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**Research Article**

# Effects of Rosemary (*Rosmarinus officinalis L.*), Onion (*Allium cepa L.*) Extracts and their Mixtures on White Leghorn Layers Performance and Blood Profile

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**ABSTRACT**

**Introduction:** The consumer's requirements for the highest quality animal products should be achieved by the application of appropriate health-safe animal diets. The study was conducted to evaluate the effects of rosemary and onion extracts on performances, and some hematological and serum biochemical parameters of White Leghorn.

**Materials and methods**: One hundred twenty White Leghorn layers at 32 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 control group without any addition of feed additives (T1), basal diet supplemented with 4 milliliters of onion extract in water (T2), basal diet supplemented with 4 milliliters of rosemary extract in water (T3), and basal diet supplemented with 4 milliliters of onion and rosemary extract mixture in water (T4). The data on performance parameters such as feed intake, body weight change, body weight gain, feed conversion ratio, egg production, egg mass, and serum biochemistry and hematology were evaluated.

**Results:** The daily feed intake of chickens in T2 was significantly higher than the feed intake of chickens in T1, T3, and T4. There was no significant difference in packed cell volume percentage but there was a significant difference in total serum cholesterol in T1 compared to T4. Layers supplemented with onion extract had lower blood total cholesterol levels compared to other groups. Results revealed that the use of onion extract (T2) in drinking water recorded significantly higher daily feed intake, body weight change, and gain compared to other groups. Layers fed on onion blended with rosemary extract in drinking water recorded the highest feed conversion compared to T1, T2, and T3 treatments.

**Conclusion:** The use of rosemary, onion extracts, and their mixtures in drinking water significantly influenced most of the studied hematological and serum biochemical parameters of White Leghorn layers except packed cell volume and serum albumin.

## 1. Introduction

Consumer requirements for the highest quality animal products should be achieved by the application of appropriate health-safe animal nutrition systems[1]. For several decades, some feed additives such as antibiotics have been vastly used in poultry rations[2]. Due to the potential for bacterial resistance and antibiotic residues in animal products[3] and drug residue in the body of the birds nowadays, some attempts have been made to replace these additives with herbs such as onion, rosemary, cinnamon, and garlic. Thus, the use of antibiotics as a feed additive is no longer acceptable and it is prohibited in developed countries[4]. 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[5]. As a consequence, medicinal plants and their bioactive components are attaining importance in animal and poultry production as well as health care systems because of their broad beneficial effects in promoting growth and production, immune enhancement, and safeguarding health[6,7]. Natural medicinal products originate

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from herbs and spices which are used as feed additives for poultry[8]. These plant-derived products have proven to be natural, less toxic, and residue-free, and are thought to be ideal feed additives in animal feed production[9].

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[10]. Phenol compounds in 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[7,11]. The anti-oxidative activity of rosemary is due to Phenolic terpenes, rosmanol, and rosmarinic acid which lead to an improvement in the oxidative stability of poultry meat. The onion (*Allium cepa L.*) is well known for its very effective in the prevention and treatment of diseases by antioxidant antihypertensive, antithrombotic, antibiotic, and anti-carcinogenic effects with its variable biochemical functions[12]. It contains Sulfur-containing compounds, which are sources of methionine, cysteine, and amino acids and have effects in lowering the level of cholesterol in blood plasma or serum and an important for the growth of the birds[13]. However, in Ethiopia, there is limited research conducted to determine the combined effects of rosemary leaf and onion extract mixture on the productive performance of layers. Therefore, this study was conducted to evaluate the effects of rosemary, onion, and their extract mixtures with drinking water on White Leghorn layers' performance and some blood profiles.

## 2. Materials and Methods

### *2.1. Ethical approval*

The study was conducted at Haramaya University Poultry Farm Research Center, Ethiopia and were following the international guiding principle for Biomedical Research Involving Animals listed under Article 2012 of the International Council for Laboratory Animal Science (ICLAS).

