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Effects of Microorganism Supplementation on Egg Quality and Production

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ABSTRACT

Effective microorganism (EM) is a combination of more than 80 different types of beneficial microorganisms contributing to a wide range of applications in medicine, environment, and agriculture (livestock sector, crop sector, and forestry). This experiment was conducted to evaluate the effects of EM supplementation on hen day egg production and egg quality traits of Bovans Brown laying hens. At the age of 16 weeks, 144 pullets were purchased from small-scale poultry farms in Debre Markos. The chickens were divided into four treatments, each of which was replicated three times and contained 12 chickens. The treatment groups were T1 (control, commercial ration only), T2 (supplemented 16 ml EM per liter of drinking water), T3 (supplemented 5% Bokashi in feed), and T4 (supplemented 16 ml EM per liter of drinking water and 5% Bokashi in feed). Prior to collecting the actual data, the layer chickens had 2 weeks of adaptation. The hen day egg production was evaluated, and laboratory analysis was conducted to detect the external and internal egg quality. The results showed no significant difference among treatments on hen day egg productions, which ranged from 74 to 80 percent. Among the external egg quality traits, T4 had the highest egg weight, compared to other treatment groups. The T3 and T4 treatments had the highest shell weight. The shell thickness ranged from 0.37 to 0.39 mm. The shape index ranged from 76.81 to 79.11%, with no difference among the groups. Moreover, T4 had a significantly higher egg mass than T1 and T2. The specific gravity of an egg ranged from 1.06 to 1.08 g/cm^3. Among the internal egg quality traits, the albumin weights of T3 and T4 were significantly higher than those of T1. The highest and the lowest Hough units were observed in T4 and T1, respectively. The highest yolk weight was observed in T4 among the groups. The yolk index ranged from 0.45 to 0.49. The yolk color ranged from 5.27 to 7.33. Finally, overall egg quality parameters in T4 were better than in non-supplemented groups.

Keywords: Bovans Brown laying hen, Effective microorganism, Egg quality traits, Hen day egg production

INTRODUCTION

Ethiopia currently has an estimated 57 million chickens, the majority of which are laying hens (34.26%), followed by chicks (32.86%). According to breed, 78.85%, 12.02%, and 9.11% of the total poultry were reported to be native, hybrid, and exotic, respectively (CSA, 2020; 2021). Due to various limitations, poultry has a very small economic impact in Ethiopia. The cost and accessibility of ingredients for feed are the main limitations (Yitbarek, 2013). The low immune capacities of chickens and poor management techniques are responsible for the decreased performance of the bird as a whole (Arif et al., 2021). The production performance of native and exotic chicks is improved by the use of effective microorganisms (EMs), better feeding practices, and better health care (Atsbeha and Hailu, 2021).

The EM is a mixed cell culture made up of photosynthetic bacteria, actinomyces, yeast, *Lactobacillus*, and fungi (EMRO, 2010). The microorganisms used in EM are not genetically modified; rather, they are a combination of more than 80 different kinds of beneficial microorganisms that contribute to the broad range of applications. Pathogens cannot survive in EM because it is self-sterilizing, and the pH ranges from 3.4 to 3.7 (EMROSA, 2006).

In order to feed poultry without endangering the environment or the general public's health, EM is crucial

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(Ferri et al., 2017). In a previous study, it was found that including 2% EM in a layer's diet increased egg production, reduced feed consumption, improved feed conversion ratio, increased egg specific gravity, and increased Haugh Units (Atsbeha and Hailu, 2021). The use of EM supplementation has the potential to enhance the performance of layers; however, there is limited research on its application for laying hens in Ethiopia. Moreover, EM is readily available in significant quantities at a low cost in various regions of the country. Therefore, the purpose of this study was to evaluate the effects of EM supplementation on hen day egg production and egg quality traits of Bovans Brown laying hens.

MATERIALS AND METHODS

Ethical approval

The study was carried out in accordance with the "Act of the protection of animals used for scientific purposes [and feed legislation if appropriate] of the Federal Democratic Republic of Ethiopian, which compiles with EU legislation for scientific purposes [and feed legislation if appropriate]". The university's ethical review board gave its approval to all procedures.

