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Effects of Dietary Inclusion of Different Levels of Roselle (*Hibiscus sabdariffa*) Seeds on Performance and Gut Bacterial Load of Broilers

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Abstract

The aim of the study is to assess the effects of dietary Inclusion of different Levels of Hibiscus seeds on performance, gut health and fatty acid profile of broilers. The Roselle (*Hibiscus Sabdariffa*) seeds were incorporated in broiler rations at levels of (k0%, k3%, k5% a n d k7%) the study was run in 6 weeks period using four experimental diets. The experimental diets were formulated iso-energetic and iso-nitrogenous to meet or exceed the requirements of broilers according to (NRC, 1994). The experiment was carried out in a complete randomized design (CRD). A total of 120 one day-old (Ross308) broilers were allocated to the four treatments (K0 (control), K3, k5 and k7) and each treatment was further divided randomly into three replicates with ten birds each Feed consumption (FC), weight gain(WG) and feed conversion ratio (FCR) were recorded. At the end of the experiment, two birds from each experimental unit were selected according to their closed to average weight and slaughtered. Carcass weight, weights of some internal organs and cuts were measured. Total bacterial load and some pathogenic bacteria were investigated in Gizzards and cecum of birds to assess the antimicrobial effects of Roselle seeds inclusion in broilers rations on bird's gut heath. All data was statistically analyzed using ANOVA. Result showed that there were no significant differences (P>0.05) in broilers performance but numerically decrease in feed intake, weight gain and increase feed conversion with the increase of Roselle seeds in the diets and there was no effects on the mortality rate of birds. The weights of internal organs results showed that there were no significant differences among treatments except kidney weight. Bacterial account was highly significant differ ($p \leq 0.05$) and without effect on total bacterial load due to the inclusion of Roselle seeds in broiler rations. While there no presence significant differences on *E. Coil* and *Staphylococcus aureus* presence in gizzard and cecum samples among treatments. Dietary Inclusion of different Levels of Hibiscus Seeds had no deleterious effect on broiler performance or total bacterial, there for it recommended to include Roselle seeds 3% and do not up to 7% in broiler diets.

Keywords: Roselle seeds, performance, gut bacterial load, broilers.

Introduction

Poultry meat is an important food in human nutrition which characterized by moderate energy content, highly digestible proteins of good nutritional quality, unsaturated lipids, fat-soluble and B-complex vitamins as well as minerals make poultry meat a valuable food. (Donma *et al*., 2017) [17]. The development of poultry industry in the world is many problems mainly the feed, which represents about 75% of the total cost of production due to demand competition between human and especially poultry, scarce in crop production and human population growth. Protein and energy are the costliest component in poultry fed, particularly the plant protein. (Mukhtar, 2007) [31]. This challenge has resulted in great effort is needed to be introduced from researchers for explore alternative, cheap, locally and available nonconventional ingredients without negatively influencing the performance traits and meat quality traits of the birds so less competition (Mohamed, 2018) [27]. Preventive measures against risky aspects of the matter should be developed. Since the use of antibiotics leads to the development of antibiotic resistant pathogens, antibiotics must be replaced by herbs and spices with growth promoting effects, antimicrobial properties, and other health related useful to solve the problem. (Donma, 2017) [17]. A promising alternative crop that can be exploited for this purpose is Roselle (*Hibiscus Sabdariffa*). Roselle seeds are known as highly nutritional, antibacterial, antifungal and anti-parasitic actions (Singh *et al*., 2017) [40]. Antibacterial effects of this plant extract against *Escherichia coli, P. aeruginosa and S. aureus* suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhea in man and skin diseases (Rogger *et al*., 1990) [38].

Roselle is a medicinal plant with a worldwide is rich in organic acids such a citric, malic, and tartaric and allo-hydroxycitric acids. The plant is also known for its Beta carotene, vitamin C, protein and total sugar. Roselle, having various medically important compounds called photochemical, is well known for its nutritional and medicinal

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(Arvind, 2011) [9]. Roselle seeds nutritionally is containing high amounts of protein, fiber and minerals (phosphorus, calcium and magnesium). According to (Manita–Mishra, 1999) [24] the number of compounds have been isolated and characterized from Roselle including flavonoids, anthocyanin‟s, triterpernoids, steroids and alkaloids. Many studies have been done for investigating on the raw seeds contain such as hibiscin, hydroxyl flavones and tannins as anti-nutritional factors (Mahadevan *et al*., 2009) [23]. The most common anti-nutritional factors such are total phenols, tannins and phytic acid and these have had adverse effects on the health and performance of animals (Diarra *et al*., 2011) [16]. Traditionally in Sudan, Roselle has been useful for respiratory tract infection, tuberculosis, pneumonia, and laryngitis to kill the microbes (Aziz *et al*., 2007) [11].

