



## Original research article

The effects of *Lippia javanica* dietary inclusion on growth performance, carcass characteristics and fatty acid profiles of broiler chickensDavid A. Mpofo<sup>a, b</sup>, Upenyu Marume<sup>a, b, \*</sup>, Victor Mlambo<sup>a, b</sup>, Arno Hugo<sup>c</sup><sup>a</sup> Department of Animal Science, Faculty of Agriculture, Science and Technology, North-West University, Mmabatho 2735, South Africa<sup>b</sup> Food Security and Safety Niche Area, Faculty of Agriculture, Science and Technology, North-West University, Mmabatho, 2735, South Africa<sup>c</sup> Department of Microbial, Biochemical and Food Biotechnology, University of Free State, Bloemfontein 9300, South Africa

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## ABSTRACT

This study was conducted to determine the effect of inclusion of fever tea (*Lippia javanica*) leaf meal in broiler diets on growth performance, carcass characteristics and fatty acid (FA) profiles over a 42-day feeding period. One hundred and eighty, one-day-old, broiler chicks were randomly allocated to the following four treatments: 1) negative control (commercial broiler diet only [Negcontrol]); 2) positive control (commercial broiler diet + prophylactic antibiotics [Poscontrol]); 3) commercial broiler diet without prophylactic antibiotics + 5 g of *L. javanica* per kg of feed (Ljav5) and 4) commercial broiler diet without prophylactic antibiotics + 12 g of *L. javanica* per kg of feed (Ljav12). Body weights (BW) and feed intake (FI) were recorded weekly and used to calculate feed conversion ratio (FCR) and average daily weight gain (ADG). At the end of the trial (day 42), all chickens were slaughtered at a local commercial abattoir for assessment of carcass characteristics and FA profiles of meat. The broilers fed *L. javanica* had significantly ( $P < 0.05$ ) lower FI compared with the other two groups. However, the broilers in the Poscontrol and Ljav5 treatment groups had higher ( $P < 0.05$ ) ADG, lower FCR and higher slaughter weights. *L. javanica* inclusion had no effect on the breast weight, thigh weight, carcass weight, and dressing percentage of the broilers. Most of the n-3 FA were not affected by diets except for the docosapentaenoic, which was found to be higher ( $P < 0.05$ ) in the Ljav12 treatment group and the lowest in the Negcontrol. The broilers in the Negcontrol and Poscontrol groups had higher ( $P < 0.05$ ) total saturated fatty acids (SFA). On the contrary, the *L. javanica* fed broilers had higher ( $P < 0.05$ ) total polyunsaturated fatty acids (PUFA), total n-3 FA and PUFA:SFA ratio and also had significantly lower n-6:n-3 ratios compared with the other two treatment groups. No differences were observed with regards to total monounsaturated fatty acids (MUFA) and total n-6 FA. Overall, the findings from the study showed that inclusion of *L. javanica* in broiler diets at 5 g/kg feed has positive influences on growth performance, carcass characteristics and FA profiles of broiler meat.

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## 1. Introduction

Over the years, antibiotic growth promoters have been used successfully in poultry to increase growth rates through improved gut health and better nutrient utilisation (Landy et al., 2011). However, the continuous use of these growth promoters has been observed to be consequential to increased bacterial resistance and persistence and accumulation of residues in meat, and hence increasing health risks in humans. This has resulted in a ban on the sub-therapeutic use of antibiotics, firstly in Europe and worldwide in general (Toghyani et al., 2010). There is, therefore, an urgent need to explore alternatives to antibiotics that can be used to improve

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growth and end product quality in broiler production. Natural alternatives that have received increased attention include the phyto-genic plants and other herbal products (Toghyani et al., 2010; Landy et al., 2011; Ghalamkari et al., 2012; Hong et al., 2012). Phyto-genic plants, including *Lippia javanica*, and herbal products have been traditionally used in South African households and across the world to treat some animal ailments (Viljoen et al., 2005; Ghalamkari et al., 2012).

A natural herbal plant that has been popularised in many communal households of South Africa in the treatment of livestock ailments is *L. javanica* (fever tea) (Viljoen et al., 2005). The plant is widely distributed in Southern Africa and across the world and it has been used extensively as a medicinal plant for both animals and humans (Viljoen et al., 2005; Oliveira et al., 2007). It contains secondary plant metabolites, particularly terpenoids that have been reported to possess analgesic, anti-inflammatory and antipyretic activities (Abena et al., 2003). The terpenoids were also observed to have inhibitory effects on cultures of *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Manezhe et al., 2004). The plant has been used as an anthelmintic (Mabogo, 1990) and has also been found to be active against *Plasmodium falciparum* and *Klebsiella pneumoniae* (Nkhumeleni et al., 2004; Viljoen et al., 2005).

Information is available on the use of phyto-genic plants, with similar medicinal properties to *L. javanica*, as alternatives to antibiotics in broilers and laying hens. For example, the inclusion of *Mentha pulegium* L. in broiler diets has been observed to improve performance and carcass quality (Modiry et al., 2010; Ghalamkari et al., 2012), while neem (*Azadirachta indica*) was observed to favourably influence the immune response of broilers without any adverse effects on growth and carcass quality (Landy et al., 2011). In addition to the increase in antioxidant activity of meat, phyto-genic plants like *L. javanica* have been observed to influence meat quality (chemical composition and colour stability) as well as fatty acid (FA) composition of meat (Sreelatha and Padma, 2009; Qwele et al., 2013). Moreover, the phyto-genic compounds in meat have the benefit of increasing conjugated linoleic acid concentration and yield meat of lighter colour (Mapiye et al., 2011). However, despite the potential of *L. javanica* as growth promoting feed additive in broilers, information on its influences on growth, carcass characteristics and FA profiles in broiler meat is unavailable. Therefore, the current study was conducted to assess the effect of *L. javanica* leaf meal on growth performance, carcass characteristics and FA profiles of broiler chickens.

