Citrullus lanatus essential oils inclusion in diets elicit nutraceutical effects on egg production, egg quality, and physiological characteristics in layer hens

U. Marume,[*,†,1] J. M. Mokagane,[*,†] C. O. Shole,[*,†] and A. Hugo[‡]

*Department of Animal Science, School of Agricultural Sciences, Faculty of Agriculture, Science and Technology, North West University 2046, Mmabatho, South Africa; †Food Security and Safety Niche area, Faculty of Agriculture, Science and Technology, North West University, Mmabatho 2735, South Africa; and ‡Department of Microbial, Biochemical and Food Biotechnology, University of Free State 9300, South Africa

ABSTRACT The study evaluated the effects of *Citrullus lanatus* essential oils inclusion in diet on egg production, egg quality, and physiological parameters in layer hens. A total of 72 White Leghorn point-of-lay hens at 18 wk were used for the study. The hens were randomly allocated to following 3 dietary treatments: 1) commercial layer diet (control), 2) commercial diet + 1 g *C. lanatus* essential oil/kg feed (**1gCL**), and 3) commercial diet + 2 g *C. lanatus* essential oil/kg feed (**2gCL**). Each treatment was replicated 8 times arranged in completely randomized design. From the results, an increase in total weight gain, average daily feed intake, and average daily gain was observed with inclusion of the *C. lanatus* essential oil. In addition, the inclusion of *C. lanatus* in diet improved the egg mass and feed conversion ratio (**FCR**). Hens fed *C. lanatus*–containing diets had higher egg mass (1gCL, 53.35 g ± 0.71; 2gCL, 53.99 ± 0.71 g) compared with the control (52.90 ± 0.71). The *C. lanatus* containing diets also had lower FCR than the control (1gCL [2.18 ± 0.22] and 2gCL [2.16 ± 0.22] vs. control [2.20 ± 0.71]). With regards to egg quality parameters, inclusion of *C. lanatus* oil appeared to reduce shell weight and shell ratio, while increasing albumen height and Haugh unit. Diets containing *C. lanatus* had significantly higher amounts of stearic acid, and linoleic acid was highest ($P < 0.05$) in the 2gCL fed hens. Birds fed the 2gCL diet (15.29 ± 0.60) had the highest total polyunsaturated fatty acids, total n-6 fatty acids (14.81 ± 0.59) and also had the highest n-6/n-3 ratio. An increase hematological values was observed with inclusion of *C. lanatus* essential oils in diets. Moreover, tibia bone parameters were also significantly improved with inclusion of *C. lanatus* essential oils in diets. It can be concluded that *C. lanatus* essential oil positively affected egg production, and quality and health of layer hens can be used successfully as a natural feed additive.

Key words: essential oils, egg production, egg quality, health, bone development

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INTRODUCTION

In commercial egg farming, intensive production is practiced where layers are kept in battery cages. Their diet is often composed of medicated feeds with the inclusion of feed additives that increase growth and improve health status of chickens and efficiency of production (Dhama, et al., 2014). Nevertheless, there have been concerns about the use of the commercial feed additives including antibiotics that might affect human health (Bozkurt et al., 2014; Albour-Elkhair et al., 2018). Use of natural feed additives such as phytogenic plants with medicinal benefits has been suggested as possible alternatives to the commercial feed additives (Karori et al., 2007; Bozkurt et al., 2014; Omonijo et al., 2017). Natural growth enhancers differ from synthetic supplements in terms of typical working mechanisms. Whereas commercial antibiotic can directly affect harmful as well as, inevitably, some useful bacteria, natural antibiotic works by not only simply killing the bacteria but also enhancing the body's natural capacity to fight off such bacterial infections in the future (Omonijo et al., 2017; Albour-Elkhair et al., 2018).

The use of natural plant extracts, such as essential oils as alternatives, has received much attention because of their chemical composition and characteristics that includes among others, the immunoboosting, antioxidative,

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[1]Corresponding author: upenyu.marume@nwu.ac.za

and antimicrobial properties (Landy et al., 2011a, b; Hong et al., 2012; Wang et al., 2019). Several reports have described the potential effects of various phytogenic additives, particularly essential oils, on poultry production. In one study, phytogenic plant extracts were reported to increase egg production, reduce cholesterol content of serum and yolk, and improve immune response in layers (Azeke and Ekpo, 2009). In another, phytogenic feed additives were reported to have viably positive effect on performance, ovarian morphology, serum lipid parameters, egg sensory quality in laying hens, egg production, and general poultry performance (Ghajarbeygi et al., 2015; Wang et al., 2019). Such reports on the potential of phytogenic extracts on layer hen performance has therefore stimulated an upsurge of interest in the evaluation of plant essential oils as sources of natural antioxidants and immunopromoting agents (Bozkurt et al., 2014; Albour-Elkhair et al., 2018; Zhai et al., 2018).

