Iranian Journal of Applied Animal Science

Substitution Effect of Noug Seed (*Guzoitia abyssinica*) Cake with Various Levels of Samma (*Urtica simensis*) Leaf Meal on Egg Production and Egg Quality Parameters of Commercial Layer Hens

Research Article

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Received on: 13 Nov 2018
Revised on: 8 Feb 2019
Accepted on: 16 Feb 2019
Online Published on: Dec 2019

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Online version is available on: www.ijas.ir

ABSTRACT

The substitution effect of noug (*Guzoitia abyssinica*) seed cake with samma (*Urtica simensis*) leaf meal (SLM) was investigated on egg production and egg quality parameters in commercial layer hens. Five treatment (T) diets were formulated to contain SLM at 0% (T1), 3% (T2), 6% (T3), 9% (T4) and 12% (T5) by substituting noug seed cake. One-hundred fifty Isa Brown layer pullets were randomly allocated to the treatment diets, replicated thrice consisting of 10 hens each. The results indicated that feed intake did not vary among treatment diets. The individual final body weight (g) of hens in T1, T2, T3, T4 and T5 was 1786, 1804, 1804, 1819, 1858, respectively, being the highest ($P<0.05$) for T5. The average egg weight (g) of hens fed with T1, T2, T3, T4 and T5 diets was 45.0, 47.2, 48.9, 53.5 and 55.5, respectively, being ($P<0.05$) different for T4 and T5. The hen-housed egg production (%) for hens fed with T1, T2, T3, T4 and T5 diets was 44.3, 49.5, 59.4, 70.9 and 80.5%, respectively and differed ($P<0.05$) from each other. The respective individual daily egg mass output (g) in hens fed with T1, T2, T3, T4 and T5 diets was 20.0, 23.4, 29.1, 37.9, and 44.7, being ($P<0.05$) different from each other. The feed conversation ratio (kg feed/kg egg mass) was 5.20, 4.18, 3.55, 2.83 and 2.40 for hens reared in T1, T2, T3, T4 and T5, respectively and being lowest ($P<0.05$) for those of T4 and T5. Hens fed with T3, T4 and T5 diets had higher ($P<0.05$) shell thickness than those of T1 and T2. The yolk index was higher ($P<0.05$) for hens reared in T5 than those of T1 and T3 diets. In conclusion, the substitution of noug seed cake with SLM improved the egg production and most egg quality parameters. We recommend further studies to corroborate the effect of samma leaf on total cholesterol and triglycerides concentrations of egg yolk and meat.

KEY WORDS egg production, egg quality, Isa Brown hens, noug seed cake, samma leaf.

INTRODUCTION

With steadily growing population of Ethiopia (current census indicate over 100 million), there is an increasing demand for animal products (personal observation). However, the livestock sector could not be able to meet this growing demand mainly due to limited availability of animal products and / or high prize of animal feed resources. This has led to shortage of high quality protein from animal products to the fast growing population by widening the gap between estimated protein requirement and actual protein consumption. It is therefore vital that some efforts must be undertaken towards improving the per capital animal protein consumption of the country. However, scarcity of animal feed resources, particularly during the dry season, is a major constraint to livestock production in the country. Animal feed supplements such as sunflower seed cakes are too expensive for most farmers. Therefore, those relatively less expensive but locally available alternative feed resources need to be investigated. Among those locally available unconventional protein feed resources, the samma (*Urtica simensis*) leaves could be used as one of alternative feed resources to monogastric nutrition. It is one of species of stinging nettle, which belongs to the genus *Urtica* of the family *Urticaceae*.

Samma is endemic in Ethiopia and grows all year round in the mid- and highlands of the country. Recent studies have revealed that samma leaves are excellent and easily available source of protein as well as minerals. According to the reports of various scholars, the CP content of samma leaves ranged from 263-318 g/kg DM (Eskedar *et al.* 2013; Dereje *et al.* 2016; Melesse *et al.* 2018). The samma leaves contained high calcium (58.6 g/kg DM), Iron (1186 mg/kg DM) and manganese (433 mg/kg DM). Most of the essential amino acids were also available in appreciable concentrations (Melesse *et al.* 2018). Moreover, Eskedar *et al.* (2013) and Melesse *et al.* (2018) reported negligible levels of total phenols and condensed tannins (below 0.72 % and 0.028%, respectively).

