NUTRIGENETIC EFFECT OF *Centella asiatica* AND *Capsicum annum* ON BODY WEIGHT AND CARDIOVASCULAR RISK IN CHICKENS

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ABSTRACT

A set of 80 birds (40 Marshall broiler chicks of mixed sexes and 40 Harco black layer lineage chicks also of mixed sexes) were used in a 2 x 2 x 2 Factorial (Genotype x Dietary Centella x Dietary Cayenne Pepper) layout of treatments within a Completely Randomized Design to evaluate the effects of Genotype, dietary *Centella asiatica* at 0 and 0.2 % levels, and *Capsicum annum* at 0 and 0.2 % levels, and their two- and three-way interactions modulation of cardiovascular disease risk through their effects on performance (body weight), fatness (Body Mass Index), lipid profile, inflammation / rheology and insulin secretion and action (Intraperitoneal Glucose Tolerance, IPGTT) in chickens. Body weight at age 1, 4 and 7 weeks was significantly ($p<0.05$) determined by Genotype and Genotype x Cayenne interaction. Fasting glycaemia in the IPGTT was significantly determined by Genotype, Cayenne, Centella x Cayenne and Genotype x Centella x Cayenne factor and factor interactions. These data point to significant benefits of *Centella asiatica* and Cayenne pepper nutriceuticals for reducing cardiovascular risk in chickens raised as broilers. *Centella asiatica* should be used as a nutraceutical dietary supplement at the level examined, to reduce cardiovascular risk over a 7 week production cycle through its effect in transiently reducing body weight and improving insulin secretion and action as measured by glucose tolerance. Cayenne pepper should be used as a nutraceutical dietary supplement at the level examined, to reduce cardiovascular risk over a 7 week production cycle through its effect in reducing inflammation. Given the nutrigenetic (Genotype x Nutraceutical interaction) effects observed here, care should be exercised in use of *Centella asiatica* and Cayenne pepper for the prescribed purposes, ensuring that the desired effects are present within the genotype background context in which they are used.

Keyword: Cayenne pepper, *Centella asiatica*, nutraceutical, nutrigenetic, broiler, cardiovascular risk.

INTRODUCTION

Broiler meat is popular because it is cheaper, more versatile, and is perceived to give more health benefits than red meat (Chang, 2007). Concerted genetic improvements through breeding and selection has increase the rate of growth and weight at market age 5 – 8 weeks; Wepruk (2003) reported that in 1976, it took 63 days for a broiler to reach a market weight of 2kg while in 2000 it took just 36days. Selection for efficient productivity is often accompanied by correlated changes in other genetic traits, which may be of a positive or negative impact to the welfare of these animals (Rauw *et al.*, 1998); among of which is metabolic syndrome (a cluster of many health concerns including obesity, oxidative stress, hyperlipidimea, low grade inflammation etc) which thus predisposes the animal to cardiovascular disease. The control of cardiovascular risk factors including body weight/fatness (obesity), hyperlipidaemia, elevated blood glucose and low grade inflammation through medication, diet and lifestyle changes has been shown to reduce morbidity and mortality associated with cardiovascular disease. With respect to use of medicinal agents in reducing cardiovascular risk, use of natural medicinal products is viewed as more attractive than synthetic products because of the negative impact associated with continuous use of the latter. While claims have been made about the effectiveness of many botanicals in the modulation of cardiovascular risk factors, few have been validated through scientific methods of hypothesis testing. Studies that examine whether and to what extent botanicals are efficacious in controlling cardiovascular risk will increase the options of proven natural products available to farmers for controlling cardiovascular disease risk. In the current study, the singular and combined effects of two botanicals (*Centella asiatica* and *Capsicum annum*) on cardiovascular risk were examined. *Centella asiatica* was examined because it contains asiaticoside (Liang *et al.*, 2008) which has anti-inflammatory, and antioxidant properties (Kimura *et al.*, 2008) through which it may possibly reduce cardiovascular risk. Other properties (anxiolytic, antiulcer, and wound-healing) have been described. Cayenne pepper was examined because it also has antioxidant activity of about 172.00mg GAE/100g and can serve as scavenger of free radicals that have been reported to be associated with occurrence of chronic and degenerative diseases such as cardiovascular diseases (Ogunlade *et al.*, 2012). Additionally, the present study examined whether and to what extent the singular and combined effects of *Centella asiatica* and *Capsicum annum* were genotype dependent.