Hibiscus sabdariffa commonly named as “red sorrel” or “rosella”, in English, in Sudan is called Karkadeh and in Latin Roselle jamica, the scientific name is *Hibiscus sabdariffa* L, is a member of Malvaceae family. Hibiscus is one of the short daytime plants for flowering. (Singh, *et al*., 2017) [40]. The hibiscus origin is uncertain, while others believe that its original country is West Africa (Cobley, 1975) [15], India (Abu-Tarboush *et al*., 1997) [4], Malaysia and Saudi Arabia Abd Aziz *et al*., 1985) [1]. Turkish (Mclean, 1973) [26] Sudan (Hassan, 2010) [20]

The seeds also have been used as a source of protein for broiler chicken production (Owosibo *et al*., 2017) [35]. Roselle seed cake could be used up to 30% of the diet without exhibiting any deleterious effect on broiler performance (Mohammed and Idris 1991) [28]. However, (Salih *et al*., 1990) provided evidence that Roselle seed meal can replace groundnut and sesame meals without significant reduction in feed intake, weight gain, feed conversion ratio (FCR), mortality and dressing percentage of broilers. Mohammed *et al.,* (2022) [29] Roselle seeds can be used as the protein source in broiler diets up to 15% without any adverse effects..

Staphylococcus aureus is one of the main causes of human infections. It can cause diseases ranging from minor infections such as pimples and boils to serious systemic fatal infections (Evans and Brachman, 1991) [19]. *Escherichia coli is* a Gram negative rod (bacillus) in the family Enterobacteriaceae. Most *E.coli* are normal commensals found in the intestinal tract. Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such as exotoxins. Many researchers reported that *Hibiscus sabdariffa* exhibit Antibacterial effects against *Escherichia coli*, and *S. aureus* like Olaleye (2007) and Ahsan *et al*., (2018) [5] Al-Hashimi (2012) [6] (Babayi *et al*., 2004) [12]. Suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhea (Rogger *et al*., 1990) [38] The purpose of this study was effects of dietary inclusion of different levels of roselle (*hibiscus sabdariffa*) seeds on performance and gut bacterial load

Materials and Methods

Experiment Site: This experiment was carried out at Extension and Rural Development Centre, Faculty of Animal Production, University of Gezira Elmanagil, Gezira State, Sudan.

Birds Housing and Management: The birds were kept in open sided house situated on east west direction making the long axis facing North and south wind. The house was cleaned, burned and sprayed with Cypermethrin 10% E.C. (2ml/l). The feeder and drinker were also washed and disinfected three days before the arrival of the birds and they were cleaned daily throughout the experimental period. The house was divided into 12 Pens. The dimensions of each pen were (100cm) length, (100) width was (100 cm) and (90 cm) height. Each pen contained ten chicks, one metallic tubular feeder and one plastic drinker. Fresh water and feed were supplied throughout the experimental period. The drinkers were cleaned daily. The birds were exposed to natural light during the day and the light bulb lamp during the night. The lamp of 60 watts were used as brooder to supply heat to the birds during the first two weeks by hanging them at 30 cm height, and then hanged at one-meter height to supply light during the night. The electrical appliance was checked and switched on few hours before the arrival of the birds. The house was covered with plastic curtains to remained warmth. One hundred and twenty (120) one-day old Ross308 classic strains of broiler chicks were selected from commercial broiler flock in the farm. The birds were mixed sexes and the average weight for experimental birds about (45 ±2) gram. To protect birds against Newcastle disease (N.D.) and infectious bronchitis (I.B.) dual dose at seven and twenty-one days of age using I.B. Colon 30. The birds were vaccinated against Gambro using Gumbo Best vaccine at fifteen and twenty-eight days old. All vaccines were offered via drinking water.