### *2.2. Description of the study site*

The study was conducted at Haramaya University poultry farm, which is located 525 km from Addis Ababa, Ethiopia. The site is situated at an altitude of 1980 millimeters above sea level, 9 $^0$ 26 'N latitude, and 42 $^0$ 3' E longitude. The area has an average annual rainfall of 741.6 millimeters. The mean annual minimum and maximum temperatures are 8.25 $^0$ C and 23.4 $^0$ C, respectively.

### *2.3. Preparation of experimental feed additives*

The measured amount of rosemary (1.4kg) and onion (4kg) were purchased from the Harar market. The onion bulbs were cleaned, the peeled and the root were removed, then cut into small pieces and spread on a plastic sheet for two days and make it easier to grind following the method finally grated with a mixer and put in a plastic container at room temperature[14]. The rosemary leaf was purchased from the local market, (Harar city, 25 kilometers away Haramaya University, Ethiopia) cleaned and separated from steam, and grated by a grinder to make powder following the methods[15]. 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 leveled amount of extract was divided into experimental pens (replication) for daily use[16].

### *2.4. Feed ingredients and chemical analysis*

The proportion of feed ingredients used in ration formulation was maize grain (50%), wheat short (15%), noug seed cake (15%), soybean meal (9.8%), L-lysine HCL (0.1%), Layer vitamin premix (1%), Dl-methionine (0.1%), Di-calcium phosphate (1%), salt (1%), and limestone (7%). The ration was formulated to 16.5 % crude protein and 2936.5 ME (kcal/kg) which meet the nutrient requirements of layers recommended to be iso-caloric and iso-nitrogenous with 2800-2900 kcal ME/kg DM and 16-17% Crude Protein (National Research Council[17].

### *2.5. Experimental chickens and management*

A total of 132 White Leghorns (120 layers and 12 cocks) at thirty-two weeks of age were randomly taken from Haramaya University Poultry farm and acclimatized to experimental ration for 7 days. Then, the layers were managed for 70 days. The experimental 2m by 2m wide pens, watering, feeding troughs, and laying nests were carefully cleaned and disinfected. The experimental diet and water have been provided in a group on *ad libitum* throughout the experimental period. The chickens were randomly allocated to four treatments each with three replicates. Each replication consists of 10 layers and 1 cock in a completely randomized design (CRD). All chickens in different treatment groups were fed on the same layer ration. Onion, rosemary, and their extract mixtures were allocated to treatments T1 (control group without any addition of feed additives), T2 (4 milliliters of onion extract in drinking water), T3 (4 milliliters of rosemary extract in drinking water), and T4 (2 milliliters of onion and 2 milliliters of rosemary extract in drinking water) based on previous research recommendation.

### *2.6. Data collection and measurement*

Average feed intake was calculated from daily feed intake. Feed consumed was determined as the difference between the feed offered and refusal for each replication and feed intake was then calculated from feed consumed divided by the total number of layers in the replication based on method[18]. All layers were individually weighed at the beginning and end of the experiment by sensitive balance and then the average body weight was taken for each treatment. Bodyweight (BW) change was determined

as the difference between the final and initial body weight. BW gain or loss was calculated as BW change divided by the number of experimental days[19]. All daily collected eggs from each replication were immediately weighed and the average was taken at the end for each treatment. All eggs laid every day throughout the study duration were individually weighed for each replication and the average was taken at the end for each treatment. A total of 418, 428, 419, and 415 eggs were used respectively from T1, T2, T3, and T4. Then, the average egg weight was used for the calculation of egg mass on a daily basis by multiplying the average egg weight by the percentage of hen-day egg production[20]. The feed conversion ratio was determined by calculating the weight of feed consumed and egg mass following the method of Zhenhua et al.[21]. The rate of lay for each treatment was expressed as the average percentage of hen-day egg production (HDEP %). At the end of the experiment, 5ml of blood samples (2.5ml from the right and 2.5ml from a left-wing vein) were collected from selected two layers per replication or six layers per treatment. The hematological parameters (Hemoglobin, red blood cell, white blood cell counts, and packed cell volume) were given to the hematology analyzer. About 20µl of blood mixed with diluents was analyzed by HUMACOUNT analyzer (Britain) using an electrical impedance procedure. Blood cell (WBC and RBC) was counted using an automatic blood analyzer as it produces resistance to electric signals when it passes through the solution[22]. The packed cell volume (PCV) was centrifuged at 3000 rpm for 5 minutes by microhematocrit capillary tubes. Hemoglobin concentration was determined by using acid hematin or Sahli's methods[23]. Blood collected by plain tube was used to determine the serum biochemistry (total cholesterol, HDL, LDL, total protein, albumin, and globulin) by HUMALYZER using the spectrometry procedure[24]. The serum was mixed with each respective reagent incubated and brought to the analyzer (Britain). The analyzer measures the analytes as the chemical reaction is produced and observance is detected. The globulin value was determined by the difference between serum total protein and albumin[24].