## 2. Materials and methods

### 2.1. Study site

The study was carried out at North-West University (NWU) experimental farm. The study site is located in North West Province of South Africa. The North West Province is situated between 25° and 28° south latitude and between 22° and 28° east longitude.

### 2.2. Harvesting and preparation of *L. javanica*

*L. javanica* foliage (stem and leaves) was harvested from Mafikeng Game Reserve (North West Province, Republic of South Africa). The Mafikeng game reserve is located against the municipal boundary of Mahikeng covering an area of 4,600 hm<sup>2</sup> of open Kalahari grassland and *Acacia* thorn scrub. The plant foliage was hand collected and dried to constant weight on open floors at room temperature (20–22°C) for 6 days. Collection was done from an area measuring approximately 250 m<sup>2</sup> to avoid harvesting plants from different growth environments. After drying, dry leaves were pruned from the whole plant and then ground into a powder to

pass through a 1 mm sieve producing the *L. javanica* powder. The leaf meal was then packed in white polythene plastic bags and stored under dry conditions away from direct sunlight in the laboratory pending use in feeding experiments.

### 2.3. Animal management

One hundred and eighty, one-day-old, Cobb 500 broiler chickens purchased from a commercial hatchery were randomly allocated to four diets with three replicate pens holding 15 chicks each in completely randomised design (CRD). The experimental unit was the pen holding 15 chickens. The chicks were weighed upon arrival and housed in a total of 12 pens measuring 1.5 m × 1.5 m (2.25 m<sup>2</sup>). The pens met the space requirements for the rearing of broiler chickens. Each pen was equipped with water and feeding troughs while sunflower seed hulls were used as bedding. Infra-red light was provided continuously from day 1 till day 21. On day 22 chickens were reared under a light–dark cycle (from 06:00 to 18:00 lights were switched off, then after 18:00 light were switched on) and on day 35 until the end of the experiment, the light was switched off completely.

### 2.4. Dietary treatments

The four dietary treatments were formulated as follows: 1) a negative control made up of the commercial broiler diet without prophylactic antibiotics (Negcontrol); 2) a positive control made up of the commercial broiler diet with prophylactic antibiotics (Albac and Aviax) (Poscontrol); 3) commercial broiler diet without prophylactic antibiotics but supplemented with 5 g of *L. javanica* per kg of feed (Ljav5) and 4) commercial broiler diet without prophylactic antibiotics but supplemented with 12 g of *L. javanica* per kg of feed (Ljav12). The two inclusion levels of *L. javanica* were determined based on the available information on bioactive extracts (mainly, 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one) and chemical composition of *L. javanica* (Mabogo, 1990; Manezhe et al., 2004). The chickens were reared under a 3-phase feeding program consisting of a maize–soya bean based broiler starter diet (1–15 days), grower diet (16–28 days), and broiler finisher diet (29–42 days). In all phases, the diets were formulated (Table 1) to meet the nutrient requirements for broilers at different growth phases according to Cobb Broiler Performance and Nutrition Supplement (2008). The formulated diets were obtained from a commercial feed milling company, Nutrifeds (Mafikeng, SA). Feed and water were offered *ad libitum*. Ethical clearance for the study was obtained from the North-West University Ethics Committee (Ethics Clearance number: NWU-0099-14-S9) and experimental conditions conformed to the national and international standards governing research using animals.

The experimental diets were milled through a 1-mm screen then analysed for dry matter content (DM) (AOAC, 2005; method No. 930.15) and nitrogen (N) (AOAC, 2005; method No. 984.05). Crude protein (CP) was then calculated as N × 6.25. Neutral detergent fibre (NDF) and acid detergent fibre were determined using the ANKOM2000 fibre analyser (Ankom Technology, NY USA), while ether extract was determined using the ANKOM XT10 extractor (ANKOM Technology, NY, USA) (Table 2).

### 2.5. Growth performance

All the birds were weighed individually at the beginning of the experiment (initial body weight), then weekly at 7, 14, 21, 28, 35 and 42 days of age. Feed intake was measured weekly per pen calculated as weight of feed offered less the amount of feed refused (measured the following morning). Feed conversion ratio was

