Growth and Metabolic Indices of Furazolidone-induced Cardiomyopathy in the Pearl Grey Guinea Fowl

Collins N Khwatenge, Kellee N Hill and Samuel N Nahashon*
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Abstract

Dilated cardiomyopathy (DCM) is a naturally occurring heart disease associated with high mortality in rapidly growing poultry. The disease is common in broilers and its causes are least understood. This condition has not been reported in guinea fowl hence, the purpose of this study was to induce DCM in guinea fowl and elucidate physiological changes associated with onset of DCM. In three replications 5 week-old Pearl gray guinea fowl keets were fed corn-soy diets containing 0 (control), 400, 600 and 800 ppm furazolidone for four weeks. The experimental diets were fed in mash form and contained 3,000 kcal of metabolizable energy (ME)/kg diet and 24% crude protein (CP) at 0-5 weeks of age (WOA) and 3,100 ME kcal/kg and 24% CP at 5-9 WOA. After nine WOA, experimental birds were selected at random, blood samples were collected, the birds were then euthanized and liver and heart tissue samples were collected and immediately frozen in liquid nitrogen. Total RNA was extracted from the tissues, reverse transcribed and quantified to evaluate the expression of cardiac and liver troponin (TNT) and phospholamban (PLN) genes which serve as markers for DCM. Feeding 800 and 600 ppm furazolidone successfully induced DCM in Pearl gray guinea fowl. Induction of DCM was associated with a significant decrease in feed consumption and body weight gain and a significant reduction in feed efficiency. Increasing furazolidone concentration from 0-800 ppm significantly down regulated both TNT and PLN in the heart and liver. Liver hyperplasia, severe ascites, a decrease in serum creatinine, bilirubin, glucose, protein, and alkaline phosphatase, and an increase in serum glutamic pyruvate transaminase were observed. For the first time we report induction of DCM in the guinea fowl by feeding either 600 or 800 ppm furazolidone. DCM was characterized by metabolic changes and down regulation of heart and liver TNT and PLN.

Keywords: Dilated cardiomyopathy; Poultry; Guinea fowl; Furazolidone

Introduction

Dilated cardiomyopathy (DCM) is a form of heart failure which occurs naturally in broilers and turkeys whose cause remains obscure [1,2]. According to Olkowski [3], some of the modern strains of fast-growing meat type poultry are highly susceptible to DCM. Earlier, this disease was known to primarily affect birds that were raised in high altitudes and over the past decade this has become a nation-wide epidemic termed ascites instead of DCM [4-6].

DCM falls under the umbrella of congestive heart failures [7]. Wu et al. [6] documented DCM as an ischemic disease of the heart, due to its role in limiting supply to the heart muscles as well as the weakening of the ventricles of the heart that leads to a thickening or stiffness of the heart, limiting the heart’s ability to properly pump blood. The dead heart muscle is replaced by fibrous (scar) tissue and the remaining uninjured heart muscle stretches and thickens (hypertrophies) to compensate for the lost pumping action at a declining pace leading to onset of DCM and heart failure [8].

It has been speculated that DCM may be attributed to several mitigating factors such as genetic anomalies, drastic changes in physical environment, nutritionally incomplete diets, toxins and many other unknown factors. Several genes including phospholamban (PLN) and cardiac troponin (TNT) have been reported to influence spontaneous DCM and therefore they may serve as markers of onset of DCM [9-11]. According to Liew and Dazu [12], mutations in PLN affect calcium transport in cells leading to abnormal myocardial function. Parvari and Levitas [13], Murphy and Starling [14] attributes most DCM cases to the direct response of genetic mutations that code for cytoskeleton, contractile or other proteins found in myocardial muscles. However, variations of these factors do not occur consistently, making the identification of an exact cause of DCM difficult.

Idiopathic or spontaneous DCM occurs in 1-4% of normal turkeys; therefore, this condition is of significant concern to the poultry industry [15]. In broilers DCM is one of the leading causes of death and is responsible for the onset of ascites, accumulation of fluid in the abdominal cavity and a sign of severe liver damage [1]. The ascites syndrome which is common in broilers at 21-37 days of age is synonymous to DCM [5,6]. Consequently, the etiology of spontaneous DCM is still unknown. In order to better understand the possible causes of DCM, successful models of induced DCM in turkeys and broiler chickens using the drug furazolidone have been reported [1,16,17].