Experimental Diet

Collection and Preparation Roselle Seeds

Roselle seeds were collected from Elmanagil market. Roselle seeds were cleaned and milled by electrical miller, and sample of Roselle seed flour (RSF) was taken for the Proximate analysis table (1). According to the result of proximate analysis, four experimental diets were formulated to meet the requirements of broiler chicks (NRC, 1994) [34].

Table 1: Proximate analysis of Roselle seeds

Parameters%	Moisture%	Dry mater%	Crude protein%	Ash%	Ether extract %	Crude fibre%	NFE%
Roselle	7.4	92.6	29.67	4.4	16.67	15.27	26.06

Table 2: Chemical Analysis and Nutrients composition of rations containing deferent levels of Roselle Seeds

Parameters	Treatments			
	K0	K3	K5	K7
Dry matter (%)	96.50	94.50	95.50	96.75
Crude protein (%)	22.75	22.85	22.90	22.95
E.E (%)	3.35	3.25	3.35	3.30
NFE (%)	61.50	58.60	59.35	61.10
Crude fiber (%)	3.95	4.15	4.05	4.25
Ash (%)	4.75	5.65	4.05	4.25

Table 3: Composition of experimental diets (% As Fed) containing different levels of Roselle Seeds during starter period (1-3weeks).

Ingredients	Treatments			
	Control 0%	Karkade 3%	Karkade 5%	Karkade 7%
Sorghum	57.88	57.18	56.16	55.09
Ground nut meal	34.50	32.43	31.40	30.40
Wheat bran	0.10	0.00	0.00	0.00
Karkade seed	0	3	5	7
Super concentrates	5.00	5.00	5.00	5.00
Di calcium	0.72	0.72	0.72	0.72
Grits	0.30	0.30	0.30	0.30
Na cl	0.25	0.25	0.25	0.25
Lysine	0.50	0.50	0.50	0.50
Methionine	0.10	0.10	0.10	0.10
Vegetable groundnut Oil	0.40	0.27	0.32	0.39
Premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated Analysis				
Protein %	22.91	22.83	22.87	22.92
ME(Kcal/kg	3197.64	3190.38	3190.03	3198.64

[1]Super concentrate contained 37% protein, 10% Moisture, 4% Fat, 28% Ash, 7% fiber.
[2]vitamin = mineral premix provided the following per kilogram of diet: vitamin (retinyle acetate), 10.000IU; cholecalciferol, 2.500IU;" tocopheryl acetate, 60 mg; mendione sodium bisulfide complex, 15mg; thiamine hydrochloride, 2 mg; riboflavin, 8 gram pyridoxine hydrochloride, 4 mg; cyanocobalamin., 04 mg; pantothenic acid 15 mg; nicotinic acid, 40 mg folic acid 1.5 mg; biotin, 2 mg; choline chloride, 200mg; iron, 50 mg; manganese, 50 mg; copper, 10 mg; zinc, 50 mg; calcium 352 mg; iodine, 1.46 mg; cobalt. 5 mg; selenium. 2 mg; values and Metabolizable energy were calculated according to (Suleiman and Mabrouk 1999).

Table 4: Composition of experimental diets (%As Fed) containing different levels of Roselle Seeds during Finisher period (3-6weeks).

Ingredients	Treatments			
	Control 0%	Karkade 3%	Karkade 5%	Karkade 7%
Sorghum	57.88	57.18	56.16	55.09
Ground nut meal	26.50	24.70	23.40	22.40
Wheat bran	8.10	7.50	7.82	7.89
Karkade seeds	0	3	5	7
Super concentrates	5.00	5.00	5.00	5.00
Di calcium	0.72	0.72	0.72	0.72
Grits	0.30	0.30	0.30	0.30
Na cl	0.25	0.25	0.25	0.25
Lysine	0.50	0.50	0.50	0.50
Methionine	0.10	0.10	0.10	0.10
Vegetable groundnut Oil	0.40	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated Analysis				
Protein %	20.57	20.53	20.50	20.56
ME(Kcal/kg	3229.02	3233.51	3231.51	3228.19

[1]Super concentrate contained 37% protein, 10% Moisture, 4% Fat, 28% Ash, 7% fiber.
[2]vitamin = mineral premix provided the following per kilogram of diet: vitamin (retinyle acetate), 10.000IU; cholecalciferol, 2.500IU;" tocopheryl acetate, 60 mg; mendione sodium bisulfide complex, 15mg; thiamine hydrochloride, 2 mg; riboflavin, 8 gram pyridoxine hydrochloride, 4 mg; cyanocobalamin., 04 mg; pantothenic acid 15 mg; nicotinic acid, 40 mg folic acid 1.5 mg; biotin, mg; choline chloride, 200mg; iron, 50 mg; manganese, 50 mg; copper, 10 mg; zinc, 50 mg; calcium 352 mg; iodine, 1.46 mg; cobalt. 5 mg; selenium. 2 mg; values and Metabolizable energy were calculated according to (Suleiman and Mabrouk 1999).

