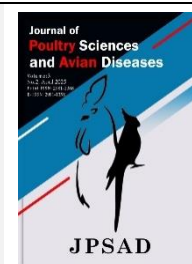


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Effects of Rosemary (*Rosmarinus officinalis* L.), Onion (*Allium cepa* L.), and their mixtures as Natural Feed Additives on Egg Quality, Hatchability, Embryonic Development, and Chicks Quality of White Leghorn



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ABSTRACT

The study evaluated the effects of rosemary, onion, and their mixture extracts on egg quality, fertility, hatchability, embryonic mortality, and chick quality of White Leghorn. One hundred twenty White Leghorn layers at thirty-two weeks of age were randomly allocated to four treatments, each replicated three times with ten layers and one cock per replication and managed on a deep litter system for 70 days. The treatments were T1(control group without any addition of feed additives), T2 (water with 4 milliliters of onion extract), T3 (water with 4ml of rosemary extract), and T4 (water with 2ml of onion and 2ml of rosemary extract mixture). Seventy-two eggs were randomly taken and evaluated for egg quality parameters such as eggshell and weight, albumen quality, and egg yolk quality. Three hundred sixty eggs were incubated and evaluated for fertility, hatchability, embryonic mortality, and chick quality. Results revealed that the use of Rosemary, Onion, and their mixtures significantly ($P<0.05$) higher on egg weight, albumen weight, albumen ratio, yolk index, shell weight, hen-day egg production, and egg mass in which the highest values were recorded in T2 compared to other treatments. Layers drunk on water diluted with onion extract produced eggs with the heaviest weight.

Keywords: Egg Quality, Extracts, Hatchability, Onion, Rosemary

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1 Introduction

Feed additives originating from natural medicinal products such as herbs and spices have been used to formulate poultry ration (1). Feed additives derived from plant products have proven to be natural, less toxic, and residue-free and are thought to be ideal feed additives in animal feed production (2). Although the discovery antibiotics used in the development of medicine to cure diseases and promote growth applied to human food and animal feed, nowadays, they produce significant negative effects on animal products' qualities and hygiene due to drug resistance (3). The antibiotics used in the animals may pass without being absorbed by them and are excreted from their bodies. The amounts of antibiotics dumped into the animal manures and feces as an antibiotic residue in pigs and chickens, with a varied amount posing serious threats to the terrestrial environment (4). After the ban on antibiotics, animal nutritionists tried to find alternative feed additives to replace them and use medicinal herbs, which are used as feed additives for better-growing conditions and product quality improvement (5).

This has increased the pressure on the poultry industry to find adequate alternatives, such as medicinal plants, that can be used instead of antibiotics in animal nutrition. Herbal feed additives improve nutrient utilization and absorption or stimulate the immune system (6). Plant herbs such as rosemary and onion are widely used, especially in tropical regions. Rosemary (*Rosmarinus officinalis*. L.) and onion (*Allium cepa* L.) are among the herbal medicinal plants that exert a potential effect on animal feed industry development (7).

Phenol compounds of rosemary, such as diterpenes, carnosol, carnosic acid, methyl carnosic, rosmarinic, and caffeic acid, play a vital role in antioxidant and antimicrobial activity against bacterial growth (8). The onion (*Allium cepa* L.) is well known for its effective prevention and treatment of diseases by antioxidant, antihypertensive, antithrombotic, antibiotic, and anti-carcinogenic effects with its variable biochemical functions (9). It contains Sulfur-containing compounds, which are sources of Methionine, cysteine, and amino acids. It has the effect of lowering the level of cholesterol in blood plasma or serum and is important for the growth of birds (10).

Despite these herbs' beneficial effects, limited research has been conducted to determine the combined effects of a rosemary and onion extract mixture on egg and chick quality. Therefore, this study evaluated the effects of rosemary and onion extracts on egg quality, hatchability, embryonic mortality, and chick quality of White Leghorn.

2 Materials and Methods

2.1 Description of Study Area

The study was conducted at Haramaya University poultry farm, 525 km from Addis Ababa. The site is situated at an altitude of 1980 millimeters above sea level, 9 ° 26 ' N latitude, and 42 ° 3' E longitude. The area has an average annual rainfall of 741.6 millimeters. The mean annual minimum and maximum temperatures are 8.25° C and 23.4° C, respectively.

2.2 Commercial Layers' Ration and Preparation of Feed Additives

Table 1. The proportion of ingredients used in formulating layers' rations.