Study area

This study was conducted in Debre-Markos, East Gojjam Zone, Amharra Region, Ethiopia. The geographical coordinates of the town are 10°21' latitude North and 37°43' longitude East. The town is about 16000 hectares in size and is 2420 meters above sea level. The mean annual rainfall and temperature is 1308 mm and 16°C, respectively.

Ration and experimental supplementations

In this experiment, the commercial ration was supplemented with 16 ml of activated EM solution (lactic acid bacteria, yeasts, photosynthetic bacteria, actinomyces, enzymatically active fungi) added to a liter of drinking water, Additionally, 5% Bokashi was incorporated into the feed. The stock EM was purchased from Woljeejii Agricultural Industry PLC (WAI), Debre Zeit, Ethiopia. The stocked EM contained lactic acid bacteria (*Lactobacillus* and *Pedicoccus*) at 1 x 10^5 CFU/ml suspension, yeast (*Sacharomyces*) at 2 x 10^6 CFU/ml suspension, photosynthetic bacteria (*Rhodopseudomonas palustrus* and *Rhodobacter spaeroides*) at 1 x 10^3 CFU/ml suspension, actinomycetes (*Streptomyces albus*) at 3 x 10^3 CFU/ml suspension, fermenting fungi (*Aspergillus oryzae*) at 1.1 x 10^5 CFU/ml suspension. To prepare 10 kg of feed mash, 100 ml of EM solution, 100 ml of molasses, and 1000 ml of water were necessary (APNAN, 1995). The steps for making Bakashi were by dissolving the recommended amount of molasses in the recommended amount of water to make molasses solution, adding the recommended EM to the molasses solution, and then spraying the EM and molasses solution mixture onto the recommended amount of feed while thoroughly mixing it. The mixed feed should then be sealed in an airtight polyethylene bag and left for 8 days to ferment and develop a sweet smell (Atsbeha and Hailu, 2021). The proximate analysis of the commercial ration contained 90% dry matter, 16.5% crude protein, 9% crude fiber, 5% crude fat, and 2800 kcal ME/kg DM. The ration was formulated to contain 3.75% calcium, 0.7% phosphorus, 0.75% lysine, 0.36% methionine, and 0.25% vitamin premix in addition to the main feed ingredients. The layers were fed this commercial ration throughout the experimental period.

Management of layers, design, and treatments

Before the commencement of the experiment, the experimental pens, waterers, feeders, and laying nests were thoroughly cleaned and disinfected with two teaspoons of magic disinfectant per 4.5 liters of water, and sprayed 0.6 ml Diazinol per liter of water again. A total of 144 Bovans Brown grower chickens at the age of 16 weeks with an average body weight of 1280 g were purchased from small-scale commercial poultry farms in Debre Markos town. The chickens were kept in a deep litter experimental house and managed in the intensive production system. The average temperature and humidity of the house were 20^{O}C and 60%, respectively. The house was partitioned into 12 pens (each pen 2.4 m^2 per 12 chickens) by wire mesh. The pens were covered with a litter of dry teff straw, reaching a depth of approximately 8 cm. In each pen, 12 grower chickens were randomly distributed. The chickens were adapted to experimental supplementations for 2 weeks before recording the actual data. The chicken was vaccinated with the recommended vaccines based on vaccine programs. The chicken was vaccinated Lasota vaccine with drinking water at 17 weeks of age against Newcastle disease. At 40 weeks of age, the layer was vaccinated with the infectious bronchitis virus (IBV) vaccine (V-IBV) with drinking water for infectious bronchitis. All health precautions and disease control measures were carefully followed throughout the experimental period. The fluorescent lamp was suspended on the ceiling of the experimental house to provide 14 hours of light daily for laying hens. The layers were fed a measured amount of feed in groups twice a day at 6:00 AM and 6:00 PM throughout the experimental period. A measured amount of clean tap water free from chlorine (expose the water for 24 hours to evaporate chlorine) was used for 336 days. The feed was offered on hanging feeders. The water was provided in plastic fountains, and the watering troughs were cleaned every morning.

A total of 144 layers were randomly distributed in 12 pens using a completely randomized design in four treatment groups and three replicates for each treatment. The treatment groups were control (commercial ration only, T1), supplemented 16 ml EM per liter of drinking water (T2), supplemented 5% Bokashi in feed (T3), and Supplemented 16 ml EM per liter of drinking water and 5% Bokashi in feed (T4).

