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Effects of Dietary Inclusion of Different Levels of Roselle (Hibiscus Sabdariffa) Seeds on Blood Parameters and Fatty Acids Profile of Broilers

[*1] Mohamed Elkhatim Ibrahim Abdelmageed, [2]Amjad Basheir Suliman Haj Babiker, [3]Mutaz Saeed Babiker Mahmoud, [4]Elhashmi YH and [5]Hassan HE

[*12,4,5] Box (20), Department of Meat Production and Technology, Faculty of Animal Production, University of Gezira, Al-Managil, Gezira State, Sudan.

[3]Box (20), Department of Poultry Production, Faculty of Animal Production, University of Gezira, Al-Managil, Gezira State, Sudan.

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***Corresponding Author**
Mohamed Elkhatim Ibrahim Abdelmageed
Box (20), Department of Meat Production and Technology, Faculty of Animal Production, University of Gezira, Al-Managil, Gezira State, Sudan.

Abstract

The aim of the study is to assess the effects of dietary Inclusion of different Levels of Hibiscus (Karkade) seeds on blood parameters and Fatty Acids profile of Broilers. The Roselle (*Hibiscus Sabdariffa*) seeds were incorporated in broiler rations at levels of (k0%, k3%, k5% a n d k7%) in broiler rations the study was run in 6 weeks‟ period using four experimental diets. The experimental diets were formulated to be 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. At the end of the experiment, two birds from each experimental unit were selected according to their closed to average weight of birds in particular pen and slaughtered. Blood samples were collected for hematological profile and constituents. Abdominal fat pad samples from birds were taken to assess the effects on fatty acid profile of birds. All data was statistically analyzed using ANOVA. Result showed that. Hematological parameters were not affected by experimental treatments except white blood cells (WBC) ($p \leq 0.01$). Blood Cholesterol and triglycerides were affected by experimental treatments. General characteristic of Fatty acid results showed that there were high significant differences except some very long chain fatty acids was not significantly defer among treatments. Main unsaturated fatty acids in fat were Oleic, Linoleic and Elaideic acid and the saturated acids were Palmitic, stearic and Myristic acid. Omega3 (167.9). omega 6 (43.77) and omega 9 (55.08). The ratio of saturated FA to unsaturated FA 2:1 and omega 6 to omega 3 was 1:3. Dietary Inclusion of different Levels of Hibiscus Seeds improved Broiler Fatty acid profile and reduced of blood Cholesterol there for it recommended to include Roselle seeds 3% and do not up to 7% in broiler diets.

Keywords: Roselle seeds, blood parameters, fatty acids, profile, broilers.

Introduction

Poultry meat is an animal product important for human nutrition such as it is low in fat and cholesterol and it is healthier than other animal particularly red meat. Howe, *et al.*, (2006). Poultry meat has been considered one of the solution of the cardiovascular disease which is containing a higher amount of PUFA particularly n-3PUFA. In recent researches dietary supplement have been tested in broiler diet content low fat and cholesterol of broiler meat. Adam (2021). The substitution of red meat with poultry decreases the risk of developing type 2 and gestational diabetes mellitus, improves glycemic control and cardiovascular risk factors. Low-fat diets supported poultry instead of red meat yields cardiovascular health benefits. Donma (2017).

The major factor limiting the growth of the poultry industry is the high prices of balanced feed which represents about 75% of the total cost of production due to demand competition between human a n d a n i m a l s especially poultry Oloyed *et al*., (2010). Broilers are more efficient in converting raw feedstuffs into high protein food which is urgently needed to improve the human nutrition. Therefore, recently the poultry nutritionists keep looking for economic alternatives of protein sources Mukhtar *et al*., (2012).