Experimental Deign

The experimental period was divided into two periods on age bases. The first phase is starter (1-3 weeks) and finisher (4-6 weeks). In completely randomized design (C.R.D), four dietary inclusion of Roselle seeds 0% (k0), 3% (k3), 5% (k5) and 7% (k7) were adopted as treatments and replicated three times with ten birds each. The experimental diets were formulated iso-energetic and iso-nitrogenous to meet or exceed the requirements of the Roselle according to (NRC, 1994) [34]. Table (2) and (3) showed the diets composition of starter and finisher periods. The birds had free access for feed and water throughout the experimental period.

Data Collection

Performance

Measurements were taken weekly for life body weight, feed intake, feed conversion ratio

Slaughter Performance

At the end of the experimental period two birds from each experimental unit (replicate) were selected according to their closed average weight to their respective group. Before birds slaughtering they were fasted overnight. They were slaughtered according to the Islamic traditions by Jugular veins serving. The slaughtered (scaled) were dipped into hot water (70ºC-80ºC), manually scalded (de-feathering) and eviscerated. The head and legs were removed and hot carcasses weighted was obtained and then stored one bird at 1Cº. Each other bird was eviscerated. Also, the internal organs (liver, kidney, heart, lungs, gizzard, glandular stomach, abdominal fat, pancreas and the length of intestine) were dissected out from the experimental birds. The organs were weighed in grams.

Chemical Composition of Roselle Seeds

Proximate Analysis of Roselle Seeds

Roselle seeds samples were subjected to proximate analysis at laboratory of Animal nutrition for the International Laboratory Centre in Soba. Dry matter, (D.M.), crude protein (C.P.), fat, crude fiber (C.F.) and ash content were determined according to (AOAC, 2005) accordingly, were included to form experimental diets. Table 1.

Microbial Analysis

After slaughtering and the bird was eviscerated then collected fresh Gizzards and Cecum sample, weighted and chilling. Instruments such as loops; spoons were sterilized by direct flaming. Hot air oven (160˚C-170˚C) was used for serialization of glassware such as Petri dishes, pipettes, tubes and flask. Autoclaving was used for sterilization of media and distilled water, the exposure time were 15-20 minutes at 121˚C under 15 pounds/inch

Culture Media

The cultured media contains the required nutrients in the correct amount, suitable osmotic pressure and pH. Microorganism were incubated in an atmosphere and temperature most suitable to their metabolism (Cheesbrough, 2000) [14].

Media Preparing Steps: All media were prepared according to the manufactures instruction as follows: the powder was weighed, dissolved in distill water and heated, sterilized by using autoclave and dispensed in petri dishes.

Total Viable Count of Bacteria: A weight of 0.1ml from10^{-2} was placed in serial Petri dishes then melted plate count agar was added, after solidification plate was inverted. All cultured media were incubated at 37˚C for 24 hours.

Techniques Used for Identification: Ten grams from each sample were weighed by a sensitive balance, then dissolved with 100 ml sterile distill water in sterile test tube. All the samples were cultured in Mac Conkey, Manitol, S agar and Potato dextrose agar.

Serial Dilution (10^{-1}, 10^{-2}) of the Sample

One ml of the dissolved sample was added to tube No. 1 which contains 9 ml of distilled water then serial dilution was made.

Gram Stain: The gram stain was used to identify the bacteria according to morphology (bacilli and cocci) and according to the Gram reaction (Gram + ve and Gram –ve).

Statistical Analysis

Experimental data are presented as mean values ± standard errors of the mean ±St. statistical analysis was carried out by using the spss 2010 program package. The significance of the differences among the groups has been determined by Duncan's multiple range Tests (Petric and Watson, 1999) [36].

