

KAEMPFEROL IMPROVED LIVER ENZYMES IN TWO WEEKS OLD BROILER CHICKENS WITH EXPERIMENTAL *EIMERIA TENELLA* INFECTION

*Muhammad, Y¹., Jatau, I. D²., Chiroma, M. A.³ Umar, A.M¹., & Dahiru, M¹

¹Department of Animal Health and production, Binyaminu Usman Polytechnic, Hadejia, Jigawa State.

²Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria.

³Department of Veterinary Pathology, University of Maiduguri, Borno State, Nigeria.

Corresponding author: Email: yamohad@gmail.com, phone number: 07063249774

ABSTRACT

The effect of kaempferol on the concentration of liver enzymes in two weeks old broiler chickens with experimental *Eimeria tenella* infection was evaluated in this study. Sixty day old broilers were randomly allotted into six groups (I-VI) of ten broilers each and brooded for two weeks. At exactly two weeks of age, broilers in group I served as non-infected control. Broilers in groups II- VI were infected with *Eimeria tenella* sporulated oocyst (10^4 /ml) via oral inoculation. After infection was established, broilers in groups II-IV were treated *per os* with 1 mg/kg, 1.5 mg/kg and 2 mg/kg of kaempferol respectively. Broilers in group V were treated for five days with amprolium, 1.25 g/L in drinking water. Broilers in group VI served as infected untreated control. Five days post infection; all broilers were sacrificed by severing their jugular veins. Blood sample was collected from each broiler in a plane sample container devoid of anticoagulant so as to harvest serum for serology. Concentration of liver enzymes (ALT, AST and ALP) in the serum was determined. Data obtained were analyzed using pad prism version 5.0. There were statistically significant ($P < 0.05$) reductions in the mean values of liver enzymes (ALT, AST, ALP) in groups II, III and IV in a dose dependent manner when compared to group VI. It was observed in this study that kaempferol improved the serum level of liver enzymes and reduced the level of pathology in the liver and caecum of broilers infected with *Eimeria tenella* in this study, and therefore recommends that it should be incorporated in bird's feed.

Key words: Amprolium, Broilers, Diminazene aceturate *Eimeria tenella*, kaempferol, liver enzymes,

INTRODUCTION

Each year, over 50 billion chickens are raised as a source of meat, accounting for over one-third of protein food for humans (Bampidis *et al.*, 2005), however, this practice is often confronted by so many problems of avian coccidiosis (Coccidia subclass) (Chapman *et al.*, 2010). So far, nine *Eimeria* species, *E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix*, *E. praecox*, *E. mitis*, *E. tenella*, *E. mivati*, and *E. hagani*, have been identified from chickens (Chapman *et al.*, 2010). These parasites can infect and multiply within the mucosal epithelia in different parts of bird guts, which cause severe gut damage (i.e., inflammation, hemorrhage, diarrhea, etc.), morbidity, and mortality (Calderon-montana *et al.*, 2011). It also causes a global loss of over 2.4 billion US dollars in the poultry industry, including poor growth performance, replacement of chicks, and medication (Lilleheoj and Trout, 1996). Anticoccidial chemicals, coccidiocides, coccidiostats, and ionophores, have long been used as a mainstream strategy to control avian coccidiosis in modern poultry production and birds have developed resistance to them (Chapman, 2014)

Antioxidant compounds like kaempferol are well known to have a cellular protective action against oxidative stress and reduce the severity of *Eimeria tenella* infection by altering the degree of intestinal lipid peroxidation¹. (Allen *et al.*, 1998). Most of the antioxidants occur as dietary constituents³, as shown by many researches, antioxidants deposited in broiler meat following oral ingestion delay lipid peroxidation in the muscles and improve it quality (Bampidis *et al.*, 2005), these important role, made us conduct this study with aim of determining the ameliorative effect of kaempferol in stress induced by experimental *Eimeria tenella* infection in two weeks old broiler chickens.

MATERIALS AND METHODS

Study area

This research was conducted in the Departments of Animal Health and Production (poultry unit), Binyaminu Usman Polytechnic, Hadejia, Jigawa State, Nigeria (10°02' 28.14'' E Longitude and 12°27' 12.49 N Latitude)

Drugs, Sources and Preparations

Kaempferol was sourced from whitehead scientific (pty) limited, South Africa, it came along with the following details; CAS number (520-18-3), Catalog number (3603), EC number (208-287-6) and batch number (3).

***Eimeria tenella* Isolate**

The *Eimeria tenella* isolate used in this study was obtained from the parasite bank of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The oocysts were propagated in two week-old coccidian-free chickens before use. The unsporulated oocysts collected from the caecal content on the seventh day post-infection was purified and preserved in 2.5% potassium dichromate solution to induce sporulation at 28° C. The sporulated oocysts were kept at 4° C until use.

Experimental birds and grouping

Sixty day old broiler chicks obtained from a reputable hatchery were used in this study. The chicks were randomly allotted into six groups (I-VI) of 10 birds and brooded for 2 weeks on concrete floor (using wood shaven as litter material) under strict biosecurity measures and as routine were adequately vaccinated against Newcastle disease and infectious bursal disease. They were fed with commercial broiler feed (Vital feed®) and water which were provided *ad libitum*.

Experimental infection and treatments

At exactly two weeks of age, birds in Groups II-VI were orally inoculated with 10⁴ sporulated oocysts of the *E.tenella* isolate, and then treated as follows:

Group I: Birds in this group served as non-infected control.

Group II: Birds in this group were treated with 1 mg/kg of kaempferol *Per os* for 5 days.

Group III: Birds in this group were treated with 1.5 mg/kg of kaempferol *Per os* for 5days.