### *2.7. Statistical analysis*

All data collected were analyzed using SAS software version 9.4. The significant differences among treatment means were located by the least significant difference. The significance was declared when $p < 0.05$. The following statistical model was used for data analysis. $Y_{ij} = \mu + t_i + e_{ij}$; Where *Yij was* the response variable, $\mu$ was the overall population means, $t_i$ was the treatment effect (rosemary, onion, and their mixtures), $E_{ij}$ was an experimental error.

## 3. Results and Discussion

### *3.1. Production performance of white leghorn layers*

### *3.2. Feed intake*

The effects of onion, rosemary, and their combination extract infusion offered in drinking water on production performances are shown in Table 1. During the entire experimental period, there was no record of experimental extract water left over which means all amounts of the extract were used by the chickens. The feed intake was significantly higher in T2 compared to T3 and T4 the result of the inclusion of onion powder increased the feed consumption of layers ($P< 0.05$). This was due to the bioactive S-compounds of onion such as cysteine, methionine, and amino acids which might enhance feed efficiency and nutrient utilization. The increased feed intake of layers by using onion extract in drinking water was also reported[25-27]. However, T1, T3, and T4 were statistically similar. The lower feed intake in T3 and T4 might be due to the bitter test produced by rosemary extract which reduces the feed consumption. This is recognized that the effect of the onion extract might help in maintaining the beneficial gut microorganisms and improve nutrient absorption[28], who reported the effects of onion extract on egg laying performance due to some of its compounds such as phenols, polyphenols, terpenoid, polypeptides, lectin, alkalis, and essential oils that stimulate digestion and promote growth. It is used to stimulate the synthesis of bile acid and pancreatic enzyme activity, mainly lipase, and amylase, ultimately improving fat digestion. The aromatic oils present in onion facilitate and enhance the digestion process on layers. The study reported that onion administration enhanced villus height and crypt depth and decreased epithelial thickness and goblet cell numbers in the duodenum, jejunum, and ileum of birds[29].

### *3.3. Bodyweight change and gain*

This study revealed that the inclusion of feed additives in drinking water may boost the body weight change and gain which recorded T2 and T3 2 ml each respectively than in T1 and T4 ($p < 0.05$). This could be associated with efficient nutrient utilization because of the biological activities of Phenolic compounds found in onion and rosemary extracts[30,31].and also reported higher body weight gain for layers consumed onion extract. The study of Aji et al.[32] also noted higher body weight gain in chicks supplemented with 50 and 100 mg of garlic and onion powder than in the control group. In addition, onion can reduce blood glucose stimulating the nervous system for higher feed intake, which can lead to increased weight gain. The metabolic process comprises acts on excess abdominal fat distribution, abnormal insulin and glucose metabolism, disturbed blood lipids, pro-inflammatory state, and hypertension[32].

The use of onion in diet can reduce blood glucose and hypoglycemia and can stimulate the nervous system for higher feed intake. The lower BW gain was recorded in T4. This might be due to the bitter test produced by the combination of the onion and rosemary extracts resulting in decreased feed consumption.