One of the medicinal plants and herbs commonly used in communities with great potential is *Citrullus> lanatus* (Kalahari melon) (Liu et al., 2009). Kalahari melon seeds oil has been used traditionally in Southern Africa as a cosmetic product, primarily as a face and body scrub which is said to impart a blemish-free complexion to the skin (Vermaak et al., 2011). The seeds are also rich in fat and protein and are an excellent source of energy (African Origin Oil, 2014). Moreover, the seed essential oils contain large amounts of some essential fats including palmitic, stearic, oleic, and various linoleic acids isomers (Berry, 2015). In particular, conjugated linoleic acids have been reported to have a wide range of health-beneficial effects, including anticarcinogenic, antiatherogenic, anthelmintic, antidiabetic, and immune stimulatory effects (Suksombat et al., 2006; Liu et al., 2017). They may also have some positive effects of fatty acid composition of meat and egg yolk and bone parameters (Kolakshyapati et al., 2019). Despite the potential application of *C. lanatus* essential oils in poultry production, currently no studies have attempted to assess the influence of *C.lanatus* essential oils on egg production and quality and physiological responses in commercial layer hens. Much of the available information is on chemical composition, in vitro bacterial effects, and antioxidant effects (Nyam et al., 2009; Jarret and ILevy 2012; Liu et al., 2017). We therefore postulate that *C. lanatus* essential oils in layer diets may positively influence egg production performance and general health of the hens. The study therefore assessed the efficacy of utilization of *C. lanatus* essential oil on egg production performance, egg quality, and physiological characteristics of layer hens.

MATERIALS AND METHOD

Description of the Study Area

A feeding trial was conducted at the North West university farm (Molelwane). The study site is situated in South Africa, North West province. The geographical location of the farm is situated between 25°86′00″5, 25°64′32″E. The average rainfall ranges from 300 to 600 mm per annum. Temperatures range from 22°C to 38°C in tot wet season (November-March) and 2°C to 25°C during cold dry season (May-October).

Table 1. Commercial layer diet ingredients and treatments.

Ingredients	Total g/kg feed
Corn	555.7
Soybean meal	220
Soybean oil	30
Wheat Bran	62.9
Monocalcium phosphate	15.7
Calcium carbonate	106.1
Common salt	2.7
DL-Methionine	1.7
L-lysine	0.2
Threonine	0.2
NaHCO3	2
Vitamin premix	2.5
Mineral premix	2.5
Nutrient composition	
Metabolizable energy (MJ/kg)	11.6
Crude protein (g/kg)	154.5
Ether extract (g/kg)	53.7
Lysine (g/kg)	7.5
Methionine (g/kg)	4.4
Threonine (g/kg)	5.7
Available phosphorus (g/kg)	4.7
Calcium (g/kg)	42.9

Treatments: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg *C. lanatus* oil; 2gCL, commercial layer diet mixed with 2 g/kg *C. lanatus* oil.

C. lanatus *Essential Oil and Feed Component*

The essential oil were purchased from Nutrica Organics (Pvt Ltd), South Africa. The essential oil was extracted from the *C. lanatus* seeds by using the cold pressing method. All other dietary components were obtained from Opti Feeds, Lichtenburg (**SA**).

Animals, Treatments, and Experimental Design

A total of 72 White Leghorn layer hens at 18 wk purchased from Mimosa Chicks were used for the study. The

Table 2. Analyzed bioactive compounds in *C. lanatus* essential oil.

Compound	Amounts
Phenolics (mg/100g)	
Garlic acid	0.23
Vanillic acid	0.74
Caffeic acid	0.42
Ferulic acid	0.18
Sterols (mg/100g)	
Squalene	160.32
Campesterol	35.03
Sitosterol	405.21
Total flavonoids (mg RE/kg)	163.5
Principal fatty acids (%)	
Palmitic	11.6
Stearic	8.32
Oleic	13.81
Linoleic	65.17