Measurements

Feed intake

The feed offered and rejected for each replicate was recorded and multiplied by the respective DM content. The difference between the offered and rejected feed served as the basis for calculating the amount of feed consumed.

$$\text{Feed intake per layer} = \frac{\text{Feed offered- feed refusal}}{\text{Duration of experiment} \times \text{Number of layers}}$$

Body weight

In order to track changes in body weight, each layer's weight was recorded individually at the start of the experiment (initial body weight) and every two weeks till the end of the study period. By deducting the initial weight from the final weight and dividing it by the number of experimental days and layers. An average body weight gain was calculated for each replicate.

Egg production

At 9:00 AM, 11:00 AM, and 3:00 PM, eggs were recorded from each pen three times daily. The quantity of eggs laid that day was the sum of the three recorded numbers. It was also recorded how many birds were alive in each replicate on each day. The average percentage of hen-day egg production was used to represent the rate of lay for each replicate. According to Hunton (1995), hen-day egg production was calculated by multiplying the average daily egg production by 100 and dividing that result by the typical daily number of birds alive.

$$\text{HDEP} = \frac{\text{Total number of eggs produced on a day}}{\text{Total number of hens present on that day}} \times 100$$

Egg quality parameter measurements

For each replicate, parameters relating to egg quality were measured. Measurements were made of egg quality traits such as egg weight, shell weight, shell thickness, yolk height, yolk weight, yolk color, yolk index, yolk diameter, albumen height, albumen weight, and Haugh Unit Score (HUS). An electronic Digital Caliper (Mitutoyo, Japan) was used to measure the egg's length and width as well as its weight, which was measured in grams by digital balance.

The weight of the egg shell and its membrane were measured after the eggs were broken, and the egg shell thickness was then determined after the membrane had been taken off. Using a micrometer gauge, the egg's shell thickness was measured on three of its sides: the large end (the top or pointed part), the narrow end (the bottom or round part), and the middle portion. The thickness of the egg shell was determined by averaging the three sites.

The albumen was separated from the yolk, its height was measured using a tripod micrometer called a Spherometer, and its weight was determined using a sensitive balance. Egg weight and thickness of thick albumen were correlated using the Haugh Unit measurement technique. Following is the formula used to calculate the Haugh Unit (Haugh, 1937).

$$\text{HU} = 100 \log (\text{AH}–1.7\ \text{EW}^{0.37} + 7.6)$$

Where, HU is Haugh Unit, AH signifies Albumen Height in millimeters, EW denotes Egg weight in grams.

The yolk's diameter and height were measured using a ruler and a tripod micrometer after it had been separated from the albumen. Sensitive balance was used to measure the yolk's weight. Using this formula, the yolk index was calculated.

Yolk index = Yolk height/ Yolk diameter

The yolk color was assessed using a Roche fan, equipped with 1-15 strips representing a spectrum of hue from pale to orange yellow. The yolk membrane (vitelline membrane) was first removed, followed by a thorough mixing of the entire yolk, and then a yolk sample was taken on a piece of white paper and compared with Roche fan measurement strips (Vuilleumier, 1969). The shape index (percent) was calculated as 100 times egg width divided by egg length. Albumen percentage was determined by 100 times the albumen ratio to egg weight. Yolk percent was calculated as 100-time yolk weight divided by egg weight. Shell weight was calculated by egg weight minus (Albumen weight plus yolk weight). Shell percentage was determined by 100 times Shell weight divided by egg weight. Egg specific gravity (gm/cm3) was determined by egg weight (gm)/egg volume (cm3), after determining egg volume. Egg volume (cm3) was obtained by 0.524 LB^2.

Where, 0.524 is a constant value, L stands for egg length (cm), B determines egg breadth (cm).

Statistical analysis

The Statistical Analysis Systems Software (SAS, 2008) Version 9.2 was employed to perform analysis of variance (ANOVA) on the collected data using the general linear model (GLM) procedure. Tukey HSD test was performed for mean separation when treatment effects were significant ($p < 0.05$).

RESULTS

Performance of Bovans Brown laying hen supplemented with EM in drinking water and Bokashi in feed is indicated in Table 1. A group supplemented in T4 and T1 had the highest and the lowest feed intake, respectively. The initial body weight, final body weight, the total body weight gains and the hen day egg production of laying hens did not significantly differ among the treatment groups ($p > 0.05$). The hen day egg production across different age groups on a weekly basis is presented in Graph 1.

