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# Effect of Herbal Supplement on the Laying Performance and Antioxidant Status of Serum and Egg Yolk of Laying Birds

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors OTD and AOJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ODO and SOA managed the analyses of the study. Author OTD managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The experiment was carried out to investigate the effect of herbal supplement on laying performance and antioxidant status of serum and egg yolk of laying birds.
**Study Design:** The experiment employed a completely randomised design, all data generated were subjected to analysis of variance, P =0.05.
**Methodology:** A total of ninety-six (26-week old laying birds) of Isa-brown laying birds were used in a 120-day feeding trial. The birds were assigned to 4 dietary treatments replicated eight times with 3 hens per replicate and were fed the control diet and diets supplemented with 5 g/kg garlic powder, 5 g/kg moringa leafmeal and 5 g/kg ginger powder as diets 2,3,4, respectively. The birds were fed with experimental diets for the 120-day period during which data were obtained on laying performance, internal and external egg qualities and antioxidant status of serum and egg yolk.

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**Results:** There was no significant difference in hen day production, feed intake in birds fed control diet and diets supplemented with herbs. Final live-weight and weight change decreased ($p<0.05$) with dietary herbal supplementation. Supplementation of diets with herbs resulted in a significant increase in egg weight, eggshell weight, eggshell thickness, eggshell surface area compared to the control diet. The yolk weight and albumen weight were significantly ($p<0.05$) influenced by the dietary herbal supplement. Serum superoxide dismutase (SOD) activity increased in the dietary herbal supplement and was maximised (288 $\mu ml^{-1}$) at diet 3 likewise, the egg yolk superoxide dismutase (SOD) activity increased ($p<0.05$) in the dietary herbal supplement and was maximised (9.21 $\mu ml^{-1}$) at diet 3.
**Conclusion:** Diet supplemented with 5 g/kg moringa leafmeal gave optimum performance, 5g/kg moringa leafmeal may be used as feed additive to improve performance, egg qualities characteristics and antioxidant status of serum and egg yolk of laying birds.

*Keywords: Laying birds; egg qualities; garlic; moringa and ginger.*

## 1. INTRODUCTION

There is an increasing trend towards the use of alternative or complementary medicines for general health maintenance, immunomodulation and therapeutic purposes for treating various diseases and disorders due to the emergence of drug resistant micro-organisms, side- effects of antimicrobials and the harmful residual toxicity effects of drugs observed in food chain. Particularly the utilisation of the plants' herbs, fruits and vegetables are becoming popular due to their low toxicity, fewer side effects and other beneficial advantages for safeguarding the health of humans and their companions animals including poultry [1,2,3].

Herbs, roots and ethno-veterinary medicines, possessing therapeutic effects have been used from time immemorial for strengthening the body its immune system and to prevent or fight against diseases [4,5]. People around the world are now aware of the limitations of synthetic drugs and chemicals regarding higher cost, toxicity and adverse effects [6]. Nowadays, there has been an increase in demand for natural antioxidants in food due to its health benefits against oxidative stress and several diseases. Plant-derived antioxidants are gaining more demand for poultry nutrition because their meat has a high content of polyunsaturated fatty acids and susceptible to lipid oxidative [7]. Many plants have been identified as excellent poultry antioxidants, important among which are rosemary, moringa, olive leaves e.t.c. [8,9]. Studies showed that active ingredients of some plants have strong antioxidative effects including neutralisation of superoxide, hydrogen peroxide and nitric oxide either by scavenging for radicals or by increasing the production of superoxide dismutase (SOD) and glutathione peroxidase (GPx) [10,11]. Ginger contains some essential metabolites and alkaloids like gingerol, shogaol, gingerdione and other phenolics compounds which have antioxidativ e properties [12,13]. The phytochemicals (flavonoid) such as quercetin and kaempterol were identified as the most potent antioxidant in moringa leaves. Their antioxidative activity was higher than the conventional antioxidants such as ascorbic acid which is also present in large quantity in moringa leaves [14]. Hence this study was carried out to investigate the effect of herbal supplement on laying performance, egg quality characteristics and antioxidant status of serum and egg yolk of laying birds.