Same authors also reported below 9% crude fiber content of samma leaves. This makes the samma leaves to qualify as alternative suitable feed resources for feeding monogastric animals such as chickens.

Previous works have revealed that the addition of 1 to 2% stinging nettle (*Urtica dioica*) to broiler diet resulted in increased body weight gain and improved feed utilization (Kwiecien and Mieczan, 2009; Safamehr *et al.* 2012).

Broilers and layer hens fed diets supplemented with 2 to 3% stinging nettle also showed lower (P<0.05) concentrations of serum cholesterol and triglyceride compared with control birds (Mansoub, 2011a; Mansoub, 2011b). In pigs, the use of nettle extract had positive effects on meat quality, improving oxidative stability and the polyunsaturated/saturated fatty acid ratio (Hanczakowska *et al.* 2007). The use of stinging nettle alcoholic extract in growing chickens stimulated the innate cell mediated immune response that could result in higher resistance to diseases (Sandru *et al.* 2016). Moreover, Poudel and Khanal (2011) reported that the supplementation of nettle improved the immune status of laying hens

In Ethiopia, few research works are available on samma and are limited to medicinal uses. In some parts of Ethiopia, young shoots of samma leaves are eaten as a leafy vegetable (personal observation). There is no information available in the literature with regard to the utilization of samma leaves in layer hens' diet. Moreover, both noug seed (*Guizotia abyssinica*) cake and samma leaves contain comparable values of crude protein (noug seed cake 28-30% and samma leaves 31.8%) on dry matter basis.

However, they differ considerably in their crude fiber content being highest in noug seed cake (24.5%) compared to that of samma leaves (9.12%). Moreover, the samma leaves are very rich in calcium (5.86%) compared to that of noug seed cake (0.5%). Noug seed cake is considered in Ethiopia as one of the most common protein sources in poultry nutrition (personal observation). However, the cost of noug seed cake has been consistently rising over the last many years making it inaccessible to smallholder poultry producers.

Moreover, due to its high crude fiber content (Melesse *et al.* 2018) feeding high levels of the noug seed cake pose serious problems on the feed consumption and subsequently on the performance of chickens. Consequently, substituting the noug seed cake with other suitable cheap protein and calcium sources becomes justifiable in monogastric nutrition such as poultry, particularly for layer hens. The main objective of this research work was thus to evaluate the nutritional potential of leaf meal prepared from samma as alternative protein and mineral sources on feed consumption, egg production and egg quality parameters of commercial layer hens.

MATERIALS AND METHODS

Description of the study area

The experiment was carried out at poultry farm of School of Animal and Range Sciences, College of Agriculture, Hawassa University. Geographically it is situated at 7˚ 4'N latitude and 38˚ 31'E longitude at an altitude of 1650 m above sea level. Rainfall is bi-modal and ranges between 674 and 1365 mm. The mean temperature ranges between 12 ˚C and 27 ˚C.

Samma leaf preparation

Fresh samma leaves were collected from farmers residing near Boditti town of Wolaita Zone that grow the plant materials abundantly. The leaves were removed from the stem and then dried under the shade. During the drying process, regular turning of leaves was done to prevent the possible growth of moulds. The dried leaves were then transported to the research site and processed into samma leaf meal through grinding which is referred hereafter as samma leaf meal (SLM).

Ingredients of the experimental diets

The dietary ingredients used in this experiment were maize, roasted soybean seed (*Glysine max*), wheat bran, noug seed (*Guizotia abyssinica*) cake, meat and bone meal, limestone flour, poultry premix and salt. All ingredients were purchased from the local market.

The soybean seed was roasted for 5 minutes until the beans became brown to deactivate trypsin inhibitor (Negesse and Tera, 2010; Navicha *et al*. 2017) and milled in sieve size of 5 mm separately. All other feed ingredients were also milled with similar sieve size. All ingredients were then mixed according to the formulated experimental diets. Moreover, representative samples from each treatment diets were taken for the determination chemical and mineral compositions.

Experimental design

After having results on chemical and mineral analysis of each feed ingredients, five iso-nitrogenous and iso-caloric experimental diets were formulated to contain SLM at 0% (treatment 1=T1), 3% (treatment 2=T2), 6% (treatment 3=T3), 9% (treatment 4=T4) and 12% (treatment 5=T5) by substituting noug seed cake of the control diet. Initially, 161 (12 weeks of age) Isa-Brown pullets were purchased from Alema Poultry Farm Limited Company (Debrezeit, Ethiopia). At the age of 19 weeks (at the start of egg lay), 150 Isa Brown layer pullets were randomly assigned to each of the three replicates of the five dietary treatments in a completely randomized design (Table 1). The experiment lasted for 60 days.