**Table 1**  
Composition of starter, grower and finisher diets (%/kg feed).

Ingredients	Diet <sup>1</sup>											
	Starter				Grower				Finisher			
	Negcontrol	Poscontrol	Ljav5	Ljav12	Negcontrol	Poscontrol	Ljav5	Ljav12	Negcontrol	Poscontrol	Ljav5	Ljav12
Fine yellow maize	60.1	59.4	59.7	59.3	62.8	62.6	62.5	62.1	73.0	72.9	72.6	72.1
Prime Gluten 60	6.06	6.05	6.03	5.98	6.46	6.45	6.43	6.38	6.76	6.75	6.72	6.68
Wheat bran	8.01	8.0	7.97	7.91	8.01	8	8.00	8.00	–	–	–	–
Full fat soya	1.20	1.20	1.20	1.19	1.00	1	1.00	0.99	–	–	–	–
Soya bean meal	16.7	16.7	16.6	16.5	13.1	13.1	13.0	12.9	13.1	13.1	12.9	12.9
Sunflower oilcake	4.00	4.0	3.98	3.96	5.01	5	5.00	5.00	3.40	3.4	3.39	3.36
Limestone powder	1.20	1.2	1.20	1.19	1.20	1.2	1.20	1.19	1.10	1.1	1.10	1.09
K <sub>2</sub> CO <sub>3</sub>	0.16	0.16	0.16	0.16	0.07	0.07	0.07	0.07	0.15	0.15	0.15	0.15
MCP	1.34	1.34	1.33	1.33	1.16	1.16	1.16	1.15	1.10	1.1	1.10	1.09
Salt-fine	0.31	0.31	0.31	0.31	0.32	0.32	0.32	0.32	0.30	0.3	0.30	0.30
Soya oil	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.40	0.4	0.40	0.40
Bicarbonate	0.19	0.19	0.19	0.19	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Choline powder	0.08	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.08	0.08	0.08	0.08
Lysine	0.30	0.30	0.30	0.30	0.25	0.25	0.25	0.25	0.31	0.31	0.31	0.31
Methionine	0.04	0.04	0.04	0.04	0	0	0	0	0.01	0.01	0.01	0.01
Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Albac	–	0.07	–	–	–	0.07	–	–	–	0.07	–	–
Aviav	–	0.05	–	–	–	0.05	–	–	–	0.05	–	–
<i>Lippia javanica</i>	–	–	0.50	1.19	–	–	0.50	1.19	–	–	0.50	1.19

MCP = mono calcium phosphate; K<sub>2</sub>CO<sub>3</sub> = potassium carbonate.

<sup>1</sup> Negcontrol = commercial broiler diet without prophylactic antibiotic; Poscontrol = commercial broiler diet with prophylactic antibiotic; Ljav5 = *L. javanica* leaf powder 5 g/kg; Ljav12 = *L. javanica* leaf powder 12 g/kg.

**Table 2**  
Analysed chemical compositions of experimental diets and *Lippia javanica* on dry matter basis.

Dietary treatment <sup>1</sup>	ME, MJ/kg	CP, g/kg	CF, g/kg	Ash, g/kg	NDF, g/kg	ADF, g/kg	Ca, g/kg	P, g/kg
Starter (1–14 days)								
Negcontrol	12.1	250.5	29.9	60.9	110.3	36.4	10	10
Poscontrol	12.1	250.2	30.1	60.8	109.9	36.1	10	10
Ljav5	12.3	251.1	31.1	61.6	112.3	41.6	12	12
Ljav12	12.4	251.3	31.6	61.7	113.1	43.4	13	11
Grower (15–28 days)								
Negcontrol	12.8	220.1	31.8	58.8	111.5	38.6	10	8.1
Poscontrol	12.8	220.2	31.6	60.1	111.9	39.8	10	8.3
Ljav5	13.3	221.3	32.3	61.6	114.6	43.2	11	9.6
Ljav12	13.5	221.5	32.7	61.7	116.1	46.1	13	9.8
Finisher (29–42 days)								
Negcontrol	13.2	220.1	31.9	57.8	111.1	43.5	10	8.0
Poscontrol	13.3	220.3	31.9	59.4	109.3	44.1	10	7.8
Ljav5	13.8	221.4	32.4	61.1	112.6	49.6	12	9.6
Ljav12	14.1	221.5	32.5	61.4	113.9	51.2	13	9.7
<i>Lippia javanica</i> <sup>2</sup>	–	106.2	32.5	8.1	262.1	160.3	4.1	1.1

ME = metabolisable energy; CP = crude protein; ADF = acid detergent fibre; NDF = neutral detergent fibre.

<sup>1</sup> Negcontrol = commercial broiler diet without prophylactic antibiotic; Poscontrol = commercial broiler diet with prophylactic antibiotic; Ljav5 = *L. javanica* leaf powder 5 g/kg; Ljav12 = *L. javanica* leaf powder 12 g/kg.

<sup>2</sup> *Lippia javanica* (Condensed tannins: 0.14%; Total phenolics: 0.49%).

calculated as feed intake divided by weight gain over the experimental period. All the cages were checked three times a day for mortality and any dead chickens were removed from the cages. In case of mortalities, feed intake and feed conversion efficiency were adjusted accordingly.

## 2.6. Determination of carcass traits

At the end of the feeding trial, chickens were fasted for 13 h to allow for the emptying of the gut. Thereafter, all chickens were taken to a commercial abattoir (Agrichicks, SA) for slaughter following the standard procedures for stunning, exsanguination, and de-feathering. At slaughter, the carcasses from different treatments were identified using woollen fibre tied to the feet which different colours of the fibre representing different treatments. Thereafter all the birds were taken back to the university laboratory for evisceration and carcass characteristics measurements. The following organs were removed before being weighed:

proventriculus, gizzard, breast, thigh, liver, pancreas, heart, caecum, and spleen. The length of small intestines was also measured and recorded. The carcass weight of each chicken was also recorded and dressing out percentage was calculated. Breast muscles were carefully removed, vacuum packed and sent for analysis of FA at the Food Science Division, University of Free State (SA).