There were no significant differences in the percent hen day egg production among all age groups ($p > 0.05$). At weeks 20-23 of age, the percent hen day egg production was very low. In the age of week 24-27, the percent hen day production indicated an increase. The maximum hen day egg production was registered in weeks 28-31, 32-35, 36-39, and 40-43. The percent hen day egg production revealed a diminishing order at 44-47 weeks of age and beyond this until the end of the experimental period.

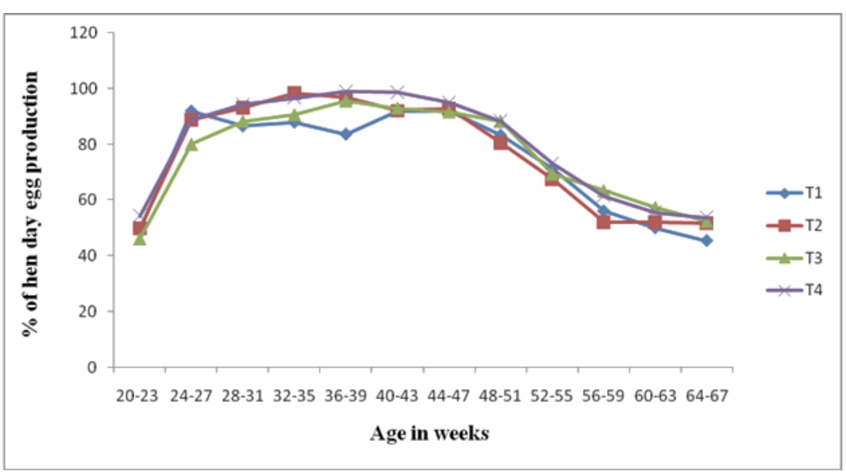
External and internal egg quality traits

The characteristics of the internal and external egg quality are shown in Table 2. The lowest egg weight among the external egg quality traits was observed in T1, compared to other treatment groups ($p < 0.05$). The T3 and T4 recorded the highest shell weights. However, there was no significant difference in the treatments for shell thickness, shape index, egg volume, or egg specific gravity ($p > 0.05$). The egg mass of T4 was significantly higher than T1 and T2 ($p < 0.05$). The albumin weight of T3 and T4 was significantly higher than T1 under the internal egg quality traits ($p < 0.05$). The highest ($p < 0.05$) and the lowest ($p < 0.05$) Hough Unit were seen in T4 and T1, respectively. Compared to the other treatment groups, T4 had the significantly highest yolk weight ($p < 0.05$). The treatment group of T1 had the significantly least amount of yolk color, compared to the other groups ($p < 0.05$).

Table 1. Performance of Bovans Brown laying hen supplemented with effective microorganisms in drinking water and Bokashi in feed from 20-67 weeks of age

Parameters	T1	T2	T3	T4	SEM	p-value
Average feed intake (g)	119.67[c]	122.36[b]	122.37[b]	124.07[a]	0.167	<0.001
Initial body weight (g)	1547.10	1545.53	1597.27	1592.63	13.265	0.395
Final body weight (g)	1775.80	1875.80	1909.47	1901.57	31.434	0.457
Total body weight gain(g)	228.70	330.30	312.20	308.97	35.030	0.746
Hen day egg production (%)	74.00	76.33	76.33	80.00	2.819	0.276

[abc]Means with a different superscript in a row are significantly different ($p < 0.05$); T1: Control, T2: 16 ml EM/L of drinking water, T3: 5% Bokashi per quintal of feed, T4: 16 ml EM/L of drinking water + 5% Bokashi per quintal of feed, SEM: Standard error of the mean



Graph 1. The hen day egg production of Bovans Brown laying hens on a weekly basis. T1: Control, T2: 16 ml EM/L of drinking water, T3: 5% Bokashi per quintal of feed, T4: 16 ml EM/L of drinking water + 5% Bokashi per quintal of feed.