A promising alternative crop that can be exploited for this purpose is Roselle (Hibiscus Sabdariffa) seeds are known as highly nutritional, antibacterial, antifungal and anti-parasitic actions Singh *et al*., (2017). Roselle plants are suitable for tropical and sub-tropical climate mainly for production of calyxes, many studies had shown that Roselle seeds contained high mounts of protein used in some African countries for human consumption. Abdalla, (2013) Roselle is so rich that in organic acids, minerals, amino acids, carotene, vitamin C and total sugar in its calyx, leaves and seeds at variable levels depending on the variety and geographical area Mady *et al*., (2009).

The hibiscus origin is uncertain, while others believe that its original country is India Abu-Tarboush *et al*., (1997), West Africa Cobley (1975), Sudan Abdalla (2013) Turkish Mclean (1973) Malaysia and Saudi Arabia Abd Aziz *et al*., (1985).

Roselle also contributes in the field such as veterinary medicine to experiments with domestic fowl showed that the aqueous extract of calyces is effective against Ascaris gallinarum (Hassan 2010). Roselle seeds have been used as a source of protein for broiler chicken production Owosibo *et al*., (2017).

Roselle seed meal nutrients content were comparable to those of groundnut meal Mohammed and Idris, (1991). Certainly, both contained reasonable levels of crude protein, high enough to allow the inclusion of these meals in broiler diets as protein sources.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. ELtoum, (1992) concluded that feeding graded levels of Roselle seed (0, 7.5, 15 and 22.5%) to broiler chicks showed a linear reduction in feed intake whereas body weight and mortality rate at four weeks of age. Bakheit, (1993) showed a linear increase in feed intake and suggested that Roselle seed has no effect on feed intake when fed (5, 10, 15 or 20%) level on layer performance.

Materials and Methods

Experimental Site

This experiment was carried out at Extension and Rural Development Centre, Faculty of Animal Production, University of Gezira Elmanagil, Gezira State, Sudan. This site lies between latitudinal 13.30 and 14.45 longitudinal and 32.45 and 33.15 horizontally at the Centre of 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 housing system used was deep litter system. The dimensions of the house were length was (21 meters), width was (6 meters) and the height of each longitudinal side was (3meters) with 80 centimeters height of bricks wall and the rest of the area was made of wire mesh. The roof and the walls were painted with white paint to reflect the solar radiation the floor was made of concrete and it was covered with thick layer of sawdust to remain always dried, the roof was made of corrugated iron sheets. 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 (100cm) 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 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).

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			
	K 0%	K 3%	K 5%	K 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
Roselle 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	0.27	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

Table 4: Composition of experimental diets (%As Fed) containing different levels of Roselle Seeds during starter period (1-3weeks). (4-6weeks).

Ingredients	Treatments			
	K 0%	K 3%	K 5%	K7%
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
Kerkrade 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

1Super concentrate contained 37% protein, 10% Moisture, 4% Fat, 28% Ash, 7% fiber. 2vitamin=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; calcium352 mg; iodine, 1.46 mg; cobalt. 5 mg; selenium. 2 mg; values and Metabolizable energy were calculated according to (Suleiman and Mabrouk1999).

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). Table (2) and (3) showed the diets composition of starter and finisher periods. Three levels of Roselle seeds were incorporated with other ingredients as alternative protein source as follows: k0 diet was0% (control), k3 diet was 3%, k5 diet was 5% and k7 diet was 7%. The birds had free access for feed and water throughout the experimental period.

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 and the weight of carcass cuts (chest, thigh, drum stick and wing).

Chemical Composition

Proximate analysis of Roselle seeds and pictorials muscle

Samples of Roselle seeds was 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).

Blood Samples

Four samples were kept (from each experiment) which were already prepared to be slaughtered at the end of the experiment after two hours feed withdrawal period to decrease the effect of feeding on blood parameters. Samples were collected from the jugular vein for determination of metabolites and hematological profile.

