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MIXED-SPICES SUPPLEMENTATION SUPPRESSES FAT DEPOSITION AND CAUSES NO ALTERATIONS IN LIPID AND RED BLOOD CELL PROFILES IN BROILER CHICKENS

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ABSTRACT

This research was conducted to determine the carcass traits, lipid and red blood cell indices of broiler chickens fed mixed spices-supplemented diet. Driedand pulverized spices; *Allumsativum* (garlic), *Zingiberofficinale* (ginger) and *Punicagranatum* (pomegranate) were mixed in ratio of 2:2:1 respectively.The mixed-spices were combined with Starter and Finisher to formulate four (4) diets; A was non-supplemented Starter, B was 2% mixed spices-supplemented Starter, C wasnon-supplemented Finisher and D was 2% mixed spices-supplemented Finisher. In the first phase of the experiment, 150 chicks were divided into groups 1 and 2 of 75 birds each, and randomly assigned to diets A and B. Groups 1and 2were respectively maintained on diets A and B for 3 weeks. In the second phase, group 1 in the first phase was sub-divided into groups A and B while group 2 was sub-divided into groups C and D and fed *ad libitum* for additional 3 weeks. The feed intake, carcass traits, lipid and red blood cell indices were determined. Increase in weight and feed intake were significantly lower ($p<0.05$) in the birds fed 2% mixed spices supplemented Starter. All the test groups had significantly lower ($p<0.5$) percentage fat to carcass weight as compared to the control (A). Other evaluated carcass traits did not vary significantly ($p>0.5$). All evaluated red blood cell indices, lipid and artherogenic indices did not significantly differ ($p>0.5$) from the control. Conclusively, the spices could suppress fat accumulation in broiler chicken, without affecting other carcass traits, lipid and red blood cell indices.

Keywords: Broiler; Garlic; Ginger; Pomegranate; Spices

INTRODUCTION

Poultry are susceptible to stress induced as a response to common management practices such as vaccination, feed withdrawal, and rearing on reused litter as well as severe stressors such as high environmental temperature. These stressors can result in economic costs to producers that are associated with poor growth and disease (Jill *et al*., 2018).Further, the excessive abdominal fat deposit in broilers (Liang,2015) causes increase in feed cost and economic loss to producers (Wu *et al*., 2006). Consumption of diet high in fat could lead to various metabolic alterations such as hyperphagia in humans (Westerterp *et al*., 2008). This has led to a growing increase in consumer aversion to excess fat deposition in food animals.

The addition of sub-therapeutic amount of antibiotics to the feed of poultry and swine was observed more than 50 years ago to have a distinct growth promoting effect on the animals and thus increase their production efficacy (Halldor, 2012). Sooner after that, the use came under criticism due to its potential toxic properties in human (Puvaca *et al*., 2013), though scientific evidence that the use of antibiotics in food-producing animals is contributing to the antibiotic resistance issues in human is quiet not strong (Cox *et al*., 2007; Phillips, 2007 and Phillips *et al*., 2007). Challenges with the control of diseases of broiler chickens while minimizing the use of antibiotics, excess fat control and improvement of meat quality make necessary the need for alternative means of disease prevention and control.

Spices have widespread beneficial effects and safety margin, and are considered potential alternatives. Having antioxidant (Gardzielewska *et al*., 2003), antibacterial and anti-inflammatory properties (Mikaili *et al*., 2013; Gebreyahonnes and Gebreyahonnes, 2013), some spices such as ginger (*Zingiberofficinale*), garlic (*Allium sativum*), and pomegranate (*Punicagranatum*), have been reported to individually and in some combinations with one another improve health (Lawal *et al*., 2016; Idoko *et al*., 2018). This research was designed to compare broilers raised with or without mixed spices (ginger, garlic and pomegranate) vis-à-vis the carcass trait, lipid and erythropoietic profiles.