Table 2. External and internal egg quality traits of Bovans Brown laying hens supplemented with effective microorganisms from week 20 to week 67

Parameters	T1	T2	T3	T4	SEM	p-value
External egg quality traits						
Egg weight (g)	57.63^{b}	59.19^{ab}	61.44^{a}	62.47^{a}	1.356	0.003
Egg Breadth (mm)	43.33	43.13	43.87	44.42	0.513	0.067
Egg length (mm)	54.97^{b}	56.17^{a}	56.78^{a}	57.72^{a}	0.927	0.034
Shell weight (g)	5.25^{b}	5.39^{ab}	5.59^{a}	5.61^{a}	0.123	0.014
Shell thickness (mm)	0.38	0.37	0.39	0.39	0.016	0.527
Shell (%)	9.1	9.1	9.1	9.0	0.284	0.251
Shape index (%)	79.11	76.81	77.25	77.71	1.539	0.476
Egg volume (cm^3)	54.19	54.77	57.25	55.93	1.484	0.184
Egg specific gravity (g/cm^3)	1.07	1.08	1.07	1.06	0.019	0.793
Egg mass (g)	42.67^{b}	45.00^{b}	46.67^{ab}	50.00^{a}	1.509	0.008
Internal egg quality traits						
Albumen weight (g)	34.87^{b}	35.93^{ab}	37.35^{a}	38.04^{a}	0.825	0.001
Albumen height (mm)	6.49^{b}	6.98^{ab}	7.27^{a}	8.03^{a}	0.271	<0.001
Albumen length (mm)	60.66^{a}	60.13^{b}	63.05^{a}	63.43^{a}	1.275	0.024
Hough Unit	80.82^{b}	83.58^{b}	84.71^{b}	89.76^{a}	1.805	<0.001
Albumen (%)	60.5	60.7	60.8	60.9	1.016	0.253
Yolk weight (g)	17.51^{b}	17.87^{b}	18.51^{b}	18.81^{a}	0.413	0.011
Yolk height (mm)	18.13^{b}	19.00^{ab}	18.27^{ab}	19.13^{a}	0.398	0.026
Yolk length (mm)	40.04	39.37	40.81	41.16	0.626	0.027
Yolk color	5.27^{c}	7.33^{a}	6.80^{b}	6.27^{ab}	0.216	< 0.001
Yolk Index	0.46	0.49	0.45	0.47	0.017	0.217
Yolk (%)	30.4	30.2	30.1	30.1	0.604	0.241

^{abc}Means with a different superscript in a row are significantly different ($p < 0.05$); T1: Control, T2: 16 ml EM/L of drinking water, T3: 5% Bokashi per quintal of feed, T4: 16 ml EM/L of drinking water + 5% Bokashi per quintal of feed, SEM: Standard error of the mean

DISCUSSION

Egg production

At the age of week 20, both treatment groups in this study laid their first eggs. The first egg is laid by chickens when they are 21 weeks old (Anene, 2020). According to Abera et al. (2021), Brown hens start laying their first eggs between 18 and 20 weeks of age. In contrast to previous studies where the age of the first egg laying hen in control and EM-supplemented groups ranged between 179 and 186 days (Simeamlak et al., 2013), the current study yielded different results. The way chickens were treated during the experimental period might have made a difference. According to Atsbeha and Hailu (2021), chickens provided with EM in water and feed began laying eggs at 161 days, those given EM in water started at 166 days, control group chickens initiated laying at 168 days, and chickens receiving EM in feed commenced laying at 175 days. This suggests that EM supplementation positively influenced the age at first lay by promoting early maturity. The observed improvement in age at first lay may be attributed to the accelerated growth of chickens facilitated by the presence of beneficial microbes in the gut. This, in turn, enhances the rapid absorption of essential nutrients (Gnanadesigan et al., 2014). Both the health and the microscopic structure of the ileum and ceca were improved by dietary probiotic supplementation (Xiang et al., 2019).

Between 241 and 268 eggs were produced overall in this study during the experimental period (20-67 weeks). It was revealed that commercial egg type layers began laying eggs at the age of 20-21 weeks and produced 277 eggs till 72 weeks of their production cycle (Petek, 1999). Similarly, North (1984) reported that during the 52-week laying period, 266 eggs were produced. The average number of eggs laid per 100 hens per day in 2020 was 81, as reported by the USA (2020). Better nutrition, genetics, health and disease prevention, and flock management all contributed to the hens' higher productivity.