## 2.7. Proximate and fatty acid analysis

Total lipids from *L. javanica* were extracted with a Soxhlet extraction according to AOAC (2003) procedures for the determination of fats. Total lipids from muscle samples were quantitatively extracted, according to the method of Folch et al. (1957) using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene, was added at a concentration of 0.001% to the chloroform: methanol mixture. A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as a

moisture adsorbent. The total extractable fat was determined gravimetrically from the extracted fat and expressed as percent fat (wt/wt) per 100 g tissue. The extracted fat from feed, subcutaneous fat and muscle was stored in a polytop (glass vial, with a push-in top) under a blanket of nitrogen and froze then it was stored at  $-20^{\circ}\text{C}$  pending FA analyses.

A lipid aliquot (20 mg) from feed, subcutaneous and muscle lipid was transferred into a Teflon-lined screw-top test tube by means of a disposable glass Pasteur pipette. Fatty acids were trans-esterified to form methyl esters using 0.5 mol/L NaOH in methanol and 14% boron trifluoride in methanol (Park and Goins, 1994). Fatty acid methyl esters (FAMES) from subcutaneous fat, feed and muscle were quantified using a Varian 430 flame ionisation GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2  $\mu\text{m}$  film thicknesses). Analysis was performed using an initial isothermic period ( $40^{\circ}\text{C}$  for 2 min). Thereafter, temperature was increased at a rate of  $4^{\circ}\text{C}/\text{min}$  to  $250^{\circ}\text{C}$ . Finally an isothermic period of  $230^{\circ}\text{C}$  for 10 min followed. Fatty acid methyl esters n-hexane (1  $\mu\text{L}$ ) was injected into the column using a Varian CP 8400 Auto sampler. The injection port and detector were both maintained at  $250^{\circ}\text{C}$ . Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Galaxy Chromatography Software recorded the chromatograms.

Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma–Aldrich Aston Manor, Pretoria, South Africa). All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, and Johannesburg, South Africa). Individual FA were expressed as a proportion of total FA present in the sample. The following FA combinations were calculated: omega-3 (n-3) FA, omega-6 (n-6) FA, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA:SFA ratio and n-6:n-3 ratio, atherogenicity index.

## 2.8. Statistical analysis

Data on growth performance (weekly feed intake, body weight changes, and feed conversion efficiency) was analysed using the mixed procedures for repeated measures (PROC MIXED) of SAS

(2008) while data on overall feed intake, weight gain, carcass traits and FA profiles were subjected to analysis of variance using the general linear model procedure (PROC GLM) of SAS (2008). For statistical analysis, the pen means served as the experimental units. Comparisons of means were done using the probability of difference (PDIFF) option of SAS (2008). The model used was as follows:

$$Y_{ij} = \mu + D_i + E_{ij},$$

where  $Y_{ij}$  = response variable (growth performance, carcass traits, and FA profiles),  $\mu$  = overall mean,  $D_i$  = effect of diet level  $i$ ,  $E_{ij}$  = random error.

## 3. Results

### 3.1. Growth performance

The effect of including *L. javanica* in the broilers diets on live weights is presented in Fig. 1. At the beginning of the experiment, all chicks in the different treatment groups had similar initial BW (52 g/chick). Between week 1 and week 3, a gradual increase in live weights of the chicks was observed across treatments. From week 3 to the end of the experiment at week 6 a sharp and dramatic increase in the live weights was observed. Poscontrol, Ljav5 and Ljav12 treatment groups had significantly higher ( $P > 0.05$ ) weekly weights than the Negcontrol treatment group throughout. The average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and slaughter weights are presented in Table 3. The broilers fed *L. javanica* had comparable ADFI, ADG and FCR to the Poscontrol group at all feeding phases. However, the broilers in the Negcontrol group had significantly ( $P < 0.05$ ) lower ADG and higher FCR than those in other groups at different feeding phases. Slaughter weights were also higher ( $P < 0.05$ ) in the Ljav5 and Poscontrol treatment than the other two treatment groups. The broilers in the Negcontrol had the lowest average slaughter weights. In terms of mortalities only a single death was recorded in the Ljav12 treatment group.

### 3.2. Carcass characteristics

The results for the relative organ sizes are presented in Table 4. The weights of proventriculus, gizzard, small intestine, liver,