MATERIALS AND METHODS

Pelletized Starter's and Finisher`s mash, product of ChikunVital Feed were purchased from dealers in Vital Feeds in Dutsin-Ma Local Government of Katsina State. One hundred and fifty (150) chicks at a day-old were purchased from Chi Farms Limited Ibadan, Nigeria. Garlic and pomegranate powder were both purchased from Al-Halal Islamic store in Katsina, Katsina State. Dried ginger was bought from Dutsin-ma Central market, screened thoroughly for unwanted particles and stones, milled to flour using a mechanical grinder to pass a 0.5-mm mesh sieve and stored inside a clean vessel. The pomegranate, ginger and garlic were respectively combined in the ratio of 1:2:2 and thoroughly mixed to achieve homogeneity. The mixed-spices were mixed with the Starter and Finisher`s feeds to formulate four (4) experimental diets;

A was non-supplemented Starter feed.
B was 2% mixed spices-supplemented Starter feed.
C was2% mixed spices-supplemented Finisher feed.
D was non-supplemented Finisher feed.

Experimental design and animal management

Starter phase: In this phase of the experiment, the 150 chicks were divided into two groups of 75 birds each and randomly assigned to diets 1 and 2;

Group 1 was maintained on non-supplemented Starter feed (control).

Group 2was maintained on 2% mixed spices-supplemented Starter feed.

Finisher phase: In the second (Finisher) phase of the experiment, group 1 in the first phase was sub-divided into groups A and B while group 2 in the first phase was sub-divided into groups C and D as follows;

Group A was maintained on non-supplemented Starter and non-supplemented Finisher feeds (control).

Group B was maintained on non-supplemented Starter but 2% mixed spices-supplemented Finisher feeds.

Group C was maintained on 2% mixed spices-supplemented Starter but non-supplemented Finisher feeds.

Group D was maintained on 2% mixed spices-supplemented Starter and 2% mixed spices-supplemented Finisher feeds.

The broilers were kept in a warm (35°C-38°C) and appropriately disinfected environment. They were maintained *ad libitum* on their respective experimental Starter feeds for three (3) weeks and Finisher feeds for another three (3) weeks after seven days of acclimatization period. The birds received gumboro disease vaccines on the 10^{th} and 24^{th}days. Newcastle disease vaccine (Lasota strain) was administered on the $17^{th}$and 31^{st} days.

Determination of feed intake and growth pattern

The quantity of feed left over was subtracted from the quantity of feed served on daily basis to determine the daily feed consumed. The average weekly feed consumptions were calculated from the daily consumptions. The change in weight was estimated by weighing the chicks at the commencement of the experiment and thereafter on weekly basis (Idoko *et al*., 2016).

Assessment of carcass performance

The sacrificed birds were scalded and plucked. They were cut open and eviscerated. The ready-to-cook carcass was obtained by removing the head, neck, legs, heart, liver, gizzard, intestines, proventriculus, gall bladder, spleen, esophagus, full crop and abdominal fat. The dressing, carcass, giblets and fat pad percentages as well as percentage organ to body weight were then computed following the methods adopted by Isa (2011) as follows:

Dressing % = Carcass weight/live weight × 100

Giblets % = (Liver+Gizzard+Heart) weight/Live weight ×100

Abdominal fat pad % = (Fat pad weight/ Live weight)×100

Determination oflipid and red blood cellindices

At the age of 42 days, 5broiler birds were selected randomly from each group after being subjected to twelve-hour fasting. The birds were weighed and sacrificed by incising the jugular vein. Blood was collected into EDTA-treated and into plain tubes. Serum was processed from the blood in the plain tubes following the method of Akanji and Ngaha (1989). The red blood cell indices of the whole blood such as haemoglobin (Hb), packed cell volume (PCV) and red blood cells (RBC) were carried out at the Federal University Dutsin-Ma Clinic using an automated haematology analyser. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were computed from the Hb, PCV and RBC (Zahra *et al*., 2013);

