Effect of Prophylactic Vitamin C Administration On The Efficiency of Florfenicol Or Sulfadiazine-Trimethoprim Antimicrobial Therapy In Chickens With Staphylococcal Arthritis

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Abstract

Septic arthritis (SA) shows improper response to antibacterial therapy. This study evaluates the effect of prophylactic vitamin C administration on the efficiency of sulfadiazine-trimethoprim (SDT) or florfenicol (FF) in broilers with experimental SA. Broilers (210) were randomly allocated into 7 equal groups: I. Negative control (NC) (normal birds). II. Positive control (PC) that rendered arthritic by injection of multi drug resistant *S. aureus* in tibiotarsal joint at the age of 35 days. III. Vehicle control (injected with sterile medium). IV. Arthritic FF-treated (20 mg/kg/day). V. Arthritic vitamin C+FF-treated (as above+vitamin C at 15g/100L of D.W. from day 25 of age). VI. Arthritic SDT-treated (35 mg/kg/day). VII. Arthritic vitamin C+SDT-treated. Antibacterials started at day 39 of age and lasted for 5 days. Samplings were performed at the age of 44 and 54 days. A long lasting SA with severe fibrinoheterophilic synovitis and reduced body weights developed in PC broilers as compared to NC group (p<0.05). Oxidative stress was present at sampling 1. Arthritis was not reflected in IL-6 levels in synovial fluid. None of the antibacterials resulted in completely successful treatment. Vitamin C did not appreciably improve lameness and arthritis scores, although it decreased lipid peroxidation and improved weights of FF treated-arthritic birds. For SDT-treated birds, vitamin C only ameliorated histopathological changes. In conclusion, except for improving body weight in FF-treated birds, prophylactic administration of vitamin C is not associated with improvements in clinical outcome of antimicrobial therapy of broilers with SA, although it ameliorates oxidative stress and some histopathological changes.

Introduction

Septic arthritis (SA) with resultant lameness in broiler chickens compromises the birds’ welfare and imposes economic loss to poultry industry due to reduced birds’ performance and increased mortality and carcass condemnation in slaughterhouses. Among bacterial causes of SA, *Staphylococcus aureus* is considered as a major culprit which is also important from public health view (Marcon et al. 2019).

The bacteria are associated with chondronecrosis with osteomyelitis in chickens that most commonly affects proximal end of the femur and tibiotarsus in birds around 35 days of age (McNamee and Smyth 2000).

Different predisposing factors are involved in the development of the disease which include stress, immunosuppression, vascular disorders, nutritional deficiencies etc (Wijesurendra et al. 2017).

Unfortunately, when established the disease shows improper response to antibacterial therapy, due to the fact that the bacteria remain in sites which are usually inaccessible to antibiotics and even some immune components of the host (Wideman Jr 2016). Hiding from immune system and antibacterial agents may result in recurrence of the disease even after antimicrobial therapy. In 2016, Mosleh and colleagues (Mosleh et al. 2016) evaluated the therapeutic efficiency of four different antibacterials including sulfadiazine-trimethoprim (SDT), florfenicol (FF), oxytetracycline and enrofloxacin in the treatment of experimentally-induced staphylococcal arthritis in broilers. They found that none of the antibiotics results
in completely successful therapy of affected birds although they suggested SDT as the preferred agent with regard to most of the assayed criteria.

Vitamin C or ascorbic acid, as a well-known antioxidant, is routinely administered to stressed broilers such as those under heat stress since under stressful conditions the need of bird is not met by internal synthesis of this vitamin (Khan et al. 2012, Abidin and Khatoon 2013).

This vitamin is also famous for its role in bone and cartilage development and health (Aghajanian et al. 2015). It has been clearly demonstrated that vitamin C supplementation during rearing period improves bone mineralization and resistance in broilers (Franchini et al. 1994).

