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Efficacy of *Hermetia illucens*-derived Fatty Acids and/or Florfenicol in Broiler Chickens Infected with *Escherichia coli*

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ABSTRACT

Hermetia iIlucens fat includes balanced and unique medium-chain fatty acids (MCFAs), which act as a prophylactic agent against many microorganisms. MCFAs are one of the pioneering ways to act as antimicrobial fatty acids. *Escherichia coli* (*E. coli) is* one of the *Enterobacteriaceae* which threaten broiler production. *H. illucens* oil (HIO) is enriched with MCFA, mainly lauric acid (LA), which has antibacterial activity. Florfenicol (FFC) is a broad-spectrum antibiotic that works against Gram-negative bacteria. Excessive use of antibiotics induces bacterial resistance, which is a serious problem facing animal health. In the present study, we aim to evaluate the influences of *H. illucens* MCFAs as a prophylactic agent versus chemical treatment by FFC on the growth performance and histomorphological character of the intestine in broilers infected by *E. coli* O_{157} strains. One-hundred-day-old chicks were divided into 5 groups, 20 chicks for each. Group 1 was assigned as control negative and administered only saline. Group 2 was a control positive (non-treated) and infected with *E. coli* serotype O_{157}. Group 3 was infected by *E. coli* and administered *H. illucens* fat. Group 4 was infected and FFC-treated. Group 5 was infected and treated with both FFC + *H. illucens* fat. The results showed that MCFAs in *H. illucens* fat promote the growth rate of broilers, provide significant body weight gain, and improve their intestinal mucosal structure compared to the control positive group. In conclusion, *H. illucens* MCFA can be used as a natural growth promoter instead of the chemical antibiotic FFC.

1. INTRODUCTION

In poultry production, antibiotics are widely used to control many diseases. *E. coli* is a gram-negative bacterium that affects the GIT inducing digestive problems such as diarrhea, a breakout of the mucosal barrier of the intestine, increases GIT permeability, and inflammation (Barnes et al., 2008). In turn, it causes loss in body weight, and negatively affects growth performance. Florfenicol (FFC) has antimicrobial properties against Gram-negative bacteria such as *E. coli* and Salmonella spp. It can prevent protein synthesis by inhibiting peptidyl transferases enzyme (Shen et al., 2003; Ismail & El-Kattan, 2009). It belongs to wide-spectrum antibiotics with a structure like chloramphenicol. However, unlike chloramphenicol, it does not cause aplastic anemia for a lack of O-nitro group and is effective in the treatment of gastrointestinal and respiratory infectious diseases in domestic animals (Ben et al., 2019). The excessive use of antibiotics causes bacterial resistance, which is a serious problem facing animal health. The resistance to *E. coli* is related to drug resistance genes and developed by commonly used clinical antibiotics (EFSA, 2008).

The Black soldier fly larvae (*Hermetia illucens: H. illucens*) can convert organic wastes into nutrient-rich biomass suitable for animal and poultry feed, which could be a way to achieve a well sustainable food production. Because of their nutritional composition, *H. illucens* larvae have been used as a valuable feed ingredient for poultry and fish. *H. illucens* oil (HIO) provides antibacterial activity due to the high content of MCFA, which contains mainly lauric acid (LA), providing healthy digestive tract organs (Taulescu et al., 2010; Khatun et al., 2018). MCFA (chain lengths of 6 to 12 carbon atoms) have effective absorption and metabolic properties. Additionally, they have antimicrobial properties, enhancing GIT health and growth parameters in broilers (Zentek et al., 2011). Lauric acid and its monoglyceride derivative, monolaurin, are generally recognized as safe by the United States food and drug administration. They have a strong antimicrobial property by destabilizing the bacterial cell membrane. They are natural fatty acids, promising candidates for other chemical antimicrobials, getting rid of bacterial resistance (Schlievert and Peterson, 2012; Yoon et al., 2018; Dabbou et al., 2020; Jackman et al., 2020). Lauric acid and monolaurin (C12:0), which constitute up to 60% of the total saturated fatty acid composition of the Hermetia illucens offer benefits to broilers production, enhancing intestinal histomorphology as well as broiler body performance (Fortuoso et al., 2019; Londok and Rompis, 2019). Partial or total replacement of soybean oil with *H.*