Hematological Parameters

Blood samples for hematological parameters were collected in tubes containing ethylene diamine tetra acetic acid (EDTA). Red Blood Cells (RBC), White Blood Cells (WBC), Packet Cell Volume (PCV), Hemoglobin (Hb) concentration were expressed as milligrams per 100 ml and determined according to (Jain, 1986). PCV was determined by micro hematocrit method. Hb concentration was measured spectrophotometrically by cyanomethaemoglobin method using SP6-500 UV spectrophotometer (Pye UNICAM ENGLAND). The RBC and WBC count were estimated using haemocytometer (Schalm *et al.*, 1975).

Serum Biochemical Indices

Samples with anti-coagulant were centrifuged in test tube for ten minutes (hetlich EBA 20- Germany) at 2000 rpm at room temperature to separate the cells from the plasma for serum biochemical indices. Serum samples were collected and kept for analysis. All tested blood cholesterol; tri-glycerides, blood protein, calcium and phosphorus were determined spectrophotometrically by using commercial kits.

Cholesterol

The cholesterol was determined using enzymatic, liquid colorimetric test CHOD/PAP method as described by (Trinder., 1969 and Richmond., 1973).

Triglyceride MR

The method on enzymatic hydrolysis of serum or plasma triglyceride to free fatty acids (FFA) by lipoprotein lipase (LPL).

Total Protein

Photometric colorimetric test-biuret method was used to determine the total protein (Tietz., 1986).

Glucose

The quantitative determination of glucose DIV was used to determined glucose content.

Fatty Acid Determination

Collection the Sample Data

After slaughtering and the bird was eviscerated then collected fresh abdominal fat sample, weighted and chilling.

The Equipment

The used equipment was: Gas Chromatography Mass Spectrometer (GC.MS), Detector: Mass Spectrometer. Model: GC.MS, q p. 2010, Company: Shimadz Country: Japan, Carrier gas mobile phase: Helium, Column: (1. Name: Rtx. 50, 2. Diameter: 0.25 Mm, 3. Thickness: 0.25), Injection temp300C, Injectio mode: split, pressure: 82.6 KPa, Total flow: 50.0 mL minG1, Column flow: 1.34 mL mi.

Methylation Steps

Two grams from the sample (abdominal fat bad of broiler chicken from each treatment) was put in a test tube. Then 7 mL from alcoholic sodium hydroxide was added 2ml of the sample was mixed thoroughly with 7ml of alcoholic sodium hydroxide (NaoH) that was prepared by dissolving 2 g in 100 ml methanol. 7 ml from alcoholic sulfuric acid (1ml H2SO4 to 100 ml methanol) was then added. The mixture was then shake for 5 minutes. The content of the test tube was left to stand overnight.1 ml of Super saturated sodium chloride (Na Cl) was then added and the contents being shaken. 2ml of normal hexane was added and the contents were shaking thoroughly for three minutes. Then the n hexane layer (the upper layer of the test tube) was taken using disposable syringe. 5 µl from the n-hexane extract was diluted with 5 ml of diethyl ether. Then the mixture was filtered through syringe filter 0.45 µm and dried with 1g of anhydrous sodium sulphate as drying agent and 1µl of the diluted sample was injected in the Gas Chromatography Mass Spectrometer GC.MS instrument (GC.MS), Detector: Mass Spectrometer, Model: GC.MS, q p. 2010, Company: Shimadz Country: Japan, Carrier gas mobile phase: Helium, Column: (1. Name: Rtx. 50, 2. Diameter: 0.25 Mm, 3. Thickness: 0.25), Injection temp300C, Injectio mode: split, pressure: 82.6 KPa, Total flow: 50.0 mL minG1, Column flow: 1.34 mL min.

Results and Discussion

Effect of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broiler Hematological Profiles Table (8) showed the effect of inclusion of different dietary levels of Roselle seed treatment by0%, 3%, 5% and 7% as a source of protein on broiler hematology. The hematological parameters were not affected by experimental treatments. The only exception was reported with white blood cells (WBC) cells ($p \leq 0.01$). All the birds diet containing Roselle higher values of white blood cells had 5% (98,65) than those bird's diets on control diets, follow 3% (95.15). However, the birds diet had 7% (93.80) the lowest white blood cells. It may be due to some antigenic proteins which could be found in major leguminous plants resulting in immunomodulation (Huisman and Tolman 1990). Although the total WBC affected significantly by experimental treatments the differentials of WBC were numerically different affected between treatments. This result indicates when bird diet Roselle seed have a good immunity. The current results disagree with (Amer *et al*., 2022) reported that there were no significant WBC ($P > 0.05$) and significant ($p \leq 0.01$) RBC.