hens were randomly allocated to 3 dietary treatments in a completely randomized design with each treatment having 8 replicate cages. The experimental unit was a cage containing 3 birds. Layers were kept in cages in a conventional battery cage layer unit normally used for commercial later egg production that has curtains for control of environmental conditions. All experimental cages were equipped with a nipple drinker and a separate feeder. Feed, in mash form, and water were provided ad libitum for 8 wk. The dietary treatments were formulated as follow: treatment 1—control (Commercial layer diets); treatment 2—**1gCL** (Commercial diet + 1 g oil/kg feed); treatment 3—**2gCL** (Commercial diet + 2 g oil/kg feed). The 2 inclusion levels of *C. lanatus* were based on the available information on bioactive extracts and chemical composition of the plant. The oil was added to the mash, then evenly and finely mixed using an electrical feed mixer. The composition of the diet is presented in Table 1, whereas Table 2 shows some important bioactive compounds of the *C. lanatus* essential oil. The experimental procedures were approved by the NWU-ANIMPROD Research Ethics Committee of North-West University and the Ethics number granted was NWU-00516-16-S9.

Feed Intake and Body Weight Changes

Feed intake was measured daily as the difference between feed offered and the refusals. The hens were weighed at the beginning of the experiment subsequently weekly thereafter using a TSW equipment weighing scales/Adam equipment, SA. Average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed conversion ratio (**FCR**) were calculated as follows:

$$\text{ADFI} = \frac{\text{Feed offered} - \text{feed refused}}{\text{60 days}} \quad (1)$$

$$\text{ADG} = \frac{\text{Finish weight} - \text{Start weight}}{\text{Age (days)}} \quad (2)$$

Egg Production Indices

The number of eggs produced was recorded daily including those that were broken. Hen-day egg production (**HDEP**), average egg mass (**AEM**), FCR (feed efficiency per kg egg mass), and net feed efficient index (**NFEI**) were calculated as follows:

$$\text{HDEP} = \text{Total number of eggs produced in a day/Total number of hens present} \times 100\% \quad (3)$$

$$\text{AEM} = \%\ \text{HDEP} \times \text{average egg weight} \quad (4)$$

$$\text{FCR(per kg egg mass)} = \text{kg of feed consumed/kg eggs produced} \quad (5)$$

$$\text{NFEI} = (\text{AEM} + \text{ADG})/\text{ADFI} \times 100\% \quad (6)$$

Egg Quality Measurements

Egg linear parameters (length and width) were measured with the vernier calliper, and egg shape index was calculated as the ratio of egg width over egg length expressed as a percentage. Egg shell weight was measured after removal of the shell membrane using an analytical scale. Egg shell thickness was then measured using a micrometer screw gauge at three (3) points (blunt, middle, and sharp). Egg shell index was calculated as the ratio of shell weight over egg weight expressed as a percentage. With regards to internal parameters, 80 eggs from each treatment were collected and marked differently, weighed individually, and broken onto a petri dish to determine the following measurements: yolk height, yolk diameter, yolk color, yolk weight, Haugh units (**HU**), albumen height, albumen length, albumen width, albumen weight, and thickness. Albumin ration and index and yolk ratio and index were ultimately calculated from these measurements.

Fatty Acid Analysis in Egg Yolks

Total extracted fat from egg yolk was determined gravimetrically and expressed as percent fat (w/w) per 100 g egg yolk. The fat-free dry matter (**FFDM**) content was determined by weighing the residue on a preweighed filter paper, used for Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as % FFDM (w/w) per 100 g tissue. The moisture content of the muscle and BF was determined by subtraction (100% - % lipid - % FFDM) and expressed as % moisture (w/w) per 100 g tissue. Fatty acid methyl esters from egg yolk were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thicknesses). Fatty acid methyl ester samples were subsequently identified by comparing the retention times of fatty acid methyl esters 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, Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. The following fatty acid combinations were calculated: omega-3 (n-3) fatty acids, omega-6 (n-6) fatty acids, total saturated fatty acids, total monounsaturated fatty acids, polyunsaturated fatty acids (**PUFA**), PUFA/ total saturated fatty acids ratio, and n-6/n-3 ratio.

Hematological Analysis

At the end of the trial, blood samples were collected from 15 randomly selected birds per treatment. Blood samples (1 mL) were drawn from the brachial vein of birds using a needle and a syringe and was immediately transferred into 2 a purple tube with anticoagulant for hematological analysis. The Idexx lasercyte (Hematology analyzer) was used to analyze the full blood count.

Internal Organs

At the end of the trial, 16 birds per treatment were randomly selected and sacrificed for assessment of internal organs. At necropsy, the following organs were also removed and weighed: livers, spleens, and intestines. Length of small intestines (jejunum, duodenum, and ileum) were measured and recorded. The hepatosomatic index was calculated as the ratio of liver over the final bird weight, whereas the spleen index was calculated as the ratio of spleen weight over the final bird weight.