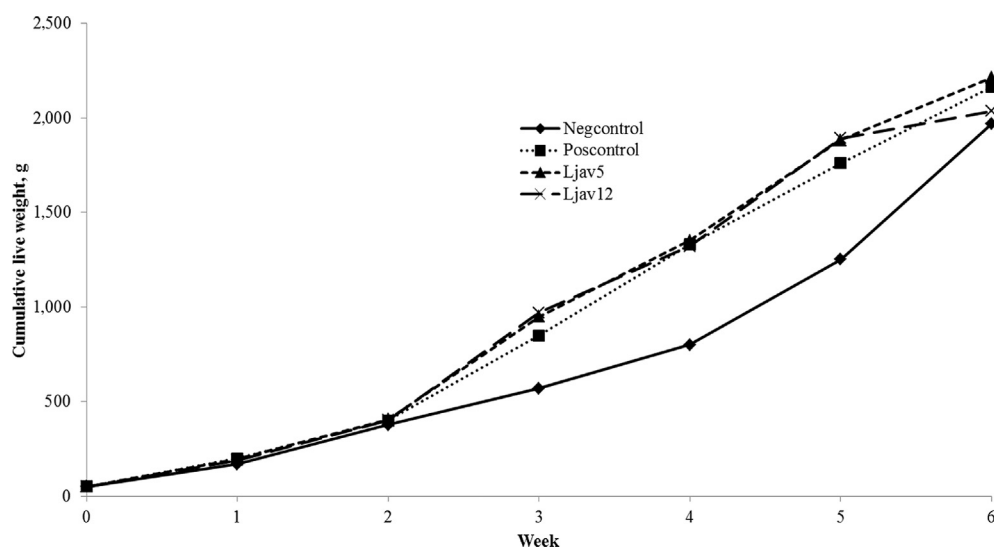


Fig. 1. Cumulative live weights for broilers receiving different levels of *L. javanica* leaf powder. Dietary treatments: Negcontrol = commercial broiler diet without prophylactic antibiotic; Poscontrol = commercial broiler diet with prophylactic antibiotic; Ljav5 = *L. javanica* leaf powder 5 g/kg; Ljav12 = *L. javanica* leaf powder 12 g/kg.

pancreas, caecum and the small intestine length were significantly ( $P < 0.05$ ) affected by diet. Broilers that were fed 12 g/kg of *L. javanica* leaf meal had the highest weights for the proventriculus and gizzard as well as the longest small intestines ( $P < 0.05$ ). Nevertheless, the broilers in the Ljav12 treatment group had the lowest weights for the liver and pancreas while the broilers in the Poscontrol group had the highest ( $P < 0.05$ ). No differences were observed across the treatments in terms of heart and spleen sizes. *L. javanica* inclusion had no effect on the breast weight, thigh weight, carcass weight, and dressing percentage of the broilers. However, significant differences were observed on the weight of abdominal fat pad, with broilers that received *L. javanica* leaf meal showing significantly higher abdominal fat weight compared with those whose diets did not contain *L. javanica* leaf meal.

### 3.3. Proximate composition and fatty acid profiles

The effect of diet on proximate composition of the intramuscular fat content of broilers is presented in Table 5. Diet significantly ( $P < 0.05$ ) affected intramuscular fat % (IMF%). Birds on the Poscontrol group had the highest IMF% while those on the Ljav12 diet had the lowest IMF%. Diet, however, had no effect on fat free dry matter (FFDM) and moisture. The results on the composition of individual FAs in the breast muscle of the broilers are presented in Table 6. Across treatments, oleic (30.9%–33.9%), followed by palmitic (23.9%–25.2%) and linoleic acid (15.8%–16.6%) were the main FAs found in the breast muscle of the broilers. Diet had no effect on most of the FAs except for heptadecenoic (C17:1c10), eicosatrienoic [C20:3c8, 11, 14 (n-6)], docosadienoic [C22:2c13,16 (n-6)] and docosapentaenoic [C22:5c7, 10,13,16,19 (n-3)] acids. The negcontrol group had the highest value ( $P < 0.05$ ) for heptadecanoic acid while Ljav5 group had the lowest.

With regards to eicosatrienoics [C20:3c8, 11, 14 (n-6)], the Ljav12 group had the highest proportion ( $P < 0.05$ ) whilst the poscontrol had the lowest proportion. Additionally, the proportions of docosadienoic [C22:2c13, 16(n-6)], another n-6 FA, were the highest ( $P < 0.05$ ) in the poscontrol group and the lowest in the Ljav5 and negcontrol groups. Most of the n-3 FAs were not affected by diets except for the docosapentaenoic [C22:5c7, 10,13,16,19 (n-3)], which was found to be higher ( $P < 0.05$ ) in the Ljav5 treatment group and the lowest in the negcontrol. The effects of diet on total saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), omega-6 (n-6) FAs, omega-3 (n-3) FAs, PUFA:SFA and n-6:n-3 ratios of broiler breasts are presented in

Table 7. The broilers in the Negcontrol and Poscontrol groups had a significantly ( $P < 0.05$ ) higher total SFA compared with the *L. javanica* fed broilers. On the contrary, the broilers in the *Lippia*-containing treatment groups had higher ( $P < 0.05$ ) total PUFA, total n-3 FAs and PUFA:SFA ratio and also had significantly lower n-6:n-3 ratios compared with the other two treatment groups. No differences were however, observed with regards to total MUFA and total n-6 FAs.

## 4. Discussion

### 4.1. Growth performance

The current study was conducted to evaluate the potential of *L. javanica* as an alternative antibiotic and growth promotant in broilers. The results revealed that the broilers fed *L. javanica* had comparable ADFI, ADG and FRC to the positive control in all feeding phases. This indicates that *L. javanica* improved the feed efficiency given the reduced feed intake and improved the ADG and ultimately slaughter weights. Phytochemicals in *L. javanica* leaf meal can stimulate glycolysis and increase utilisation of for energy production and ultimately growth. This is consistent with the findings of Hong et al. (2012) that made similar observations in broilers fed essential oils from some natural plants (oregano, anis and citrus peel). Toghyani et al. (2011) also demonstrated that natural plant supplements such as cinnamon in broilers significantly influenced growth performance indices and have the potential to be applied as alternative for in-feed antibiotics.