Ingredients	Amount (%)	Amount(Kg)
Maize	50	600
Noug Seed Cake	15	180
Limestone	7	84
Wheat Short	15	180
Premix	1	12
Salt	1	12
Soybean Meal	9.8	117.6
Lysine	0.1	1.2
DL-Methionine	0.1	1.2
Ca phosphate	1	12
Total	100	1200

The Haramaya University animal feed processing plant purchased the commercial layers' ration. The ration consists of maize, wheat short, soybean meal, Noug seed cake,

limestone, salt, and vitamin premix, which were indicated with their proportion and amount in (Table 1). The diet was formulated based on isocaloric and isonitrogenous with

approximately 2900 kcal ME/kg DM and 16.5% CP to fulfill the nutrient requirement of layers.

The 1.4kg of rosemary and 4kg of onion were purchased from the local market (Harar). The onion bulbs were cleaned, the peels and root were removed, then cut into small pieces and spread on a plastic sheet for two days to dry and make it easier for grinding and to make powder following the method of (11) and finally grated by mixer and put in a plastic container at room temperature. The rosemary leaf was cleaned, separated from stem, and grated by a grinder to make powder following the methods (12). Then, 20g of powder was weighed from each spice and mixed with one liter of distilled water in a separate plastic container. The mixture was shaken thoroughly to get the diluted solution of

the juice. The obtained solution was kept overnight for about 12 hours at room temperature, then filtered and poured into a separate plastic container. Then, each amount of extract was divided into experimental pens (replication) for daily use (13).

2.3 Commercial Layers' Ration

The commercial layers' ration was purchased from the Haramaya University animal feed processing plant (Table 1). It was formulated based on isocaloric and isonitrogenous with approximately 2900 kcal ME/kg DM and 16.5% CP to fulfill the layers' nutrient requirements.

Table 2. Dietary Composition of Commercial Layers Ration

Ingredients	DM%	CP%	EE%	CF%	Ash%	Metabolizable energy(kcal/kg)
Maize	90.10	8.50	11.50	3.90	3.80	3595.5
Noug seed cake	93.50	29.40	12.00	11.50	10.20	3130
Wheat short	90.50	150	3.50	5.80	3.50	2955
Soybean meal	92.50	38.40	3.80	5.60	5.50	3410

DM=Dry matter, CP = Crude protein, EE = Ether extract. CF = Crude fiber, ME = Metabolizable energy, kcal= kilo calorie, and kg= kilogram.

Table 3. Chemical Composition of onion and rosemary and treatment ration

Spices	DM%	CP%	EE%	Ash%	CF%	ME(Kcal/kg DM)
Onion	81.54	1.17	1.65	4.64	5.013	3406.79
Rosemary	91.74	9.68	5.95	8.16	25.03	7121.59
Layer ration	90.50	16.5	4.80	11.35	8.50	2936.45

DM=Dry matter, CP = Crude protein, EE = Ether extract. CF = Crude fiber, ME = Metabolizable energy, kcal= kilo calorie, and kg= kilogram.

2.4 Preparation of Feed Additives

A measured amount of rosemary (1.4kg) and onion (4kg) were purchased from the local market (Harar). The onion bulbs were cleaned, the peels and root were removed, then cut into small pieces and spread on a plastic sheet for two days to dry and make it easier for grinding and make powder following the method of (14) and finally grated by mixer and put in a plastic container at room temperature. The rosemary leaf was cleaned, separated from stem, and grated by a grinder to make powder following the methods of (6). Then, 20g of powder was weighed from each spice and mixed with one liter of distilled water in a separate plastic container.

The mixture was shaken thoroughly to get the diluted solution of the juice. The obtained solution was kept overnight for about 12 hours at room temperature and then filtered and poured into separate plastic containers. Then, each amount of extract was divided into experimental pens (replications) for daily use (15).

2.5 Experimental Design and Treatments

The chickens were randomly allocated to four treatments, each with three replicates. Each replication consists of 10 layers and one cock in a completely randomized design (CRD). The four experimental dietary treatments were prepared as follows:

Table 4. Experimental Layout

Treatments	Proportion of extracts	Replication	Cocks	Layers	Total
T1	Control group	3	1	10	33
T2	4 ml of onion extract in one litter of water	3	1	10	33
T3	4ml of rosemary extract in one litter of water	3	1	10	33
T4	2ml onion+2ml of rosemary extracts	3	1	10	33

T1=Treatment one, T2=treatment two, T3=Treatment three, and T4=Treatment four

2.6 Experimental Chickens and Management

At thirty-two weeks of age, 132 White Leghorns (120 layers and 12cocks) were taken from Haramaya University Poultry Farm. The chickens were acclimatized to the experimental diet for seven days and managed for 70 days. The experimental pens, watering, feeding troughs, and laying nests were carefully cleaned and disinfected. The experimental diet and water were provided in a group *ad libitum* basis throughout the experimental period.