MCV = PCV× 10/RBC
MCH = Hb×10/RBC
MCHC = Hb×10/PCV

Serum lipid(Total cholesterol(TC), Triglyceride and HDL-C)concentrations were determined enzymatically using lipid profile enzyme assay kits from Randox Laboratories Ltd. while LDL-C was determined using the formula TC-TG/2.2-HDL (Friedewald *et al*., 1972). Atherogenic indices were computed from the determined lipid profiles (Ikewuchi and Ikewuchi, 2009);

Cardiac Risk Ratio (CRR) = TC/HDLC
Atherogenic Coefficient (AC) = (TC– HDLC)/HDLC
Atherogenic Index (AI) = log(triglyceride/HDL-C).

Statistical analysis

Results were expressed as means ± SEM. Results obtained in the starter phase were subjected to Student t-test but to one-way ANOVA in the Finisher phase to assess statistical significance where $p < 0.05$ was considered significant. SPSS (16.0 version) was the statistical package program used for the analysis.

RESULT AND DISCUSSION

Feed intake and growth pattern of broiler chickens fed with or without mixed spices supplemented diet: The growth and average weekly weight gain in broiler chicks fed with or without spices supplemented diets during the Starter phase are respectively presented in figure 1 and table 1. The chicks fed with mixed spices-supplemented Starter experienced significantly lower growth ($p<0.5$) during the third week of the experimental period. On the whole, the broilers fed with mixed spices-supplemented Starter had significantly lower average weekly weight gain but had lower mortality rate. During the Finisher phase however, there was no significant variations ($p>0.5$) in feed intakes, feed conversion ratio and weight gain among the four experimental groups. There was no mortality in the group maintained on 2% mixed spices-supplemented Starter but non-supplemented Finisher feed (C) and group maintained on 2% mixed spices-supplemented Starter and 2% mixed spices-supplemented Finisher (D) (Table 2).The lower weight gain in the birds fed

on mixed spices supplemented diet during the Starter phase is attributable to lower feed intake by the birds rather than to the quality of the feed. This is inferred from the non- significant difference in the feed conversion ratio. The lower feed intake could be due to the mixed spices in the diet of the test group. Some spices had been reported to cause decrease in feed intake due to some of the bioactive compounds in them. Ajoene, an organo-sulfur compound found in garlic (*Allium sativum*) is reported to exert inhibitory effect on adipogenesis and to induce apoptosis (Ambati *et al*., 2009).The oil in garlic has been reported to have inhibitory effect on nitric oxide which is thought to be associated with improved GLUT4 expression (Cheng-Tzu *et al*., 2012).The potential of ginger in weight management is due to its role in enhancing thermogenesis and reducing feeling of hunger (Muhammad *et al*., 2012). Also, Mei *et al*. (1980) showed that raw garlic homogenate effectively improves insulin sensitivity. However, Gamal *et al*. (2015) reported non-significant effect of pomegranate on feed intake during the first stage of the growth experiment (1-14 days). This is consistent with our finding as depicted in figure 1 which shows that the decrease in weight of the birds on mixed spices became significant during the third week of the experiment. In the second stage (Finisher stage), the feed intake and FCR were not affected, hence the change in weight was also not affected. The decreased mortality could be due to health benefits of the spices which are largely due to their antioxidants and anti-inflammatory constituents. Ginger root contains many compounds which have biological activities such as antioxidant, antimicrobial and pharmacological effects (Gebreyahonnes and Gebreyahonnes, 2013). Garlic, according to Mikaili *et al*. (2013) is effective in boosting immune systems in humans which is attributable to its anti-microbial, antihypertensive, anti-cancer, immunomodulatory and hypolipidemic properties. Pomegranate is rich in antioxidant phenols (Heena *et al*., 2018) and thus plays significant role in maintenance of health.