On the hand, there are evidence that indicate immunomodulatory (both cell-mediated and antibody-mediated pathways), and anti-inflammatory properties of vitamin C in poultry (Shojadoost et al. 2021). Interestingly, vitamin C has shown inhibiting effects on \textit{in vitro} growth of \textit{S. aureus} by affecting metabolic pathways of the bacteria (Kallio et al. 2012, Golonka et al. 2017). The synergistic effects of vitamin C with antibacterials is another intriguing property of this vitamin that has been reported against \textit{P. auroginosa} and \textit{H. pylori} (Cursino, Chartone-Souza, and Nascimento 2005, Kaboli et al. 2009).

These previously reported properties of vitamin C accompanied by its common use in poultry farms, encouraged us to evaluate the plausible effect of prophylactic administration of this vitamin on the efficiency of two different antibacterials (SDT or FF) in a challenge study on chickens with experimental staphylococcal arthritis. Clinical and histopathological features of the disease as well as the antioxidant status and synovial fluid concentration of IL-6 in infected birds were evaluated.

\textbf{Materials And Methods}

\textit{Bacteria isolation, identification and antibacterial sensitivity test}

The \textit{S. aureus} bacteria that were used to challenge birds were isolated from bumble foot lesions of poultry in a local farm. In biochemical tests the bacteria were beta hemolytic, catalase and coagulase positive. The genus and species of the bacterium was confirmed by molecular methods including the polymerase chain reaction (PCR) to amplify a species-specific region of the DNA coding for rRNA by using the primer pair of (Sau 327 S. aureus GGA CGA CAT TAG ACG AAT CA) and (Sau 1645 S. aureus CGG GCA CCT ATT TTC TAT CT) as described previously by (Riffon et al. 2001). Moreover, PCR method with primers mecA1 (GTA GAA ATG ACT GAA CGT CCG ATA A) and mecA2 (CCA ATT CCA CAT TGT TTC GGT CTA A) (Jonas et al. 1999) was used for to determine resistance to methicillin. To further characterize the bacteria multilocus sequence typing (MLST) was performed by primers specific to seven housekeeping genes as described by Enright et al., 2000 (Enright et al. 2000).

Kirby-Bauer disk diffusion susceptibility test on Mueller-Hinton agar was performed based on Clinical and Laboratory Standards Institute (CLSI) guidelines. All disks were prepared by Padtan Teb Co., Tehran, Iran.

\textit{Study design}
Two hundred and ten Ross 308 chickens from both sexes were reared at the same condition as instructed in the manual. The birds had access to commercial feed and tap water during the experiment *ad libitum*. All birds were randomly allocated into 7 equal groups with 30 birds each and treated as follows:

I. Negative control (NC) group: normal birds that received no treatment. II. Positive control (PC) group: arthritis was induced in this group at the age of 35 days by injecting 1ml of a suspension of *S. aureus* bacteria containing $1.4 \times 10^8$ CFU in TSB medium in right tibiotarsal joint as described previously by Mosleh et al., 2016. The proper bacterial concentration for injection was determined in a pilot study. III. Vehicle control (VC) group: 1 ml of sterile TSB medium was injected in right tibiotarsal joint. IV. Arthritic FF-treated (FF group): arthritic birds that received FF (Fluorfen® 10%, Rooyan Darou, Semnan, Iran) at 20 mg/kg/day in drinking water. V. Arthritic vitamin C + FF-treated (VitC + FF group): The treatment of these birds was like FF group except that vitamin C (vitamin C 50%, Rooyan Darou, Semnan, Iran) at the dosage of 15g/100L of drinking water (equivalent with 11.25 mg/kg body weight of vitamin C) as the routine dosage of vitamin C for chickens in stressful conditions was administered from day 25 of age and continued to the end of the experiment. VI. Arthritic SDT-treated (SDT group): The arthritic birds received SDT (Kimiaprim 48®, sulfadiazine 400 mg/ml + trimethoprim 80 mg/ml, KimiaFam Pharmaceutical Co., Tehran, Iran) at 35 mg/kg in drinking water. VII. Arthritic vitamin C + SDT-treated (VitC + SDT group): The treatment was the same as SDT group except for administration of vitamin C as stated previously.