2.7 Egg Quality Parameters

For egg quality parameters such as egg weight, albumen quality (weight, height ratio), shell quality (weight and thickness), yolk quality (weight, height, diameter, index, ratio, and color), and Haugh unit score, a total of three (3) from each replication or nine (9) samples of eggs were randomly taken and assessed. One hundred eighty medium-sized eggs (15 from each replication or 45 from treatment) were used to evaluate fertility and hatchability. From each replicate, three (3) chicks were randomly selected, and their quality was measured using three different methods, which include visual scoring and measuring day-old chick weight and length. Finally, all collected data were analyzed using SAS software version 9.4 (14), and significant differences between treatment means were located using the least significant difference (LSD) method. The following statistical model was applied to the experimental data analysis (16).

$$y_{ijk} = \mu + t_i + e_{jk}$$

Where:- y_{ij} = the response variable

μ = the overall population means,

T_i = treatment effect (t = rosemary, onion, and their mixture effects as feed additives),

E_{jK} = experimental error.

3 Results and Discussion

3.1 Egg production and Egg mass

Total egg number (EN) and egg mass (EM) were significantly ($P < 0.05$) higher compared to the control group and treatment T4 by administration of onion and rosemary extract (Table 3). Higher EN and EM were recorded in T2 compared to T3 and T4. Adding onion extract in layers of drinking water significantly ($p < 0.05$) improved the number of eggs/hen, percentage of egg production, and egg mass/hen compared with the control group. The highest hen-day egg production (HDEP) was recorded in T2, whereas the lowest was in T1 and T4. The blended use of the two spice extracts has resulted in lower HDEP than the separate use. The HDEP of T3 and T4 sharply increased during the first two weeks but slowly increased up to the fifth week, and then, after, it slowly declined (Figure 1). This was consistent with the findings of (17), who reported subsequent egg production because of the phytogetic impact of onion as feed additives. This could be attributed to the positive effects of this additive in modulating gut microbiota, enhancing nutrient digestibility and absorption, and improving ovarian characteristics, resulting in better health status.

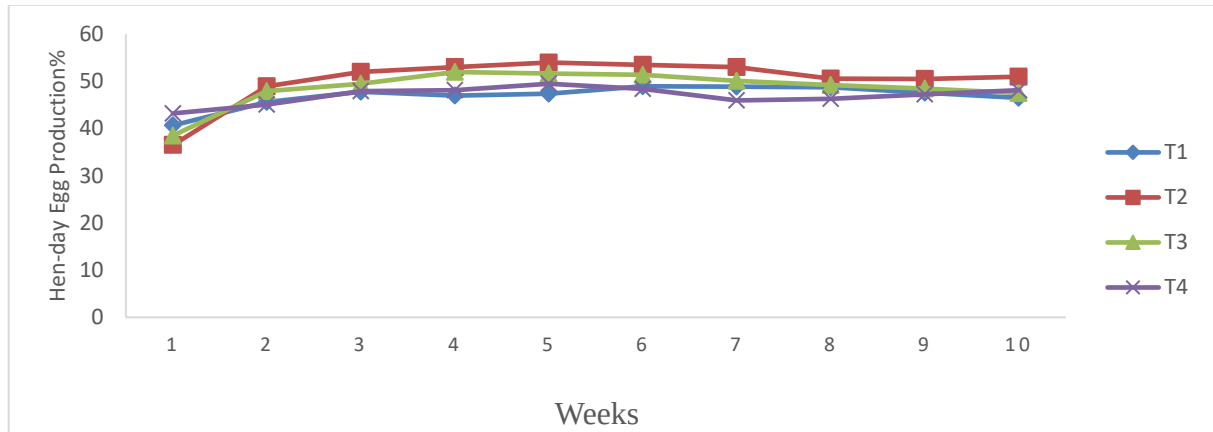


Figure 1. Weekly HDEP% of White Leghorn offered onion and rosemary extract in drinking water.

3.2 Egg weight and eggshell quality

The highest numerical value of egg weight was observed in T₂, while T₁, T₃, and T₄ showed the same statistical results. This might be due to the effective feed conversion ratio and nutrient utilization of the bioactive components found in onions. Contradictory reports by (18) indicated that adding rosemary powder to the diet increased egg production and weight.