## 2. MATERIALS AND METHODS

The experiment was conducted at the poultry unit of Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti. The experiment was carried out using a set of 96 (ninety-six) Isa brown pullets purchased at 16 weeks of age and reared for 8 weeks prior to data collection in a two-tier cage with a cell dimension of 38×40cm. Three birds were enclosed in each cell of the cage in an open-sided house in the Teaching and Research farm. Four experimental diets were formulated to be iso-nitrogenous and iso-caloric to provide 16.05% crude protein and 2518kcal/kg metabolizable energy [15]. The diets were adequately furnished with minerals and vitamins. Ninety-six laying pullets aged 26 weeks (4weeks in -lay) were randomly placed into 4 experimental diets each replicated eight times with three birds per replicate. The birds had an average initial weight of 1.41±0.01 kg and were fed experimental diets and water *ad-libitum*.

**Experimental diets:** Four experimental diets used for this study were compounded in the

premises of the Teaching and Research farm, Federal Polytechnic, Ado-Ekiti.

Diet 1= control diet (diet without supplementation).

Diet 2= garlic layermash containing 5 g/kg garlic powder.

Diet 3=moringa layermash containing 5 g/kg moringa leaf meal.

Diet 4= ginger layer mash containing 5 g/kg ginger powder.

**Garlic preparation:** Fresh garlic bulbs were obtained from a local market and peeled. The peeled garlic bulbs was homogenised with an equivalent weight in a food blender for 1 minute. This was mixed thoroughly into the already prepared layer mash.

**Ginger preparation:** Fresh matured ginger roots were obtained from a local market. They were washed, sliced and sun dried. The dried ginger was subsequently milled with grinding machine and later mixed with diet.

**Moringa Preparation:** The test ingredients *Moringa oleifera* leaf meal was prepared by harvesting daily the fresh and matured leaves. Air drying was done for 4 days, and the leaves were manually turned using a rake to guarantee even drying.

### 2.1 Data Collection

The birds were weighed at the beginning and at end of the experimental period to obtain the change in body weight during the experiment. The procedure was adopted to prevent stress. Feed intake was taken by subtracting the weight (g) of the leftover feed from the weight (g) of the feed initially offered. Feed efficiency was recorded as the gram of feed consumed per gram of egg produced.

**Table 1. Composition of experimental diets (g/100 g) for laying birds**

| Ingredients | Diets | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | Control diet | Garlic - | Moringa | Ginger |
| Maize | 50.00 | 50.00 | 50.00 | 50.00 |
| Wheat offal | 18.00 | 18.00 | 18.00 | 18.00 |
| Soyabean meal | 21.00 | 21.00 | 21.00 | 21.00 |
| Bone meal | 3.00 | 3.00 | 3.00 | 3.00 |
| Oyster shell | 7.00 | 7.00 | 7.00 | 7.00 |
| Layers premix | 0.20 | 0.20 | 0.20 | 0.20 |
| Methionine | 0.30 | 0.30 | 0.30 | 0.30 |
| Lysine | 0.20 | 0.20 | 0.20 | 0.20 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 |
| Garlic | - | 0.005 | - | - |
| Moringa | - | - | 0.005 | - |
| Ginger | - | - | - | 0.005 |
| Total Calculated composition | 100.00 | 100.00 | 100.00 | 100.00 |
| Crude protein (%) | 16.05 | 16.05 | 16.05 | 16.05 |
| Crude fibre (%) | 3.89 | 3.89 | 3.89 | 3.89 |
| Metabolisable energy | 2518 | 2518 | 2518 | 2518 |
| Lysine | 1.02 | 1.02 | 1.02 | 1.02 |
| Methionine | 0.56 | 0.56 | 0.56 | 0.56 |
| Calcium | 3.72 | 3.72 | 3.72 | 3.72 |
| Phosphorus | 0.82 | 0.82 | 0.82 | 0.82 |