Management of chickens

Pullets were already vaccinated by the company against major poultry viral and bacterial diseases including Marek's disease, Newcastle, infectious bursal disease (Gumboro), fowl typhoid and fowl pox diseases. After arrival, pullets were leg-tagged, weighed and randomly distributed to the already prepared experimental pens with a dimension of 3 m^2 (2×1.5 m) per replicate. The concrete floor was covered with wood shavings at a depth of 5 cm. The pullets were fed with pullet and pre-lay rations until the age of 20 weeks. They were then fed with layer rations starting from the 20^{th} weeks of age until end of the experiment (Table 2). Clean water and feed was provided *ad libitum* throughout the experimental period.

Data collection protocols

Feed intake and egg production

Chickens were fed on replicate basis and each day a measured amount of feed was offered in the morning (between 7.00 and 8.00 a.m.) and late afternoon (between 4.00 and 5.00 p.m.) and refusals were always collected and weighed in the morning of the following day before feed is offered. Feed intake on group basis was then computed by subtracting the feed refusal from that of feed offered. Eggs were collected daily and egg production rate was calculated on hen-housed basis by considering the number of hens that were housed initially.

Egg weight was determined on weekly basis and the average egg weight was calculated. Total egg mass was computed by multiplying the average egg weight with total number of eggs produced. Daily egg mass per hens was computed through dividing the total egg mass by the number of hens that were initially housed and total number of days in which the hens were in lay. Feed conversion ratio was calculated as grams of feed: grams of egg mass output. Body weight was taken at the beginning and end of the experimental period and then total weight gain was calculated by subtracting the initial body weight from that of the final.

Egg quality parameters

Internal and external egg quality parameters were assessed in all birds at the age of 23 and 28 weeks and values were averaged. Two eggs from each hen were collected over two consecutive days and the following parameters were determined: egg weight, egg length and breadth, shell thickness, albumen and yolk heights, yolk diameter and colour. Eggs were weighed using triple beam balance. Egg length and width and yolk diameter were measured using electronic digital caliper.

A tripod micrometer was used to measure the heights of albumen and yolk. Yolk color was measured subjectively with Roche yolk color fan by matching the yolk color on a glass plate with the 15 bands of the color fan. Standard color score was from 1 (extremely pale) to 15 (deeply yellow-orange). Shell thickness was determined by taking the average thickens of the broad end, the center and narrow end. Haugh unit (HU) was calculated according to Haugh (1937) by fitting the average albumen height and egg weight in to the following the equation:

$$HU = 100 \times \log [\text{albumen height} + 7.57 - 1.7 (\text{egg weight}^{0.37})]$$

Chemical analysis

Analyses of proximate nutrients were performed as outlined by AOAC (2005). Samples of samma leaf meal and feeds offered were analyzed for dry matter (DM, method 950.46), ether extract (EE, method 920.39), crude fibre (CF, method 962.09) and ash (method 942.05). The crude protein (CP) was assessed using Kjeldahl procedure (method 954.01) and the nitrogen content was multiplied by 6.25 to obtain the CP.

Calcium (Ca) was determined by atomic absorption spectrophotometer and phosphorus by colorimetrically methods as described by AOAC (2005). The metabolizable energy (ME) of diets was estimated based on the feed composition tables of tropical feeds for poultry. All the samples were analyzed in duplicates at Animal Nutrition Laboratory of Hawassa University.

Table 1 Experimental design of the feeding trial with Isa Brown layer hens

Treatment	Inclusion rate of SLM (%)	Number of replicates	Hens per replicate	Hens per treatment
T1	0	3	10	30
T2	3	3	10	30
T3	6	3	10	30
T4	9	3	10	30
T5	12	3	10	30
Total (N)	-	-	-	150

SLM: samma (*Urtica simensis*) leaf meal.
T1: contain 0% SLM; T2: contain 3% SLM; T3: contain 6% SLM; T4: contain 9% SLM and T5: contain 12% SLM.