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illucens fat changes positively the fatty acid profile of broiler chickens. (Schiavone et al., 2017). *H. illucens* fat has an inhibitory effect against some gram-negative bacteria like *E. coli* and Salmonella sp. (Auza., 2020). We aimed to evaluate the influences of *H. illucens* MCFA as a prophylactic agent versus chemical treatment by FFC on the growth performance and histomorphological character of the intestine in broilers infected by *E. coli* O157 strains.

2. MATERIAL AND METHODS

2.1. Drugs and chemicals

H. illucens oil was obtained from EGYMAG company, Egypt. The dose administered was 3 ml/kg of drinking water (Fortuoso et al. 2019). Florfenicol ($C_{12}H_{14}Cl_2FNO_4S$) a fluorinated derivative of thiamphenicol (Floromed® 10%) was obtained from ARABCOMED company, Egypt. The dose administered was 3 ml/ L of drinking water for 3 days.

2.2. Microorganisms

E. coli serotype O_{157} was obtained, prepared to use, and identified in Animal Health Research Institute, Giza Branch, Egypt. The infection dose was 0.5 ml/bird of bacterial suspension (3×10^8 CFU/ml) orally.

2.3. Chicks

One hundred one-day-old chicks were divided into 5 groups, each of 20 chicks. Group (1) was a non-infected and non-treated group and took only saline. Group (2) was infected at 2 days of age with isolates of *E. coli* O157 and did not receive any treatment. Group (3) was infected as in group (2) and treated with 3ml/ kg of *H. illucens* fat orally. Group (4) was infected as in group (2) and treated with florfenicol orally at a dose of 3 ml/ kg for 3 days. Group (5) was infected as in group (2) and treated orally with both florfenicol and *H. illucens* fat as mentioned.

2.4. Body Growth performance

The first body weight was recorded on 1st day. The infection occurred on the 2nd day of age, then the body weight was determined at 4, 7, 10, and 14 days of age. The body weight gain calculation (BWG, g/bird) was calculated as follows: BWG = W2 -W1, where W2 is the final body weight at the intended period, and W1 is the initial body weight in the same period.

2.5. Histopathology study

Autopsy specimens of the intestine were taken from each sacrificed tested chick 5 days post-treatment. Specimens were collected from the intestine and fixed directly in formalin 10 % for histopathological assessment.

2.6. Statistical analysis

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS Inc. Released, 2009) applied to data to determine any variables differed between groups, according to Snedecor and Cochran (1989). Means comparisons were conducted by one-way ANOVA and subsequent Duncan's multiple range test (Duncan, 1955). Probability values of less than 5% ($P < 0.05$) were considered significant.

3. RESULTS

The body growth weight and weight gain were evaluated every 3 days, and the data were recorded in tables (1 and 2) and figures (A and B). The present results revealed a significant increase in body weight and body weight gain of the groups treated with HIO compared to the control positive group. There is a beneficial effect in the group treated with both HIO and FFC when compared with either the negative control group (non-infected and non-treated) or with the infected non-treated.

Table 1 Effect of FFC (3ml/L for 3 days) and or HIO (3ml/kg) on body weight (gm) of broiler chickens infected with *E. coli*. Data are presented as Mean ±SEM, (n=10).