### *3.4. Feed conversion ratio, egg production, and egg mass*

Layers of drunken water (T4) contained extracts of

**Table 1.** Effects of onion and rosemary extracts on the performance of White Leghorn managed for 90 days

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SD | SL |
|---|---|---|---|---|---|---|
| Feed Intake (g/bird/day) | 94.65$^{ab}$ | 97.59$^{a}$ | 94.14$^{b}$ | 93.39$^{b}$ | 0.72 | ** |
| Initial BW (g/bird) | 1209.30 | 1178.30 | 1205.00 | 1198.30 | 12.23 | NS |
| Final BW(g/bird) | 1292.70 | 1271.70 | 1296.70 | 1280.00 | 11.11 | NS |
| BW Change (g/bird) | 83.40$^{bc}$ | 93.40$^{a}$ | 91.7$^{ab}$ | 81.70$^{c}$ | 5.07 | * |
| BW gain(g/bird) | 1.30$^{bc}$ | 1.46$^{a}$ | 1.43$^{ab}$ | 1.27$^{c}$ | 0.04 | * |
| Total egg/hen | 41.83$^{ab}$ | 42.83$^{a}$ | 41.96$^{b}$ | 41.50$^{b}$ | 0.41 | * |
| HDEP (%) | 50.63$^{ab}$ | 52.49$^{a}$ | 51.21$^{ab}$ | 49.21$^{b}$ | 0.65 | * |
| Egg mass (g/hen/day) | 29.79$^{b}$ | 35.44$^{a}$ | 28.63$^{b}$ | 21.00$^{b}$ | 0.43 | * |
| FCR (g feed/g egg) | 3.19$^{b}$ | 2.75$^{b}$ | 3.26$^{b}$ | 4.41$^{a}$ | 0.00 | * |

$^{a,b,c}$ Means within a row with different superscripts are significantly different, *: Significant at ($p < 0.05$), **: Significant at ($p < 0.01$), SL: Significant level, SD: Standard Deviation, g: Gram, BW: Body weight, HDEP: Hen day egg production, FCR: Feed conversion ratio, EM: egg mass, T1: Control group( No additive), 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 extracts mixture in one liter of water.

onion and rosemary mixtures rerecorded the highest feed conversion ratio (FCR) than T2 and T3 (Table 1). The smallest and the best FCR was observed in T2. This is an indication of the better production performance of white leghorn layers[33]. Alicen is the bioactive found in onion that improves and regenerates the physiological structure of the intestinal epithelium layer and enhances crypt depth and villus height, which ultimately supports its digestive capacity through increased absorption of nutrients and assimilation. The additives may act in different mechanisms, affecting feed intake and conversion, stimulating the secretion of digestive enzymes and gastrointestinal motility, as well as immune and endocrine functions in addition to their antioxidant, antimicrobial, antiviral, anti-inflammatory, ant-helminthes, and coccidiostats activities[28]. These compounds increased feed efficiency by decreasing the harmful microbial population in the gut and improving healthy levels[34]. The variation in feed conversion ratio in this study was mainly due to egg production increment as a result of onion and rosemary administration in drinking water. This idea is in line with the report of Mehmet et al.[34], who showed variation in feed conversion efficiency because the feed conversion ratios highly dependent on the number of eggs produced, feed consumption, and egg weight in layer hens.

Total egg number (EN) and egg mass (EM) were significantly higher with onion and rosemary extract administration (T2) and (T3) when compared with control (T1) and the mixture of the two spices (T4, $p < 0.05$). This is consistent with the Belenli et al.[35], who noted the effects of polyherbal medicinal plants on layers' egg production performance. The highest hen-day egg production (HDEP) was recorded in T2 whereas the lowest was in T1 and T4 (Table 1). The blended use of the two spice extracts has resulted in lower HDEP than the separate effects. The improved HDEP was obtained due to the biological activities of Phenolic compounds found in onion and rosemary, which cause feed utilization improvement or feed efficiency. This is in line with the result of Betul et al.[36], who found improved HDEP because of the addition of rosemary to layers diet. The improvement in egg production with photogenic additives may be due to the provision of certain compounds that improve digestion and absorption of nutrients in the digestive tract. The extracts of medicinal plants increased the secretion of digestive enzymes, so enhanced nutrient digestibility, and improved the egg production performance of layers[37]. This finding indicated that the inclusion of onion and rosemary extract in the drinking water of layers could give the best overall production performance of layers.

