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An in Vitro Study on the Antibacterial Activity of Kefir Milk, Labzyme, Biozyme and Some Antibiotics against *Staphylococcus aureus* and *Escherichia coli*causes Lameness in Broiler Chickens

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Abstract: ***Bacterial chondronecrosis with osteomyelitis (BCO) is the most common cause of lamenessin commercial broilers. Bacteria entering the blood viatranslocation from the respiratory system or gastrointestinaltract spread hematogenously to the proximalepiphyseal-physeal cartilage of rapidly growing femoraand tibiae, causing BCO. We tested the hypothesis that probiotic kefir milk might attenuate bacterial translocation and thereby reducethe incidence of BCO. This study demonstrates that kefirpossesses antibacterial effect against Staphylococcus aureus and Escherichia coli as compared with Labzyme. Biozyme and some antibiotics, Pencilin, Gentamycin, Tetracyclin and Newmycin. The results indicated that the Newmycin was the best significantly (p < 0.05) against Staphylococcus aureus and Escherichia colibacteria compared with others antibiotics. Labzyme. Biozyme and Kefir. Kefir was a highly significant (p < 0.05) to inhibit the growth of Staphylococcus aureus and Escherichia coli bacteria as compared with Labzyme, Biozyme. Pencilin, and Tetracyclin.***

Keywords: Kefir fermented milk, probiotic, antibiotics, *Staphylococcus aureus*. *Escherichia coli*

1. Introduction

Worldwide surveys revealed the prevalence of lameness in poultry, especially broiler chickens. In a study conducted in the United Kingdom, the rate of lameness in broiler chickens was 27.6%, of which 3.3% of birds are not fully able to move, and about 12.5 billion chickens suffering from lameness worldwide and similar prevalence rates were estimated in Denmark and the United States. It was observed that 70-90% of these cases were due to Bacterial Chondronecrosis with Osteomyelitis (BCO). (Knowles et al., 2008). BCO is one of the most common causes of lameness in broiler chickens. Symptoms usually begin after the third week of life and may result increased mortality up to 5%. The disease was first diagnosed in 1972 in Australia and later mentioned in the United States, Canada and Europe. At the end of the 20th century, high rates of BCO were detected in broiler chickens in the United Kingdom, where a group of bacteria causing the disease were isolated, including *Staphylococcus aureus, Escherichia coli, Salmonella spp*. (Dinev, 2009 : Joiner et al., 2005). The negative impact of the lamination on the economics of broiler chickens is clearly observed through the difficulty of movement due to pain, hence the reduction of feed and water consumption, resulting in poor production (Wideman *et al*., 2012).

The rapid rate of growth and increase of the weights of chicken broilers are among the main causes of BCO. This high rate and rapid growth leads to fractures and fine cracks in the osteoocytes. These cracks lead to closure of the blood vessels in the affected area and thus slow flow For blood. (Kense and Landman, 2011). The Mucosal disorder of the gastrointestinal tract due to intestinal microflora disturbance and invasion of intestinal pathogens is an additional factor in the occurrence of BCO.Knowles et al., 2008).This imbalance leads to the transmission of the pathogenic bacteria from the gastrointestinal tract to the bloodstream and the bacteria in the blood vessels settle in the infected area, which in turn leads to blood clotting and therefore the lack of bone supply in the blood leading to necrosis of the bones. In addition, transient pathogens attach to cartilage joints and colonize areas affected by necrosis, making it difficult for immune cells to reach infected areas due to lack of blood flow due to vascular closure. This immune deficiency can be exacerbated by any general immune deficiency which may Occurs as a result of certain diseases such as Cumboro, environmental factors or the presence of fungal toxins in feed (wideman et al., 2012).The use of antibiotics may help to reduce the incidence of lameness problems in poultry, but the Europeans and Americans band using antibiotics in poultry fed (Rigobelo *et al*., 2011; Bitterncourt et al., 2011). Hence, the use of alternative antibiotics as a controlling health problem, including lameness, has been promoted globally. The use of probiotics is an effective alternative that can be used in the prevention of lameness in broiler chickens. The use of probiotic milk kefir is one of the most effective alternatives that can be used to prevent lameness in broiler chicks. (Wideman *et al*., 2012; Toghyani et al., 2015).