Furazolidone is an antibiotic that is commonly used at therapeutic levels to reduce salmonella in the intestinal tracts of poultry. The mechanism by which furazolidone induces DCM is still unknown, although it has been speculated it may be associated with the inhibition of the conversion of pyruvate to acetyl CoA that hinder many biochemical reactions such as metabolism of protein, carbohydrate and lipid that interferes with energy production hence inducing DCM [15,18]. Czarnecki [19] evaluated the mechanisms that underlie cardiac hypertrophy and congestive heart failure in furazolidone-induced DCM in the turkey. They reported that myelin fibers and glycogen

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deposits were observed in mitochondria of the right ventricular wall and damaged myofibers of DCM-affected birds. It was postulated that furazolidone affected the membrane system of the inhibition that leads to alteration of the mitochondrial and myofibrillar components with a consistent increase in cytoplasmic glycogen. Modeling DCM using diverse avian species will provide better understanding of the causes of DCM which will also allow informed design of potential mitigation strategies. Emerging avian species, such as the guinea fowl, add to the diverse gene pool, among the animal models of DCM to further explore the mechanisms behind its etiology through comparative studies. Since DCM is linked to genes that are considered hereditary, models of DCM in diverse avian species would be beneficial in elucidating such causes.

The guinea fowl is a common commercial avian species across Africa and Europe and its popularity as a commercial meat-type bird in the United States has gained traction [20-22]. Guinea fowl are susceptible to similar disorders as most avian species, and therefore they could be susceptible to DCM as well, which could curtail current progress in commercialization. The guinea fowl has not been used in studies of DCM or ascites. Recent studies have shown that there are differences in susceptibility to pathogenic microorganisms between guinea fowl and chickens [23]. Previous reports have shown that guinea fowl adapt well to harsh environmental conditions, and they are less susceptible to poultry diseases [24].

The guinea fowl has retained many of the characteristics of its wild ancestors (including feather morphology, social behavior, and hardness) no matter how intensive the rearing methods are including battery cages and artificial insemination [21]. These characteristics provide a unique gene pool that has not been heavily influenced by excessive environmental stimulus or genetic drift that would produce a significant amount of deleterious familial genes. Therefore, using the guinea fowl as a new animal model will allow in depth view of the manifestation of this DCM in vivo and the mechanisms that influence its onset. Therefore, the objectives of this research were to: (1) induce DCM in guinea fowl using furazolidone; (2) monitor performance of birds subjected to furazolidone treatment; and (3) elucidate physiological changes that may serve as biomarkers for the presence of DCM in birds.

Materials and Methods

Birds and treatments

Five hundred and seventy six straight-run day-old guinea keets of the Pearl grey variety were obtained from Ideal Poultry Breeding Farms (Cameron, Texas, USA). The dietary treatments fed from 0-5 and 5-9 weeks of age (WOA) had 3,000 and 3,100 kcal of ME/kg of diet, respectively, and each contained 24% CP (Table 1). From 5-9 WOA, experimental birds were randomly assigned to four dietary treatments in which furazolidone was incorporated at 0 (control), 400, 600 and 800 ppm. Each dietary treatment was replicated four times with 36 birds per replicate. The diets were fed in mash form and feed and water were provided at free choice throughout the experimentation period. The use of animals was approved by Tennessee State University Institutional Animal Care and Use Committee (IACUC).

Management of experimental birds

At day-old experimental birds were weighed individually and randomly assigned to electrically heated, temperature controlled Petersime battery brooders (Petersime Incubator Company, Gettysburg, OH) equipped with raised wire floors for the first four WOA. The battery cages measured 99 × 66 × 26 cm and each housed

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>0-5</th>
<th>5-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, Kcal/kg</td>
<td>3,000</td>
<td>3,100</td>
</tr>
<tr>
<td>CP%</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

Ingredients

- Corn, yellow # 2 (8% CP) | 44.93 | 42.03 |
- Soybean meal (48% CP) | 42.7 | 43.3 |
- Alfalfa Meal (17% CP) | 1 | 1 |
- Meat and Bone meal (50% CP) | 3 | 3 |
- Poultry blended fat | 5.8 | 8 |
- Dicalcium Phosphate (18% P, 22% Ca) | 0.9 | 1.2 |
- Limestone flour (38.8% Ca) | 0.9 | 0.7 |
- Salt | 0.37 | 0.37 |
- Vitamin-Mineral premix1 | 0.25 | 0.25 |
- DL-methionine (98%)2 | 0.15 | 0.16 |

Calculated levels

- Metabolizable Energy (Kcal/kg diet) | 3,000 | 3,100 |
- Crude fat | 7.7 | 9.56 |
- Crude Protein | 24 | 24 |
- Calcium | 1 | 1 |
- P, total | 0.72 | 0.72 |
- Available P | 0.48 | 0.48 |
- Methionine | 0.53 | 0.53 |
- Methionine+Cystine | 0.92 | 0.92 |
- Lysine | 1.46 | 1.46 |