*Vitamins 12,000,000, vit. E 30,000 mg, vit. K 2,500 mg, folic acid 1,000 mg, niacin 40,000, folic acid 1,000 mg, vit B 5000 mg, vit $B_{12}$ 20 mg, vit $B_6$ 3,500 mg, biotin 80mg and antioxidant 125,000 mg, cobalt 250 mg, Selenium 250 mg, iodine 1,200 mg, iron 40,000 mg, manganese 70,000 mg, copper 8,000 mg, zinc 60,000 mg, chloride, 200,000 mg*

During the last week of each month, two eggs from each replicate were collected for determination of egg external and internal qualities. The egg length and diameter were determined by measuring the egg length and diameter with vernier caliper The percentage hen-day production was computed as follows:

$$\frac{Total\ number\ of\ egg}{Total\ number\ of\ days\ \times\ no\ of\ hens} \times 100$$

Eggshell surface Area (ESSA) was calculated according to the formula using [16] as $EW^{0.667}$×4.67 where EW is egg weight. The egg shape index was calculated as a ratio of egg diameter to egg length [17]. The shell egg weight (SEW) was expressed as the percentage of egg weight.

$$\text{Shell egg weight} = \frac{Shellweight}{Eggweight} \times 100$$

The two egg membranes were pulled off the shells immediately and the shells so peeled were air-dried after which the eggshell thickness was determined with a micrometer screw gauge.

The egg was carefully broken on a glass plate (35×25 cm) to measure internal egg quality characteristics. Yolks were separated from albumen. Eggshells were cleaned of any adhering albumen. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight. The yolk index as the average yolk height divided by yolk diameter (mm) following removal of the yolk from the albumen. Yolk height was measured by means of tripod micrometer reading to the nearest 0.01mm, while a vernier caliper measured yolk diameter to the nearest 0.05 mm. The Haugh unit was calculated as Haugh units (%) = 100 ×log ($H+7.57-1.7W^{0.37}$) where H is the height of the albumen and W is the weight of the egg [18].

For antioxidant assays, serum samples 5 ml were taken from the wing vein of 3 birds per treatment on the last day of each month of the experiment using sterilised needles and non-heparinised tubes. The serum was assayed for enzymatic activity of superoxide dismutase (SOD) activity in the serum was determined through xanthine oxidase method. Samples were taken to detect absorbance at 550 nm with a spectrophotometer. μ/ml nitrate unit expressed the calculated results. Glutathione (GSH-PX) activity was measured by dithio-dinitrobenzoic acid method at the absorbance of 412 nm and malondialdehyde (MDA) concentrations by spectrophotometric methods using a spectrophotometer (Hitachi, Japan). SOD activity was measured by the xanthine oxidase method, which monitors the inhibition of reduction of nitro blue tetrazolium by the sample. The GSH concentration was analysed according to [19]. The MDA level was analysed with 2-thiobarbituric acid (TBA), monitoring the change of absorbance at 532nm with a spectrophotometer [20]. The egg yolk homogenate was assayed for contents of SOD, GSH and MDA subjected to the same assay as for serum samples. The activities of antioxidant enzymes and the contents of MDA were expressed as units per millimeter for serum and units per milligram for egg yolk.

## 2.2 Statistical Analysis

All data collected in this studies were subjected to analysis of variance method for a completely randomized design using SPSS package. Duncan's Multiple Range Test of one way Anova (SPSS 17.0 for windows, SPSS Inc. Chicago IL, USA) was used to analyse the mean difference of the same parameter. Significant differences were considered where necessary at 95% level.

