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Assessment of in Vivo Antimicrobials Effect of some Medicinal Plants Seeds on Broilers Meat Keeping Quality

Mohamed Elkhatim (Corresponding Author)
Department of Meat Production and Technology, Faculty of Animal Production, University of Gezira, Elmanagil, Sudan
Email: abuelhadi@hotmail.com

Abdelmageed Hyder O. Abdalla
Department of Animal Production, Faulty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan

Omer H. Arabi
Department Basic Sciences, Faculty of Animal Production, University of Gezira, Elmanagil, Sudan

Babiker M. S.
Department of Poultry Production Faculty of Animal Production University of Gezira, Elmanagil, Sudan

Abdel Moneim E. Sulieman
Department of Food Engineering, Faculty of Engineering and Technology, University of Gezira, Wad Medani, Sudan

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Abstract

The aim of the study is to evaluate the in vivo antimicrobial effect of some natural products, that include seeds of: Black cumin Fenugreek, *Moringa olefera, Clitoria ternatea*, Sunflower and Vit E on broiler meat keeping quality. One hundred and five one day old male chicks were used. They were allotted to 7 treatments with 3 replications each in CRD design .The chicks were fed balanced rations supplemented with tested seeds and Vit E. In vivo antimicrobial effects of tested seeds were measured using the serial dilution method on chickens breast samples at fresh, - 4^{o}C and at – 20^{o}C for 30 days. The tested seeds had an *in vitro* antifungal and antimicrobial effect on Gram + ve and Gram -ve bacteria. The *in vivo* antimicrobial effects of tested seeds showed no significant at fresh and -20^{o}C storage conditions; however, there was a significant difference among treatments at – 4^{o}C-storage condition. Nevertheless, there was a significant difference among storage conditions. Generally, the results demonstrated that the tested seeds can be used as an *in vitro* antimicrobial natural source.

Keywords: Antimicrobial; Coliform; *Salmonella typhyi; Staphylococcus aureus*.

1. Introduction

Today, birds grow much faster, and reach higher market weights than ever before, not only because of the exceptional genetic improvement but also through the feed formulation and management practices. Feed additives of special interest in poultry feeds include enzymes, amino acids, pigments, minerals, vitamins and antibiotics [1]. Growth promoters, similar to chemical products, antibiotics, enzymes assume a functioning job in the production of poultry [2].

Antibiotics in sub-therapeutic levels have been used as feed additives for decades to control pathogen bacteria in the gut. However widespread use of antibiotics has led to the emergence of antimicrobial drug resistance organisms [3]. There for, due to the public concern about antibiotic residues in animal products and the potential evolving of antibiotic resistant bacteria, many countries banned their use as growth promoters. The ban forced on their utilization constrained the researchers to scan for characteristic choices that can be utilized for keeping up health and and enhancing performance of animals.

Herbs, spices and their extracts were already used thousands of years ago in Mesopotamia, Egypt, India China and old Greece, where they were appreciated for their specific aroma and various medicinal properties [4].

Feed supplements with growth promoting activity increase stability of feed and valuably impact the gastrointestinal ecosystem for the most part through growth inhibition of pathogenic microorganism's growth.

Herbs and flavors help to expand the obstruction of the creatures presented to various pressure circumstances and increment the assimilation of fundamental supplements, along these lines enhancing the development of the creatures, likewise they go about as antimicrobial operators by changing the qualities of cell films, and causing particle spillage, subsequently making microorganisms less destructive.

Herbs and spices help to expand the resistance of the animals exposed to various pressure circumstances and increase the assimilation of fundamental supplements, along these lines enhancing the growth of the animals, likewise they go about as antimicrobial agents by changing the characteristics of cell membranes, and causing ion spillage, subsequently microbes less virulent bacteria [5]. The objective of the present study is to evaluate the *in vivo* antimicrobial effect of selected natural products on broiler meat keeping quality.

2. Material and Methods

2.1. Experimental Birds

One hundred and five (105) one day old male broiler chicks (Ross308) strain were selected from a commercial broiler flock at Elbashair farm, Wadmedani. The chickens were divided into equal groups, a control group and 6 experimental groups, each group was subdivided into three group with 5 chicks each in a Completely Randomized Design (CRD).