The dosage of antibacterial agents was based on label instructions. Administration of both antibacterials was started at the age of 39 (four days after induction of arthritis when birds showed fulminant clinical signs of arthritis) and lasted for 5 consecutive days.

Two samplings were performed, first at the age of 44 (10 birds of each group) and the second at the age of 54 (the remained birds), respectively. Birds were weighed and monitored for clinical signs. Lameness in birds was scored by defining 0: normal movement, 1: slight lameness, 2: difficulty in moving, 3: difficulty in moving and a tendency to sit, 4: severe lameness and 5: debilitating lameness with the bird remained in sitting position for most of the time (Knowles et al., 2008). Severity of arthritis was also scored as described by Bremell et al., 1992 (Bremell, Abdelnour, and Tarkowski 1992) by macroscopic inspection with a score of 0 to 3 for the right tibiotarsal joint (0, normal; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; and 3, marked swelling and occasional ankylosis). Blood samples were collected from wing vein of birds from each group for determination of oxidative stress parameters. Then birds were euthanized by decapitation and samples from synovial fluid of right tibiotarsal joint was collected for determination of IL-6 level. Moreover, the joint was fixed in 10% neutral buffered formalin for histopathological evaluation.

*Determination of serum malondialdehyde (MDA) concentration and total antioxidant capacity (TAC)*

Blood samples were coagulated at room temperature and sera were harvested after centrifugation at 3000 rpm for 10 min. Harvested sera were kept at -40°C until use.
Serum MDA concentration and TAC were determined by using kits prepared by Zell bio, Germany based on colorimetric assays as described by manufacturer.

**Determination of IL-6 concentration in synovial fluid**

A sandwich ELISA kit intended for quantitative measurement of IL-6 in chicken's biological fluids was used for determination of IL-6 in synovial fluid samples. The kit was prepared by Bioassay Technology Laboratory, China with intra-assay and inter-assay CVs of < 8% and < 10%, respectively. The protocol was performed as described by the manufacturer.

**Histopathological evaluation**

After decalcification in 5% nitric acid, samples trimmed and routinely processed, embedded in paraffin, 5 µm sections from paraffin blocks were taken and stained with hematoxylin and eosin for light microscope examination.

**Statistical analysis**

All data were presented as mean ± SD for all groups. Statistical analysis was performed by one-way ANOVA followed by Tukey's multiple comparison test with p < 0.05 as the level of significant difference. Data analysis and generation of graphs were performed by Graph Pad Prism 6 software.

**Results**

**Bacterial strain and sensitivity pattern**

Based on bacteriological tests and MLST the bacterium was ST-5732 disseminated clonal complex 5 (CC5) methicillin-resistant *S. aureus*. The bacteria were sensitive to SDT, FF, neomycin, chloramphenicol, nitrofurantoin, furazolidone and tiamulin. Moderate sensitivity was observed against umequine, enrofloxacin, danofloxacin and sulfamethoxazole-trimethoprim. The bacteria were resistant against methicillin, ciprofloxacin, oxytetracycline, erythromycin, tylosin, nalidixic acid, colistin, penicillin G and bacitracin.

**Body weight and clinical outcome parameters**

As shown in Fig. 1, birds in PC group had significantly lower body weight as compared to NC birds in both sampling (p < 0.0001 in both cases) while VC birds showed statistically the same weight in comparison with NC group (p > 0.05). Interestingly, administration of SDT was associated with a significant increase in body weight of birds at both samplings as compared to PC birds (p < 0.05 for both comparisons), however FF treatment had no significant effect on this parameter at first sampling while decreased the body weight of birds in sampling 2 as compared to PC group (p < 0.01). Although prophylactic vitamin C administration was not associated with a significant change in body weights of birds that received antibiotics at first sampling, birds that received vitamin C and FF showed significantly higher body weight as compared to FF group in sampling 2 (p < 0.01). The values for SDT and VitC + SDT groups remained statistically the same at the second sampling.
Induction of staphylococcal arthritis resulted in severe lameness in birds of PC group at both samplings. Lameness scores were statistically similar among PC birds and birds in groups that received antibiotics with or without prophylactic vitamin C administration in sampling 1 (p > 0.05). In sampling two, birds in VitC + SDT group showed significantly lower lameness score as compared to PC group (p < 0.01), while prophylactic administration of vitamin C slightly decreased lameness scores as compared to birds that only received antimicrobial therapy (Fig. 2).