Calculated levels

- Crude Fat | 7.64 | 9.49 |
- CP | 23.96 | 23.94 |

1Provided per kg of diet; Retinyl acetate: 3, 500 IU; Cholecalciferol: 1,000 ICU; DL-α-tocopheryl acetate: 4.5 IU; Menadione sodium bisulfite complex: 2.5 mg; Vitamin B12: 5.0 mg; Riboflavin: 2.5 mg; Pantothentic acid: 4.0 mg; Niacin: 15.0 mg; Choline: 172 mg; Folic acid: 230 mg; Ethoxyquin: 56.7 mg; Manganese: 65 mg; iodine: 1 mg; Iron: 54.8 mg; Copper: 6 mg; Zinc: 55 mg; selenium: 0.3 mg; 2Degussa Corporation Kennesaw GA

Table 1: Composition of experimental diets fed to Pearl grey guinea fowl from hatch to 9 weeks of age.

at least 15 birds. At day old, the brooder temperature was maintained at 32.2°C for the first week and reduced gradually by 2.8°C every week until 23.9°C and at this point on no artificial heating was provided to the birds. At five WOA the guinea keets were transferred into growing batteries which were not supplied with supplemental heating. However constant room temperature was maintained at 21°C. The growing cages measured 163 × 69 × 33 cm and each house seven to eight birds from 5-9 WOA. The birds received 23 h constant lighting from 0-9 WOA. Ventilation within the growing cages was maintained by thermostatically controlled exhaust fans. Body weight and feed consumption were measured weekly from hatch to nine WOA. Mortality was recorded as it occurred.

Tissue collection

At 9 WOA blood samples were collected by left brachial venipuncture from all experimental birds into 0.5 mL tubes and immediately placed in the refrigerator at 4°C for eight hours prior to serum collection after centrifugation at 8,000 rpm for 10 minutes. The serum samples were stored at -20°C until analyzed for metabolic profiles (Vanderbilt University Medical Center’s Pathology lab, Nashville, TN 37232). The heart and liver of one hundred and seventeen birds (20% of experimental birds per treatment group) were excised, weighed and immediately five-gram samples of the tissues were snap-frozen in liquid nitrogen and then transferred and stored at -80°C for subsequent evaluations of the level of expression of genes associated with DCM.
Total RNA extraction, reverse transcription and primer design

Approximately 5 to 10 mg each of frozen liver and heart tissues of seven birds per replicate were excised and placed rapidly into 300 µl of cold phosphate buffered saline (PBS). Total ribonucleic acid (RNA) was extracted using the Bio-Rad total RNA extraction kit* (Bio-Rad Laboratories, Alameda, California, USA) and stored at-80°C until use. Reverse transcription was performed on the total RNA samples of each replicate and tissue type using the Promega RevTrans System† (Promega Corporation, Madison, WI) and Eppendorf Mastercycler® gradient thermal cycler according to the suppliers’ recommendations. Nucleotide sequences for genes of interest were obtained from the National Center for Biotechnology Information’s (NCBI) GeneBank. Primers were designed from published guinea fowl sequences to amplify guinea fowl TNT and PLN respectively, common to each of heart and liver tissues using the Primer3® Software provided by the Massachusetts Institute of Technology’s Whitehead Institute for Biomedical Research (Cambridge, MA) (Table 2). The primers were synthesized by Invitrogen Corporation (Carlsbad, CA). The guinea fowl TNT and PLN genes were employed in assaying the expression of TNT and PLN in the heart and liver of guinea fowl. An additional primer pair was synthesized to amplify the guinea fowl glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, which served as ‘house-keeping’ gene in the qPCR assays. Listings of the designed primer sets, including base sequences of primers used in this study are presented in Table 2. T-RNA and cDNA concentrations were measured with NanoDrop spectrophotometer (Nano Drop Technologies, Wilmington, DE). The cDNA samples were diluted to a final concentration of 500 ng/µl prior to gene expression assays.

Real-time gene expression assays

Quantitative real-time polymerase chain reaction (RT-PCR) assays were performed using Qiagen’s Quantitect SYBR® Green PCR Kit (Qiagen Incorporated, Valencia, California, USA) on ABI PRISM 7000® Sequence Detection System (Applied Biosystems, Foster City, California, USA) in accordance with the manufacturers’ protocol. Seven samples from each replicate were analyzed in duplicates by qPCR. The primer pair for the chicken GAPDH housekeeping gene was used as internal control for determination of relative gene expression. Applied Biosystems’ PRISM 7000® Sequence Detection System [10] was used to perform RT-PCR programmed to the following cycling parameters: initial denaturation at 95°C for 15 minutes; followed by 40 cycles of denaturation at 94°C for 15 seconds; primer annealing at 55°C for 30 seconds; and final extension at 72°C for 30 seconds. The amount of fluorescence and cycle at which fluorescence was detected by the instrument was used to calculate threshold cycle (Ct). To minimize the bias caused by T-RNA extraction, mean Ct of the house-keeping gene GAPDH was used as the internal control to calibrate RNA quality and quantity of the specific tissues. Fold change was calculated using the comparative Ct method discussed by Schmittgen and Livak [25].