Table 5: Effect of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broiler Hematological Profiles

Parameter	Treatment				SE	Sig
	K0%	K3%	K5%	K7%		
Pcv(%)	29.00	28.00	28.50	31.00	1.82	NS
Hb (%)	11.15	12.15	11.20	12.10	1.23	NS
WBCs	95.25^{b}	95.15^{b}	98.65^{a}	93.80^{b}	0.80	*
RBCs	2.34	2.28	2.32	2.55	0.14	NS
Lymphocyte (L)	91.50	89.50	91.00	92.50	1.30	NS
Main cell volume (M	3.50	3.00	3.50	3.00	0.61	NS
Hetrophils (N)	3.50	5.50	3.50	2.00	0.97	NS
Eosinophil (E)	1.00	1.50	1.50	1.50	0.34	NS
Basophil (B)	0.50	0.50	0.00	1.00	0.35	NS
Platelet	36.50	51.00	40.50	46.00	14.23	NS

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

Effect of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broiler Blood Constituents Table (9) showed the effect of inclusion of different dietary levels of Roselle seed treatment by 0%, 3%, 5% and 7% as a source of protein on broiler blood constituents. Blood glucose was not affected by dietary treatments. Blood triglycerides were affected the birds diet with5% (96.50) diets had the highest, while birds diet on 3% (82.50) and 7% (69.50) respectively the lowest values. The highest total blood protein was reported with bird's diet on 5% whereas the birds fed with 3% and 7% showed the lowest values. Blood Cholesterol was affected the highest was observed with birds fed on 5%, follow 3% and 7% was the lowest. Cholesterol and triglyceride decrease because Roselle had considerable good ratio soluble to in soluble fraction in dietary fiber contain when a higher dietary fiber content its reduce dietary fat utilization and that might reduce fat absorption through the gut. These results agreed with (Hainida 2008) and also (Lairon, *et al*., 1996) who reported that, the presence of non-starch polysaccharides fraction in the diets could affect the metabolism, digestibility and absorption of lipids and cholesterol. Costa, *et al*., (994) found, the soluble non-starch polysaccharides of legume seeds are known to be an effective cholesterol-reducing agent. Also, cholesterol was affected by the high content of unsaturated fatty acids which found in Roselle seed. The current dis agree with (Aziza *et al*., 2011) who reported that there were no significant Cholesterol (P>0.05). And agree with him there are significant (p≤0.01) in TG and no significant in glucose.

Table 6: Effect of Dietary Inclusion of Different Levels of Hibiscus (Karkade) Seed on Broiler Blood Constituents

Parameter	Treatment				SE	S
	K0	k3	k5	k7		
Cholesterol	122.00 ^{a}	82.50 ^{c}	96.50 ^{b}	69.50^{d}	5.65	*
Glucose	142.50	184.50	149.50	158.00	23.08	NS
Ph4	5.00 ^{a}	4.22 ^{ab}	3.51 ^{c}	4.07^{b}	0.20	*
T. Protein	2.37	2.13	2.16	1.71	0.38	NS
Triglyceride	16.50^{c}	19.80^{b}	34.55^{a}	17.45^{c}	.091	*

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 Seeds on Broiler fatty acid profile Table (8) showed the effect of dietary inclusion of different levels of Roselle on fatty acid such as saturated and un saturated fatty acid, Polyunsaturated to saturated fatty acids, Essential Fatty Acids, Omega – 3, Omega – 6, Omega – 9, ratio of Omega-6/Omega3 and ratio of saturated/un saturated fatty acid.