Result and Discussion

Effect of Dietary Inclusion of Hibiscus Seeds on Broiler Performance during Starter Period (1-3Weeks)

Table (5) shows the effect of dietary inclusion of different dietary levels of Roselle used by control k0%, k3%, k5% and k7% respectively on broiler performance. The results showed that there were no significant ($p>0.05$) differences in all investigated parameters (Weight gain, feed consumption and feed conversion ratio).

Table (5) showed the amount of dietary levels of feed consumed in the starter period was not significantly affected by the levels of added hibiscus seeds, although there was no significant ($p>0.05$) difference among the average levels of hibiscus seeds and the control group 0%. The largest amount of feed was consumed by birds fed with diet containing hibiscus seeds level 3% (602.44g), and the lowest amount of feed was consumed by birds fed with diet containing hibiscus seeds used by 7% (589.18g). The weight gained of broiler chickens in the starter period was not significantly ($p>0.05$) affected by the levels of added hibiscus seeds, the greatest weight gain was reported with birds fed on 3% (359.33g), The birds fed on diets containing 7% (331.39g) had the lowest weight gain value. The levels of addition hibiscus seeds did not significantly ($p>0.05$) affect the rate of food conversion in the starter period. The best feed conversion ratio values were reported with 5% and7% for both, but the table show this trait decrease compared to feed conversion ratio number was the best in 0% and 3% treatments (1.63) for both, followed by 5% and 7% treatments (1.74) the both. The current results agreed with the findings of Mukhtar, (2007) [31]. Abu Elgasim *et al*., (2008). (Yagoub and Abdallah, 2007) and (Kwari *et al*., 2011) [21] who reported that when broiler fed by diets contained Roselle that feed intake and body weight gain decreased as the level of Roselle seed increased and dis agree with feed conversion. Also agree to (Mateos *et al.,* 2012) [25] reported higher crude fiber contents of the Roselle seed based diets, voluntary feed intake was not depressed. It has been demonstrated that the inclusion of moderate amounts of different fiber sources in the diet improves digestive organ development and increases HCl, bile acids, and enzyme secretion.

Table 5: Effect of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broilers Performance during Starter Period (1-3 Weeks)

Parameters	Treatment				S.E	Sig
	K0%	K3%	K5%	K7%		
Feed consumption (g)	616.83	602.44	596.18	589.18	73.58	N.S
Weight gain (g)	370.17	359.33	335.11	331.39	33.80	N.S
Feed conversion ratio	1.63	1.63	1.74	1.74	0.06	N.S

NS= not significant

Effect of Dietary Inclusion of Different Level of Hibiscus (Karkade) Seeds on Broiler Performance during Finisher Period (4-6 Weeks)

Table (6) showed that the broiler performance fed varying dietary levels of control (k0%) and treatment (k3, k5 and k7) Roselle as a source of protein during finisher period. The results showed that there were no significant (p>0.05) differences in all investigated parameters. Although feed consumption, weight gain and feed conversion ratio was deferring numerically without significant differences. Table (6) also showed the mount of dietary levels of feed consumed in the finisher period was not significantly affected by the levels of added hibiscus seeds, although there was no significant (p>0.05) difference among the average levels of hibiscus seeds and the control group 0%. The largest amount of feed was consumed by birds fed with diet containing hibiscus seeds used by3%, compared to amount consumed 5% and the lowest amount of feed was consumed by birds fed with diet containing hibiscus seeds used by 7%. The weight gained of broiler chickens in the finisher period was not significantly (p>0.05) affected by the levels of added hibiscus seeds the greatest weight gain was reported with birds fed on 3%, compared to weight gain was reported with birds fed on 5%, The birds fed on diets containing 7% had the lowest weight gain value. The levels of added hibiscus seeds did not significantly (p>0.05) affect the rate of food conversion in the finisher period. The best feed conversion ratio values were reported with 7%, compared to feed conversion ratio values 5% and the lowest feed conversion ratio values diet containing hibiscus seeds used by 3%. There was a gradual decrease in weight gains the inclusion of raw Roselle seed in feed increased. The decrease in weight gain could be attributed to the effects of raw Roselle seed (Wang *et al*., 1999) [41]. It could also be due to the fact that Roselle seed has a lot of anti-nutritional factor such as phytate, tannins, saponins, trypsin and hydrogen cyanide (Qlogundudu and Obi, 2005; Abu-Tarboush and Ahmed 1997) [37, 4] which aided decrease in feed consumption as the concentration of raw Roselle seed meal progressively increase (El-Adawy and Khalil, 1994) [18] and (Mukhtar, 2007) [31]. Kwari *et al*., 2011) [21] reported that Roselle seeds contains11.98% insoluble fiber which decreases feed consumption and growth rate. (Mateos *et al.,* 2012) [25] reported despite higher crude fiber contents of the Roselle seed based diets, voluntary feed intake was numerically depressed. It has been demonstrated that the inclusion of moderate amounts of different fiber sources in the diet improves digestive organ development and increases HCl, bile acids, and enzyme secretion so improvement in. In nutrient digestibility, growth performance gastrointestinal tract health and eventually animal welfare