Statistical analyses

Performance data were analyzed as repeated measurements using the Two-way ANOVA option of the general linear model of SAS/STAT software as completely randomized design with dietary furazolidone concentrations as main effects [26]. The statistical model used was:

\[ Y_{ijkl}=\mu+F_i+P_j+FP_{ij}+R_{ijk}+g_{ijkl} \]

where \[ Y_{ijkl}=\text{response variables from each individual bird}, \mu=\text{the overall mean}, F_i=\text{the effect of dietary furazolidone concentration}, P_j=\text{the effect due to time period of measurements in weeks}, FP_{ij}=\text{interaction between performance traits and time period}, R_{ijk}=\text{the inter-experimental unit (replications) error term}; \text{and } g_{ijkl}=\text{the intra-experimental unit error term.} \]

Comparisons of least significant differences in performance traits between treatment means were made for main effects when there was a significant F-value. Significant differences in gene expression between furazolidone concentrations were compared using the Student’s t-test. Differences in mortality among dietary furazolidone concentrations were analyzed using the chi-square method. Significance implies (P<0.05) unless stated otherwise.

Results and Discussion

The average feed consumption was not different (P>0.05) between birds fed diets containing 400 ppm furazolidone and the control throughout the feeding trial (Table 3). However, birds fed the 400 ppm furazolidone diets consumed more feed than those on the 600 and 800 ppm furazolidone diets. On the other hand, during the first week of the feeding trial (6 WOA) birds on the 800 ppm furazolidone diet consumed 29% less feed than those fed the control diet. Throughout the study period there was an inverse relationship between the dietary concentration of furazolidone and feed consumption. Previous studies have demonstrated depression in feed consumption with exposure to extremely toxic level of this antimicrobial agent especially in quail [27]. Dietary furazolidone has also been linked to anorexia in chickens and turkeys which may be linked to the thiamin status of birds [28,29]. Combs suggested that thiamin or B₁ vitamin is crucial for neural function and metabolism of carbohydrates and its deficiency can lead to myriads of problems including wasting or “acute malnutrition” as observed in birds fed the high doses of furazolidone.

The trend in total feed consumption among the dietary treatments was also consistent with the weekly feed consumption in a dose

<table>
<thead>
<tr>
<th>Gene bank Accession number</th>
<th>Gene¹</th>
<th>Primer sequence¹</th>
<th>Amplicons length (bp)</th>
<th>Tm² (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XM_021377413.1</td>
<td>TNT</td>
<td>Forward: 5' CCCTCCTAGGCCCAACTCTG 3' Reverse: 5' CGGTGCTCGATGCTGTC 3'</td>
<td>290</td>
<td>57</td>
</tr>
<tr>
<td>XM_021392861.1</td>
<td>PLN</td>
<td>Forward: 5' AGGACTCAAAAGCGAGGCTCAC 3' Reverse: 5'TCTCCATGGCCAGCAAGAAATG 3'</td>
<td>145</td>
<td>56</td>
</tr>
<tr>
<td>NM_204305.1</td>
<td>GAPDH</td>
<td>Forward: 5' AGACACACATATCCGGAGGTGCC 3' Reverse: 5' CGCACTGCTAGGCTGAAAAC 3'</td>
<td>133</td>
<td>56</td>
</tr>
</tbody>
</table>

¹Genes: TNT: Troponin T; PLN: Phospholamban; ²GAPDH was used as internal controls

Table 2: Sequences of primers used in the amplification of the troponin, phospholamban and GAPDH genes of guinea fowl fed diets containing furazolidone from 5 to 9 weeks of age.
10 days significantly (P>0.05) reduced their feed intake and growth. Ali and Bartlett [28] reported that feeding diets containing furazolidone can tolerate higher doses of the furazolidone than the guinea fowl can and may suggest that furazolidone may be an effective therapeutic dose to promote health and beneficial effects in the guinea fowl. Hence, 400 ppm dietary furazolidone implied that the 400 ppm of furazolidone was sufficient to confer similar beneficial effects in the guinea fowl. However, in contaminated environments, the medication may be an effective therapeutic dose to promote health and beneficial effects in the guinea fowl. Wilson and Harrison [33] that feeding low doses (400 ppm) of furazolidone to Rhode Island Red chickens improved growth. On the other hand, feeding chicks more than 500 ppm furazolidone significantly (P<0.05) in feed conversion ratios among guinea fowl fed the various levels of furazolidone and the control. However, at 7 and 9 weeks of age (5 and 8 WOA, respectively), there were no significant differences (P>0.05) in feed conversion ratios among guinea fowl fed the various levels of furazolidone. The poor feed conversion of the birds fed the 600 and 800 ppm furazolidone was 4.15 and 4.90, respectively, and the control and 400 ppm furazolidone was 2.53 and 2.65, respectively, and the control and 400 ppm furazolidone may be due to consumption of the high and toxic levels of the antimicrobial. The depressed body weight gain was also associated with a significant reduction in feed consumption and in most part an increase in the amount of furazolidone consumed (Table 3). Overall, body weight gain through the entire experimentation period was consistent with the weekly pattern such that the control=400 ppm=600 ppm>800 ppm furazolidone.