Group IV: Birds in this group were treated with 2 mg/kg of kaempferol *Per os* for 5 days.

Group V: Birds in this group were treated with 1.25 g/L of amprolium in drinking water for 5 days.

Group VI: Birds in this group served as infected untreated control.

Determination of Serum level of liver enzymes

Serum level of alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were determined using chemical auto-analyzer (Bayer® Clinical Chemistry Analyzer, Germany) as described by manufacturer.

Statistical Analysis

Data obtained were expressed as means $\pm$ standard errors of mean (S.E.M), subjected to one-way analysis of variance (ANOVA) and compared with Tukey post-hoc test. The level of significance was set at $p < 0.05$.

RESULTS

Effect of Kaempferol on the Activities of Liver Enzymes

Figure 1 shows the effect of kaempferol on the activities of liver enzymes in two weeks old broilers challenged with *Eimeria tenella*. There were significant ($P < 0.05$) increases in mean AST, ALT and ALP

values in the infected untreated broilers (group VI) when compared with the infected broilers and treated with different doses of kaempferol (group II, III and IV) and those that were treated with Amprolium (group V). But no significant ($P>0.005$) difference in the mean values of AST, ALT and ALP was observed in broilers treated with 2 mg of kaempferol and those that were treated with amprolium.

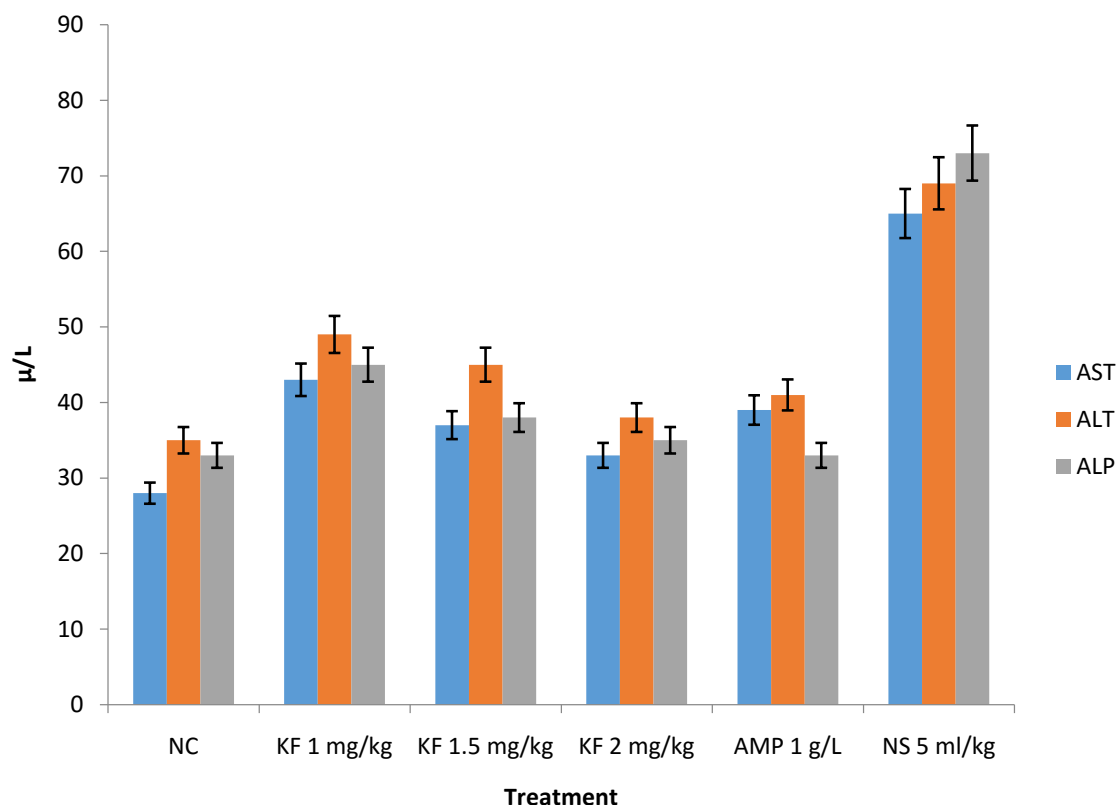


Figure 1: Effect of kaempferol on the activities of liver enzymes (ALT, AST, ALP).

Key: NC=Neutral control, KF=Kaempferol, AMP= Amprolium and NS=Normal Saline
 $P\leq 0.05$ considered significant.

DISCUSSIONS

The present study revealed significant reduction in the concentration of liver enzymes (ALT, AST and ALP) in groups (II, III and IV) treated with kaempferol when compared to infected untreated broilers (group VI). No significant difference in the concentration of liver enzymes (ALT, AST and ALP) was recorded between group III, IV and V.

The increased levels of ALT, AST and ALP in the infected untreated broilers agrees with the earlier reports of Srivastava *et al.*, 2012, who attributed the elevation of ALT, AST and ALP to inflammation in the host, particularly of the liver. Another possibility of increased levels of ALT and AST is the activities of free radicals generated at different stages of the infection (Murali, 2014). Therefore, serum levels of ALT and AST are two of the most useful indications of liver cell injury, although the AST is less liver specific than is ALT (Kim and Choi, 2013). Oyewale 1991) attributed the increase in the liver enzymes to inflammatory condition involving liver and gastro-intestinal tract¹⁸

CONCLUSION AND RECOMMENDATION

It was therefore concluded in this study that, the oral administration of kaempferol had ameliorated stress induced by the experimental *Eimeria tenella* infection in two weeks old broilers via improving the serum level of liver (ALT, AST and ALP) enzymes

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