### *3.5. Serum biochemistry and hematology*

#### *3.5.1 Serum biochemistry analysis*

The blood serum biochemistry is indicated in Table 2. There was a significant difference in total blood protein concentration in which the highest value was recorded in

**Table 2.** Effects of onion, rosemary, and their extract mixtures on layers of blood profile

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SD | SL |
|---|---|---|---|---|---|---|
| Serum biochemistry | | | | | | |
| Total cholesterol (mg/dl) | 164.80$^{a}$ | 159.98$^{ab}$ | 160.50$^{ab}$ | 158.98$^{b}$ | 1.07 | * |
| HDL-C(mg/dl) | 94.87$^{ab}$ | 93.28$^{ab}$ | 90.82$^{b}$ | 98.22$^{a}$ | 1.76 | * |
| LDL-C (mg/dl) | 46.43$^{ab}$ | 43.42$^{ab}$ | 41.97$^{b}$ | 48.75$^{a}$ | 1.75 | * |
| TP (g/dl) | 4.13$^{b}$ | 4.56$^{a}$ | 4.50$^{a}$ | 4.16$^{ab}$ | 0.06 | * |
| Albumin (g/dl) | 2.73 | 2.90 | 2.93 | 2.85 | 0.09 | NS |
| Globulin (g/dl) | 1.40$^{b}$ | 1.67$^{a}$ | 1.60$^{a}$ | 1.31$^{b}$ | 0.06 | * |
| Hematology | | | | | | |
| RBC ($10^6$/µl) | 2.65$^{a}$ | 2.57$^{a}$ | 2.51$^{ab}$ | 2.34$^{b}$ | 0.07 | ** |
| WBC ($10^4$ /µl) | 1.85$^{a}$ | 1.65$^{ab}$ | 1.87$^{a}$ | 1.45$^{b}$ | 0.16 | * |
| Hb (g/dl) | 10.22$^{b}$ | 10.62$^{a}$ | 10.28$^{b}$ | 10.16$^{b}$ | 1.69 | * |
| PCV (%) | 33.17 | 33.17 | 33.00 | 31.67 | 0.66 | NS |

$^{a,b}$Means within a row with different superscripts are significantly different; HDL-C: High-Density Lipoprotein cholesterol, LDL-C: Low-Density Lipoprotein Cholesterol;*: Significant at $P < 0.05$; *: Significant at $P < 0.01$; NS: Non-significant. TP: Total protein, RBC: Red Blood Cells, Hb: Hemoglobin, PCV: Packed Cells Volume, WBC: White Blood Cells, TP: Total Protein, SD: Slandered deviation, SL: Significance level, T1: Control group (No additive), T2: 4 ml of onion extract added in one liter of water, T3: 4 ml of rosemary extract added in one liter of water, T4: 2 ml of rosemary and 2 ml of onion extracts mixture in one liter of water.