## 3. RESULTS AND DISCUSSION

The effect of the dietary herbal supplement on the performance characteristics of laying birds during the experimental period is shown in Table 2. Feed intake, hen day production were not influenced ($p>0.05$) by the experimental diets. The final live-weight, feed efficiency and weight change of birds on diets 2,3 and 4 were similar ($p>0.05$) but significantly ($p<0.05$) lower than birds on control diet.

The experimental diets containing garlic, moringa and ginger were consumed without any palatability problems. The results of the current study agreed with the findings of [21] who reported that phytogenic feed additives supplementation to layers diet had no effect on feed intake compared with an unsupplemented diet. This result conforms with [22] who noted that phytogenic feed additives improved feed efficiency while feed intake was not statistically affected.

Medicinal plant supplementation to poultry diets can improve feed efficiency [23]. Herbal supplementation to layer diets reduced the final live weight of birds on garlic, moringa and ginger

which consequently affect weight change of the birds.

The effect of the dietary herbal supplement on external egg qualities parameters is illustrated in Table 3. Addition of dietary herbal supplement to laying birds diets resulted in a significant (P<0.05) increase in egg weight, eggshell weight, eggshell thickness and eggshell surface area. This study agreed with [24] who reported that supplementation of laying birds diet with herbal product improved egg weight, egg shell weight and egg shell thickness. Increased shell thickness by addition of moringa leaf meal may be attributed to some antioxidant properties like high nutritive values, richness of its leaves in minerals, vitamins and other essential phytochemicals [25]. Hitherto, information about garlic, moringa and ginger supplement in diets of laying birds concerning external egg qualities has not been presented in the literature.

The effects of dietary herbal supplementation on internal egg quality parameters are illustrated in Table 4. Addition of moringa leaf meal to laying bird diets resulted in a significant increase in yolk weight (p<0.05). However, the albumen weight of birds on diet 3 (moringa leafmeal) was significantly (p<0.05) higher than birds on other experimental diets. The Haugh unit, yolk index and albumen height were not significantly (p>0.05) influenced by the experimental diets though dietary herbal supplementation increased the values recorded for Haugh unit and yolk index, this study agreed with the report of [26] that there is a positive correlation between Haugh unit and quality of egg component (yolk and albumen). Egg albumen height and egg weight are indices for evaluation of Haugh unit. Increase in egg weight is related to increase in weight of albumen. Increase in egg weight due to increase in weight of albumen and yolk might be the main cause of improvement in Haugh unit in this study. The range of Haugh unit observed in this study was 75.11 in diet 4 to 76.69 in control diet. The higher the value of Haugh unit, the better the quality of eggs which are classified according to the United State Department of Agriculture (USDA) as AA (100 to 72), A (71 to 60) , B (59 to 30) and C (Below 30) (USDA, 2000) in which the refrigerated egg are classified as AA as [27].

**Table 2. Effect of dietary herbal supplement on performance characteristics of laying birds (26-42 week)**

| | Diets | | | | |
|---|---|---|---|---|---|
| **Parameters** | **1** | **2** | **3** | **4** | **±SEM** |
| | **Control diet** | **Garlic** | **Moringa** | **Ginger** | |
| Initial weight (kg) | 1.42 | 1.42 | 1.41 | 1.41 | 0.01 |
| Final liveweight (Kg) | $1.77^{a}$ | $1.72^{b}$ | $1.72^{b}$ | $1.73^{b}$ | 0.01 |
| Henday production (%) | 77.25 | 76.88 | 77.25 | 77.38 | 1.39 |
| Feed intake (g) | 113.63 | 114 | 113.75 | 113.75 | 0.45 |
| Feed efficiency | $2.04^{a}$ | $1.89^{b}$ | $1.87^{b}$ | $1.87^{b}$ | 0.04 |
| Weight change | $0.35^{a}$ | $0.30^{b}$ | $0.31^{b}$ | $0.31^{b}$ | 0.01 |

*Means with different superscript on the same row differ significantly (p<0.05); SEM: standard error of mean*