As shown in Fig. 3, birds in all groups with staphylococcal arthritis showed moderate to marked swellings at tibiotarsal joint with a statistically similar arthritis severity scores among these groups in sampling one (p > 0.05). In sampling 2, mild to moderate swelling was still present in these birds. Administration of antibiotics with or without prophylactic administration of vitamin C was not associated with a significant decrease in this parameter as compared to PC group. Interestingly, birds that were treated with SDT or VitC + SDT had significantly lower scores as compared to birds that received FF as the single agent (p < 0.0001 and p < 0.01, respectively). Vitamin C administration with antibiotics did not change this parameter as compared to birds that were only treated with antibiotics (p > 0.05).

**Serum oxidative stress parameters**

Induction of arthritis was associated with a significant increase in serum MDA concentration of birds in PC group as compared to NC birds in sampling 1 (p < 0.01). Prophylactic administration of vitamin C resulted in a significant decrease in serum MDA in birds of VitC + FF group as compared to birds that were treated only with FF (p < 0.01), however birds in VitC + SDT group showed statistically the same level of serum MDA in comparison with SDT birds (p > 0.05). In second sampling, serum MDA concentration of PC birds showed no significant difference with NC or VC groups, while birds in FF and VitC + FF groups had lower serum MDA concentrations as compared to PC group (p < 0.05 and p < 0.01, respectively). Prophylactic administration of vitamin C was associated with a significant decrease in this parameter in FF-treated groups when compared with birds that only received antimicrobial therapy (p < 0.01) in sampling 1 (Fig. 4).

As shown in Fig. 5, PC birds had significantly increased TAC as compared to NC or VC groups in sampling 1 (p < 0.001 and p < 0.01, respectively), while this parameter was statistically the same among above mentioned groups in second sampling (p > 0.05). Moreover, no significant difference was observed among vitamin C plus antibiotic-treated groups with those that only received antibiotic in both samplings.

**Synovial fluid level of IL-6**

In both samplings, birds in PC group showed statistically the same level of IL-6 in synovial fluid as compared to NC or VC birds (P > 0.05). Administration of vitamin C did not result in a significant change in this parameter in both antibiotic-treated groups when compared to administration of antibiotic as the single agent (Fig. 6).

**Histopathological findings**

Normal histological features of tibiotarsal joint including articular cartilage, joint capsule and synovial membrane were observed in birds of NC and VC groups at both samplings while the histopathological
lesions were severe in PC birds as follows. Degeneration and necrosis were vastly seen in synovial membrane cells accompanied by hyperemia, edema, infiltration of inflammatory cells mostly heterophils, deposition of fibrin as severe fibrinoheterophilic synovitis (Fig. 7). Erosions were observed on the surface of articular cartilage in some samples.

The articular capsule was thickened with edema and fibrinoheterophilic exudate containing intralesional bacterial colonies.

At first sampling, birds of FF group showed moderate degree of degeneration and necrosis in synovial cells accompanied by edema, fibrin deposition and infiltration of heterophils and mononuclear inflammatory cells (Fig. 8). The changes were also moderate in VitC + FF group. Edema and infiltration of heterophils and mononuclear cells were mostly seen in perivascular regions. In second sampling, birds of FF group showed only mild changes with infiltration of few heterophils and mononuclear inflammatory cells in perivascular regions. Similar degree of changes was present in birds of VitC + FF group at this sampling point.