T2 and T3 ($p < 0.05$). The data on serum albumin content showed no statistical difference ($p < 0.05$). The inclusion of onion, rosemary, and their extract mixtures offered through drinking water had a significant effect on serum total cholesterol, HDL-cholesterol, LDL-cholesterol, and total protein (TP) of the laying hens. The inclusion of onion, rosemary, and the combined extracts in drinking water was found to cause decreased values of total cholesterol levels, and the lowest value was recorded in T4 as compared to T1, T2, and T3 ($p > 0.05$). This might be due to the synergetic effect of the combination of onion and rosemary extracts. HDL-cholesterol and LDL-cholesterol concentrations were shown significantly lowest values in T3. The reduced content of total cholesterol and LDL may reflect the hypercholesterolemia properties attributed to the defatted part of the leaves which are rich in fibrous content and may block intestinal cholesterol absorption. The study by Ayorind et al.[38] consumed rosemary extract showed a decrease in serum cholesterol, LDL-cholesterol, and lipid concentration levels and increased triglyceride levels. Moreover, Omer et al.[39] concluded that rosemary extracts numerically decrease the serum cholesterol level and serum triglycerides, cholesterol, and HDL and LDL cholesterol[40]. Moreover, some scholars noted reduced serum triglycerides, total cholesterol, and LDL-cholesterol concentrations in layers fed on diets enriched in rosemary powder[41]. The increased level of HDL-cholesterol was reported by Hashemipour et al.[42]. This could be associated with the rosemary oil that might be producing hypoglycemic activity by a mechanism of independent insulin secretion resulting in the inhibition of endogenous glucose production or the inhibition of intestinal glucose absorption which reduces LDL cholesterol accumulation[43]. Furthermore, some scholars noted reduced serum triglycerides, total cholesterol, and LDL-cholesterol concentrations in layers fed on diets enriched in onion powder[44]. This could be related to the sulfur-containing of onion which oxidizes thiol compounds either present free or combined with a protein which is necessary for lipid synthesis[44].

### *3.5.2 Hematological analysis*

The blood hematological analysis is indicated in Table 2. There were significant ($p < 0.05$) differences in RBC, WBC, and Hb concentration but the PCV percentage was not impacted by the use of the spices ($p < 0.05$). The probable reason for the significant result could be the effects of bioactive compounds of onion and rosemary on hematological and serum biochemical effects. The bioactive sulfur organic compounds including S-Methyl cysteine sulfide and S-allyl cysteine sulfide found in onion can affect the concentration of blood lipid, protein, and glucose by acting as antioxidant activity. Enoka et al.[45] noticed that alliums enhanced the relative weight of the spleen and thymus were increased by garlic and onion supplementation, which was recognized to increase lymphocyte proliferation and lead to increased WBC counts. The lowest results of RBC and WBC are shown in T4. On the contrary, Granstad et al.[46] reported that medicinal plants and their components could activate immune functions such as lymphocyte proliferation, phagocytosis, RBC, and Hb and WBC. The Hb was significantly highest in T2. This could be due to the Phenolic compounds found in onion and rosemary as good sources of iron which were the components of Hb and determine the oxygen-carrying capacity of red blood cells. This result is in line with the findings of Mehmet et al.[34], who reported that a feeding diet with rosemary leaves significantly increased Hb, RBC, WBC, lymphocytes, and monocytes compared to the control group. Similarly, Malematja et al.[47] reported that supplementation of onion in poultry diets was used as an iron source which was an important component of Hb and used to carry and transport oxygen in RBCs.

## 5. Conclusion

The use of onion, rosemary, and their extract mixtures in drinking water significantly influenced feed intake and conversion ratio, body weight gain, egg mass and production, and the most of blood profiles in layers. Generally, it is concluded that the inclusion of 4 ml of onion extract in drinking water is recommendable based on the overall performance of the White Leghorn layer whereas the use of rosemary is the best cause of lowering the LDL-cholesterol concentration compared to individual onion extract and the blended extracts. Indeed, further detailed research is needed to assess the identification of active chemical compounds in onion and rosemary as well as their effects at higher proportions in drinking water on the performance of layers.

## Declarations

### *Competing interest*

The authors declared that there are no competing interests.

### *Authors' contribution*

Seyoum Bekele first author, generates the primary data, manipulates, organizes, and analyzes by an SAS computer and interprets. Meseret Girma (PhD, Associate Professor) designed the manuscript for publication format, translated, and corrected the English language format. Ewunetu Kebede (Assistant Professor) designed the manuscript for publication format, translated, and corrected the English language format. All authors read and approved the final edition of the article.

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### *Availability of data and materials*

Requests for permission for extended quotation from or

### *Ethical considerations*

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the author.

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