MATERIALS AND METHODS

A total of 40 Marshall broiler and 40 Harco black chicks were examined. Cayenne pepper was purchased at the Oja-oba Market, Ilorin; samples were taken for identification at the herbarium of the Plant Biology Department University of Ilorin. The sorted pepper was air dried and oven dried at 60ºC until crisp. The dried pepper was then ground using a Barr mill. Milled pure (100%) *Centella asiatica* sold under the proprietary name Centella lujica (NAFDAC Registration number B1-4305) was purchased. Four feed of isonitrogenous and isocaloric were formulated (Table 1). The control diet contained neither Cayenne pepper nor *Centella asiatica*, the second and third group contained 0.2g Cayenne pepper and 0.2g *Centella asiatica* singly while the fourth group contained both 0.2g *Centella asiatica* and 0.2g Cayenne pepper. Birds of each Genotype were divided into four (4) groups, making a total of eight (8) treatment groups. Standard broiler starter was given for the first four weeks while broiler finisher was given from the beginning of week five till the end of the experiment. Body weight was carried from day one of the experiment and at weekly interval up till week 8. Body Mass Index was computed as done by Mendes *et al.*, 2007. Intraperitoneal Glucose Tolerance Test was conducted at week 6, Five animals were randomly selected from each group and were weighed. The baseline glucose level (T = 0) was taken for each animal with the aid of a glucose meter and corresponding glucose strip. A 20% glucose solution (20% w/v glucose, 0.9% w/v NaCl) was injected into the peritoneal cavity of each animal at 2g glucose/kg body weight. A drop of blood was then taken from each bird at 15, 30 and 75 minutes following glucose injection (T15, T30 and T75 respectively) by wing venipuncture and used to determine blood glucose on a point of care device (Accu-check glucometer, Roche).

At 7th week of the experiment, birds were fasted overnight. Five birds were randomly selected for collection of blood. Blood was collected from the wing vein of the selected birds with the aid of needle and syringe. Blood collected from each chicken was transferred immediately into a set of sterile plastic bottles with and without anti-coagulant for haematological and serum biochemical tests respectively. The blood samples were analyzed at the University of Ilorin Teaching Hospital Haematology and Chemical Pathology Laboratory. Factor effects (Genotype, *Capsicum Annum* and *Centella asiatica*) and the interactions were determined by statistical analysis of variance (ANOVA), within the General Linear Model (GLM) module of SPSS19. For each parameter measured, significantly different means was separated by the use of the Duncan's Multiple Range procedure (SPSS19, IBM).

RESULTS AND DISCUSSION

The genetically fast growing Marshall breed has higher cardiovascular risk as measured by consistently higher body weight at age 1, 4 and 7 weeks than the genetically slow growing Harco breed (Table 1). The genetically fast growing Marshall breed has higher cardiovascular risk as measured by consistently higher Body Mass index (a surrogate for Obesity/fatness) at age 1, 4 and 7 weeks than the genetically slow growing Harco breed (Table 1). The genetically slow growing Harco breed has higher cardiovascular risk as measured by Lipideaemia than the genetically fast growing Marshall breed in as much as the latter has higher Total Cholesterol in blood plasma than the former, while HDL, LDL and Triglycerides in blood plasma were not significantly different between groups (Table 1). The genetically fast growing Marshall breed has higher cardiovascular risk as measured by white cell count in blood (a surrogate for inflammation) than the genetically slow growing Harco breed (Table 1). The genetically fast growing Marshall breed has higher cardiovascular risk as measured by red blood cell count (a surrogate rheological markers of cardiovascular risk) than the genetically slow growing Harco breed (Table 1). The genetically slow growing Harco breed has higher cardiovascular risk as measured by insulin secretion and action in as much as it had higher fasting glycaemia, T15, T75 post prandial glycaemia and Cumulative Glycaemia than the Marshall breed (Table 2).

Centella asiatica transiently lowers cardiovascular risk by lowering body weight at age 4 weeks. *Centella asiatica* does not significantly (>0.05) modulate cardiovascular risk through effects on body mass index (Table 1). *Centella asiatica* does not significantly (>0.05) modulate cardiovascular risk through effects on lipidaemia (TC, HDL, LDL, Triglycerides and the HDL:LDL ratio) as shown in Table 1. *Centella asiatica* produces mixed effects on cardiovascular risk as measured by inflammation in as much as it raised neutrophil differential white blood cell counts while lowering lymphocyte differential white blood cell counts (Table 1). *Centella asiatica* lowers cardiovascular risk as measured by insulin secretion and action through its effect in reducing post-prandial glucose (T75) as shown in Table 2.