In SDT group at first sampling, moderate fibrinoheterophilic synovitis including degeneration and necrosis of synovial membrane cells, hyperemia, edema, fibrin deposition and infiltration of heterophils was observed (Fig. 9). These changes were also present in second sampling but at a moderate degree. Moderate hyperemia, edema and fibrin deposition were present at both samplings. The histopathological changes that were observed in birds of VitC + SDT group were almost the same as SDT group at first sampling, but in sampling 2, fibrin deposition was absent and the degree of inflammation including edema, hyperemia, and heterophils infiltration were milder in these birds (Fig. 10) as compared to SDT group.

**Discussion**

In this challenge study, we evaluated the possible effects of prophylactic vitamin C administration on the efficiency of two common antibacterials in broilers with a multi drug and methicillin-resistant *S. aureus* induced-SA.

Staphylococci are ubiquitous bacteria in the poultry farm environment which can cause opportunistic infections in poultry. *S. aureus*, as the most pathogenic Staphylococcus species, results in clinical symptoms often seen in bones, joints, and tendon sheaths with usually a chronic nature (Szafraniec, Szeleszczuk, and Dolka 2020).

As it was expected, we observed that the induction of the arthritis is associated with a negative effect on birds’ weight of PC group at both samplings that can be described by the arthritis-associated pain and discomfort that limits the birds’ tendency to move. Although less severe, the arthritis was still present in sampling 2 (20th day after induction) with severe lameness that shows the long term nature of the disease. The histopathological findings also confirmed the presence of inflammatory reactions accompanied by degeneration and necrosis in synovial membrane cells and damage to articular cartilage
at sampling 2. Moreover, intralesional bacterial colonies were present that reflect the incapability of immune system for clearing the persistent infection. In fact, it has been demonstrated that the damage associated with SA is not only due to the bacterial invasion, but also the immense response by immune system that result in a sustained inflammation with destructive consequences in joints (Corrado et al. 2016).

Administration of SDT (but not FF) resulted in better weight gain of arthritic birds especially in sampling 2 where body weight of birds in SDT group was statistically the same with NC birds. Notably, administration of FF was associated with body weights statistically lower than PC group at sampling 2 (10 days after the last treatment). Consistently, in a study by Hassanin et al., 2014 (Hassanin, Abdallah, and Awad 2014) on broilers that were challenged with \textit{E. coli} and received FF for 5 consecutive days, the authors reported a decreasing effect of FF administration on body weight at 7 and 14 days after stopping treatment.

In the present study, although administration of vitamin C did not result in enhancement of weight gain in birds that received SDT, this vitamin significantly increased the weight of birds in VitC + FF group in comparison with those that only received FF in sampling 2. This effect was not associated with better results regarding lameness scores and therefore the ability of birds to reach food. Consistently, Njoku 1986 (Njoku 1986) reported a positive effect of dietary vitamin C supplementation on body weight of broilers in tropical environment without affecting their feed intake. In a study by Gouda et al., 2020 (Gouda et al. 2020), it was demonstrated that vitamin C can positively affect broiler growth performance under heat stress by improving hormonal and health status parameters of birds. The reason behind the positive effect of vitamin C on broiler weight in VitC + FF group might be related to better hormonal parameters although it needs to be confirmed in future studies.

When established, SA is not easily treated with antibacterials, which can be related to the characteristics of infection site as well as the possibility of multi drug resistance of the pathogen. At sampling 2 of our study, SDT showed better efficiency in reducing arthritis severity scores compared to FF. This observation is consistent with the study performed by Mosleh et al., 2016 (Mosleh et al. 2016), on the comparative efficiency of different antibiotics (including FF and SDT) in broilers with staphylococcal SA where SDT generally showed better therapeutic outcome than other antibacterials.