Broiler chickens that were fed 12 g/kg of *L. javanica* leaf meal showed the highest feed intake during the finisher phase. This could be attributed to the increased amount of fibre in the diet, which could have affected feed intake. With high fibre diets, it has been observed that broilers tend to increase feed intake as a way to compensate for the reduced nutrient concentration in feed (Walugembe et al., 2014). However, the increased feed intake coupled with reduced degradation of the fibrous diets in chickens inevitably results in increased bulk of digesta in the intestinal tract. This ultimately leads to the withdrawal from the feed by the broilers and hence feed intake is affected (Walugembe et al., 2014). This might imply that at higher inclusion levels, growth parameters may be affected and hence the need to use them with caution. The inclusion levels of *L. javanica* in the current study were, however, too low to raise any alarm with regards to digestion and absorption of feed. Generally, fibre levels above 100 g/kg have been observed to affect feed utilisation (Hetland et al., 2002). The fact that a single

**Table 3**  
Effects of feeding *L. javanica* leaf meal on growth parameters of broiler chickens at different growth phases.

Growth efficiency parameters	Phase	Dietary treatments <sup>1</sup>				SEM
		Negcontrol	Poscontrol	Ljav5	Ljav12	
Average daily feed intake, g/day						
d 1–14	Starter	64 <sup>c</sup>	51.1 <sup>b</sup>	49.1 <sup>b</sup>	47.1 <sup>a</sup>	10.4
d 15–28	Grower	83.1 <sup>a</sup>	136.1 <sup>c</sup>	132.1 <sup>c</sup>	123.7 <sup>b</sup>	11.2
d 29–42	Finisher	106 <sup>a</sup>	109.8 <sup>b</sup>	109.5 <sup>b</sup>	120.5 <sup>c</sup>	11.4
Average daily gain, g/day						
d 1–14	Starter	23.3 <sup>a</sup>	24.9 <sup>b</sup>	25.3 <sup>b</sup>	25 <sup>b</sup>	0.63
d 15–28	Grower	30.2 <sup>a</sup>	66.1 <sup>b</sup>	67.4 <sup>b</sup>	65.6 <sup>b</sup>	0.64
d 29–42	Finisher	55.6 <sup>a</sup>	53.6 <sup>a</sup>	55.7 <sup>a</sup>	64.2 <sup>b</sup>	0.64
Feed conversion ratio						
d 1–14	Starter	2.4 <sup>c</sup>	1.9 <sup>b</sup>	1.7 <sup>a</sup>	1.6 <sup>a</sup>	0.07
d 15–28	Grower	2.5 <sup>b</sup>	1.9 <sup>a</sup>	1.9 <sup>a</sup>	1.8 <sup>a</sup>	0.06
d 29–42	Finisher	2.1	1.9	1.9	1.8	0.07
Average slaughter weight, g		1,967 <sup>a</sup>	2,163 <sup>b</sup>	2,213 <sup>c</sup>	2,035 <sup>a</sup>	29.63

<sup>a,b,c</sup> Means in the same row with different superscript are significantly different ( $P < 0.05$ ).

<sup>1</sup> Dietary treatments: Negcontrol = commercial broiler diet without prophylactic antibiotic; Poscontrol = commercial broiler diet with prophylactic antibiotic; Ljav5 = *L. javanica* leaf powder 5 g/kg; Ljav12 = *L. javanica* leaf powder 12 g/kg.



**Table 4**  
Effects of feeding *L. javanica* leaf meal on relative organ weight of broiler chickens.

Parameters	Dietary treatments <sup>1</sup>				SEM
	Negcontrol	Poscontrol	Ljav5	Ljav12	
Proventriculus, g	9.20 <sup>a</sup>	10.50 <sup>a</sup>	9.60 <sup>a</sup>	12.70 <sup>b</sup>	0.850
Gizzard, g	35.40 <sup>a</sup>	34.10 <sup>a</sup>	32.00 <sup>a</sup>	46.00 <sup>b</sup>	3.400
Small intestine weight, g	47.70 <sup>b</sup>	33.00 <sup>a</sup>	40.70 <sup>a</sup>	54.00 <sup>b</sup>	4.062
Small intestine length, cm	153.80 <sup>b</sup>	135.60 <sup>a</sup>	121.00 <sup>a</sup>	160.20 <sup>b</sup>	6.598
Liver, g	36.70 <sup>b</sup>	41.90 <sup>b</sup>	36.30 <sup>b</sup>	27.00 <sup>a</sup>	2.371
Pancreas, g	3.40 <sup>a</sup>	5.20 <sup>a</sup>	4.30 <sup>b</sup>	3.00 <sup>a</sup>	0.376
Heart, g	10.90	12.10	11.20	10.20	0.956
Caecum, g	15.40 <sup>b</sup>	12.60 <sup>a</sup>	15.50 <sup>b</sup>	13.65 <sup>a</sup>	0.851
Spleen, g	2.10	1.90	2.20	1.90	0.206
Breast, g	355.6	401.3	384	357.8	36.6
Thigh, g	237.3	232.6	251.2	209.6	14.6
Abdominal fat, g	36.4 <sup>a</sup>	33.5 <sup>a</sup>	40.5 <sup>b</sup>	39.2 <sup>b</sup>	5.66
Carcass weight, g	1,490	1,510	1,470	1,480	0.06
Dressing, %	77.7	79.9	76.8	78.5	9.21

<sup>a,b</sup> Means in the same row with different superscript are significantly different ( $P < 0.05$ ). Means represent 6 chicks per treatment.

<sup>1</sup> Dietary treatments: Negcontrol = commercial broiler diet without prophylactic antibiotic; Poscontrol = commercial broiler diet with prophylactic antibiotic; Ljav5 = *L. javanica* leaf powder 5 g/kg; Ljav12 = *L. javanica* leaf powder 12 g/kg.