Cayenne pepper transiently raises cardiovascular risk through effects in raising body weight at ages 1 and 4 weeks, though by age 7 weeks the effect had disappeared (Table 1). Cayenne pepper does not significantly (>0.05) modulate risk through effects on body mass index. *Centella asiatica* does not significantly (>0.05) modulate cardiovascular risk through effects on lipidaemia (TC, HDL, LDL, Triglycerides and the HDL:LDL ratio) as shown in Table 1. Cayenne pepper lowered cardiovascular risk as measured by inflammation in as much as it lowered lymphocyte differential white blood cell counts while all other haematological markers were unaffected (Table 1). Cayenne pepper produces mixed effects on cardiovascular risk as measured by insulin secretion and action in as much as it lowers risk by lowering fasting glycaemia but raises risk by raising postprandial glycaemia (Table 2)

Centella asiatica produces a genotype dependent effect on white blood cell count. Cayenne pepper produces a genotype dependent effect on body weight, body mass index, blood haemoglobin concentration and white blood cell count, and glucose tolerance (T75 and K15-75 glucose).

The interaction between *Centella asiatica* and Cayenne pepper contributed significantly to variation in Body Mass Index, total Cholesterol, White blood cell count and lymphocyte differential white blood cell count, and glucose tolerance (T0, T75 and K15-75 glucose). A 3-way interaction between body weight, *Centella asiatica* and Cayenne pepper contributed significantly to variation in body mass index, total white blood cell count, and neutrophil and lymphocyte differential white blood cell count, and glucose tolerance (T0 glucose).

RECOMMENDATION

Centella asiatica should be used as a nutraceutical dietary supplement in the diet of chickens (raised for meat) at the level examined, to reduce cardiovascular risk over a 7 week production cycle through its effect in transiently reducing body weight and improving insulin secretion and action as measured by glucose tolerance. Cayenne pepper should be used as a nutraceutical dietary supplement in the diet of chickens (raised for meat) at the level examined, to reduce cardiovascular risk over a 7 week production cycle through its effect in reducing inflammation.

Given the Genotype x Nutraceutical interaction effects observed here, care should be exercised in use of *Centella asiatica* and Cayenne pepper for the prescribed purposes, ensuring that the desired effects are present within the genotype background context in which they are used.

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Table 1. Effect of Genotype, *Centella asiatica*, Cayenne pepper and their 2- and 3-way interactions on cardiovascular risk related traits in chickens.

Trait	Breed		*Centelle asiatica*		Cayenne pepper		Interaction			
	Marshall	Harco	0%	0.2%	0%	0.2%	G x Ca	G x Cy	Ca x Cy	G x Ca x Cy
Body weight										
Body Weight wk1	179.74 ± 3.46*	75.79 ± 1.85	131.05 ± 9.09	125.9 ± 8.76	124.86 ± 8.06	131.75 ± 9.58*	NS	*	NS	NS
Body Weight wk4	1021.79 ± 18.15*	289.46 ± 10.33	711.08 ± 64.28*	621.79 ± 59	641.62 ± 57.52	687.69 ± 65.54*	NS	*	NS	NS
Body Weight wk7	2067.78 ± 55.97*	541.89 ± 15.79	1363.24 ± 131.47	1223.61 ± 136.24	1291.08 ± 125.34	1297.78 ± 143.19	NS	NS	NS	NS
Obesity/Fatness										
BMI_WK1	0.45 ± 0.02*	0.24 ± 0.01	0.36 ± 0.02	0.34 ± 0.02	0.36 ± 0.03	0.33 ± 0.02	NS	NS	NS	NS
BMI_WK4	0.79 ± 0.02*	0.39 ± 0.01	0.62 ± 0.03	0.58 ± 0.03	0.59 ± 0.03	0.61 ± 0.04	NS	*	*	NS
BMI_WK7	0.94 ± 0.02*	0.43 ± 0.01	0.7 ± 0.04	0.66 ± 0.05	0.69 ± 0.04	0.68 ± 0.05	NS	NS	NS	*
Lipidaemia										
TC (mmol/l)	3.02 ± 0.14	3.4 ± 0.11*	3.18 ± 0.12	3.24 ± 0.14	3.28 ± 0.13	3.14 ± 0.13	NS	NS	*	NS
LDL (mmol/l)	0.82 ± 0.05	0.94 ± 0.04	0.86 ± 0.05	0.9 ± 0.05	0.91 ± 0.05	0.86 ± 0.05	NS	NS	NS	NS
HDL (mmol/l)	1.42 ± 0.09	1.58 ± 0.05	1.52 ± 0.08	1.48 ± 0.06	1.54 ± 0.06	1.46 ± 0.08	NS	NS	NS	NS
TRIG (mmol/l)	0.64 ± 0.06	0.61 ± 0.05	0.63 ± 0.05	0.62 ± 0.06	0.56 ± 0.03	0.69 ± 0.07	NS	NS	NS	NS
HDL:LDL	1.77 ± 0.07	1.72 ± 0.06	1.79 ± 0.06	1.71 ± 0.07	1.75 ± 0.07	1.75 ± 0.06	NS	NS	NS	NS
Rheology / Inflammation										
Packed Cell Volume (%)	24.8 ± 0.87	22.6 ± 0.76	23.65 ± 1.02	23.75 ± 0.64	22.85 ± 0.65	24.55 ± 0.98	NS	NS	NS	NS
Hemoglobin (g/dL)	10.69 ± 0.23	10.37 ± 0.19	10.55 ± 0.24	10.5 ± 0.18	10.37 ± 0.21	10.69 ± 0.21	NS	*	NS	NS
White Blood Cell ($\times10^9$/L)	8.38 ± 0.2*	8.14 ± 0.14	8.35 ± 0.2	8.17 ± 0.14	8.26 ± 0.18	8.26 ± 0.17	*	*	*	*
Red Blood Cell ($\times10^{12}$/L)	5.64 ± 0.13*	5.27 ± 0.11	5.4 ± 0.16	5.5 ± 0.1	5.28 ± 0.13	5.62 ± 0.12	NS	NS	NS	NS
Neutrophil (%)	38.35 ± 1.14	37.45 ± 0.65	36 ± 0.92	39.8 ± 0.72*	37.15 ± 1.12	38.65 ± 0.65	NS	NS	NS	*
Lymphocyte (%)	60.3 ± 1.14	60.9 ± 0.68	62.5 ± 0.96*	58.7 ± 0.7	61.45 ± 1.11*	59.75 ± 0.68	NS	NS	*	*
Eosinophil (%)	1.61 ± 0.18	1.65 ± 0.13	1.68 ± 0.17	1.58 ± 0.14	1.47 ± 0.12	1.79 ± 0.18	NS	NS	NS	NS