Total Saturated Fatty Acid (T.S. FAs)

As results in table (8) show all treatment showed significant differences P≤0.05 were k3, k5 and k7 lead to decrease to increase Roselle total saturated fatty acids in k3, k5 and k7 recorded (83.14), (32,70) and (35,39) respectively. S.F. As like palmitic acid C16:0 $C_{17}H_{34}O_2$, Myristic acid (C15:0) $C_{15}H_{30}O_2$, Stearic acid (C18:0) $C_{18}H_{36}O_2$, Nonadecylic acid (C19:0) $C_{19}H_{38}O_2$and Cerotic acid (C24:0) $C_{26}H_{52}O_2$. saturated fatty acids associated with coronary heart disease and hyper cholesterol (Ismail *et al*., 2008) who reported Roselle is containing to anti-hyper cholesterolemia properties to solved this problem.

Total Unsaturated Fatty Acids (T.U.S. FAs)

As the results in Table (8) shows all treatment showed significant difference P≤0.05where k3, k5 and k7 to lead decrease to increase Roselle total unsaturated fatty acid is K3 had a highest (61.93) and th K5 had lowest (16.07). U.S. FAs like C18:2n6 linoleic acid $C_{19}H_{34}O_2$, C18:1 oleic acid $C_{19}H_{36}O_2$, C20:4 n-6 Arachidonic $C_{20}H_{32}O_2$ and C20:3 n-6 Eicosatrienoic $C_{20}H_{34}O_2$. Polyunsaturated fatty acids (PUFA) in the diet for decreasing the risk of vascular diseases. The ratio saturated to unsaturated 2:1 this result disagrees with (El-Aldawy *et al*., 1994) reported 1:2.

Mono-Unsaturated Fatty Acids (M.U.S. FAs)

As the results in Table (8) shows all treatments affected significant differences P≤0.05. by dietary inclusion of Roselle lead to decrease M.U.S. FAs w h e n dietary inclusion Roselle increase. Total unsaturated fatty acid is K3 had a highest (47.80) and the K7 had lowest (1.29). M.U.S. FAs like Oleic acid (C18:1) $C_{19}H_{36}O_2$ were the main monounsaturated fatty acids. They have major role this result.

Poly-Unsaturated Fatty Acids (P.U.S. FAs)

As the results in Table (8) shows all treatments affected significant differences P≤0.05. by dietary inclusion of Roselle lead to increase P. U. S. FAs w h e n dietary inclusion Roselle increase. Poly-un saturated Fatty Acid is K7 had a highest (15.14) and the K5 had lowest (14.10). P. U. S. FAs like (C18:2 n-6) $C_{19}H_{34}O$ alpha linoleic acid This result agrees with (Peng., 2019) has significant and di a gree with (El-Aldawy *et al*.,1994) who reported unsaturated fatty acid more than 70%. of poly un saturated (Peng., 2019) reported Important biological role of n-3 and n-6 polyunsaturated fatty acids are essential components of the human diet because humans are unable to synthesize these fats. Moreover, linoleic fatty acid ($C_{19}H_{34}O_2$) is very useful for the healthy growth of human skin.

PUSFAs/SAFAs

As the results in table (8) shows all treatments affected significant differences (p≤0.5) by dietary inclusion of Roselle lead to increase PUFA/SFA w h e n dietary inclusion Roselle increase were recorded k3 had lowest level (.17) and k5and k7 both had a highest (.43). Low ratios of polyunsaturated to saturated fatty acids (PUFA/SFA) in Western diets have been considered as a big risk factors for cardiovascular diseases, which are important causes of human death. An intake of it in the ratio of 5 to 10 has been recommended by world health organization WHO/FAO, 2003).