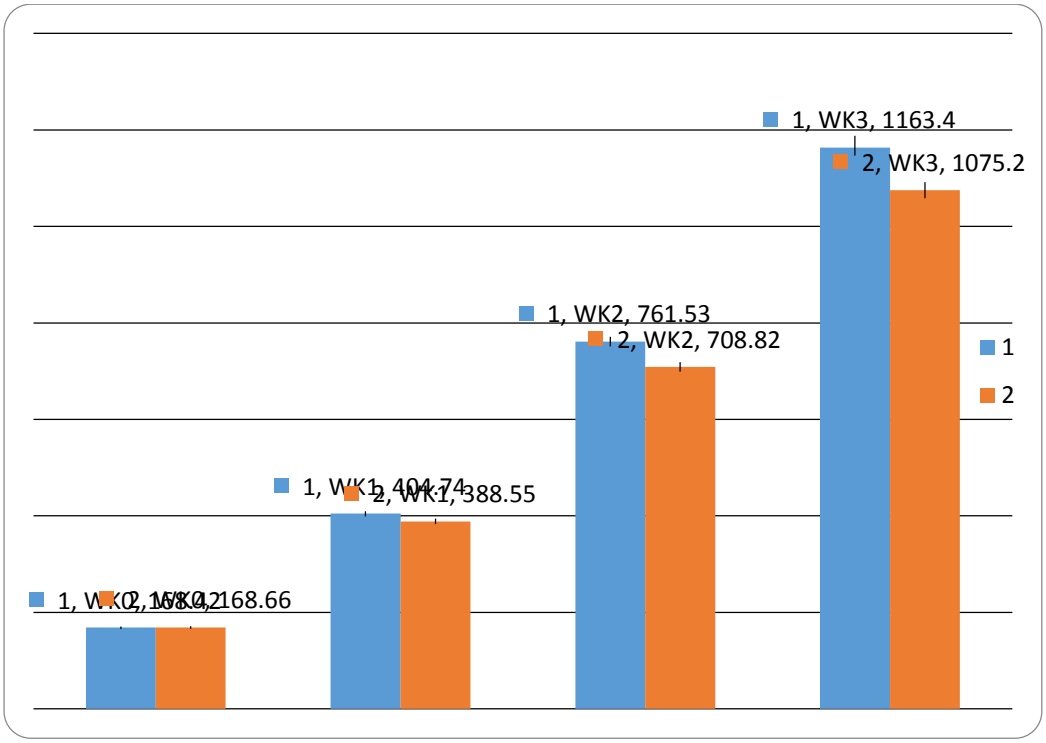

Figure 1: Growth chart of chicks fed with or without mixed spices-supplemented Starter
1: Group maintained on non-supplemented Starter feed (control).
2: Group maintained on 2% mixed spices-supplemented Starter feed.

Table 1: Weight gain by broiler chicks fed with or without mixed spices-supplemented Starter

	1	2
Weight gain (g)	331.66±49.41[a]	302.18±43.24[b]
Feed intake (g)	88.57±20.88[a]	81.32±16.53[b]
FCR	0.26±0.03[a]	0.26±0.02[a]
Mortality (%)	4.6%	2.7%

Results excluding Mortality are means of 3 determinations ± SEM Values along the same row with the same superscript are NOT significantly different (P>0.05), but are significantly different if the superscripts are different
FCR: Feed conversion ratio
1: Group maintained on non-supplemented Starter feed (control).

2: Group maintained on 2% mixed spices-supplemented Starter feed.

Table 2: Weight gain by broiler chickens fed with or without mixed spices-supplemented finisher feed

	A	B	C	D
FI (g)	190.14±21.78[a]	182.37±18.38[a]	172.56±14.58[a]	163.466±13.74[a]
FCR	0.36±0.05[a]	0.37±0.03[a]	0.36±0.09[a]	0.34±0.07[a]
WT gain (g)	543.54±82.53[a]	488.25±17.14[a]	538.53±129.86[a]	537.95±131.62[a]
Mortality	9.4%	3.03%	0	0

Results excluding Mortality are means of 3 determinations ± SEM Values along the same row with the same superscript are NOT significantly different (P>0.05), but are significantly different if the superscripts are different
WT: Weight
FI: Feed intake
FCR: Feed conversion ratio
A was maintained on non-supplemented Starter and non-supplemented Finisher feeds (control).
B was maintained on non-supplemented Starter feed but 2% mixed spices-supplemented Finisher feed.
C was maintained on 2% mixed spices-supplemented Starter feed but non-supplemented Finisher feed.
D was maintained on 2% mixed spices-supplemented Starter and 2% mixed spices-supplemented Finisher feed.