G = Genotype, Ca = *Centella asiatica*, Cy = cayenne Pepper (*Capsicum annum*), WK=Week, BMI= Body Mass Index, TC – Total Cholesterol, LDL = Low Density Lipoprotein cholesterol, HDL = High Density Lipoprotein cholesterol, TRIG = Triglycerides, * = significant ($p<0.05$), NS = Not significant ($p>0.05$).

Table 2. Effect of Genotype, *Centella asiatica*, Cayenne pepper and their 2- and 3-way interactions on insulin secretion and action as revealed in an Intraperitoneal Glucose tolerance test.

Trait	Breed		*Centelle asiatica*		Cayenne pepper		Interaction			
	Marshall	Harco	0%	0.2%	0%	0.2%	G x Ca	G x Cy	Ca x Cy	G x Ca x Cy
T 0 (mM)	10.33 ± 0.25	11.52 ± 0.16*	10.75 ± 0.3	11.06 ± 0.21	11.29 ± 0.21*	10.54 ± 0.26	NS	NS	*(0.140)	*(0.139)
T 15 (mM)	16.36 ± 0.52	19.09 ± 0.82*	17.96 ± 0.89	17.38 ± 0.59	17.53 ± 0.74	17.76 ± 0.74	NS	NS	NS	NS
T 30 (mM)	17.98 ± 0.86	19.2 ± 0.73	19.14 ± 0.81	18.04 ± 0.81	17.98 ± 0.93	19.08 ± 0.7	NS	NS	NS	NS
T 75 (mM)	13.11 ± 0.38	16.34 ± 0.75*	15.37 ± 0.89*	14.03 ± 0.4	15.2 ± 0.92	14.19 ± 0.36	NS	*(0.176)	*(0.195)	NS
CumG	1157.1 ± 34.43	1270.89 ± 34.16*	1225.58 ± 39.68	1200.15 ± 33.67	1186.82 ± 40.27	1236.98 ± 32.3	NS	NS	NS	NS
K15to75	-0.07 ± 0.01	-0.03 ± 0.03	-0.04 ± 0.03	-0.06 ± 0.01	-0.03 ± 0.03	-0.07 ± 0.01	NS	*(0.181)	*(0.209)	NS
K15to30	0.11 ± 0.05	0.01 ± 0.11	0.07 ± 0.11	0.04 ± 0.05	0.03 ± 0.11	0.09 ± 0.05	NS	NS	NS	NS
DeltaG	382.73 ± 34.2	407.21 ± 40.16	419.53 ± 39.92	371.03 ± 33.8	339.71 ± 37.88	446.85 ± 32.42*	NS	NS	NS	NS