Table 2 Feed ingredients (g/kg) and nutrient compositions (g/kg DM) of the control diet and diets containing different levels of samma (*Urtica simensis*) leaf meal of the layer ration

Ingredients	Treatments				
	T1	T2	T3	T4	T5
Maize	480	467	472	477	480
Roasted soybean	200	200	190	190	190
Wheat bran	100	113	120	115	112
Meat and bone meal	20	20	18	18	18
Noug seed cake	120	90	60	30	0
Samma leaf meal	0	30	60	90	120
Lime stone flour	65	65	65	65	65
Vitamin and mineral premix[1]	10	10	10	10	10
Salt	5	5	5	5	5
Total	100	100	100	100	100
Analyzed composition (%)					
Metabolizable energy (kcal/kg)	2780	2780	2770	2760	2750
Ash	88.9	89.5	93.0	96.6	100
Crude protein	174	174	175	175	176
Crude fiber	66.4	62.6	57.8	52.9	48.6
Fat	34.5	34.5	34.0	34.0	34.2
Calcium	27.2	28.8	30.2	31.8	31.5
Phosphorous	6.3	6.3	6.2	6.1	6.0

[1] Vitamin premix per kg of diet: vitamin A: 2.7 mg; vitamin D_3: 0.05 mg; vitamin E: 18 mg; vitamin K_3: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Panthothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg; Antioxidant: 100 mg; Fe: 50 mg; Mn: 100 mg; Zn: 100 mg; Cu: 10 mg; I: 1 mg and Se: 0.2 mg.

Statistical analysis

Data on feed intake (g), body weight, feed conversion ratio, egg production and egg quality parameters were subjected to one-way ANOVA by fitting treatment diets as fixed factors.

The general linear model (GLM) procedures of SAS (SAS, 2012) were used to analyze the collected data and treatment means were compared using Tukey's studentized range (HSD) test. Comparisons with P < 0.05 were considered as significant. The following statistical model was used to analyze the data:

$$Y_{ij} = \mu + A_i + D_j / A_i + e_{ij}$$

Where:
Y_{ij}: observed values of the dependent variables (feed intake, body weight, egg production, egg quality).
μ: overall mean of the response variable.
A_i: effect of the i^{th} samma leaf meal level (i=1, 2, 3, 4, 5) on the dependant variables.
D_j / A_i: effect of the j^{th} replicate (j=1, 2, 3) within the level of the i^{th} samma leaf meal.
e_{ij}: residual error due to random effects.

RESULTS AND DISCUSSION

Nutrient compositions of samma leaf meal and experimental diets

Diet compositions of the treatment diets are presented in Table 2. The ash and Ca concentrations showed an increasing trend from T1 through T5 due to inclusion rate of SLM in the experimental diets. However, the CF and the calculated ME values consistently reduced with increasing levels of SLM. As shown in Table 3, SLM contained appreciable amounts of ash, CP and Ca.

Egg production

The total egg number differed (P<0.001) among treatment diets being higher in hens reared in T5 than those of other treatments (Table 4). Hens fed with T4 diet were better (P<0.05) in total egg number than those reared in the other treatments. The rate of hen-housed egg production also showed similar trend as that of total egg number being higher (P<0.001) in hens fed with T5 as compared to those reared in the other treatment diets. The average egg weight from hens reared on T4 and T5 diets was higher (P<0.05) than those of T1, T2, and T3.

Table 3 Analyzed values of samma (*Urtica simensis*) leaf meal

Nutrients	Values [g/kg dry matter (DM)]
Ash	117
Crude protein	294
Ether extract	62.0
Crude fiber	87.1
Calcium (mg/kg DM)	54.6
Phosphorous (mg/kg DM)	5.60

Table 4 The substitution effect of noug seed cake with different levels of samma (*Urtica simensis*) leaf meal (SLM) on performance of Isa Brown layer hens (Mean±SD)

Parameters	Treatments				
	T1	T2	T3	T4	T5
Total egg number	239±8.01^{e}	267±12.6^{d}	321.0±12.2^{c}	383±11.7^{b}	435±6.12^{a}
HHE production (%)	44.3±1.45^{e}	49.5±2.36^{d}	59.4±2.23^{c}	70.9±2.13^{b}	80.5±1.11^{a}
Average egg weight (g)	45.0±0.43^{c}	47.2±1.90^{bc}	48.9±1.93^{b}	53.5±0.31^{a}	55.5±0.72^{a}
Total EMP (kg)	10.8±0.45^{e}	12.6±1.07^{d}	15.7±1.21^{c}	20.5±0.73^{b}	24.1±0.67^{a}
Daily EMP per hen (g)	20.0±0.85^{e}	23.4±1.93^{d}	29.1±2.25^{c}	37.9± 1.41^{b}	44.7±1.17^{a}
Total feed intake (kg)	56.0±3.29	52.2±5.75	55.6±3.07	58.0±0.17	57.9±0.15
FCR	5.20±0.40^{a}	4.18±0.80^{b}	3.55±0.26^{bc}	2.83±0.01^{cd}	2.40±0.07^{d}