Mean feed conversion ratios of Pearl grey guinea fowl fed diets containing varying concentration of furazolidone from 5-9 WOA are presented in Table 4. During the first and third week of the study (5 and 8 WOA, respectively), there were no significant differences (P>0.05) in feed conversion ratios among guinea fowl fed the various concentrations of furazolidone and the control. However, at 7 and 9 WOA birds fed diets containing the 600 and 800 ppm furazolidone exhibited significantly higher (P<0.05) feed conversion ratios than those on the control and 400 ppm furazolidone diets. Over the entire experimentation period, average feed conversion ratio of feed fed the control and 400 ppm furazolidone was 2.53 and 2.65, respectively, and that of birds fed the 600 and 800 ppm furazolidone was 4.15 and 4.90, respectively. The poor feed conversion of the birds fed the 600 and 800 ppm furazolidone relative to the control and those fed the 400 ppm furazolidone diets is in part attributed to poor body weight gain and feed consumption. Consistent with these observations, Geyani [15] reported lower average body weight in turkey pouls fed furazolidone-containing diets with the decline in body weight increasing with age. The decline in body weight gain and feed efficiency can be a key indicator of physiological or metabolic disorders attributed to DCM.

### Table 3: Consumption of feed and furazolidone of Pearl grey guinea fowl fed diets containing furazolidone from 5 to 9 weeks of age.

<table>
<thead>
<tr>
<th>Weeks of age</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9 (TFC)</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9 (TFZC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furazolidone (ppm)</td>
<td>(grams feed/bird/week)</td>
<td>(mg Furazolidone/bird/week)</td>
<td>(grams feed/bird/week)</td>
<td>(mg Furazolidone/bird/week)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>309a</td>
<td>279a</td>
<td>322a</td>
<td>289</td>
<td>1119a</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>400</td>
<td>323a</td>
<td>294a</td>
<td>338a</td>
<td>275</td>
<td>1230a</td>
<td>129a</td>
<td>120a</td>
<td>140a</td>
</tr>
<tr>
<td>600</td>
<td>279a</td>
<td>184a</td>
<td>263a</td>
<td>236</td>
<td>962a</td>
<td>167a</td>
<td>110a</td>
<td>160a</td>
</tr>
<tr>
<td>800</td>
<td>218a</td>
<td>103a</td>
<td>189a</td>
<td>241</td>
<td>751a</td>
<td>174a</td>
<td>80a</td>
<td>150a</td>
</tr>
</tbody>
</table>

### Table 4: Body weight gain and feed conversion ratios of Pearl grey guinea fowl fed diets containing furazolidone from 5 to 9 weeks of age.

<table>
<thead>
<tr>
<th>Weeks of age</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9 (TWG)</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9 (AFE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furazolidone (ppm)</td>
<td>(grams)</td>
<td>(g feed/g wt. gain)</td>
<td>(grams)</td>
<td>(g feed/g wt. gain)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>135a</td>
<td>98a</td>
<td>120</td>
<td>132a</td>
<td>485a</td>
<td>2.3</td>
<td>2.9a</td>
<td>2.7</td>
</tr>
<tr>
<td>400</td>
<td>138a</td>
<td>118a</td>
<td>126</td>
<td>108a</td>
<td>488a</td>
<td>2.3</td>
<td>2.9a</td>
<td>2.7</td>
</tr>
<tr>
<td>600</td>
<td>108a</td>
<td>63a</td>
<td>98</td>
<td>24a</td>
<td>293a</td>
<td>2.7</td>
<td>2.9a</td>
<td>3.4</td>
</tr>
<tr>
<td>800</td>
<td>61a</td>
<td>26a</td>
<td>94</td>
<td>30a</td>
<td>210a</td>
<td>3.7</td>
<td>4.0a</td>
<td>3.6</td>
</tr>
</tbody>
</table>

### Notes:
- a,b,c means within columns with no common superscripts differ significantly (P<0.05).
- Total feed consumption; Total furazolidone consumption; Parts per million; Pooled standard error of mean.