Groups	1 day-old	4 days-old	7 days-old	10 days-old	14 days-old
Control negative	39.4 ±0.8	86.6 ±2.06^a	173.1 ±4.19^b	231.7 ±3.13^b	318.2 ±9.22^a
Control positive	38.65 ±0.97	80.5 ±3.11^a	154.5 ±6.51^a	214.4 ±3.64^a	303.3 ±2.7^a
Treated with HIO	40.2 ±0.81	92.4 ±1.5^b	203.5 ±2.08^d	302.4 ±2.91^d	431.9 ±5.5^c
Treated with FFC	39.5 ±0.80	84.6 ±3.9^a	187.5 ±5.64^c	256.3 ±6.81^c	347.8 ±7.94^b
Treated with HIO+ FFC	40.6 ±1.00	97.8 ±1.99^b	205.2 ±4.80^d	312.4 ±6.6^d	445.7 ±8.36^c
F-calculated (significant level)	0.740 (0.570)	6.572 (0.001)	19.181(0.001)	76.160 (0.001)	84.219 (0.001)

a, b, c, d significant difference between different letters using Duncan Multiple Range Test for Comparative of Means at $P < 0.05$.

Table 2 Effect of FFC (3 ml/ L for 3 days) and HIO (3 ml/ kg orally) on body weight gain (gm) of broiler chickens infected with *E. coli*. Data are presented as Mean ±SEM, (n=10).

Groups	4 days-old	7 days-old	10 days-old	14 days-old
Control negative	47.2 ± 1.34^a	86.5 ± 3.9^a	58.6 ± 4.82^a	86.5 ± 9.73^a
Control positive	41.85 ± 2.43^a	74 ± 7.79^a	59.9 ± 4.3^a	88.85 ±2.74^a
Treated with HIO	52.2 ± 1.41^b	111.1 ± 2.39^b	98.9 ± 3.6^b	129.5 ± 5.31^b
Treated with FFC	45.1 ± 2.74^a	102.9 ± 5.56^b	68.8 ± 5.3^a	91.5 ± 4.83^a
Treated with HIO+ FFC	57.2 ± 1.21^b	107.4 ± 4.4^b	107.2 ± 5.99^b	133.3 ± 4.73^b
F-calculated (significant level)	5.284 (0.001)	9.325 (0.001)	21.938 (0.001)	15.493 (0.001)

a, b, c, d significant difference between different letters using Duncan Multiple Range Test for Comparative of Means at $P < 0.05$.

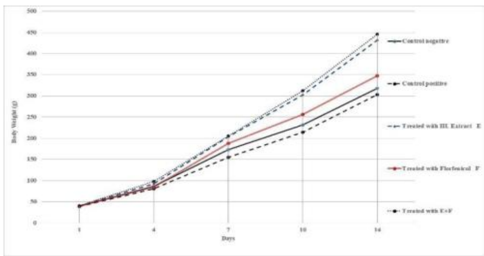

Figure (A): Effect of FFC (3 ml/L for 3 days) or HIO (3 ml/L) on body weight (gm) of broiler chickens infected with *E. coli.* Data are presented as Mean ±SEM, (n=10).

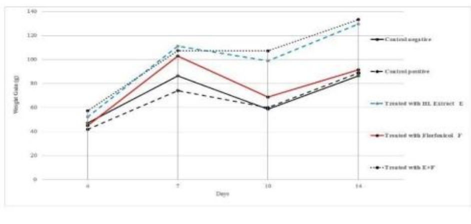

Figure (B): Effect of FFC (3ml/L for 3 days) or HIO (3 ml/L orally) on body weight gain (gm) of broiler chickens infected with *E. coli.* Data are presented as Mean ±SEM, (n=10).

Histopathological findings

Microscopic examination of the small intestine of control infected chicks showed severe degeneration, necrosis, and detachment of the mucosal epithelium with heavy proprial inflammatory cells infiltration (Figure 1). Concerning the small intestine of control infected chicks that were treated with the extract, generally good restoration was observed, with near to normal appearance of the intestinal epithelium, only scars inflammatory cells infiltrating the proprial layer, and mild degeneration of few mucosal cells (Figure 2) The small intestine of control infected chicks that treated with Florfenicol showed a decreased intensity of inflammatory reaction represented by restoration of most of the mucosal epithelium with a moderate degree of necrosis and detachment as well as moderate inflammatory cells infiltration (Figure 3). Regarding the combined treatment with the extract and Florfenicol, the small intestine of those chicks showed a moderate degree of vacuolar degeneration and necrosis of the mucosal epithelium and mild inflammatory cells infiltration (Figure 4).