**Table 3. Effect of dietary herbal supplement on external egg quality of laying bird fed experimental diets (26-42 week)**

| | Diets | | | | |
|---|---|---|---|---|---|
| **Parameters** | **1** | **2** | **3** | **4** | **±SEM** |
| | **Control diet** | **Garlic** | **Moringa** | **Ginger** | |
| Egg weight (g) | $60.19^{b}$ | $62.09^{a}$ | $62.12^{a}$ | $62.25^{a}$ | 0.31 |
| Egg length (mm) | 57.84 | 58.61 | 58.76 | 57.95 | 0.23 |
| Egg diameter (mm) | 50.45 | 50.38 | 50.47 | 50.37 | 0.08 |
| Egg shell weight (g) | $6.43^{c}$ | $6.61^{b}$ | $6.93^{a}$ | $6.64^{b}$ | 0.16 |
| Egg shell thickness (mm) | $0.34^{b}$ | $0.36^{ab}$ | $0.38^{a}$ | $0.36^{ab}$ | 0.36 |
| Shell egg weight (%) | 11.18 | 11.16 | 11.41 | 11.34 | 0.14 |
| Egg shell surface area | $71.83^{b}$ | $73.29^{a}$ | $73.51^{a}$ | $73.40^{a}$ | 0.32 |
| Egg shape index | 0.86 | 0.86 | 0.86 | 0.87 | 0.02 |

*Means with different superscript on the same row differ significantly (p<0.05) SEM: standard error of mean*

**Table 4. Effect of dietary herbal supplement on internal egg quality of laying bird fed experimental diets (26-42 week)**

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| Parameters | Diets | | | | ±SEM |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| | Control diet | Garlic | Moringa | Ginger | |
| Haugh unit | 76.69 | 75.21 | 75.32 | 75.11 | 0.98 |
| Yolk weight (g) | 12.46$^{b}$ | 12.71$^{b}$ | 12.96$^{a}$ | 12.40$^{b}$ | 0.08 |
| Yolk index | 50.14 | 50.65 | 50.71 | 51.54 | 0.17 |
| Albumen weight (g) | 30.83$^{c}$ | 30.40$^{c}$ | 40.15$^{a}$ | 32.61$^{b}$ | 0.78 |
| Albumen height (mm) | 6.02 | 5.91 | 5.93 | 5.91 | 0.10 |

*Means with different superscript on the same row differ significantly (p<0.05) SEM: standard error of mean*

The antioxidant properties of herbs need special attention because undesirable oxidation produces changes in odour, colour, and other undesirable effects. The effect of herbal supplement on antioxidant parameters including serum activity of superoxide dismutase (SOD) and concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) of laying bird is illustrated in Table 5. Serum SOD activity was (P<0.05) increased in birds fed dietary herbal supplemented diets and reached a maximum (288μml$^{-1}$) with moringa leaf meal diet while the least activity was recorded for birds on control diet at a minimum (192μml$^{-1}$) . The GSH concentration of birds on moringa leafmeal (diet 3) was significantly (p<0.05) higher than GSH concentration of birds on control diet, garlic and ginger diets. The MDA concentration of birds on diet 3 (Moringa leafmeal) was significantly (p<0.05) lower than MDA concentration of birds on control diet, garlic and ginger supplementation. This result conformed with the report of [28] that the intake of herbs or their contents resulted in an increase in serum antioxidant enzyme activities such as SOD and GSH and a decrease in the MDA concentration. Elevated levels of antioxidant enzymes may improve the steady state of the system of poultry. The results of this study demonstrated that phytochemicals (flavonoids) such as quercertin and kaempterol were identified as the most potent antioxidants in moringa leaves. Their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid which is also present in large amounts in moringa leaves [29]. [30] and [25] reported that moringa leaves possess some antioxidative properties with high nutritive values, the leaves are rich in minerals, vitamins and other essential phytochemicals. The leaves have appreciable amounts of saponins and tannins. Although there are several enzyme systems within the body which scavenge free radicals, the natural (vitamins) antioxidant are vitamin E, beta-carotene and vitamin C [31]. These micronutrient antioxidants (SOD) may be used as defence system to prevent free radicals from damaging the animal's body thereby providing protection to animals against infections and degenerative diseases [30,32]. From this findings, supplementation with ginger powder increased the activities of SOD, GSH but decreased the MDA content in the serum of laying birds in this study is consistent with that of (12) who also observed increased activities of SOD and GSH and reduced MDA content in the serum of birds. The higher activities of SOD and GSH in groups supplemented with ginger powder (apart from Moringa) in this study indicated the higher capacity of laying birds to clear out the oxygen free radicals and the lower MDA content indicated a lower extent of lipid peroxidation. The enhanced antioxidant status by ginger powder supplementation is likely attributed to the antioxidant compounds such as gingerols, shogoals, gingerdiol, gingerdione and some relating phenolic ketone derivatives [33].