**Table 5**  
Effects of feeding *L. javanica* leaf meal on proximate fat composition (%) of the breast muscle of broiler chickens.

Parameter	Dietary treatments <sup>1</sup>				SEM
	Negcontrol	Poscontrol	Ljav5	Ljav12	
IMF	1.64 ± 0.12 <sup>b</sup>	1.88 ± 0.13 <sup>c</sup>	1.59 ± 0.12 <sup>b</sup>	1.47 ± 0.13 <sup>a</sup>	0.06
FFDM	22.3 ± 0.29	22.1 ± 0.29	23.5 ± 0.29	22.3 ± 0.29	0.42
Moisture	76.1 ± 0.34	76.1 ± 0.34	75.3 ± 0.34	76.3 ± 0.34	1.63

<sup>a,b,c</sup> Means in a same row with different superscript are significantly different ( $P < 0.05$ ). IMF = intramuscular fat; FFDM = fat free dry matter.

<sup>1</sup> Dietary treatment: Negcontrol = commercial broiler diet without prophylactic antibiotic; Poscontrol = commercial broiler diet with prophylactic antibiotic; Ljav5 = *L. javanica* leaf powder 5 g/kg; Ljav12 = *L. javanica* leaf powder 12 g/kg.

**Table 6**  
Effect of feeding *L. javanica* leaf meal on total fatty acids (%) of breast muscle.

Fatty acids	Dietary treatments <sup>1</sup>				SEM
	Negcontrol	Poscontrol	Ljav5	Ljav12	
C14:0	0.3	0.32	0.33	0.3	0.01
C14:1c9	0.05	0.08	0.07	0.05	0.01
C15:0	0.1	0	0.01	0	0.01
C16:0	24.3	23.9	25.2	24.0	0.51
C16:1c9	5.15	5.74	5.28	4.97	0.35
C17:0	0.04	0.04	0.05	0.03	0.01
C17:1c10	0.51 <sup>c</sup>	0.39 <sup>b</sup>	0.27 <sup>a</sup>	0.49 <sup>c</sup>	0.05
C18:0	8.8	7.9	8.59	9.09	0.43
C18:1t9	0.01	0.04	0.02	0.03	0.01
C18:1c9	30.9	33.9	31.9	31.2	1.18
C18:1c7	5.05	5.23	5.16	5.1	0.10
C18:2c9,12 (n-6)	16.6	15.8	15.8	16.2	0.49
C20:0	0.14	0.09	0.1	0.13	0.04
C18:3c6,9,12 (n-6)	0.11	0.1	0.12	0.09	0.01
C20:1c11	0.3	0.29	0.31	0.31	0.02
C18:3c9,12,15 (n-3)	0.46	0.53	0.52	0.44	0.04
C20:2c11,14 (n-6)	0.31	0.2	0.27	0.33	0.03
C22:0	0.01	0.01	0.01	0.01	0.01
C20:3c8,11,14 (n-6)	1.06 <sup>b</sup>	0.78 <sup>a</sup>	0.91 <sup>a</sup>	1.09 <sup>b</sup>	0.09
C22:1c13	0.01	0.01	0.01	0.01	0.003
C20:4c5,8,11,14 (n-6)	4.89	3.71	4.11	4.96	0.47
C22:2c13,16 (n-6)	0.02 <sup>b</sup>	0.01 <sup>a</sup>	0.02 <sup>b</sup>	0.01 <sup>a</sup>	0.04
C20:5c5,8,11,14,17 (n-3)	0.39	0.23	0.36	0.39	0.05
C22:5c7,10,13,16,19 (n-3)	0.33 <sup>a</sup>	0.42 <sup>b</sup>	0.44 <sup>b</sup>	0.47 <sup>c</sup>	0.05
C22:6c4,7,10,13,16,19 (n-3)	0.21	0.18	0.23	0.24	0.04

<sup>a,b,c</sup> Means in the same row with different superscript are significantly different ( $P < 0.05$ ).

<sup>1</sup> Dietary treatments: Negcontrol = commercial broiler diet without prophylactic antibiotic; Poscontrol = commercial broiler diet with prophylactic antibiotic; Ljav5 = *L. javanica* leaf powder 5 g/kg; Ljav12 = *L. javanica* leaf powder 12 g/kg.

**Table 7**  
Effects of feeding *L. javanica* leaf meal on total fatty acids and fatty acid ratios.

Fatty acids	Dietary treatments <sup>1</sup>				SEM
	Negcontrol	Poscontrol	Ljav5	Ljav12	
Total SFA	33.5 <sup>ab</sup>	34.3 <sup>b</sup>	32.3 <sup>a</sup>	33.5 <sup>ab</sup>	0.62
Total MUFA	42.1	45.7	42.9	42.3	1.34
Total PUFA	22.3 <sup>a</sup>	22.1 <sup>a</sup>	24.7 <sup>b</sup>	24.2 <sup>b</sup>	1.02
Total n-6	22.8	20.6	21.1	22.6	0.99
Total n-3	1.59 <sup>a</sup>	1.37 <sup>a</sup>	1.65 <sup>b</sup>	1.64 <sup>b</sup>	0.08
PUFA:SFA	0.66 <sup>a</sup>	0.68 <sup>a</sup>	0.73 <sup>b</sup>	0.72 <sup>b</sup>	0.03
n-6:n-3	14.3 <sup>b</sup>	15.36 <sup>b</sup>	12.9 <sup>a</sup>	13.9 <sup>a</sup>	0.82

<sup>a,b</sup> Means in the same row with different superscript are significantly different ( $P < 0.05$ ). Fatty acids: SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-6 = omega-6 fatty acids; n-3 = omega-3 fatty acids.