Tibia Linear Parameters and Bone Breaking Strength

At necropsy on day 56, 10 right tibia from each treatment were surgically removed, defleshed, and cleaned on all tissue including cartilage caps (periosteum) by hand and weighed to obtain the tibia weight (**TW**), tibia diameter proximal end (**TDPE**), tibia diameter width, tibia length, and tibia diameter distal end were determined using a Toshiba-Rotanode X-ray (Toshiba Electron Tubes and Devices, Tochigi, Japan) connected to the point-of-care (CR140) system for digital imaging. After linear measurement each of the tibiae was placed on an adjustable 3-point bend/snap fixture fitted on a heavy duty **TA XT** platform of a texture analyzer (model TA-XT plus, Stable Microsystems, Surrey, UK) and broken with a 6 cm flat head probe attached to a 50 kg load cell reporting the breaking force in Newtons. The distance between the bone supports was 50 mm. The breaking bone strength (**TBS**) was recorded as the peak load before the bone breakage.

Statistical Analysis

Data on body weight changes, egg production indices, internal and external egg quality and fatty acids, hematology, bone parameters, and internal organ parameters were analyzed using the **GLM** procedure of SAS (2010) with diet as the fixed effect. The Proc univariate procedure of SAS (2010) was used to test for normality. The probability differences option of SAS (2010) was used to perform pairwise comparisons of the least square means, whereas contrasts (SAS, 2010) were used to determine the specific effects of the essential oil on different parameters. Where significant, the quadratic response surface regression was used to obtain the optimum inclusion level of the essential oil. The statistical model used was as follows

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (7)$$

where: Y_{ij} = observation (egg production indices, internal and external egg quality and fatty acids, bone parameters and internal organ parameters), μ = population mean constant common to all observations, T_i = effect of diet, and ε_{ij} = random error term.

RESULTS

Feed Intake, Body Weight Changes, and Egg Production

The effect of *C. lanatus* inclusion in diets on feed intake and body weight changes is presented in Table 3, whereas Table 4 presents the effects of diet on egg production parameters. From the results, hens fed diets containing *C. lanatus* essential oils had significantly higher ($P < 0.05$) final weights, total weight gain, and ADFI. Average daily gain was also higher ($P < 0.05$) in the diets containing *C. lanatus* compared with the control. In all instances, the 2gCL treatment group had the highest values for all parameters followed by the 1gCL treatment group. Diet had no effect on total number of eggs produced, average daily egg production, and HDEP. However, diet had a significant effect ($P < 0.05$) on egg mass and FCR. Hen fed the 2gCL diet had the highest egg mass and lowest FCR followed by the 1gCL fed hens. In all instances where diet effects were significant, linear relationships were observed between the essential oils inclusion levels and the egg production parameters.

Table 3. Effects of *C. lanatus* essential oil inclusion in diets body weight changes.

	Diet[1]			
Parameters[2]	Control	1gCL	2gCL	SEM
Initial weight (g)	1610.2	1600.27	1604	30.03
Final weight (g)	1730.52[a]	1750.5[b]	1755.5[b]	50.01
Total weight gain (g)	120.32[a]	150.37[b]	151.5[b]	5.57
ADFI (g)	115.48[a]	118.35[b]	118.57[b]	2.47
ADG(g)	1.73[a]	2.69[b]	2.71[b]	0.04

[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$).

[1]Diets: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg *C. lanatus* oil; 2gCL, commercial layer diet mixed with 2 g/kg *C. lanatus* oil.

[2]Parameters: ADFI, average daily feed intake; ADG, average daily gain.

Table 4. Effects of *C. lanatus* essential oil inclusion in diets on egg production performance.

Parameters[2]	Diet[1]			
	Control	1gCL	2gCL	SEM
NOEP	55.96	54.76	54.86	0.73
ADEP	0.98	0.96	0.97	0.13
HDEP	98.15	95.28	96.55	1.30
Egg mass	52.90[a]	53.35[b]	53.99[b]	0.71
FCR	2.20[a]	2.18[b]	2.16[b]	0.22
NFEI	45.42	45.39	45.90	0.61

[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$).
[1]Diets: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg *C. lanatus* oil; 2gCL, commercial layer diet mixed with 2 g/kg *C. lanatus* oil.
[2]Parameters: NOEP, number of eggs produced; ADEP, average daily egg production; HDEP, hen day egg production; FCR, feed conversion ratio; NFEI, net feed efficiency index.