2.2. Experimental Diets

The tested birds were fed a pre starter diet during the first week, then they received balanced starter diet (24% crude protein and 3100 kcal metabolisable energy (ME)/kg) during the next 3 weeks .Then a finisher rations were offered during the last 3 weeks , the control group receiving diet (1), feed based on iso nitrogenous iso caloric ration, and the experimental groups were fed a diet as same as the control diet supplemented with tested seeds at (5%) of the ration, and Vitamin E at (200mg/kg) of the ration as shown at Table (1) .

2.3. Management and data Collection

The chickens were offered quantity of food and free access to water. The chicken samples were taken from pectoral muscle. .Half of the samples were refrigerated at (- 4°C) and the other half were subjected to the freezing at (-20°C) for 30 days

2.4. Culture Media

The cultured media contains the required nutrients in the correct amount, suitable osmotic pressure and pH. Microorganisms were incubated in an atmosphere and temperature most suited to their metabolism [6].

Table-1. The ingredients percentage per treatment

Ingredients	Treatments						
	Fenugreek Seeds	Black Seeds	Clitoria Seeds	Moringa Seeds	Sun Flowers Seeds	Vit E	Control
S.G %	68.88	68.88	66.38	66.38	60.38	66.38	68.88
G.N.M %	14	5.8	5.8	5.8	5.8	5.8	14
M. Meal %	5	6	6.5	6.5	6.5	6.5	5
W.B %	5.8	8	10	10	16	10	5.8
S.C %	5	5	5	5	5	5	5
D. cal %	1.02	1.02	1.02	1.02	1.02	1.02	1.02
NaCl %	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Lysine %	1.17	1.07	1.15	1.09	1.15	1.14	1.17
Methionine %	0.47	0.45	0.48	0.46	0.48	0.49	0.47
Meth+Cys%	0.66	0.62	0.74	0.64	0.66	0.74	0.66

S.G: Sorghum, G.N.M: Ground Nuts Meal, M. Meal : Meat Meal ,W.B : wait bran, S.C : Super Concentrate, Dical : Di calcium NaCl: Sodium chloride

2.5. *In Vivo* Antimicrobial Activity Test

Microbial load on broiler meat was determined using serial dilution method. Mac-conkey agar (MC), Baird parker agar (BPA) and Salmonella chromogenic agar base (SA) media were used for identification of *E. coli*, *Staphylococcus aureus* and *Salmonella spp.*, respectively.

3. Results and Discussion

3.1. *IN Vivo* Antimicrobial Effects

3.3.1. *E. Coli* Detection

There were no significant differences among different treatments (tested seeds and vit E) at fresh condition when compared with the control. CLIS reduced the bacterial load by (40%). All seeds showed a positive effect on *E. coil* by lowering the bacterial load in broiler meat compared with the control (Table 2). The study was in agreement with Bölükbaşi, *et al.* [7] who found that BS (*Nigella sativa*) oil had strong potential for reducing *E. coli* in laying hens. However, Erener, *et al.* [8] found that dietary supplementation of black cumin seeds or its extract had no significant effect on coliform count in broiler meat. The results were also agreed with Subashini and Rakshitha [9]; Auwal, *et al.* [10] who reported that MORS seeds have antimicrobial effects on *E. coli* and with Abd El-Moez, *et al.* [11] who reported that moringa leaves had *in vivo* antimicrobial effects on *E. coli*. It has been stated that phenolic components were chiefly responsible for the antibacterial activities of essential oils [12]. However, Bakkali, *et al.* [13] suggested that the antibacterial properties of black cumin associated with the ability of its essential oil, which can destabilize membranes including mitochondrial membranes and also can disturb cellular integrity of bacteria and eukaryotic cells and result in cell death via necrosis and apoptos The essential oils (EO) at dose of 0.3 g/kg in mice infected with *E. coli* shows 100% inhibitory effect compared with mice who received saline. In contrast to our findings, many authors found that fenugreek seed extract had no any antibacterial effect [14-16].

The results also showed that Vit. E had antimicrobial effect on *E. coli*, which disagreed with Coetzee and Hoffman [17] who reported that aerobic plate counts were not affected regardless of the level of vitamin E supplementation. On the other hand there was a significant differences (P≤0.05) among treatments in *E. coli* at (-4°C) Storage condition. As the table shows that treatments of BS, MORS, FENS and Vitamin E, had a significant differences (P≤0.05) when they compared with the control treatment. SFS reduced the bacterial load by (33.33%). Storage conditions (-20°C) shows no significant differences in *E. coli* content, there was no growth of bacteria and that may be attributed to the fact that bacteria was damage by the frizzing. The results shows that there was a significant differences (P≤0.05) among the storage conditions. The highest bacterial count was shows in the storage condition (- 4°C) followed by the fresh condition and storage condition (-20°C) .There was no significant difference among the different treatments and that may be attributed to the presence of *E. coli* in different conditions or maybe due to the highly nutrients content of broiler meat.