<sup>1</sup> Dietary treatments: Negcontrol = commercial broiler diet without prophylactic antibiotic; Poscontrol = commercial broiler diet with prophylactic antibiotic; Ljav5 = *L. javanica* leaf powder 5 g/kg; Ljav12 = *L. javanica* leaf powder 12 g/kg.

mortality was recorded over the experimental period is commendable as this reflects the generally good hygienic and biosecurity practices that were being observed throughout the study.

#### 4.2. Carcass characteristics

The results of the relative organ weights showing the significant effect of *L. javanica* inclusion on some internal organs are contrary to previous studies, in which there were no differences in relative organ weights caused by essential oils or other herbal extracts (Çabuk et al., 2006; Ahmad et al., 2011). Nevertheless, the observed high weights of the proventriculus and gizzard as well as the longer small intestines observed in the broilers fed *L. javanica* at the rate of 12 g/kg could be a result of an adaptive mechanism to deal with the increased amounts of fibre that would ultimately optimise digestion and absorption. However, this needs further verification since the high weights of some of the internal organs are not supported by a concomitant increase in size of the liver, which plays an important role in the detoxification of increased amounts of phytochemical in the feed (Adamu et al., 2012). Alternatively, it may be suggested that the amounts of phytochemicals were insignificant to cause an increase the surface area in the liver (Dotas et al., 2014). The lack of effect of *L. javanica* inclusion levels on carcass yield, dressing percentage, thigh and breast weight is consistent

with findings from similar studies elsewhere (Nikolakakis et al., 2005; Dotas et al., 2014). Nevertheless, the observed high abdominal fat values for the broilers fed *L. javanica* could be a cause for concerns with regards to the high amounts of undesirable fat.

#### 4.3. Proximate composition and fatty acid profiles

Results for the profiles of long chain fatty acids of the broilers revealed that oleic acid (C18:1c9) was the most abundant followed by palmitic (C16:0) and linoleic acid (C18:2c9, 12). Similar observations were made in broilers fed *Camelina sativa* oil by Jaśkiewicz et al. (2014). Palmitic acid has often been found to increase blood cholesterol level, oleic has been found to have an opposite effect on the blood cholesterol levels (Peña et al., 2009). Although the inclusion level of the *L. javanica* was very low, a response to its inclusion highlighted by slight increase in C18:3c9, but a clearer increase in C20:3, C20:5, C22:5 and C22:6 was observed in the breast muscle. This can be explained by the role of C18:3c9 in the metabolism of the longer chain n-3 FA. Finding from the study also showed that the broilers receiving *L. javanica* free diets have significantly higher proportions of some n-6 FA. Interestingly and in contrast, the broilers receiving *L. javanica* diets had high proportions of some n-3 FA, which are critical with regards to human health.

Generally, plasma cholesterol concentration is influenced by the FA composition of dietary fat with high levels of long chain SFA increasing plasma cholesterol level compared to high levels of MUFA and PUFA (Grundy and Denke, 1990; Muchenje et al., 2009; Banskalieva et al., 2000; Marume et al., 2012). The present study reveals that, the broilers receiving *L. javanica* in their diets had lower total SFA, higher total PUFA and total n-3 FA which may have potential benefits to human nutrition. Moreover, the PUFA:SFA ratios were high in broilers receiving *L. javanica* whilst the n-6:n-3 ratios were significantly lower. The PUFA:SFA and n-6:n-3 ratios are commonly used to evaluate the nutritional and health value of meat for human consumption (Aldai et al., 2007; Alfaia et al., 2007). The recommended PUFA:SFA ratio in human diets should be above 0.4 (Higgs, 2002). In the present study, the PUFA:SFA ratios obtained from all treatments were above the minimum recommended values. More importantly, the higher PUFA:SFA values obtained in the broilers receiving *L. javanica* can have a desirable effect in the reduction of the chances of development of cardiovascular and some chronic conditions in humans (Grundy and Denke, 1990; Banskalieva et al., 2000; Mapiye et al., 2011). Although the *Lippia* fed broilers had significantly lower n-6:n-3 ratios than the other two, it appears that all the ratios were well above the recommended n-6:n-3 ratio of 5:1 in human diets. However, this could be characteristics of broiler meat as similar observations were made by Jaśkiewicz et al. (2014).

## 5. Conclusion

The findings from the current study revealed that supplementing feed with *L. javanica* can positively affect ADG and slaughter weights of broilers. The broilers fed Ljav5 obtained the highest slaughter weight amongst the treatment groups. The observed high weights for the proventriculus and the gizzard as well as longer small intestines observed in the broilers fed Ljav12 could be a result of an adaptive mechanism to deal with the increased amounts of fibre and phytochemicals that would ultimately optimise digestion and absorption. Broilers receiving *L. javanica* free diets have significantly higher proportions of some n-6 FA. In contrast, the broilers receiving *L. javanica* diets had high total n-3 FA which are critical with regards to human health. Overall, the findings from the study showed that use of *L. javanica* at

5 g/kg feed has positive influences, on growth performance, carcass characteristics and FA profiles of broiler meat.

## Conflict of interest

The authors declare that there have no conflicts of interest.

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