### Consumption of feed and furazolidone

<table>
<thead>
<tr>
<th>Weeks of age</th>
<th>6</th>
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<th>8</th>
<th>9</th>
<th>1 (TFC)</th>
<th>2 (TFZC)</th>
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<tbody>
<tr>
<td>Furazolidone (ppm)</td>
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</table>

### Notes:
- a,b,c means within columns with no common superscripts differ significantly (P<0.05).
- Total feed consumption; Total furazolidone consumption; Parts per million; Pooled standard error of mean.

### Body weight gain and Feed conversion ratios

<table>
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<tr>
<th>Weeks of age</th>
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<th>7</th>
<th>8</th>
<th>9</th>
<th>1 (TWG)</th>
<th>2 (AFE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furazolidone (ppm)</td>
<td>(grams)</td>
<td>(g feed/g wt. gain)</td>
<td>(grams)</td>
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<td></td>
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<tr>
<td>0</td>
<td>135</td>
<td>98</td>
<td>120</td>
<td>132</td>
<td>485</td>
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<tr>
<td>800</td>
<td>61</td>
<td>26</td>
<td>94</td>
<td>30</td>
<td>210</td>
<td>3.7</td>
</tr>
</tbody>
</table>

### Notes:
- a,b,c means within columns with no common superscripts differ significantly (P<0.05).
- Total weight gain; Average feed efficiency; Parts per million; Pooled standard error of mean.
and impaired hepatic functions which are reflected in enlarged liver, the elevated concentrations of serum glutamic pyruvate transaminase (SGPT) and reduction in concentration of serum glutamic oxaloacetic transaminase (SGOT) in birds fed diets containing the 600 and 800 ppm furazolidone though the decrease in SGOT concentrations were not significantly different (P>0.05) in these furazolidone dosage (Tables 5 and 6).

The mean weight of the liver, heart and ascites fluid, and the expression of TNT and PLN in the heart and liver of Pearl grey guinea fowl fed diets containing furazolidone are presented in Table 5. Birds fed the diets containing 800 and 600 ppm furazolidone had a 32 and 8% increase in heart weight, respectively, when compared with birds fed the control diet. The weight of the liver for birds fed 800 and 600 ppm furazolidone was not statistically different (P>0.05) from each other but the two furazolidone dosages was about 20% higher (P<0.05) than the birds fed diets containing 400 ppm furazolidone and the control. Ascites, accumulation of fluid in the peritoneal cavity also known as pulmonary hypertension syndrome has been associated with DCM in broilers [35] and rats [36]. Ascites is related to severe liver damage which in most cases is the result of heart failure. Therefore, the significantly higher (P<0.05) content of ascites fluid in guinea fowl fed the 600 ppm and 800 ppm furazolidone diets when compared to the 400 ppm furazolidone diets and the control was a sign that birds fed the 800 and 600 ppm furazolidone developed DCM. The ascites fluid was not observed in birds fed the 400 ppm furazolidone diets and the control. Previous reports have demonstrated that DCM is also associated with enlargement of the heart and liver which is also consistent with our findings [15].

The expression profiles for real time quantification of heart and liver TNT and PLN genes are presented in Figure 1. The expression of liver TNT was down regulated (P<0.05) in birds fed diets furazolidone in a dose dependent manner. Differences in the down regulation of liver TNT between birds fed diets containing the 600 and 800 ppm furazolidone were not significant (P>0.05). Previous reports that are in agreement with these observations have shown down regulation of TNT in humans and turkeys with idiopathic DCM [2,37]. The expression of heart TNT was significantly (P<0.05) down regulated at 600 and 800 ppm furazolidone as compared to the control and 400 ppm furazolidone (Figure 1B). The expression of liver PLN was down regulated significantly (P<0.05) in birds fed diets containing furazolidone in a dose dependent manner (Figure 1C). When the expression was compared with the control, the down regulation of liver PLN of birds fed the 800 ppm furazolidone was much more pronounced than those fed the 400 ppm furazolidone (6.4 and 94 folds, respectively). Heart PLN was significantly (P<0.05) down regulated in a dose dependent manner of furazolidone concentration (Figure 1D). According to Lin et al. [2] the PLN gene has been implicated in DCM and it encodes the PLN protein that regulates the sarcoplasmic reticulum Ca\textsuperscript{2+} pump and controls the size of the sarcoplasmic reticulum Ca\textsuperscript{2+} store during diastole. Our findings are supported by the report of Dash et al. [38] where low expression of cardiac PLN in transgenic mice was shown to lead to a late-onset type of DCM. Earlier reports have also demonstrated low levels of PLN mRNA in smooth muscle organs with little or no expression in non-muscle organs, such as the liver, in DCM affected individuals [39]. Mean serum constituents of pearl grey guinea fowl fed diets containing furazolidone from 5-9 WOA are presented in Tables 6 and 7. Differences in anion gap of birds fed the furazolidone diets were not statistically significant (P>0.05), but, they were significantly higher (P<0.05) than those of the control. The creatinine levels were lower (P<0.05) in birds fed the 600 and 800 ppm furazolidone diets than those in the control and 400 ppm diets. Likewise, birds on the 400 ppm furazolidone and the control also exhibited higher levels of bilirubin than those on 800 and 600 ppm furazolidone. Creatinine levels are used clinically as predictor of renal and cardiac function and thus significantly low creatinine levels can be indicative of cardiac dysfunction or damage [40]. On the other hand, bilirubin is a cellular antioxidant whose low levels may be associated with cirrhosis and severe liver failure. Low levels of bilirubin could be linked to the excessive hyperplasia and potential onset or progression of cardiovascular disease as found in birds fed diets containing the 600 and 800 ppm furazolidone [41].