Table-2. Effects of supplementation of broiler chickens rations with different seeds and the effect of different storage conditions of storage on *E. coli* in broilers meat

Storage Conditions	Treatments							Means
	BS	SFS	CLIS	MORS	FENS	Vit E	Control	
Fresh	1.00^{c} (0.00)	1.00^{c} (0.00)	1.50^{bc} (0.50)	1.00^{c} (0.00)	1.00^{c} (0.00)	1^{c} (0.00)	2.50^{ab} (1.50)	1.29^{b}
- 4 ◦c	2.50^{ab} (1.50)	1.00^{c} (0.00)	2.50^{ab} (1.50)	2.50^{ab} (1.50)	2.00^{abc} (1.00)	3.00^{a} (2.00)	1.50^{bc} (0.50)	2.14^{a}
-20 ◦c	1.00^{c} (0.00)	1.00^{c} (0.00)	1.00^{c} (0.00)	1.00^{c} (0.00)	1.00^{c} (0.00)	1.00^{c} (0.00)	1.00^{c} (0.00)	1.048^{c}
Means	1.5	1	1.68	1.5	1.33	1.78	1.68	

The data between brackets was the original data.
BS: Black seeds, **SFS**: Sun flowers seeds, **CLIS**: Clitoria seeds, **MORS**: Moringa seeds, **FENS**: Fenugreek seeds, and **Vit E:** Vitamin

3.3.2. *Salmonella Typhi* Detection

The presence of *Salmonella typhy* in different treatments indicated that there was no significant differences among treatments stored at the fresh condition, however, BS shows the highest bacterial count at the fresh condition as Table (3) show. The result disagreed with Salem [18] and Mori, *et al.* [19] who reported that BS has an antimicrobial effect on multi-antibiotic resistant organism including gram-positive and gram-negative bacteria, respectively. SFS effects in the present study results was disagreed with Subashini and Rakshitha [9] who evaluated the antimicrobial activity of methanolic extract of SFS. On the basis of the results of antibacterial activity analysis, the seeds extract shows high sensitivity to *Salmonella typhi*, the results were disagreed with study of Mhaskar, *et al.* [20] who reported that crude extract from CLIS seeds shows antibacterial activity against *S. typhi*. This result was disagreed with that of Auwal, *et al.* [10] and Jabeen, *et al.* [21] who reported that MORS seeds had antimicrobial effects on *Salmonella typhi* and with Abd El-Moez, *et al.* [11] who reported that moringa leaves had *in vivo* antimicrobial effects on *Salmonella typhi* and with Prashith, *et al.* [22] who used moringa stem. Hot water extract of fenugreek had no any effect on *Helicobacter pylori* [16]. The results agreed with Marzougui, *et al.* [15] who reported that petroluim ether and methanolic extract had no effect on *S. Typhimurium* and with Dash, *et al.* [23] who reported that SFS had effects against *Salmonella typhi.*

Table-3. Detection of *Salmonella typhy* in broiler chicken meat fed on rations supplemented with different seeds and stored at different conditions

Storage Conditions	Treatments							Means
	BS	SFS	CLIS	MOS	FENS	Vit E	Control	
Fresh	3.5 (2.5)	1.00 (0.00)	1.5 (0.50)	1.00 (0.00)	1.00 (0.00)	2.00 (1.00)	1.00 (0.00)	1.57
- 4°C	1.5 (0.50)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	2.00 (1.00)	1.00 (0.00)	1.21
-20°C	1.5 (0.5)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	2.00 (1.00)	1.00 (0.00)	1.24
Means	2.17^{a}	1.00^{b}	1.17^{b}	1.00^{b}	1.00^{b}	2.00^{b}	1.00^{b}	

The data between brackets was the original data.
BS: Black seeds, **SFS**: Sun flowers seeds, **CLIS** : Clitoria seeds, **MORS** : Moringa seeds, **FENS**: Fenugreek seeds, and **Vit E:** Vitmin E

There was no significant difference among treatments, when Vit. E was used as antimicrobial agent. There was high bacterial count which agreed with Coetzee and Hoffman [17] who reported that aerobic plate counts regardless of the level of vitamin E supplementation did not influence the bacterial load. The results shows no significant differences among storage conditions and the fresh condition shows the highest bacterial count followed by (-20°C) and then (- 4°C). There were no significant differences among the different treatments except BS treatment when compared with control treatment. That result may be attributed to the type of BS used in the experiment.