Table 6: Effect of Dietary Inclusion Different Levels of Hibiscus Seeds on Broiler Performance during Finisher Period (4-6 Weeks)

Parameters	Treatment				S.E	Sig
	K0%	K3%	K5%	K7%		
Feed consumption (g)	892.67	878.42	866.51	862.09	76.24	N.S
Weight gain (g)	460.28	444.83	424.11	420.42	28.56	N.S
Feed conversion ratio	1.89	1.92	1.98	2.00	0.06	N.S

Effect of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broiler Weights of Internal Organ (% of Body Weight) and the Length of Intestine

Table (7) showed the effect of inclusion of different dietary levels of by Roselle 0%, 3%, 5% and 7% as source of protein on broiler internal organs. As shown in Table (4.5) the length of intestine and relative weights of internal organs not affected (P≥0.05) by different experimental treatment except the Kidney weight was explain that increase dietary protein were associate with increases in urinary excretion which is influenced by inclusion of different treatment of Roselle. Which was Roselle seed like other seeds and legumes it has similar to (Liener and Kakade, 1980) [22] had reported that legumes contained protease inhibitor which causing growth reduction and declining down the nutritive values of protein. In the current study it seems that the Roselle has been used had beneficial effect on internal organs. Trypsin inhibitor which resulting in pancreas hypertrophy and hyperplasia or may be increase secretion of enzyme thus stress, when numerically increase so had no significant effect. Table (7) showed that increasing levels of Roselle did not result any significant increase in the relative weights of gizzard, Proventriculus, spleen, pancreas, abdominal fat and Heart. Spleen numerically decrease relate of immunity. The results show the unusual fatty acids namely epoxy-o leic and c y c l o p r penoid fatty acids and phenolic compound in Roselle seed caused no significant (P> 0.05) effect an increase in weights of pancreas and livers. The current results disagreed with (Bakheit 1993 and Mukhtar, 2007) [31]. There is insignificant reduction in relative weight of the livers and an increase in weights of pancreas. These results were in line with the findings of Bakheit. The results show no significant (P> 0.05) effect of the experimental treatments on gizzard, heart and abdominal fat their separable tissues due tothe level of Roselle seed. These results agree with those of (Mukhtar, 2007; Bib Baraik, 2010, Munassr, 2011, Mukhtar, 2012 and Ashom *et al.,* 2014) [31, 13, 33, 32, 10] who reported that there were no significant (P>0.05) differences were observed among the dietary treatments

Table 7: Effect of Dietary Inclusion of Different Levels of Hibiscus (karkadeh) Seed on Broiler weights of internal organs (% of body weight) and the length of intestine

Parameter	Treatment				SE	S
	K0	k3	k5	k7		
Liver (%)	1.94	1.86	1.79	1.71	.08	NS
Gizzard (%)	1.71	1.93	1.96	2.00	.14	NS
Length of small intestine (cm)	0.55	0.49	0.51	0.53	0.33	NS
Pro ventriculus (%)	0.37	0.40	0.46	0.34	0.03	NS
Spleen (%)	0.25	0.08	0.07	0.08	0.09	NS
Pancreas (%)	1.17	1.18	0.21	0.22	0.02	NS
Abdominal fat (%)	1.59	1.46	1.49	1.46	0.23	NS
Kidney (%)	0.48^{a}	0.31^{b}	0.31^{b}	0.45^{a}	0.03	*
Heart%	0.64	0.56	0.53	0.54	0.05	NS
Small intestine (%)	185.83	184.83	169.33	160.00	9.35	NS