The result of egg yolk antioxidant enzymatic and concentration of malondialdehyde in the egg yolk of laying bird fed diets supplemented with herbs is illustrated in Table 6.

The SOD activities of birds on diets 3and 4 were similar (p>0.05) but significantly (p<0.05) higher than birds on the control diet and diet 2. However, the GSH activities of egg yolk were increased (p<0.05) by moringa and ginger powder supplementation. Information on the effect of the dietary herbal supplement on antioxidant status in egg yolk is lacking. However, the enhanced antioxidants status of egg yolk by moringa leaf meal and ginger powder supplementation is attributable to the antioxidant compounds in moringa leafmeal and ginger powder. The enhanced antioxidant status by ginger powder supplementation is probably attributed to the antioxidant compounds such as

**Table 5. Effect of dietary herbal supplement on superoxide dismutase (SOD) activity, glutathione (GSH) and malondialdehyde (MDA) concentrations in serum of laying birds**

| Parameters | Diets | | | | ±SEM |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| | Control diet | Garlic | Moringa | Ginger | |
| SOD (µ/ml) | 192.00[c] | 261.00[b] | 288.00[a] | 269.66[b] | 11.05 |
| GSH (µ/ml) | 29.53[d] | 39.82[c] | 45.49[a] | 42.38[b] | 2.27 |
| MDA (nmol/ml) | 14.51[a] | 14.70[a] | 12.56[b] | 14.23[a] | 0.25 |

*Means with different superscript on the same row differ significantly (p<0.05) SEM: standard error of mean*

**Table 6. Effect of dietary herbal supplement on superoxide dismutase (SOD) activity, glutathione (GSH) and malondialdehyde (MDA) concentrations in egg yolk of laying birds**

| Parameters | Diets | | | | ±SEM |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| | Control diet | Garlic | Moringa | Ginger | |
| SOD (µ/ml) | 6.79[c] | 7.64[b] | 9.21[a] | 9.16[a] | 0.26 |
| GSH (µ/ml) | 4.53[d] | 7.52[a] | 8.18[a] | 7.55[a] | 0.57 |
| MDA (nmol/ml) | 4.84 | 4.57 | 4.12 | 4.43 | 0.07 |

*Means with different superscript on the same row differ significantly (p<0.05) SEM: standard error of mean*

gingerols, shogaols, gingerdiol and gingerdione and some relating phenolic ketone derivatives [34,33].

## 4. CONCLUSION

The results of the present study reveal that *Moringa oleifera* can boost the immunity of laying birds and can be used as alternative growth promoters. Ginger has been demonstrated to have various biological activities such as antioxidant [12], and some pharmacological effects [10]. Information on the effect of garlic on antioxidant status in serum, egg yolk and other parameter is lacking.

## ETHICAL APPROVAL

As per international standard or polytechnic standard written ethical approval has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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