T1: contain 0% SLM; T2: contain 3% SLM; T3: contain 6% SLM; T4: contain 9% SLM and T5: contain 12% SLM.
FCR: feed conversion ratio; HHE: hen-housed egg and EMP: egg mass production.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Hens fed with T3 diet produced heavier (P<0.01) eggs than those reared in T1. No significance difference was observed in egg weight between hens fed with T3 and T2 diets as well as between those reared in T1 and T2. The highest total egg mass output was obtained from hens reared in T5 followed by those raised in T4, T3, T2 and T1 diets being different (P<0.001) from each other. Similar results were also observed in the individual daily egg mass production. No difference was observed in total feed intake among treatment diets although hens reared in T2 consumed the least. A higher feed conversion ratio (FCR) was observed in hens reared in T5 and differed (P<0.05) from those fed with T1, T2 and T3 diets.

Egg quality parameters

The effect of samma leaf meal feeding on external and internal egg qualities is presented in Table 5. The average shell thickness from hens reared in T3, T4 and T5 were higher (P<0.05) than those of T1. The highest egg length was obtained from hens reared in T5 and differed (P<0.05) from those of T1, T2 and T3, which had comparable values. Layer hens reared in T5 produced eggs with higher (P<0.05) egg width values than those of T1 and T3. Similarly, the egg width values of hens reared in T2 and T4 were higher (P<0.05) than those of T1. The calculated egg shape index values from hens reared in T2 was similar to those of T1, T3 and T4 diets but was higher (P<0.05) than those of T5.

The egg weight from hens fed with T5 was heavier (P<0.05) than those of other treatments.

Hens fed with T4 diet also produced heavier (P<0.05) eggs than those of T1, T2 and T3 diets. However, no significance difference was observed in egg weight among chickens reared in T2 and T3 diets as well as those of T1 and T2. The average value of albumen height, HU and yolk width did not vary among treatment diets. However, hens reared in T5 diet had the highest (P<0.05) yolk height and its index as compared with those kept on T1 and T3. These values were similar in hens reared in T1 and T3 diets as well as between T2, T3 and T4.

The highest yolk color value was recorded in hens reared in T2 and T4 diets while the lowest from those fed on T1 and differed (P<0.05). However, the average value of yolk color was similar among hens reared in T1, T3 and T5 diets.

The CP content of SLM observed in the current study was comparable with that of reported by Melesse *et al.* (2018) for the same plant species. However, the CP content was higher than those values reported by Eskedar *et al.* (2013) and Dereje *et al.* (2016) for the same plant species. Adhikari *et al.* (2016) reported 33.8% of CP for nettle leaf powders, which are slightly higher than observed in the current study.

Such variations might be attributed to the maturity age of the plant material, difference in specie, the location where samples were collected and the methods of processing the samples for the chemical analysis. The Ca and CF contents reported by Eskedar *et al.* (2013) and Melesse *et al.* (2018) for the same plant material were consistent with the findings of the current study.

Table 5 External and internal egg qualities of Isa Brown layer hens fed with different levels of samma (*Urtica simensis*) leaf meal (SLM) by substituting the noug seed cake (Mean±SD)