Egg Quality

The effects of diet on external egg quality is presented in Table 5, whereas Table 6 shows effect of diet on internal egg quality. Diet had a significant effect ($P < 0.05$) on shell weight and shell ratio. However, diet had no effect on all other external egg quality parameters. Shell weight and shell ratio were higher ($P < 0.05$) in the control group compared with the other diets. Diet had no effect on all internal egg quality parameters part from albumin height and HU. Albumen height and HU were higher ($P < 0.05$) on the 2gCL treatment group followed by the 1gCL treatment group, respectively. Tables 7 and 8 presents the effect of diet on individual and total fatty acid indices of egg yolk. Diet had no effect on all fatty acids apart from stearic acid and linoleic acid. Diets containing *C. lanatus* had significantly higher amounts of stearic acid compared with the control. Additionally, linoleic acid was highest ($P < 0.05$) in the 2gCL fed hens and lowest in the 1gCL treatment group. In all treatment groups, the major fatty acids observed were the palmitic acid, stearic acid, oleic acid, vaccenic acid, and linoleic acid. With regards to total fatty acids, the 2gCL treatment group had the highest PUFA, total n-6 fatty acids, and also had the highest n-6/n-3 ratio, followed by the control treatment group.

Table 5. Effect *C. lanatus* essential oil inclusion in diets on the external egg quality.

Parameters[2]	Diet[1]			
	Control	1gCL	2gCL	SEM
EL	49.62	49.61	49.71	0.25
EWID	40.42	40.06	40.17	0.18
EWT	51.84	51.33	52.03	0.47
SWT	6.85[b]	6.57[a]	6.69[a]	0.06
SB	0.19	0.21	0.19	0.02
SM	0.21	0.20	0.20	0.01
SS	0.19	0.19	0.18	0.01
ESI	81.63	80.93	80.87	0.58
SR	13.27[b]	12.84[a]	12.88[a]	0.13

[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$).
[1]Diets: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg *C. lanatus* oil; 2gCL, commercial layer diet mixed with 2 g/kg *C. lanatus* oil.
[2]Parameters: EL, egg length; EWID, egg width; EWT, egg weight; SWT, shell weight; SB, shell strength blunt; SM, shell strength middle; SS, shell strength sharp; ESI, egg shell index; SR, shell ratio.

Table 6. Effect *C. lanatus* essential oil inclusion in diets on internal egg quality.

Parameters[2]	Diet[1]			
	Control	1gCL	2gCL	SEM
AH	6.87[a]	7.19[a,b]	7.33[b]	0.12
YH	17.88	18.24	18.01	0.12
YD	34.88	35.40	35.39	0.24
YWT	15.35	15.38	15.80	0.18
AL	68.74	68.26	69.18	0.37
AWID	59.25	57.94	58.14	0.50
YC	9.24	9.40	9.35	0.11
AIND	0.66	0.65	0.66	0.36
AWT	27.27	27.85	28.19	0.38
AR	52.68	54.35	54.22	0.58
YIND	51.66	51.67	51.02	0.53
YR	31.07	32.09	31.60	0.40
HU	84.81[a]	87.04[b]	87.41[b]	0.71

[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$).
[1]Diets: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg *C. lanatus* oil; 2gCL, commercial layer diet mixed with 2 g/kg *C. lanatus* oil.
[2]Parameters: AH, albumen height; YH, yolk height; YD, yolk diameter; YWT, yolk weight; AL, albumen length; AWID, albumen width; YC, yolk color; AIND, albumen index; AWT, albumen weight; AR, albumen ratio; YIND, yolk index; YR, yolk ratio; HU, Haugh unit.

Hematology and Internal Organs

Diet had a significant effect ($P < 0.05$) on all hematological parameters apart from hemoglobin and the total lymphocytes count (Table 9). Interestingly, in all the parameters measured, layers fed control diet had the lowest hematological values compared with the hens fed diets containing *C. lanatus*. In all cases, the 2gCL treatment group consistently had the highest values for total white blood cells, red blood cells, neutrophils, monocytes, and eosinophils followed by the 1gCL treatment group. With regard to the internal organs, diet only affected the intestinal length and spleen weights (Table 10). The hens fed the control diet had the longest intestines and heaviest spleen weight compared with the *C. lanatus* containing diets.

Bone Characteristics

The effects of *C. lanatus* oil inclusion in diets on tibia biomechanics of layers are presented on Table 11. From the results, diet significantly ($P < 0.05$) affected TDPE, TW, tibia ash weight (**TAW**), and TBS. The hens fed the 2gCL diet consistently had the highest values for TDPE, TW, TAW, and TBS followed by the 1gCL diet.

DISCUSSION

This study was conducted to evaluate the efficacy of utilization of *C. lanatus* essential oils as natural feed additives on the performance of layer hens. From the

Table 7. Effect of *C. lanatus* essential oil inclusion in diets on fatty acid composition (%) of egg yolks (mean ± SEM).