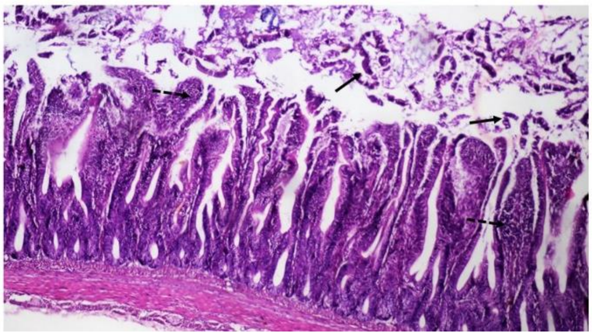

Figure (1): Small intestine of control infected chick showing severe necrosis and detachment of the mucosal epithelium (arrow) with heavy inflammatory cells infiltration (dashed arrow). (H&E, X200).

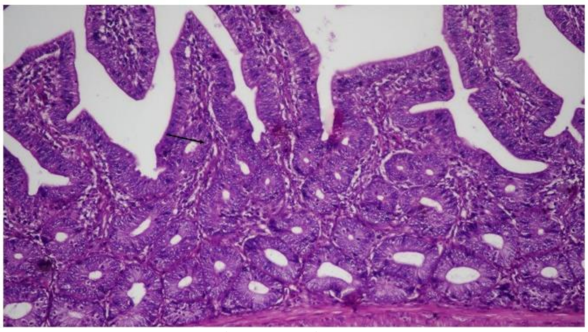

Figure (2): Small intestine of control infected chick that treated with the extract showing near to normal appearance of the intestinal epithelium with scars inflammatory cells infiltrating (arrow) the proprial layer. (H&E, X200).

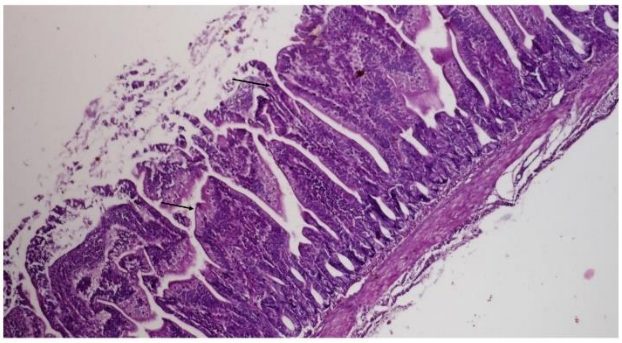

Figure (3): Small intestine of control infected chick that was treated with Florfenicol showing a decreased intensity of inflammatory reaction represented by restoration of most of the mucosal epithelium (arrow) with a moderate degree of necrosis and detachment (short arrow) and moderate inflammatory cells infiltration (dashed arrow). (H&E, X200).

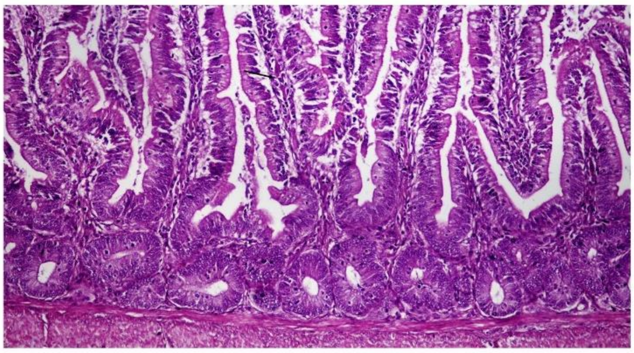

Figure (4): Small intestine of control infected chick treated with combined extract and Florfenicol showing moderate degree of vacuolar degeneration (arrow), necrosis (dashed arrow) and some sloughing (short arrow) of the mucosal epithelium and mild inflammatory cells infiltration. (H&E, X200).