Carcass performance of broiler chickens fed with or without mixed spices supplemented diet: All the test groups (B, C and D) had significantly lower (p<0.5) percentage fat to carcass weight as compared to the control (A). Other evaluated carcass traits did not vary significantly (p>0.5) (Table 3).Meat is rich in fat and cholesterol and high intakes are associated with increased blood cholesterol levels and coronary heart disease (CHD). Furthermore, consumption of diet high in fat could lead to various metabolic alterations such as hyperphagia in humans (Westerterp *et al*., 2008), reduced lipolytic activity in fat tissue, reduction in leptin secretion and/or sensitivity (Moraes *et al*., 2009) and impair mitochondrial metabolism (Pomplun *et al*., 2007).Substantial evidence shows that meat with a relatively low visible fat has lower saturated fat content and does not raise total blood cholesterol and LDL-cholesterol levels (Duo *et al*., 2005). Therefore, the decrease in the visible fat of broilers fed on the spices-supplemented diets may be advantageous.

Table 3: Carcass traits of broiler chickens fed with or without spices supplemented diets

	A	**B**	**C**	**D**
Dress%	73.72±0.67[a]	75.90±1.90[a]	75.52±0.67[a]	76.92±1.20[a]
%FW/CW	2.40±0.44[b]	1.86±0.22[a]	1.82±0.34[a]	1.88±0.22[a]
%L/CW	2.57±0.11[a]	2.37±0.09[a]	2.30±0.07[a]	2.45±0.20[a]
%H/CW	0.36±0.01[a]	0.39±0.02[a]	0.37±0.01[a]	0.40±0.04[a]
%G/CW	1.69±0.12[a]	1.85±0.12[a]	1.94±0.05[a]	1.94±0.12[a]
%(LHG)/CW	4.62±0.22[a]	4.61±0.09[a]	4.61±0.09[a]	4.78±0.30[a]

Results are means of 5 determinations ± SEM Values along the same row with the same superscript are NOT significantly different (P>0.05), but are significantly different if the superscripts are different
A was maintained on non-supplemented Starter and non-supplemented Finisher feeds (control).

B was maintained on non-supplemented Starter feed but 2% mixed spices-supplemented Finisher feed.
C was maintained on 2% mixed spices-supplemented Starter feed but non-supplemented Finisher feed.
D was maintained on 2% mixed spices-supplemented Starter and 2% mixed spices-supplemented Finisher feed.
%FW/CW: % fat to carcass weight
%L/CW: % liver to carcass weight
%H/CW: % heart to carcass weight
%G/CW: % gizzard carcass weight
%(LHG)/CW: % liver+heart+gizzard) to carcass weight

Table 4: Red blood cell indices of broiler chickens fed with or without spices-supplemented diets

	A	B	C	D
PCV (%)	27.00±0.32[a]	28.00±0.71[a]	29.20±0.86[a]	26.60±1.86[a]
HB(g/dL)	8.44±0.35[a]	9.29±0.22[a]	9.66±0.24[a]	8.88±0.61[a]
RBC(10^6/µL)	2.34±0.06[a]	2.83±0.27[a]	3.06±0.32[a]	3.06±0.40[a]
MCH(ps)	36.03±0.85[a]	33.76±3.26[a]	32.56±2.42[a]	30.17±2.40[a]
MCV(fL)	115.69±3.27[a]	101.76±9.88[a]	98.24±6.87[a]	98.26±6.94[a]
MCHC(g/dL)	31.25±1.19[a]	33.18±0.05[a]	33.10±0.25[a]	33.40±0.15[a]