Including rosemary, onion, and their combined extracts in the drinking water of laying hens had no significant effect on shell thickness. This was agreed with the study of (19), who observed that supplementation of rosemary oil to laying hen

diets did not affect eggshell thickness. Moreover, the result agreed with the report of (20), who found non-significant effects of rosemary on shell thickness. However, a significant ($p < 0.05$) difference was noticed for shell weight; the highest value was obtained from T₂. This is due to the differences in calcium, sodium, potassium, iodine, silica, iron, phosphorus, and A, B, and C vitamins of onion, which increase the whole egg weight (21). The bioactive components of feed additives improved the feed conversion ratio and increased the whole egg weight, which resulted in an increment in shell weight.

3.3 Albumen quality and Haugh unit

Table 5. Effects of onion and rosemary extracts on egg quality of White Leghorn.

Parameters	T ₁	T ₂	T ₃	T ₄	SEM	SL
Egg weight(g)	53.17 ^{ab}	55.25 ^a	50.92 ^b	51.98 ^{ab}	1.18	*
Albumen weight(g)	30.91 ^a	31.06 ^a	27.36 ^b	28.57 ^b	0.56	**
Albumen height(mm)	6.51	7.13	6.93	6.95	0.30	NS
Albumen ratio (%)	58.00 ^a	56.67 ^{ab}	55.00 ^{ab}	54.33 ^b	1.03	*
Haugh unit	90.14	93.78	94.14	93.70	1.57	NS
Yolk weight(g)	18.33	19.33	18.33	17.43	0.63	NS
Yolk height(mm)	15.58	16.21	15.89	15.45	0.24	NS
Yolk ratio (%)	34.97	35.21	36.06	33.58	1.53	NS
Yolk diameter(cm)	4.08	4.05	4.07	4.08	0.03	NS
Yolk index (%)	38.00 ^b	40.00 ^a	39.00 ^{ab}	38.00 ^b	0.05	*
Yolk color(RSP)	2.22	2.50	2.44	2.78	0.25	NS
Shell thickness(μm)	0.28	0.30	0.28	0.28	0.75	NS
Shell weight (g)	4.64 ^{ab}	4.95 ^a	4.63 ^{ab}	4.43 ^b	0.13	*

^{a-b} Means within a row with different superscripts differ ($P < 0.05$); RSP=Roche Scale Point; SEM = standard error of mean; SL=Significant Level; T₁= No additives; T₂ = 4ml of onion extract added in one liter of water; T₃ = 4ml of Rosemary extract added in one liter of water; T₄ = 2ml of rosemary + 2ml of onion extract in one liter of water.

Using rosemary and onion extracts in drinking water increased albumen weight and ratio. This difference could be due to an increment in egg weight. However, there were no statistical differences in albumen height and Haugh unit

score (Table 4). The study revealed no statistical difference ($P > 0.05$) was observed in albumen height, a good indicator of freshly laid eggs used in the treatment. The result was agreed with (22), who reported the significant effects of

adding rosemary to the layers diet on the Haugh unit and albumen height.

3.4 Egg Yolk quality

Including rosemary and onion extracts in drinking water did not affect yolk diameter, yolk color, and yolk ratio, but a higher value of yolk index was recorded in treatment two (Table 4). The highest and lowest yolk index was recorded on layers fed with onion extracts (T2) and a blend of onion and rosemary extracts (T4), respectively. Correspondingly, the report of (23) noted that the yolk index was significantly ($P < 0.05$) increased by rosemary supplementation. The

significant difference could be associated with a vitelline membrane surrounding the yolk that supported the yolk to be firm, which was in line with the findings of (20). The result showed that the freshness of the eggs ranged from 0.28 to 0.38, and the yolk index was increased by onion powder supplementation due to the formation of compacted yolk (24). There were no significant ($P > 0.05$) differences in yolk weight, height, yolk ratio, or color. This is in line with the study of (25), who showed that supplementation of rosemary powder to laying hen diets did not affect the egg yolk quality such as yolk weight, yolk height, yolk diameter, or yolk color. On the contrary, (26) reported that onion in the poultry diet improved the yolk color of eggs.

Table 6. Egg yolk color records of Roche color scale

Treatments	1	2	3	4	5	6	Total
T1	8	4	3	3	0	0	18
T2	4	4	6	4	0	0	18
T3	5	4	4	5	0	0	18
T4	5	2	7	1	2	1	18
Total	22	14	20	13	2	1	72

T1 = control group, T2 = 4ml onion extract added to one liter of drinking water, T3 = 4ml of rosemary extract added to one liter of drinking water, and T4 = 2ml of onion + rosemary extract added to one liter of drinking water.