Kefir is a fermented milk drink produced by the actions of bacteria and yeasts contained in kefir grains, and is reported to have a unique taste and unique properties. During fermentation, peptides and exopolysaccharides are formed that have been shown to have bioactive properties. (Farn worth, 2005). The Food and Agriculture Organization (WHO) and the World Health Organization (WHO) have proposed a definition of kefir based on the microbial composition (FAO / WHO, 2001). The microbial kefir contains a variety of "lactic acid bacteria. It is described as a

symbiotic association between lactic and acetic bacteria and yeast and claimed to act against the pathogenic genera Salmonella, *Helicobacter, Shigella, Staphylococcus* and *E. coli* (Arihara, 1990). The function of the microorganisms constituting the kefir may include production of lactic acid, antibiotics and bactericides, which inhibit the growth of undesirable and pathogenic microorganisms.(Angulo.1992). However scientific interest in kefir is growing due to its health benefits, a limited number of in vitro experimental studies were performed in order to understand the antimicrobial mechanism of kefir's microbial flora (Anar. 2000). It has been reported that kefir causes a bacteriostatic effect against E.coli possibly due to competition for nutrients between kefir microbiata and the test strain and/or due to substances that could appear at early stages in the fermentation of milk (Garrote *et al*., 2000).

Rodrigues, (2005) and Okda et al.(2013) assessed the antibacterial activity of kefir against *Salmonella, Shigella* and *Staphylococcus* species. Cevikbas et al. (1994) studied the antibacterial and antifungal activities of kefir and kefir grain on *Staphylococcusaureus*. The aim of this study was to competitive activity of Kefir, Labzyme, Biozyme, Gentamycin, Pencilin, Tetracyclin and Neomycin in vitro by assessing the reduction of *Staphylococcus aureus* and *Escherichia coli*on chicks tibia bone.

2. Material and Methods

Kefir Production: Experiment was conducted in Microbiology Laboratory at the Animal Production Department in the College of Agricultur - University of Basrah, for the period from 10/4/2015 to 7/5/2015 for the purpose of manufacturing a Probiotic of Kefir Milk. was manufactured according to the manufacturing steps mentioned by Auda, (2013) with some modifications to them in six stages, the first phase included the process of fermentation, The microbiology of the kefir grains was developed by using 10 g of these grains in a liter of fresh pasteurized cow's milk in a tight glass with a piece of gauze cloth, and allowed to ferment and incubate for 24 hours and at a temperature (20-25) ^{o}C. Then the kefir grains were removed using a plastic filter, to reuse the granules again. Kefir milk was kept in the refrigerator until it was used. Antibacterial activity was tested for the Efficiency of antibacterial agents in the Probiotic of Kefir Milk and compared with the Labzyme, Biozyme, Pencilin, Gentamycin, Tetracyclin, Newmycin against Staphylococcus aureus and Escherichia coli.

Preperations of bacterial solutions; *Staphylococcus aureus* and *Escherichia coli* were used as test microorganisms which cause Bacterial Chondronecrosis With Osteomyelitis BCO. They were activated innutrient broth by fermentation at 35°C for 24 hours. A loop full of the bacteria that activated and enriched in nutrient broth were transferred to sterile saline water and emulsified to a turbidity of McFarland 0.5 density. The final bacterial cell concentration approximated to 108/ml with spectrophoto metric method.

Testing antimicrobial activity; Antibiotic activity of kefir milk was evaluated using the disk diffusion method as described by Bauer et al. (1966). Probiotics Labzyme and Biozyme and antibiotic Pencilin, Gentamycin, Tetracyclin and Newmycin were used to compare the antimicrobial activity and 10µg/ml of antibiotics was pipetted on to 5 mm diameter paper disk.24 and 48 hours fermented kefir were pipette at the amount of 0.1 ml and 1.2 mg/ml as described by Rodrigues et al. (2005). The paper disks with antibiotics and experimental kefir were applied to the agar surface previously inoculated with 0.1 ml organism suspension. These plates were incubated at 37°C for 24 hours and the inhibition zones were measured at the end of fermentation period. Experiments were performed intriplicates and mean values were used.

Statistical Analysis; The test for the inhibition of the samples obtainedfrom kefir grains in relation to the Probiotics Labzyme and Biozyme and antibiotic Pencilin, Gentamycin, Tetracyclin and Newmycin was carried out with three repetitions, observing the absorbance readings at600 nm byspectrophotometry. The averages of the results for each kefir sample in relation to each pathogen were compared statistically using the RLSD test at the 5% significance level (SPSS, 2009).

3. Results and Discussion

The table (1) showed significant differences ($p<0.05$) between the regions of the microbial inhibition zones of the different treatments. The best effect of Neomycin was 19.42, 18.90 mm for the inhibition area. In the *Staphylococcus aureus* and *Escherichia coli* inhibition area respectively. The table also showed no significant differences in the treatment of Kefir milk with Gentamycin in diameter (mm) inhibition of *Staphylococcus aureus, Escherichia coli,* while significantly different with Pencilin, Labzyme, Tetracyclin, Biozymein the diameter of the inhibition of *Staphylococcus aureus* (17.15, 16.5, 16.1 and 12.47 mm respectively) and the diameter of the *Escherichia coli* inhibition area was 14.92, 14.40, 17.37 and 10.05 mm respectively, and the following images 1, 2. 3 and4 showed diameter (mm) the inhibition area of *Staphylococcus aureus* and *Escherichia coli*.