Essential Fatty Acids (E. FAs)

As the results in table (8) shows all treatments affected significant differences (p≤0.5) by dietary inclusion of Roselle lead to decrease E. FAs w h e n dietary inclusion Roselle increase. E. FAs like $C_{19}H_{36}O_2$, $C_{15}H_{30}O_2$, $C_{17}H_{34}O_2$, $C_{26}H_{52}O_2$, $C_{18}H_{36}O_2$, $C_{19}H_{34}O_2$ and $C_{22}H_{32}O_2$. E. FAs were recorded K3 had a highest level of E. FAs (145.07) and the K5 had lowest level of E. FAs (48.79) compared with control (24.45). (Peng., 2019) EFA is important for producing life energy, which human bodies could not synthesize and can be obtain from external source in diet. (Bradberry *et al*., 2013) reported Hypercholesterolemia, low immune system and low metabolism rate are related imbalances or deficiencies of these EFAs.

Omega - 3

As the results in table (8) shows all treatment affected significant differences ($p \leq 0.5$) by dietary inclusion of Roselle lead to decrease Omega – 3 w h e n dietary inclusion Roselle increase. Omega – 3 like $C_{22}H_{32}O_2$ and $C_{20}H_{34}O_2$. Omega – 3 recorded K3 had a highest level of (83.14), while K5 had lowest level of (32.70). (Ismail *et al*., 2008) reported (ALA), Docosahexaenoic acid (DHA) and Eiocosapentaenoic acid (EPA) are excellent sources. Poultry meat has been considered as one of the main sources of PUFA for human diets n-3 PUFA such useful in reducing cholesterol Docosahexaenoic acid DHA $C_{22}H_{32}O_2$.

Omega - 6

As the results in table (8) shows all treatments affected significant differences ($p \leq 0.5$) by dietary inclusion of Roselle lead to increase Omega – 6 w h e n dietary inclusion Roselle increase were recorded K7 had a highest level of (15.14), while the K5 had lowest level of (14.10) Omega – 6 compared with control (3.76). Omega – 6 like $C_{20}H_{32}O_2$ and $C_{19}H_{34}O_2$. The n-6 fatty acids are important, such as the Arachidonic acid (AA) is the most important n-6 PUFA because it is the primary precursor for the n-6 derived eicosanoids Both n-6 and n-3 fatty acids have been shown to have anti-inflammatory properties that are protective of atherogenic changes in vascular endothelial cells.

Omega-6/Omega-3

As the results in table (8) shows all treatments affected significant differences ($p \leq 0.5$) by dietary inclusion of Roselle lead to increase n-6/n-3 w h e n dietary inclusion Roselle increase were recorded k5 and K7 had a highest level of (.43), while the K3 had lowest level of (.17) compared with control (.23). Simopoulos, 2004) reported Polyunsaturated fatty acids contents of modern diets are low in n-3 fatty acids leading to high n-6/n-3 fatty acid ratios. The imbalance in the n-6 vs. n-3 proportion is responsible for the pathogenesis of many diseases, such as cardiovascular disease.

Omega - 9

As the results in table (8) shows all treatments affected significant differences ($p \leq 0.5$) by dietary inclusion of Roselle lead to decrease omega9 w h e n dietary inclusion Roselle increase were recorded K3 had a highest level of (47.80), while the K7 had lowest level of (1.27) compared with control (4.02). Omega-9 like $C_{19}H_{36}O_2$ (Massaro *et al*., 2002) reported Omega-9 is important Fatty Acids such as Oleic acid, (C18:1) $C_{19}H_{36}O_2$ Its important in dietary it‟s the main function and mechanism is to reduce LDL cholesterol and to improve lipid profile Fatty acids also are classified into 3 groups short, medium, and long chain fatty acids. (Talukdar *et al*., 2011). Long chain fatty acids (LCFAs) are fatty acids with aliphatic tails longer than12 carbons, Medium chain fatty acids (MCFAs) are fatty acids with aliphatic tails between 6 and 12 carbons and Short chain fatty acids (SCFAs) are those carboxylic acids that contain aliphatic tails less than 6 carbon atoms.