4. DISCUSSION

Related to the present study, we showed that there is a significant increase in body weight and body weight gain in infected groups treated with H.illucens fat, followed by the FFC-treated group. According to Fortuoso et al. (2019), the improvement of performance in the broilers fed *H. illucens* fat may be due to the relatively high levels of MCFA present in *H. illucens* fat, leading to an improvement of more than 11% in body weight gain and 6% reduction in FCR. This highlights That *H. illucens* demonstrated strong antimicrobial activity and growth promoter without toxicity (Fortuoso et al. 2019). There is a synergism between *H. illucens* fat and FFC-treated group, which showed a slight increase in body weight and body weight gain than other groups.

The histopathological examination in the present study showed that the group treated with *H. illucens* fat has longer intestinal villi than other groups. Much research has shown that LA and monolaurin's ability to enhance body performance, digestive and immune profile, and improve the GIT ecosystem (Mountzouris et al., 2010). LA and monolaurin have recently more importance for their antimicrobial property. The performance of GIT can be supported by monolaurin as increased BW by 4% and FCR by 12% (Çenesiz et al., 2020). LA showed improved body performance, histomorphological character, and decrease intestinal pathogens (Khosravinia et al., 2015). many studies have linked the benefit of LA with the increase in absorption of epithelial function in the upper gut. Intestinal cells can utilize monolaurin to generate energy and thus promotes the intestinal ecosystem. (Guillot et al., 1993). The addition of 3% MCFA to a broiler diet to replace part of soybean oil and animal fat has been shown to improve feed conversion efficiency (van der Hoeven-Hangoor et al., 2013). Genetically, there are 53 genes encoding apparent antimicrobial peptides present in the *H. illucens*, proven antibiotic activities when its extract is used in vitro at a MIC of 25 mg/mL. (Park et al., 2014; Vogel et al., 2018). Rats supplemented with LA diets showed a healthy GIT morphology with an increase in the mucous membrane, longer villi, shorter crypt, increased phospholipid/ protein ratio of jejunal mucous lipids, and enhanced membrane-bound enzyme activity (Takase et al., 1990). Spleen was significantly increased in weight by monolaurin; but other organ weights were not affected as reported in (Londok et al., 2018). The enhancement in the leg and breast muscles by supplementation with LA and monolaurin was linked with increased protein deposition in broilers, as total protein in serum was significantly increased. Many studies have also observed a positive performance impact of dietary inclusion of *H. illucens* extracts such as higher BW, BWG, or lower FCR in at least one phase during the experiment in broilers (Loponte et al., 2017; Khan et al., 2018; Dabbou et al., 2018; Gariglio et al., 2019). It has been stated that medium-chain fatty acids are absorbed more efficiently than long-chain fatty acids (Papamandjaris et al., 1998). MCFA in mammals has been shown their energetic utilization at high levels (Zentek et al., 2011). Additionally, effects on broiler gut health and performance may differ when fed either free or esterified MCFA. It has been shown in vitro that non-esterified lauric acid and capric acid, as well as monolaurin and monocaprin, are more effective in terms of antimicrobial activity than triglycerides (Kabara et al., 1972).

Some studies showed that adding fat rich in lauric acid and monolaurin to the diet can improve cell renewal and result in increased villi length. The enhancement in body weight gain and FCR of monolaurin may be due to an improvement in the activities of digestive enzymes, the process of digestion, and absorption (Yegani and Korver, 2008).

5. CONCLUSION

The emergence of resistant bacterial strains is a result of the extensive use of antibiotics. So, these microorganisms are no longer susceptible to currently available drugs. New natural antimicrobial agents are required to minimize bacterial resistant strains and maintain public health. This forces researchers to pursue novel antibiotics which not yet resistant to bacteria.

This study showed that MCFAs in *H. illucens* fat are natural antimicrobial fatty acids which able to promote the growth performance of broilers, improve their intestinal mucosal structure, enhance immune functions, and inhibited inflammation of broilers. They act as growth promotor has the potential to improve food safety. It is believed that bioactive components present in *H. illucens* fat can reduce the use of antibiotics which have withdrawal time and side effects on organs of birds, and improve weight gain and body performance of broilers.

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