Several authors have studied the effect of kefir andits microbial flora on the inhibition of microbial activity *in vitro*, in relation to a large variety of Gram-positive and Gram-negative bacteria as well as some fungi. Several studies have demonstrated the antagonistic effect of kefir (Saloff-Coste 1996; Garrote et al. 2000; Güven and Goles 2003; Santos *et al*. 2003; Witthuhn et al. 2004; Yuksekgag et al., 2004) demonstrated that microorganisms isolated from the kefir grains inhibited the growth of *S. aureus***.** These authors suggested that organic acids, hydrogen peroxide and other substances were responsible for the inhibition. Pathogenic bacteria such as *Shigella* and *Salmonella* do not grow in the drinks fermented with kefir grains (Koroleva 1988). *Lactobacillusacidophilus* isolated from the kefir showed inhibitory activity toward various Gram (+) and Gram (-) microorganisms (Gilliland and Speck1977; Apella et al. 1992; Gupta et al. 1996). Of the different microorganisms isolated from kefir, *Lactococcus* and the acetic acid bacteria were those which presented the maximum inhibitory effect on coliforms (Van, 2001), and they also inhibited the growth of *S. aureus*, *B.cereus*,

Clostridium tyrobutyricum and *L.monocytogenes*. The antagonistic mechanisms of lactic acid bacteria may include the activity of organic acids, hydrogen peroxide (Shahani and Chandan 1979; Juven et al. 1992), diacetyl, bacteriocins and other compounds (Helander et al.1997).The results corroborate the Hypothesis that these chemical compounds presentkefir grains were associated withAntagonistic mechanisms against the pathogens.

In conclusion, Kefir milk can be used hn broiler diets or water as antibacterial

Table 1: Effect of Antimicrobial Activity on the Treatment of Kefir Milk, Labzyme, Biozyme, Antibiotics, Gentamycin, Pencilin, Tetracyclin, Neomycin on Diameter (mm) *Staphylococcus aureus, Escherichia*

Strain	S. aureus	E. coli
Kefir	18.85b±0.26	17.88 b ± 0.38
Labzyme	16.51 d±0.23	14.40 c ± 0.34
Biozyme	12.47 f ±0.11	10.05 d± 0.29
Gentamycin	18.70 b ± 0.21	18.025 b ± 0.19
Pencilin	17.15 c ± 0.27	14.925 c ± 0.29
Tetracycllin	16.1 d± 0.39	17.37 b ± 0.53
Neomycin	19.42a± 0.042	18.90 a ± 0.102
Significant level	*	*

(*) Vertically different letters "between the averages of treatments means that there are significant differences ($p < 0.05$).

Image 1: Diameter (mm) of the inhibition areas *S. aureus*

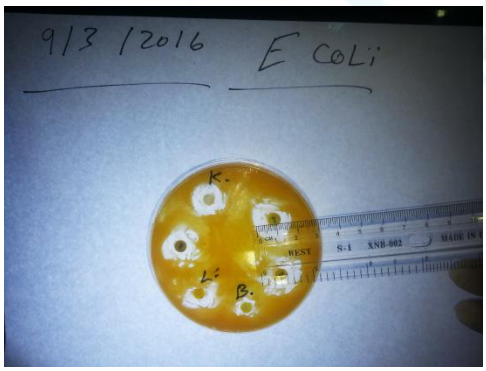

Image 2: Diameter (mm) of the inhibition areas *E.Coli*

Image 3: Diameter (mm) of the inhibition areas *S. aureus*

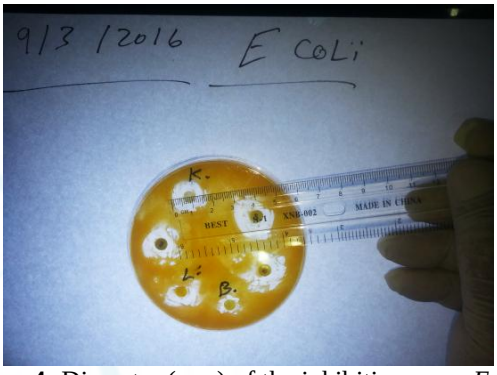

Image 4: Diameter (mm) of the inhibition areas *E.Coli*

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