3.5 Egg Fertility, hatchability, Embryonic Development, and Chick Quality

The effects of rosemary, onion, and their combined extracts in drinking water as feed additives on egg fertility, hatchability, and embryonic mortality are shown in (Table 6). All treatments had no significant differences in egg fertility, hatchability, embryonic mortality, and chick quality. Although embryonic mortality was statistically non-significant ($P > 0.05$), a greater value was recorded in T4 (27).

Infusion of rosemary extract in drinking water for layer primarily used as medicinal value due to the bioactive compounds found in their chemical constituents. The

findings of (28) concluded that dietary protein contents affected egg fertility and hatchability. According to the report of (29), poor hatching results occur when nutritionally deficient feeds are used for layers. The study of (30) also stated that inadequacy of nutrients in the breeder diets resulted in poor hatchability of fertile eggs. The greater percentage for chick quality assessment made by visual score was T2, followed by T3, and the least was for T4 and T1 (31). It was observed that rosemary (*Rosmarinus officinalis* L.) leaf extract has quality attributes for chicken growth and weight gain. Day-old chicks with T3 and T4 weights scored higher than T1 and T2 (32). Thus, the numerical difference in chick weight recorded might be due to the difference in egg weight used for incubation (33).

Table 7. Effects of onion and rosemary extracts on egg fertility, hatchability, embryonic mortality, and chick quality of White Leghorn

Parameters	T ₁	T ₂	T ₃	T ₄	SEM	SL
Fertility (%)	81.5	82.83	80.33	80.00	1.27	NS
Hatchability on fertile egg bases (%)	80.83	80.5	81.13	80.86	0.51	NS
Early E. M (%)	11.12	6.10	8.69	13.77	4.08	NS
Late E. M (%)	3.51	3.58	3.55	3.52	0.75	NS
Pip E. M (%)	1.74	2.03	1.62	1.40	0.31	NS
Chick quality						
Chick weight(g)	31.00	30.47	32.14	31.24	1.23	NS
Chick length(cm)	15.31	14.83	15.68	15.22	0.38	NS
Chick visual score (%)	74.00	85.00	80.00	77.00	3.92	NS

T1= No additives; T2=4ml onion extract added to one liter of drinking water; T3= 4ml of rosemary extract added to one liter of drinking water; T4=2ml of rosemary+2ml of onion extract added to one liter of drinking water

4 Summary and Conclusion

Onion and rosemary are natural feed additives in locally available poultry feed. They can be blended as a powder in the ration and included in extracts in drinking water to enhance the performance of layers. The study was conducted on the performances of white leghorn layers and egg quality, fertility, and hatchability parameters at Haramaya University Poultry Farm. A total of 132 (120 layers and 12 cocks) with uniform age. Treatments were T1 (treatment containing no addition of an additive, T2 (treatment containing 4ml of onion extract, T3 (treatment containing 4ml of rosemary extract, and T4 (treatment containing 2ml of onion and 2ml of rosemary extracts).

The study revealed significant ($P<0.05$) differences in hen-day egg production, egg mass, albumen weight, albumen ratio, yolk index, and shell weight. Onion and rosemary extracts did not affect fertility, hatchability, embryonic mortality, and chick quality. However, the lowest numerical value of embryonic mortality of chicks was recorded in T₃ layers fed with rosemary extracts. Indeed, further detailed research is needed to assess the identification of active chemical compounds of onion and rosemary and their effects at higher proportions layers fed in drinking water.

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Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

Seyoum Bekele, the first author, generated the primary data, which was manipulated, organized, analyzed by the SAS computer, and interpreted. Meseret Girma (PhD, Associate Professor) designed the manuscript for publication, translated it, and corrected the English language format. Ewunetu Kebede (Assistant Professor) also designed the manuscript for publication, translated it, and corrected the English language format.

Data Availability Statement

Acknowledgment and brief recognition of the first author and correspondence authors and Haramaya University Data Center is needed from the first author and correspondences for reproducing this manuscript in whole or in part.

Ethical Considerations

The study was conducted on the topic entitled: Effects of Rosemary (*Rosmarinus officinalis L.*), Onion (*Allium cepa L.*), and their mixtures as Natural Feed Additive on Egg Quality, Hatchability, Embryonic Mortality, and Chick Quality of White Leghorn at Haramaya University Poultry farm research center, Ethiopia were following the international guiding principle for Biomedical Research Involving Animals listed under article 2012 of the International Council for Laboratory Animal Science (ICLAS). Therefore, the School of Animal and Range Sciences Animal Ethics Committee and Committee for Control and Supervision of Experiments on Animals in Ethiopia approved the experimental procedure dated 5 May 2021.

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