3.3.3. Staphylococcus Arueus Detection

There was no significant difference (P>0.05) among treatments in the fresh condition, the highest bacterial count was found in MORS treatment. There was no significant difference among treatments in the storage conditions (-4°C), the highest bacterial count was found in control treatment. There was no significant difference among treatments in the storage conditions (-20°C), the highest bacterial count was found in vitamin E and control treatments (Table 4).

There was no significant differences (P>0.05), among different storage conditions. However the highest bacterial count found in storage condition (- 4°C). There was significant differences.

(P<0.05) among treatments. The highest count found in the control treatment followed by Vitamin E treatment ,while BS ,SFS ,CLIS ,MORS and FENS treatments shows no significant difference (P>0.05) among them. The results of reducing *S. aureus* total count by using the tested seeds may be due to the phytochemicals content in them (Table 4).

Table-4. Detection of *Staphylococcus spp*. in broiler chickens meat

Storage Conditions	Treatments							Means
	BS	SFS	CLIS	MORS	FENS	Vit E	Control	
Fresh	1.50 (0.50)	1.500 (0.50)	1.00 (0.00)	2.50 (1.50)	2.00 (1.00)	1.00 (0.00)	1.50 (0.5)	1.57 [b]
- 4°C	2.00 (1.00)	2.00 (1.00)	1.533 (0.533)	1.00 (0.00)	1.50 (0.5)	4.33 (3.33)	6.50 (5.50)	2.70 [a]
- 20°C	1.00 (0.00)	1.50 (0.50)	1.00 (0.00)	1.00 (0.00)	1.50 (0.50)	2.67 (1.67)	2.67 (1.67)	1.62 [b]
Means	1.50 [c]	1.66 [c]	1.18 [c]	1.50 [c]	1.67 [c]	2.67 [b]	3.56 [a]	

The data between brackets was the original data.
BS: Black seeds, **SFS**: Sun flowers seeds, **CLIS** : Clitoria seeds, **MORS** : Moringa seeds, **FENS**: Fenugreek seeds, and **Vit E:** Vitmin E

The results were in agreement with Salman, *et al.* [24] who demonstrated that BS oil was active against sensitive and multi-drug resistant strains of *Staphylococcus aureus* and with Hosseinzadeh, *et al.* [12] used essential oil (EO) at dose of 0.3 g/kg in mice infected with *S. aureus.* The treated mice shows 100% inhibitory effect compared with the control mice who received saline. The result was also, in agreement with Subashini and Rakshitha [9] who reported that polar oil from SFS shows antimicrobial activity against *Staphylococcus aureus*, the results were disagreed with study of Mhaskar, *et al.* [20] reported that crude extract of CLIS shows anti-bacterial activity against *S. aureus.* The results were in agreement with Auwal, *et al.* [10] who reported that MORS had antimicrobial effects and concluded that the minimum inhibitory concentration of the extract for all sensitive isolates is 100 mg/ml and 50 mg/ml as minimum bactericidal concentration of the extract on *Staphylococcus aureus.* The study was agreed with the result of Elnour, *et al.* [25] reported that the petroleum ether and Methanolic extract of FENS had antimicrobial effects against *S. aureus.* The results of the present study were disagreed with Coetzee and Hoffman [17] who reported that aerobic plate counts regardless of the level of vitamin E supplementation and with Coetzee and Hoffman [17] who reported that vitamin E supplementation did not influence the bacterial load.

4. Conclusions

The *In vivo* use of the different seeds exhibited a positive antimicrobial effects on the tested harmful microorganism, *E. coli, Salmonella typhi and Staphylococcus aureus.* The bacterial load of these bacteria was reduced 1in the meat) with non- significant difference ($P \geq 0.05$) among them on fresh samples. However, there was a significant difference ($P \leq 0.05$) among different seeds at (- 4°C) storage condition, while there was no growth of bacteria at (-20°C) storage condition. It is recommended to identify the active ingredients and therepatice agent which can be used as bacteriostatic and bacterocids.

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