Long Chain Fatty Acids (L.C.F. As)

As the results in table (8) shows all treatments showed significant differences ($p \leq 0.5$) by dietary inclusion of Roselle lead to decrease L.C.F. As w h e n dietary inclusion Roselle increase k3, k5 and k7 were recorded 100.00, 95.97 and 89.47 respectively. Observe lead to decrease long chain fatty acids like $C_{14}H_{30}$, $C_{15}H_{24}O_2$, $C_{15}H_{30}O_2$, $C_{16}H_{24}O_4$, $C_{16}H_{48}O_8$, $C_{17}H_{16}N_2$, $C_{17}H_{36}$, $C_{17}H_{31}F_3O_3$, $C_{17}H_{32}O_2$, $C_{18}H_{52}O_7$, $C_{18}H_{54}O_7$, $C_{18}H_{54}O_9$, $C_{19}H_{36}O_2$, $C_{19}H_{36}O_2$, and increased levels of some long chain fatty acids like $C_{17}H_{34}O_2$, $C_{19}H_{36}O_2$, $C_{19}H_{34}O_2$ and showed also increase in some very long chain fatty acids $C_{20}H_{34}O_2$, $C_{22}H_{32}O_2$, $C_{26}H_{52}O_2$and $C_{26}H_{52}O_2$. (Bos *et al*., 2016) reported that the docosahexaenoic acid (C226n-3) is indispensable for neuronal myelination, and it is an important precursor for the very long chain fatty acids synthesis found in the brain. It is also involved in neurogenesis, neurotransmission and protects the brain from the oxidative stress.

Medium Chain Fatty Acids (M.C. F. As)

As the results in table (8) shows all treatments showed differences ($p \leq 0.5$) where k3, k5 and k7 lead to decrease in medium chain (nil) while control showed the highest levels in both of tow acids ($C_{10}H_8$ and $C_{11}H_{24}$) which appeared in the samples. When our back to show table (4.3) to observe the effect of Roselle in finishing period. While 3%, 5%, and 7% compare 0% were high feed conversion to due obesity, that a gree with (Turner *et al*., 2009) who reported Medium Chain Fatty Acids MCFAs have useful use against diabetes were observed to stimulate insulin secretion. Thus dietary supplementation with it was good for preventing obesity and insulin resistance.

Short Chain Fatty Acids (S.C.F. As)

As table (8) there was a presence of Propionic acid in the samples which produced in the gut via bacterial fermentation and functions as a central metabolite of odd chain fatty acid metabolism. (Wolever *et al*., (1997) Highlighting some potential toxic effects of propionic acid, an autosomal recessive disease resulting from mutations in propionyl CoA car- boxylase. As the results in table (9) shows all treatments showed significant differences ($p \leq 0.5$) where k5 and k7 lead to increase in short chain fatty acids and recorded high levels of short chain of fatty acids 3.81 and 10.52 respectively, while the treatment of k 3 recorded the lowest content of the short chain fatty acids (0.000) value as the control recorded. When our back to show table () to observe the effect of Roselle in finishing period While 3%, 5%, and 7% compare 0% were high fibre to due weight loss this agree with (Murphy *et al*., 2010) reported that the relationship between gut flora, S. C. F. As production and obesity because a high fiber diet results in weight loss.

Table 7: Effect of Dietary Inclusion of Different Levels of Hibiscus Seeds on Broiler fatty acid profile