Results are means of 3 determinations ± SEM Values along the same row with the same superscript are NOT significantly different (P>0.05), but are significantly different if the superscripts are different
A was maintained on non-supplemented Starter and non-supplemented Finisher feeds (control).
B was maintained on non-supplemented Starter feed but 2% mixed spices-supplemented Finisher feed.
C was maintained on 2% mixed spices-supplemented Starter feed but non-supplemented Finisher feed.
D was maintained on 2% mixed spices-supplemented Starter and 2% mixed spices-supplemented Finisher feed.RBC: red blood cells HB: haemoglobin
PCV: Packed cell volume
MCV: mean corpuscular volume
MCH: mean corpuscular haemoglobin
MCHC: mean corpuscular haemoglobin concentration

Table 5: Lipid and artherogenic profiles of broiler chickens fed with or without spices-supplemented diets

	A	B	C	D
TC(mmol/l)	2.40±0.23[a]	2.44±0.25[a]	2.34±0.19[a]	2.38±0.24[a]
TG(mmol/l)	1.12±0.04[ab]	1.30±0.06[b]	1.08±0.06[a]	1.18±0.07[ab]
HDL-C(mmol/l)	0.46±0.04[a]	0.45±0.04[a]	0.43±0.04[a]	0.42±0.05[a]
LDL-C(mmol/l)	0.75±0.16[a]	0.66±0.13[a]	0.72±0.12[a]	0.69±0.16[a]
AI	0.39±0.04[a]	0.46±0.02[a]	0.41±0.04[a]	0.45±0.05[a]
CRR	5.17±0.11[a]	5.39±0.15[a]	5.53±0.28[a]	5.66±0.37[a]
AC	4.17±0.12[a]	4.39±0.15[a]	4.53±0.28[a]	4.66±0.36[a]

Results are means of 3 determinations ± SEM Values along the same row with the same superscript are NOT significantly different (P>0.05), but are significantly different if the superscripts are different
A was maintained on non-supplemented Starter and non-supplemented Finisher feeds (control).
B was maintained on non-supplemented Starter feed but 2% mixed spices-supplemented Finisher feed.
C was maintained on 2% mixed spices-supplemented Starter feed but non-supplemented Finisher feed.
D was maintained on 2% mixed spices-supplemented Starter and 2% mixed spices-supplemented Finisher feed.

TC: total cholesterol
TG: triglyceride
HDL-C: high density lipoprotein cholesterol
LDL-C: low density lipoprotein cholesterol
AI: atherogenic index
CRR: cardiac risk ratio
AC: atherogenic coefficients

Lipid and red blood cell indices of broiler chickens fed with or without mixed spices supplemented diet: All the evaluated red blood cell indices (Table 4), lipid and artherogenic indices in the test groups did not significantly differ ($p>0.5$) from the control (Table 5). The abdominal fat is known to be associated with adverse changes in lipid indicators and increased risk of coronary heart disease and hypertension. Clinical studies show strong relationships between adipokines and body composition indices (Silha *et al*., 2003; Goropashnaya *et al*., 2009 and Mutairi *et al*., 2014). Increase in adipocytes associated with development of adipose tissue inflammation and changes in leptin, resistin, tumour necrosis factor (TNF α), adiponectin, interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1) secretion leads, consequently, to obesity-mediated adverse effects on glucose and lipid metabolism (Chudek *et al*., 2006). This was however not the case here; though the control group had relatively more abdominal fat, all the groups were neither obese nor overweight and hence, the non significant change in the lipid and atherogenic indices. There were no significant ($p>0.5$) variations in the studied red blood cell indices.

Conclusion

Our investigation has shown that *Allumsativum*, *Zingiberofficinale*and *Punicagranatum*mixed in ratio of 2:2:1 respectively could suppress fat accumulation in broiler chicken, without affecting carcass traits, lipid profile and erythropoiesis in them.

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