Egg qualities	Treatments				
	T1	T2	T3	T4	T5
External egg qualities					
Shell thickness (mm)	0.32±0.07^{b}	0.36±0.06^{ab}	0.37±0.06^{a}	0.38±0.06^{a}	0.40±0.04^{a}
Egg length (mm)	53.0±1.8^{c}	53.1±1.41^{c}	54.2±1.2^{bc}	55.2±1.31^{ab}	56.7±1.76^{a}
Egg width (mm)	41.1±1.14^{c}	42.2±2.77^{ab}	41.8±0.97^{bc}	42.8±0.82^{ab}	43.1±0.91^{a}
Egg shape index	0.78±0.02^{ab}	0.79±0.05^{a}	0.77±0.02^{ab}	0.77±0.02^{ab}	0.76±0.02^{b}
Internal egg qualities					
Egg weight (g)	50.3±4.03^{d}	52.3±2.87^{cd}	53.3±2.92^{c}	56.9±2.50^{b}	59.6±3.00^{a}
Albumen height (mm)	4.97±1.70	5.32±1.76	5.03±1.63	5.11±1.91	5.68±1.72
Haugh unit	70.3±14.5	72.4±13.4	69.9±12.2	67.8±18.9	72.3±14.1
Yolk height (mm)	16.5±1.35^{c}	17.5±1.5^{ab}	16.6±1.88^{bc}	17.6±1.29^{ab}	18.1±0.68^{a}
Yolk width (mm)	37.7±2.09	37.3±1.52	37.3±1.80	37.8±1.29	37.6±0.96
Yolk index	0.44±0.05^{c}	0.47±0.04^{ab}	0.45±0.05^{bc}	0.47±0.04^{ab}	0.48±0.02^{a}
Yolk color	6.53±2.32^{b}	8.12±1.90^{a}	7.82±2.16^{ab}	8.17±2.20^{a}	7.94±2.14^{ab}

T1: contain 0% SLM; T2: contain 3% SLM; T3: contain 6% SLM; T4: contain 9% SLM and T5: contain 12% SLM.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Comparable values for ash and CF contents were also reported by Adhikari *et al.* (2016) for the leaves of common stinging nettle.

Feeding different levels of SLM to layer hens showed significant improvements in final body weight and body weight gain. The positive effect of samma leaf could be attributed to the effect of bioactive compounds (aromatic compounds and essential oils) of the plant material. Animal-based studies have proved that stinging nettle leaf extract enhanced the overall liver function (Nassiri-Asl *et al.* 2009). It has been thus speculated that herbs like samma leaves and their derivatives may possess digestion-enhancing properties by stimulating the production of endogenous secretions in the small intestinal mucosa, liver and pancreas, and thus facilitate the digestion process. However, such digestion-enhancing properties of samma leaves shall be verified in a different study.

There are limited reports on the performance of layer hens fed with leaves of samma. Increase in the weight of eggs, percentage of the egg production and improved in the FCR in the treatment groups with the samma might be attributed to the quality of nutrients supplied by the samma leaves particularly essential amino acids. As shown in Table 6, the concentration of most essential amino acids in samma leaves was comparatively higher than those found in soybean meal. The protein of SLM is notably rich in tryptophan, methionine and threonnine compared to soybean meal. The presence of methionine as a limiting amino acid as well as other essential amino acids in samma leaves might have contributed to the observed improved egg production of hens fed with the SLM diet compared to those on the control diet.

It might also be due to the influence of other substances such as the vitamins and non-specific immune-modulators in the SLM diet, which improved the efficiency of feed utilization of layer hens (Sandru *et al.* 2016). Adding dried powder of stinging nettle into laying hens diets significantly increased egg production, proved the modulating effects of the immune parameters (Mansoub, 2011a), and lowered the total cholesterol and triglycerides concentration (Mansoub, 2011b).

Nasiri *et al.* (2011) reported that the use of 1.5% of stinging nettle in starter and grower feeds showed positive effects on carcass traits of broilers without having any significant effects on performance and blood biochemical and immunity parameters. Moreover, improved FCR and growth performance was reported in broiler chickens fed with dried nettle extracts (Hashemi *et al.* 2018). Dereje *et al.* (2016) have also documented a positive effect of samma leaves on the milk yield of goats. These authors reported that replacement of concentrate supplement with different levels stinging nettle leaf meal in lactating local goats increased milk production without affecting the major milk components. Furthermore, the values of anti-nutritional factors in samma leaves have been reported to be very low as compared to other indigenous leaves of root crops (Melesse *et al.* 2018). In fact, this low concentration of condensed tannin might be an advantage for maintaining the bioavailability of other nutrients at a normal level.

The FCR was significant across treatment diets being the lowest in hens fed with T4 and T5 diets. These results indicated that SLM could possess antimicrobial effects and might explain the improved FCR in hens fed with SLM treatment diets.