Serum glucose, protein and albumin were lower in birds fed the 600 and 800 ppm furazolidone when compared with those fed the 400

<table>
<thead>
<tr>
<th>Furazolidone (ppm)</th>
<th>Heart</th>
<th>Liver</th>
<th>Ascites fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g/100 g of BW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.48± 0.59±</td>
<td>0.59±</td>
<td>0.00±</td>
</tr>
<tr>
<td>400</td>
<td>2.44± 0.59±</td>
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<td>0.00±</td>
</tr>
<tr>
<td>600</td>
<td>2.69± 0.76±</td>
<td>5.57±</td>
<td>9.95±</td>
</tr>
<tr>
<td>800</td>
<td>3.28± 0.70±</td>
<td>1.35</td>
<td>0.01</td>
</tr>
<tr>
<td>PSEM²</td>
<td>0.30</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*¹means within columns with no common superscripts differ significantly (P<0.05); *²Parts per million; *³Pooled standard error mean

Table 5: Weight of organs and tissues expression of cardiomyopathy of Pearl grey guinea fowl fed diets containing furazolidone from 5 to 9 weeks of age.

| Furazolidone (ppm) | Anion Gap | Creatinine | Bilirubin | Glucose | Protein | Albumin | CO₂ | SGPT | SGOT | AlkPhos |
|-------------------|-----------|------------|----------|---------|---------|---------|     |      |      |        |
| 0                 | 16± 0.32± | 0.45± | 330± 2.65± | 1.13± | 23 | 8.8± | 303 | 235± | 19± | 311 | 261± |
| 400               | 42± 0.33± | 0.43± | 271± 2.58± | 1.08± | 19 | 8.0± | 311 | 261± | 19± | 311 | 261± |
| 600               | 32± 0.21± | 0.38± | 247± 1.75± | 0.78± | 20 | 9.5± | 268 | 150± | 251 | 912± |
| 800               | 38± 0.24± | 0.35± | 314± 1.83± | 0.75± | 19 | 15.3± | 251 | 912± | 19± | 311 | 261± |
| PSEM² | 1.5 | 0.03 | 0.05 | 24 | 0.17 | 0.07 | 1.5 | 1.8 | 57.4 | 598 |

*¹means within columns with no common superscripts differ significantly (P<0.05); *²Carbon dioxide; *³Serum glutamic pyruvate transaminase; *⁴Serum glutamic oxaloacetic transaminase; *⁵Alkaline phosphatase; *⁶Parts per million; *⁷Pooled standard error of mean

Table 6: Serum metabolic constituents of Pearl grey guinea fowl fed diets containing furazolidone from 5 to 9 weeks of age.
and 0 ppm furazolidone. In humans, DCM has been associated with certain cardiac or systematic abnormalities such as neuromuscular disorders, glycogen storage diseases, mucopolysaccharidosis, and disorders of fatty acid metabolism [42]. The extremely low serum protein and albumin levels have been correlated with significant liver damage and a possible marker for diabetes or other glycogen disorders. Although differences in serum glucose concentrations between the control and birds fed the 400 and 800 ppm furazolidone were not significant (P<0.05), it is not clear why serum glucose levels were low in the birds fed 600 ppm furazolidone. Low levels of albumin in the blood are primarily used to diagnose malnutrition or kidney, liver or gastrointestinal disorders. Low levels of albumin may lead to leakage of fluid outside the capillaries with fluid accumulation under the skin (edema) and body spaces, which is consistent with ascites in birds fed the 600 and 800 ppm furazolidone diets in this study [43,44]. Mean serum carbon dioxide and serum glutamic oxaloacetic transaminase (SGOT) were not different among furazolidone treatments and the control. The serum glutamic pyruvate transaminase (SGPT) was significantly higher (P<0.05) and alkaline phosphatase was significantly lower (P<0.05) in birds fed diets containing 800 ppm furazolidone when compared with those fed the 600, 400 and 0 ppm furazolidone. Fluctuations in the SGPT level can signify the potential for moderate to severe damage to the liver which is common in individuals suffering from heart failure especially in DCM related incidence where hyperplasia is the most common form of liver dysfunction. Hyperplasia is also a common element that is present in individuals that have signs and symptoms of DCM as well as other myocardial disorders [15]. Previous reports suggested that liver hyperplasia may be caused by increased demand, chronic inflammatory response, hormonal dysfunctions, or compensation for damage or disease [45-47].