Fatty acid	Treatment				SE	Sig	
	K0	K3	K5	K7			
C3H8O3	.000 c	.000 c	3.81 b	10.52 a	K0.002	K3**	Glycerin
C10H8	.55 a	.000 b	.000 b	.000 b	.001	**	Naphtalene
C11H24	.47 a	.000 b	.000 b	.000 b	.001	**	Undecane
C12H24o2	2.39 a	.000 b	000 b	.000 b	.001	**	Lauric
C14H30	1.05 a	.000 b	.000 b	.000 b	.001	**	Tetradecane
C15H24O2	2.64a	.06d	2.28c	2.47b	.06	**	Butylated
C15H30O2	.000d	.39b	.33c	.60a	.005	**	Myristic
C16H24o4	.64 a	.000 b	.000 b	.000 b	.001	**	Brefedlin
C17H16N2	.45 a	.000 b	.000 b	.000 b	.001	**	Xinidamine
C17H31F3o3	.86 a	.000 b	.000 b	.000 b	.001	**	Pentadecane
C17H32O2	4.02 a	2.11 b	.000 c	.000 c	.004	**	Palmite oleic
C17H34O2	15.77 d	27.25 a	25.64 c	26.31 b	.006	**	Palmitic
C17H36	.38 a	.000 b	.000 b	.000 b	.001	**	Hepetadecane
C18H34O2	.000 b	45.66 a	,000 b	.000 b	.03	**	Elaideic
C18H36O2	.000 b	.03 a	.000 b	.000 b	.03	**	Stearic
C18H54o7	2.39 a	.000 b	.000 b	.000 b	.044	**	Phenol
C19H34O2	3.76 d	14.13 b	14.10 c	15.14 a	.006	**	Linoleic
C19H36O2	18.19 c	.53 d	43.18 a	35.18 b	.006	**	Oleic
C19H38O2	.000 d	9.63 a	6.73 c	8.48 b	.005	**	Nonadecylic
C20H32O2	4.77 a	.000 c	1.49 b	.000 c	.002	**	Arachidonic
C20H38O2	.000 b	.000 b	1.97 a	.000 b	.001	**	Gad oleic
C20H40o2	1.15 a	.000 b	.000 b	.000 b	.001	**	Arachidic
C21H26O2	.947	.000	.000	.000	.165	NS	Menaquinol
C22H32O2	.000 b	.000 b	.24 a	.000 b	.001	**	Docosahexaenoic
C22H42O2	.000 b	.000 b	.000 b	1.29 a	.002	**	Cetoleic
C23H32o2	2.44 a	.000 b	.000 b	.000 b	.001	**	Eicosatrienoic
C26H50O2	.000 b	.12 a	.000 b	.000 b	.001	**	Ceronic
C26H52O2	.000 b	.09 a	.000 b	.000 b	.003	**	Cerotic

Calculated Data				
	K0	K3	K5	K7
S. C. FAs	0.00	0.00	3.81	10.52
M. C. FAs	1.02	0.00	0.00	0.00
L. C. FAs	64.34	100.0	95.97	89.47
T.S.FAs	16.67	83.14	32.70	35.39
T.U.S.FAs	7.78	61.93	16.07	16.43
M.U.S.FAs	4.02	47.80	1.97	1.29
P.U.S.FAs	3.76	14.13	14.10	15.14
E.FAs	24.45	145.0	48.77	51.82
Omega 3	16.67	783.14	32.70	35.39
Omega 6	3.76	14.13	14.10	15.14
Omega 9	4.02	47.80	1.97	1.29
Omega 6: Omega 3	.23	.17	.43	.43
P.U.S.FAs: T.S.FAs	.23	.17	.43	.43

Where: FAs: Fatty Acids, S.C. FAs: Short Chain Fatty Acids, M.C.FAs: Medium Chain Fatty Acids, L.C.F As: Long Chain Fatty Acids T.S. FAs: Total Saturated Fatty Acid, T.U.S.F. As: Total Unsaturated Fatty Acids, M.U.S.F. As: Mono-Unsaturated Fatty Acids, P.U.S.F. As: Poly-Unsaturated Fatty Acids, EFAs: Essential Fatty Acids.

Conclusions

Dietary Inclusion of different Levels of Hibiscus Seeds improved Broiler Fatty acid profile. Also, Hibiscus sabdariffa seeds supplementation reduced triglyceride and cholesterol in blood of broiler chicken there for it recommended to include Roselle seeds 3% and do not up to 7% in broiler diets.

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