Parameter	Diet[1]		
	Control	1gCL	2gCL
Proximate composition			
Fat	32.81 ± 0.59	31.36 ± 0.46	32.82 ± 0.48
FFDM	18.72 ± 0.35	18.98 ± 0.27	18.89 ± 0.29
Moisture	48.45 ± 0.71	49.66 ± 0.55	48.29 ± 0.58
Individual fatty acids			
Myristic	0.29 ± 0.02	0.29 ± 0.02	0.30 ± 0.01
Myristoleic	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Pentadecyclic	0.005 ± 0.01	0.00	0.01 ± 0.01
Palmitic	26.34 ± 0.42	26.38 ± 0.32	26.76 ± 0.34
Palmitoleic	3.57 ± 0.24	3.41 ± 0.19	3.44 ± 0.19
Margaric	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01
Stearic acid	5.96 ± 0.22[a]	6.67 ± 0.28[b]	6.32 ± 0.23[b]
Elaidic	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Oleic	44.63 ± 0.81	45.03 ± 0.31	43.23 ± 0.66
Vaccenic	4.63 ± 0.39	4.17 ± 0.31	4.38 ± 0.32
Linoleic	13.35 ± 0.69[a]	12.64 ± 0.54[a]	14.11 ± 0.57[b]
γ-Linolenic	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
α-Linoleic	0.46 ± 0.02	0.42 ± 0.01	0.46 ± 0.02
Eicosadienoic	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
Erucic	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Arachidonic	0.58 ± 0.12	0.61 ± 0.09	0.59 ± 0.10
Docosahexanoic	0.06 ± 0.03	0.06 ± 0.02	0.03 ± 0.02

[a,b]Means in the same column with different superscripts are significantly different ($P < 0.05$).

Abbreviation: FFDM, fat-free dry matter.

[1]Diets: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg *C. lanatus* oil; 2gCL, commercial layer diet mixed with 2 g/kg *C. lanatus* oil.

results, the effects of *C. lanatus* inclusion in diet on feed intake and weight gain was apparent. The improved feed intake and weight gain may, perhaps, be attributed to the influence of the *C. lanatus* essential oil in stimulating digestive enzyme secretion of gut and microflora ecosystem stabilization. Consequently, this could have resulted in accentuation of feed utilization mechanism and suppression of possible digestion and metabolism dysregulations as observed in other studies (Bento et al., 2013; Kurekci et al., 2014; O'Bryan et al., 2015; Zhai et al., 2018; Wang et al., 2019). Although there are many contrasting observations on the effects on essential oils inclusion in diets on feed intake attributed to varied explanations including the presence of unpleasant smell (Zhang et al., 2014), in our study, the increased feed intake could have been aided by the absence of unpleasant strong smell in the *C. lanatus* oils, which could have made it more acceptable to the layers hens. Although no effects of *C. lanatus* inclusion on total number of eggs produced and HDEP were observed in the current study, the observed high egg mass and lower FCR could be confirmatory of the notion that the essential oils could have been positively influential, not only in increasing weight gain but also in the improvement of conversion of feed to egg mass. Similar observations were made in other studies (Ozek et al., 2011; Bozkurt et al., 2014; Çabuk et al., 2014). The observed linear rather than quadratic relationships observed between essential oil inclusion level and the growth and egg production parameters were rather surprising. Normally, a general quadratic response is expected. A negative relationship observed for FCR and a positive relationship for ADFI, ADG, and eggmass may imply that the essential oils can be included at higher levels than those used in the study with positive benefits. Nevertheless, inclusion of the essential oils beyond the levels used in the study may be done with extreme caution, and higher levels of some bioactive compound in the essential oil can be toxic to the birds (Bozkurt et al., 2014).

Table 8. Effect of *C. lanatus* essential oil inclusion in diets on total fatty acids (%) and ratios of egg yolks (mean ± SEM).

Parameters[2]	Diet[1]		
	Control	1gCL	2gCL
Total SFA	32.71 ± 0.48	33.45 ± 0.37	33.52 ± 0.39
Total MUFA	52.76 ± 0.90	52.74 ± 0.70	51.19 ± 0.74
Total PUFA	14.53 ± 0.73[a]	13.81 ± 0.57[a]	15.29 ± 0.60[b]
Total n-6	14.02 ± 0.72[a]	13.33 ± 0.55[a]	14.81 ± 0.59[b]
Total n-3	0.51 ± 0.03	0.48 ± 0.03	0.48 ± 0.03
PUFA:SFA	0.45 ± 0.02	0.41 ± 0.02	0.46 ± 0.02
n-6/n-3	27.34 ± 1.62[a]	28.19 ± 1.26[a]	31.19 ± 1.33[b]

[a,b]Means in the same column with different superscripts are significantly different ($P < 0.05$).