Mineral elements in serum and mortality of the pearl grey guinea fowl fed diets containing furazolidone are presented in Table 7. Differences in serum concentrations of sodium, calcium and chloride diets containing 800, 600, 400 and 0 ppm furazolidone were not significant (P>0.05) among groups fed. Serum potassium

<table>
<thead>
<tr>
<th>Furazolidone (ppm)</th>
<th>Sodium (mEq/L)</th>
<th>Calcium</th>
<th>Potassium</th>
<th>Chloride</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>153</td>
<td>11.1</td>
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<tr>
<td>400</td>
<td>182</td>
<td>11.6</td>
<td>3.85b</td>
<td>108</td>
</tr>
<tr>
<td>600</td>
<td>181</td>
<td>10.1</td>
<td>4.80*</td>
<td>117</td>
</tr>
<tr>
<td>800</td>
<td>179</td>
<td>10.1</td>
<td>7.15*</td>
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</tr>
<tr>
<td>PSEM*</td>
<td>19.5</td>
<td>0.46</td>
<td>0.91</td>
<td>5.42</td>
</tr>
</tbody>
</table>

Table 7: Mineral elements in serum and mortality of Pearl grey guinea fowl fed diets containing furazolidone from 5 to 9 weeks of age.

Figure 1: Tissue expression (fold change) of liver and cardiac TNT (A and B, respectively) and liver and cardiac PLN (C and D, respectively) of Pearl grey guinea fowl fed diets containing furazolidone from 5 to 9 weeks of age. The GAPDH ‘house-keeping’ gene was used as endogenous control. Means with different letters are significantly different (P<0.05) among bird groups (N=10 birds per group). Error bars represent SEM.
concentrations were significantly lower (P<0.05) in birds fed the 400 ppm furazolidone diets when compared with other dietary treatments. Previous reports have associated higher serum concentrations of potassium with cardiomyopathy [36]. In this study also birds fed the 800 ppm furazolidone and exhibited cardiomyopathy, had elevated levels of potassium, although these levels were not statistically different from the control. It is however not clear why this serum potassium level was low in the birds fed 400 ppm furazolidone when compared with the control, even though birds in both these groups had no signs of cardiomyopathy. Potassium is important to the regulation of the cardiac muscles which aids in continued normal electrical rhythms in the heart.

Birds fed diets containing 800 ppm furazolidone experienced high mortality throughout the experimental period. While in most part, no mortality was observed in birds fed diets containing the 400 ppm furazolidone and the control diets, average mortality was significantly higher (P<0.05) in birds fed the 800 and 600 ppm furazolidone diets. Lin et al. [2] and Gyenai [15] observed that higher levels of this antimicrobial agent can cause DCM and mortality at a more expedient rate. The feed consumption, weight gain and feed conversion of these groups were significantly depressed and that could be linked to anorexia, which is common in poultry fed furazolidone [28]. Similar observations were noted in turkeys and chicks fed purified diets containing 500 and 700 ppm furazolidone, respectively [34].

Conclusion

In conclusion, 400 ppm furazolidone was not sufficient to induce DCM in guinea fowl, however for the first time; DCM was induced in the Pearl grey guinea fowl by feeding either 600 or 800 ppm furazolidone. The condition was characterized by down regulation of heart and liver TNT and PLN, excessive accumulation of ascites fluid in the peritoneal cavity, enlargement of the heart and liver, a decrease in serum creatinine, bilirubin, glucose, protein, alkaline phosphatase and an increase in SGPT. These metabolic changes may be directly or indirectly associated with noted changes in liver and heart functions leading to DCM. DCM was also associated with a decrease in feed consumption and body weight gain and an increase in feed conversion ratios and mortality. Therefore, findings from this research provide insight to the possible pathways leading to onset of DCM in the guinea fowl and can be utilized in developing concepts of determining how the residues from antimicrobial agents could possibly induce DCM in poultry.

References

evidence as to the possible mechanism involved in the growth-promoting responses obtained from antibiotics. J Nutr 51: 507-513.