Table 6 Composition of essential amino acids of samma (*Urtica simensis*) leaf meal (SLM) leaves and stinging nettle (*Urtica dioica*) shoots (g/16 g N) compared to soybean meal

Amino acids	Soybean meal (Hossain and Becker, 2001)	Samma leaves Rutto *et al.* (2013)	Stinging nettle shoots[1]
Methionine	1.22	1.54	0.85
Cysteine	1.70	1.19	-
Valine	4.59	4.59	4.33
Leucine	7.72	6.48	6.19
Phenylalanine	4.84	4.65	3.98
Threonine	3.76	3.91	3.65
Tryptophan	1.24	2.99	-
Isolucine	4.62	3.40	3.58
Lysine	6.08	4.18	4.08
Arginine	7.13	4.47	4.98
Histidine	-	2.30	2.59

[1] Average values of fall and spring and calculated from the protein content on dry matter basis.

The overall performance of hens fed with the control diet was relatively lower than expected. This might be attributed to the composition of the diet, as the CF content was relatively high due to increased levels of noug seed cake in the control diet.

Egg weight is one of the important phenotypic traits that influence egg quality and reproductive fitness of the chicken parents (Zhang *et al.* 2005). The highest shell thickness and yolk index were observed in the hens fed with 12% of samma leaf, which agrees with the results of Mansoub (2011b). Consistent with the current results, Poudel and Khanal (2011) reported that the supplementation of nettle to layer hens increased the egg quality parameters.

The enhanced yolk index might be related to stability of yellow pigments in the membrane of the yolk among the lipid molecules, in which the antioxidants present in samma leaves might have prevented these molecules from being affected (Mavi *et al.* 2004; Kukrić *et al.* 2012).

The higher yolk color value observed in experimental diets might be due to the inclusion of SLM, which is rich in vitamin A (including vitamin A as β-carotene) as reported by Rutto *et al.* (2013). Kukrić *et al.* (2012) reported a higher content of chlorophyll, carotenoids, and proteins in young nettle leaves. Thus, the carotenoids in samma leaf such as xanthophylls might be transferred to the yolk and increase its yellowish color. Loetscher *et al.* (2013) reported that inclusion of nettle in layer diets significantly increased yellowness of yolk and was equally effective as synthetic pigmentation.

Eggshell quality as measured by shell thickness is a very important concern for consumers, as high resistance to breaking and lack of shell effects are necessary in order to protect against the penetration of pathogenic bacteria into eggs. In the present study, the highest shell thickness was observed in hens fed with T3, T4 and T5 diets, which differed significantly from those of the control diet.

This might be attributed to the high level of mineral calcium that is supplied by the samma leaf, as calcium is one of the key nutrients required for production and optimal eggshell quality of laying hens. Unbalanced diets might have deeply influenced the capacity of hens maintaining bone integrity during lay, as bones may be required to supply calcium for egg production and body maintenance, particularly when hens are still growing which is factual in the case of hens fed with the control diet in the current study.

In current study, no mortality was observed and this could be due to higher resistance to diseases as a result of developing innate immune response of hens fed with the samma leaves (Modarresi-Chahardehi *et al.* 2012; Sandru *et al.* 2016; Hashemi *et al.* 2018). Extract of stinging nettle (*Urtica dioica*) was tested for antibacterial activity against various gram positive and gram-negative bacteria and was reported minimum inhibitory concentration and minimum bactericidal concentration of the extracts (Kukrić *et al.* 2012). Studies conducted by Ngugi *et al.* (2015) demonstrated that using extracts of stinging nettle (*U. dioica*) has improved immunity of juvenile and adult fishes by making them more resistant to bacterial infections. Omar *et al.* (2018) reported reduced mortality in broilers supplemented with natural herbal extract.

CONCLUSION

The nutrient contents of SLM indicated that it is rich in CP (with better amino acid profile) and Ca, which are important for growth, skeletal development, and egg production. The substitution of noug seed cake with different levels of samma leaf has considerably improved the feed intake, egg production and egg quality parameters by providing protein and calcium as major source of nutrients. Thus, using the SLM in layer rations presents as good alternative feed resource to substitute the expensive noug seed cake in layer rations under smallholder poultry production activities. We recommend further studies to corroborate the effect of samma leaf on total cholesterol and triglycerides concentration of egg yolk in layer hens and carcass components in broilers.

ACKNOWLEDGEMENT

This research project was jointly supported by the research fund granted by the Alexander von Humboldt Foundation and Vice President for Research and Technology Transfer (VPRTT) of Hawassa University for which the authors are highly grateful. The assistance received from Mr Tadesse Bekore in analysing the chemical compositions is highly appreciated.

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