[1]Diets: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg C. lanatus oil; 2gCL, commercial layer diet mixed with 2 g/kg C. lanatus oil.

[2]Parameters: SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids; n-6, total omega-6 fatty acids; n-3, total omega-3 fatty acids.

The observations in the current study that the inclusion of *C. lanatus* oil in the diet had no effect on most internal and external egg quality parameters are consistent with previous reports (Ozek et al., 2011; Bozkurt et al., 2014). Nevertheless, from this study, the inclusion of *C. lanatus* essential oil in diets induced some variations in shell weight and shell ratio. Similar observations were also made by Bozkurt et al., 2014. More notably, the inclusion of the *C. lanatus* essential oils improved the HU in the current study. Generally, the HU are the used as best measure of albumen quality (Zhao et al., 2015). An HU that is 72 and above is normally classified as the best grade (grade AA), 60-72 as grade A and 60 and less as grade B (Zhao et al., 2015). Although the HU obtained in this study for all dietary treatments were higher than 72, it can however be concluded that inclusion of *C. lanatus* oil in diets has the potential to improve freshness and quality of eggs as reported elsewhere (Albour-Elkhair et al., 2018, Wang et al., 2019).

As observed in other studies (Galobart et al. 2001; Ding et al., 2017), the inclusion of essential oil in diets had no effect on individual fatty acid composition apart from stearic acid and linoleic acid with were higher in the 2gCL treatment group. Nevertheless, in contrast to the observation by Bolukbasi et al., (2010), an increase in total PUFA and n-6 fatty acids was observed in the current study with a decline in n-3 fatty acids. The increase total PUFAs and n-6 fatty acids could be attributed to the high amounts of some essential fatty in the oil including palmitic, stearic, oleic, and various linoleic acids isomers (Berry, 2015). Suggestions have been made that essential oil source could be

Table 9. Effects of *C. lanatus* essential oil inclusion in diets on hematological parameters in layer hens (mean ± SEM).

Parameters	Diet[1]		
	Control	1gCL	2gCL
White blood cell (x 10^9/l)	168.71 ± 24.89[a]	291.55 ± 27.82[c]	227.72 ± 22.72[b]
Red blood cell (x 10^{12}/l)	1.02 ± 0.20[a]	1.98 ± 0.17[c]	1.41 ± 0.14[b]
Platelets count	2089.80 ± 184.26[a]	2500.00 ± 206.01[b]	2500.00 ± 168.21[b]
Hemoglobin (g/dl)	8.43 ± 0.73	10.00 ± 0.63	9.42 ± 0.52
Neutrophils (x 10^9/l)	3.80 ± 4.39[a]	14.90 ± 4.39[b]	9.82 ± 3.11[b]
Lymphocytes (x 10^9/l)	92.47 ± 7.54	78.68 ± 6.53	84.07 ± 5.33
Monocytes (x 10^9/l)	2.50 ± 4.49[a]	3.58 ± 3.89[b]	3.18 ± 3.17[b]
Eosinophils (x 10^9/l)	1.13 ± 0.89[a]	2.80 ± 0.77[b]	2.70 ± 0.63[b]
Basophils (x 10^9/l)	0.10 ± 0.09[a]	0.20 ± 0.07[a]	0.32 ± 0.06[b]

[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$).
[1]Diet: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg *C. lanatus* oil; 2gCL, commercial layer diet mixed with 2 g/kg *C. lanatus* oil.

influential to the contrasting observation on yolk fatty acid responses (Ding et al., 2017). Generally, there is scarcity of empirical evidence on the effects of essential oils on fatty acid profiles of egg yolk. The current study is one of the few attempt to assess the influence of essential oils in diets on egg yolk fatty acid profiles.

Knowledge of bird hematology is a useful diagnostic tool in veterinary medicine (Merck Manual, 2012). Hematological values are commonly used as physiological indicators of health in birds housed in cage systems and can be used detect stress caused by various factors, such as environmental, nutritional, and pathological aspects (Toghyani et al., 2010; Li et al., 2015). Overall, hematological indices in all treatment groups were observed to be within the anticipated ranges for healthy layers (Merck Manual, 2012). Nevertheless, the high values in most of the hematological parameters in birds fed *C. lanatus* could imply that the inclusion of *C. lanatus* essential oil had a positive influence hematopoiesis. In fact, the desirable blood metabolites levels realized in the current study indicates the potential of *C. lanatus* essential oil with phytogenic compounds in improving the general health status of layers. In other studies, compounds of similar nature were observed to reduce the hematological disorders associated with aflatoxins and mycotoxins in feeds (Tehrani et al., 2012; Disetlhe et al., 2018).