In the present study, prophylactic administration of vitamin C was not associated with a significant improvement of the effect of both antibacterials on clinical parameters of septic arthritis, although milder histopathological changes were observed at sampling 2 in birds that received vitamin C and SDT compared with SDT group. In contrast to our results, Mal et al., 2012 (Mal et al. 2012), reported that administration of vitamin C with gentamicin enhances the reducing effect of gentamicin on inflammatory response especially in ankle joint of mice challenged with \textit{S. aureus} on days 3, 9 and 15 post arthritis induction. The difference in the antibacterial agents that were used as well as the animal species may be the major reasons for this discrepancy.
It has been demonstrated that neutrophils are the major recruited immune cells to the joint during the immune response against *S. aureus* SA and production of reactive oxygen species (ROS) is one of the major mechanisms that neutrophils use to kill bacterial pathogens (Boff et al. 2018). As previously stated, we observed vast and intense infiltration of heterophils, the avian counterparts of mammalian neutrophils, in histopathological joint samples of birds in PC group. This observation was accompanied by oxidative stress in birds of this group at first sampling as shown by an obvious increase in serum MDA levels, a primary indicator of lipid peroxidation, of PC birds compared to NC group. The increase in MDA levels was associated with increased TAC that can be considered as a compensatory response to oxidative stress. Administration of both antibacterials was not associated with a significant change in MDA levels as compared to PC birds in sampling 1, although the levels of MDA was lower in FF and VitC + FF group as compared to PC birds in sampling 2. This observation can be explained by the fact that only few heterophils were present in histopathological samples of birds in FF and VitC + FF groups at sampling 2.

In our study, vitamin C significantly lowered levels of MDA in VitC + FF group as compared to FF in sampling 1. In a study by Mosleh et al., 2018 (Mosleh et al. 2018), it was reported that 5 days of vitamin C administration to broilers under chronic heat stress can significantly reduce serum MDA levels of these birds while longer administration was not associated with appreciable effects on this parameter.

In human patients with staphylococcal SA, migration of neutrophils to infected joints is followed by activated macrophages (Boff et al. 2018). Cytokines released from macrophages such as IL-6 have a major role in cartilage and bone destruction and IL-6 is also important in differentiation of T-cells (Dey and Bishayi 2017). Consistently, *S. aureus*-dependent SA in murine knee joints has been associated with an increase of IL-6 in both blood and knee joint (Corrado et al. 2016). However, in our study, induction of arthritis did not result in significant change of IL-6 levels in synovial fluid of PC birds as compared to NC group and also no significant difference was observed among treated groups and PC birds at both samplings. This observation is consistent with the study performed by Mosleh et al., 2016 (Mosleh et al. 2016) on broilers with staphylococcal arthritis. These authors also did not find a change in serum levels of IL-6 in arthritic birds. It is noteworthy that in histopathological examination of arthritic joints we did not find many macrophages. This can describe the reason that we did not find an increase in IL-6 level in arthritic joints.

In conclusion, by injecting a multidrug resistant *S. aureus* into the joint, a long lasting SA develops in broilers which is not reflected in the level of IL-6 in synovial fluid. The therapeutic response to SDT is generally better than FF with regard to body weight and arthritis severity score while FF administration has better effects on oxidative stress parameters. Both antibacterials are not completely successful in treating the condition. Prophylactic administration of vitamin C does not appreciably enhance the therapeutic efficiency of antibacterials on clinical parameters of arthritis, although it decreases the lipid peroxidation and improves weights of FF treated-arthritic birds. For SDT-treated birds, administration of vitamin C only ameliorates histopathological changes.
Declarations

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Conflicts of interest/Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

All procedures used in this study are approved by institutional ethical guidelines (Shiraz University, School of Veterinary Medicine, No. 94GCU2M163773) for care and use of animals in experiments based on guidelines of EU Directive 2010/63/EU for animal experiments.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material (data transparency)

Data can be available on demand.