*a-b means values within rows with no common superscripts are significantly different (P≤0.05). *:significantly. NS= not significant,*

Effect of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broiler Gut Bacterial Load

Table (8) show the effect of Roselle seed were added by 3%, 5% and 7% compare 0% to the broilers diets in order to study their effects on the bacterial count show high significant (p<0.05) the highest 7% (10.875), follow 3% was higher (8.500), follow 5% is lower (8.250) and control 0% (7.500) was lowest. Roselle seed were added to the broilers diets in

order to study their effects on the bacterial load on the broilers organs (Gizzard and Cecum) at different treatment. The bacterial load and total plate count include: *E. coli* and *Staphylococcus aureus*

Table 8: Effects of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broiler Gut Total Bacterial Count

Parameters	Treatments				Sig.
	K0%	K 5%	K 3%	K 7%	
Bacterial count	7.5000^{b}	8.2500^{ab}	8.5000^{ab}	10.8750^{a}	**

a-b means values within rows with no common superscripts are significantly different (P≤0.05).

Effect of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broiler Gut Bacterial count of *E. coli and Staphylococcus aureus*

i). *E. coli*

Results showed in table (9)showed that there were *E. coli* growth in treatments of (0%, 3%, 5% and 7%. where the lowest growth recorded by treatment of 3% (3.500), followed 5% (5.500) and 7% was highest (7.500) compare the control 0% was (1.500). Study results were in agreement with Olaleye (2007) who *H. sabdariffa* have been found to exhibit antibacterial activities against *E. coli*, our results dis agreed with (Abdelmageed *et al.,* 2011) [2] who reported nil growth of *E. coli* in gizzard. Which the diet in gizzard may be contaminate and digestion is not bingeing so that count of bacteria load has been high.

Cecum results show there was no significant differences among different treatments (0%, 3%5% and 7%. However, the lowest growth recorded by treatment of 3% (2.500), follow 5% (11.000) and 7% was highest (11.500) compare the control 0% was (7.500). The growth in treatment 3% agree with Olaleye (2007) who *H. sabdariffa* have been found to exhibit antibacterial activities against *E. coli*

When the digestion finish and the effect of Roselle treatments show negative effect on *E. coil* by high range the bacterial load and reflect on bacteria count with increase Roselle percent. If Roselle has antimicrobial agent to reduce effect of *E. coli*.

ii). *Staphylococcus aureus*

Table (9) show in gizzard there was no significant difference (P>0.05) among treatments (0%, 3%, 5% and 7%. where the lowest growth recorded by treatment 5% (4.500), follow 3% (11.500) and 7% was highest (12.000) compare the control 0% was (10.000). Which the diet in gizzard may be contaminate and digestion is not bingeing so that bacteria load has been high

In cecum showed that there was no significant differences among different treatments (0%, 3%, 5% and 7%. where the highest growth recorded by treatment highest 3% (16.500), followed 7% (12.500) and 5% was lowest growth (12.000) compare the control 0% was (11.000). The results were in contrast with Olaleye (2007) and Ahsan *et al*., (2018) [5] Al-Hashimi (2012) [6] who reported that the results roselle have antibacterial activity against *S. aureus.*

When the digest finish and the effect of Roselle treatments show negative effect on *Staphylococcus aureus* by high range the bacterial load and reflect on bacteria count with increase Roselle percent. If Roselle has antimicrobial agent to reduce effect of *Staphylococcus aureus*.

The antibacterial activity of the cycle extracts of *H. sabdariffa* can be attributed to the action of the phytochemical compounds it contains (Babayi *et al*., 2004) [12].

Antibacterial effects of this plant extract against Escherichia coli, and S. aureus suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhea (Rogger *et al*., 1990) [38]

Table 9: Effects of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broiler Gut Paso genic Bacterial Load

Treatments	Organs			
	Gizzards		Cecum	
	E coli	S. AUREUS	E coli	S. AUREUS
K 0%	1.500	10.000	7.500	11.000
K 3%	3.500	11.500	2.500	16.500
K 5%	5.500	4.500	11.000	12.000
K 7%	7.500	12.000	11.500	12.500
SE	2.027	2.027	2.027	2.027
Sig	NS	NS	NS	NS

NS= not significant

Conclusions

In conclusion, supplementation diets with *Hibiscus sabdariffa* seeds based diet can be profitable because it did not have any adverse effect on bird's performance.

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