Table 10. Effects of *C. lanatus* essential oil inclusion in diets on internal organ parameters in layer hens.

Parameters	Diet[1]			
	Control	1gCL	2gCL	SEM
Intestine weight (g)	60.64	56.78	58.02	3.77
Intestine length (cm)	142[b]	134[a]	136[a]	0.05
Intestine pH	5.96	5.97	5.96	0.04
Liver weight (g)	27.39	26.53	27.85	1.67
Hepatosomatic index	0.004	0.004	0.004	0.0002
Spleen weight (g)	1.94[b]	1.73[a]	1.61[a]	0.21
Spleen index	0.0003	0.0002	0.0002	0.00003

[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$).
[1]Diet: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg *C. lanatus* oil; 2gCL, commercial layer diet mixed with 2 g/kg *C. lanatus* oil.

Changes in size and structure of internal organs can be indicative of the effect of diet and its components on the development and function of the organs. In the current study, although there were differences in sizes and weights of the internal organs, the differences were marginal. The weights of the internal organs (intestine, liver, spleen), intestinal length, intestine pH and hepatosomatic index were not significantly influenced by the diets. Generally, an increase in liver and heart sizes could be indicative of the need to deal with toxic substance in feed. On the other hand, an increase in intestinal length could be indicative of the need to increase retention time for digestion processes (Shim et al., 2013; Uphadhaya and Kim, 2017).

Bone health and metabolism of laying hens are of major concern to egg producers, veterinarians, nutritionists, and geneticists (Guz et al., 2019; Kolakshyapati et al., 2019). Tibia biomechanical characteristics such as tibia linear measurement bone breaking strength and bone ash (Świątkiewicz et al., 2014; 2018; Zhou et al., 2009) are usually used as indicators of mineral adequacy and bone development in broiler diets. In the current study, it was observed that dietary inclusion of *C. lanatus* seed oil significantly improved TDPE, TW, TAW, and TBS.

Table 11. Effects of *C. lanatus* essential oil inclusion on tibia biomechanics of layers.

Parameters[2]	Diet[1]			
	Control	1gCL	2gCL	SEM
TL (cm)	11.77	11.82	11.92	0.11[a]
TDPW (cm)	2.18[b]	2.07[a]	2.18[b]	0.04
TDW (cm)	1.10	1.10	1.07	0.01
TM (cm)	0.63	0.58	0.60	0.03
TAW	1.05[a]	1.13[b]	1.17[b]	0.05
TW (g)	8.93[a]	8.52[a]	9.82[b]	0.28
TBS (N)	128.93[a]	134.79[a]	138.84[b]	14.41

[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$).
[1]Diet: Control, commercial layer diet; 1gCL, commercial layer diet mixed with 1 g/kg *C. lanatus* oil; 2gCL, commercial layer diet mixed with 2 g/kg *C. lanatus* oil.
[2]Parameters: TL, tibia length; TDPW, tibia proximal distal width; TDW, tibia distal width; TM, tibia middle; TAW, tibia ash weight; TW, tibia weight; TBS, tibia bone strength.

In particular, the observed increase in bone breaking force with inclusion of *C. lanatus* essential oil could be reflective of the ability of the oil to improve the bone health and general welfare of the birds which are supposed to stand for long periods of their productive life (Świątkiewicz et al., 2014; Disetlhe et al., 2018; Kolakshyapati et al., 2019). To our knowledge, very few attempt have been made to assess the effects of essential oils on bone development in layer birds. Future research of essential oils in layer diets should also focus on the mechanism behind there improvement of bone development particularly bone strength as this may improve the welfare of layers hens which are normally kept in cages in entirety of their productive life.

In conclusion, although the inclusion of *C. lanatus* essential oil in layer diets did not influence most of the internal and external egg quality parameters, the positive influences on feed intake weight gain were apparent. Moreover, positive influence were also observed in terms of some important PUFAs, general health of the birds, and bone development particularly bone strength. Therefore inclusion of *C. lanatus* in doses of up to 2 g/kg feed can be beneficial to layer hens and hence can be recommended as a natural feed additive. More studies, however, need to be done to ascertain the standard/effective doses for commercial production of the *C. lanatus* feed additive.

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