Code availability

Not applicable

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Figures
Figure 1

Body weight (mean±SD) of birds in different groups at two sampling time points (day 44 and 54 of age). Different superscript letters are used to show significant difference at p<0.05. NC: negative control (no specific treatment), PC: positive control with S. aureus-induced arthritis, VC: Vehicle control, FF: birds with arthritis that treated with florfenicol, VitC+FF: birds with arthritis that received prophylactic vitamin C with florfenicol, SDT: birds with arthritis that treated with sulfadiazine/trimethoprim and VitC+SDT: birds with arthritis that received prophylactic vitamin C with sulfadiazine/trimethoprim.
Figure 2

Lameness scores (mean±SD) of birds in different groups at two sampling time points (day 44 and 54 of age). Different superscript letters are used to show significant difference at p<0.05. NC: negative control (no specific treatment), PC: positive control with S. aureus-induced arthritis, VC: Vehicle control, FF: birds with arthritis that treated with florfenicol, VitC+FF: birds with arthritis that received prophylactic vitamin C with florfenicol, SDT: birds with arthritis that treated with sulfadiazine/trimethoprim and VitC+SDT: birds with arthritis that received prophylactic vitamin C with sulfadiazine/trimethoprim.

Figure 3

Arthritis severity scores (mean±SD) of birds in different groups at two sampling time points (day 44 and 54 of age). Different superscript letters are used to show significant difference at p<0.05. NC: negative control (no specific treatment), PC: positive control with S. aureus-induced arthritis, VC: Vehicle control, FF: birds with arthritis that treated with florfenicol, VitC+FF: birds with arthritis that received prophylactic vitamin C with florfenicol, SDT: birds with arthritis that treated with sulfadiazine/trimethoprim and VitC+SDT: birds with arthritis that received prophylactic vitamin C with sulfadiazine/trimethoprim.
Figure 4

Serum malondialdehyde (MDA) concentration (mean±SD) of birds in different groups at two sampling time points (day 44 and 54 of age). Different superscript letters are used to show significant difference at p<0.05. NC: negative control (no specific treatment), PC: positive control with S. aureus-induced arthritis, VC: Vehicle control, FF: birds with arthritis that treated with florfenicol, VitC+FF: birds with arthritis that received prophylactic vitamin C with florfenicol, SDT: birds with arthritis that treated with sulfadiazine/trimethoprim and VitC+SDT: birds with arthritis that received prophylactic vitamin C with sulfadiazine/trimethoprim.
Figure 5

Serum total antioxidant capacity (TAC) (mean±SD) of birds in different groups at two sampling time points (day 44 and 54 of age). Different superscript letters are used to show significant difference at p<0.05. NC: negative control (no specific treatment), PC: positive control with S. aureus-induced arthritis, VC: Vehicle control, FF: birds with arthritis that treated with florfenicol, VitC+FF: birds with arthritis that received prophylactic vitamin C with florfenicol, SDT: birds with arthritis that treated with sulfadiazine/trimethoprim and VitC+SDT: birds with arthritis that received prophylactic vitamin C with sulfadiazine/trimethoprim.
Figure 6

Synovial fluid concentration of interleukine-6 (IL-6) (mean±SD) of birds in different groups at two sampling time points (day 44 and 54 of age). Different superscript letters are used to show significant difference at p<0.05. NC: negative control (no specific treatment), PC: positive control with S. aureus-induced arthritis, VC: Vehicle control, FF: birds with arthritis that treated with florfenicol, VitC+FF: birds with arthritis that received prophylactic vitamin C with florfenicol, SDT: birds with arthritis that treated with sulfadiazine/trimethoprim and VitC+SDT: birds with arthritis that received prophylactic vitamin C with sulfadiazine/trimethoprim.
Figure 7

Severe degree of fibrinoheterophilic arthritis associated with intralesional bacterial colony in a tibiotarsal joint of positive control group.
Figure 8

Moderate degree of degeneration and necrosis in synovial cells accompanied by edema, fibrin deposition and infiltration of heterophils and mononuclear inflammatory cells especially around vessels are seen in FF group.
Figure 9

Moderate fibrinoheterophilic synovitis including degeneration and necrosis of synovial membrane cells, hyperemia, edema, fibrin deposition and infiltration of heterophils are seen in SDT group.
Figure 10

Mild heterophilic synovitis including degeneration of synovial membrane cells, hyperemia, edema, and infiltration of a few heterophils and mononuclear inflammatory cells are seen in Vit C + SDT group after 2nd sampling.