df	Asymp. Sig. (2-sided)
Pearson chi-square	7.500[a]	6	0.277
Likelihood ratio	7.777	6	0.255
Linear-by-Linear Association	3.104	1	0.078
No. of valid cases	5		

a. 12 cells (100%) have expected count less than 5. The minimum expected count is 20.

there is no significance ($p < 0.05$) difference between the depletion rates of the drug in liver and muscle tissues.

DISCUSSION

The Delvotest SP system is a broad spectrum screening test for the detection of antibiotic residues and sulphonamides in milk (IDF, 1991; Kroll, 1999). The present research suggests that in addition to detecting antibiotics in milk, the Delvotest SP system is could also be capable of detecting the Furazolidone residues in fried chicken tissues. The in vitro studies revealed positive responses of the system to Furazolidone residues greater than or equal to 11 g/ml in liver homogenates. However, the response of the Delvotest SP system to liver homogenates was unpredictable between 2 and 10 g/ml, but concentrations below 2 g/ml were not detected. These levels of detection seem to be higher than those for another *B. stearothermophilus* based test, the two tube test which showed much lower detection levels in experiments carried out in the same laboratory (Shitandi et al., 2006).

In the *in vivo* experiment, the Delvotest SP system detected a maximum furazolidone concentration of 0.6 ug/ml in deep fried liver tissues at 1 h after treatment. The concentration of furazolidone in deep fried liver tis-sues declined steadily between 1 h and 264 h post treat-ment. The least concentration determined in deep fried liver was 0.2 g/ml 264 h post treatment. The system detected a maximum furazolidone concentration of 0.8 g/ml in deep fried muscle tissues at 1 h after treatment.

The concentration of furazolidone in deep fried muscle tissues declined rapidly between 1h and 24 h post treatment. Afterwards the concentration remained relatively constant between 24 and 168 h post treatment. This was followed by a decline to 0.2 g/ml at 264 h post treatment. The concentration detected after 1h post treatment in deep fried muscle tissue was higher than that detected in deep fried liver tissue. However, the depletion rates were not significantly different at 95% confidence interval.

The Delvotest SP system microbial assay contains a pre-defined number of *B*. *stearothermophilus* spores in agar wells which enables the test to be carried out in 2 h 30 min (at the time the negative control has been changed to yellow). The growth of the spores at 64°C initiates an acidification process which causes the turning of a pH indicator from purple to yellow. The presence of antibacterial substances will cause delay or inhibition of the spores, depending on the concentration of the residues. In the presence of residues the spores will not multiply and the pH indicator will remain purple (Food Safety Authority of Ireland, 2002).

The bioavailability and effect of cooking on AOZ was studied by McCracken and Kennedy (1997). There were no significant effects on total concentration of AOZ due to grilling, micro waving or frying. Humans may be exposed to AOZ if animals treated with Furazolidone have no adequate withdrawal periods before slaughter. Drug residues in food animals being raised for human consumption may pose a public health concern. In low income countries such as Kenya, monitoring of drug residues is not done (Shitandi and Sternesjo, 2001) and hence the risk consumers of animal products are exposed to is not known. Consumer protection can be ensured by screening such animals for residues (Anadon and Martinez, 1998; Kozarova and Mate, 2000). Most food containing drug residues is consumed after cooking or processing, yet surveillance for these residues is almost always conducted on raw tissue (Rose et al., 1997).

According to the manufacturer's instructions (Cosmos, Kenya limited), the animals to which the drug has been administered should be consumed after ten days (240 h), which is considered the safe withdrawal period. However it is apparent from the present research that even after eleven days (264 h), the drug still persists in the deep fried tissues. The fact that the Delvotest system was able to detect furazolidone residues in deep fried liver and muscle tissue for longer periods of time may reflect the ability of these tissues to retain Furazolidone or its metabolites for long periods. Furazolidone is rapidly metabolized *in vivo* (Cooper et al., *2005*). However, protein bound metabolites are formed and are detectable several weeks after administration McCracken et al.,1997*;* Okeeffe et al., 2004). The side chain residues (AOZ) persist longer than intact parent compounds. Furazolidone residues have been shown to accumulate in eggs from birds administered therapeutic dose of furazolidone in their daily feed (McCracken et al., 2001). The side chain accounts for the long lasting residues detected in deep fried chicken tissues such as muscle and liver.

The general public is becoming increasingly aware of food safety issues and the potential for chemical and microbiological hazards in foods. There is a possibility of health risk due to protein bound metabolites of drugs that are formed during metabolism as they are characterized by long half lifes (Hoogenboom et al., 2002). Considering the withdrawal time of ten days for furazolidone in chicken as prescribed by the manufacturer, residues can be expected to persist in treated animals even after cooking, indicating the consumers are not given adequate protection.

From the present research, it was apparent that furazolidone drug residues in chicken liver and muscle tissues were not destroyed by deep frying. This study further demonstrated that the depletion rates in both tissues were not significantly different at 95% confidence interval. The depletion studies suggests that the withdrawal periods for furazolidone in liver and muscle tissues may need to be adjusted above the manufacturer's recommended periods. It may be of future interest to also investigate the possible elution of the drug residues into oil that has been used to deep fry chicken liver and muscle tissues.

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