In the present study, the hen day egg production of the birds ranged from 74% to 80%. documented improved results in hen day egg production for layers treated with EM, reporting 88.28 %, 84.28 %, and 83.20 %, in three consecutive months of egg production, respectively. AL-Nasser et al. (2020) noted that the overall average hen-day egg production for brown hens was 85.6%, which was higher than that of white hens (83.2%). The percentage of hen day egg production increased significantly up to 42 weeks of age and then gradually decreased until the end of the laying period (Sibanda et al.,2020). The findings of the present study contrast with those reported by Tanaka (1993), who documented a hen day egg production of 72.5%, and North (1984), who reported a rate of 73%.

External egg quality traits

Egg weight, egg breadth and egg length

At 72 weeks of age, the mean egg weight of a Bovans Brown laying hen is 63.2 g. At the end of the experimental period in the current study, the mean egg weight of Bovans Brown layers was 57.63-62.47 g. In all age groups, the eggs weighed better when EM was added to the drinking water and Bokashi to the feed. This result coincided with Gnanadesigan et al. (2014), reporting the maximum egg weight within the range of 61.6 g when layers were fed with activated EM (5 L/ton) treated commercial feed masses and activated EM treated (2 L/1000 L) drinking water. In the first 21-28 weeks of the egg-laying period, Atsbeha and Hailu (2021) reported an average egg weight of 59.1 g in chickens given EM in water, 58.3 g in chickens given EM in feed, 60.9 g in chickens given EM in water and feed, and 57.2 g in chickens given control feed. They also noted that during the peak egg-laying period (29-39 weeks), egg weight varied according to treatments. It was found that a group of EM-treated layers achieved a maximum egg weight of 61.6 g when provided with both EM-treated feed and drinking water (Gnanadesigan et al., 2014). In contrast, Ahan et al. (2020) found different results, noting that supplemented Brown laying birds produced eggs weighing 60.72 g, which were lighter than the control group's eggs at 61.34 g. It was found that the average weight of the eggs produced by the EM-treated chickens was comparable to that of the control groups (El-Deep et al., 2011). The variations in egg weight could be brought about by variations in breed, feed composition, environment, and other management factors.

The egg breadth of Bovans brown layer chicken in this experiment ranged from 43.13 to 44.12 mm. However, Gnanadesigan et al. (2014) reported that the egg breadth of EM-supplemented layers ranged from 45.79 to 46.10 mm. In the EM-supplemented groups, the egg width was 44 mm (Kinati et al., 2021). For chickens raised in deep litter, legume pasture, and grass pasture systems, the Isa Brown chicken egg width was 40.07 mm, 40.05 mm, and 40.13 mm, respectively (Oke et al., 2014). The findings of Abanikannda et al. (2007) revealed that there was a strong correlation between egg weight and egg width (r = 0.84). The highly significant positive correlation indicated that the egg width increases with increasing egg weight.

The Bovans Brown layer chicken used in this experiment had egg length that ranged from 54.97 mm to 57.72 mm. In comparison to the control group, layers supplemented with EM in drinking water and Bokesh were higher. In the same line, Abanikannda et al. (2007) found that the egg length varied from 53.86 to 57.43mm. In the study by Gnanadesigan et al. (2014), it was reported that the range of the mean egg length in the EM-supplemented groups was 54.1 to 55.8 mm. According to Ahan et al. (2020), the control group and EM-supplemented layers both had eggs that were 55.22 mm long. Abanikannda et al. (2007) reported a strong correlation between egg weight and length (r = 0.78). They also reported that as the age of the layer increased, there was an increase in egg weight, egg width, and egg length.

Shell weight, shell percentage, and shell thickness

In this experiment, the shell weights of the eggs ranged from 5.25 to 5.61 g. When layers received EM in drinking water, Bokashi in feed, and both of these supplements, there was a significant difference in shell weight ($p < 0.05$). Similar result was reported by Atsbeha and Hailu (2021). The shell weight of eggs supplemented with EM ranged from 5.57 to 6.18 g. However, Ahan et al. (2020) noted that there was no significant difference in the shell weight between the control group (6.96 g) and the EM-supplemented group (6.94 g). In addition, Congjiao et al. (2019) reported the shell weight of the egg in EM supplemented as 5.63 g, and Şahan et al. (2020) noted that the shell weight ranged from 5.8 to 6.2 g. According to John-Jaja et al. (2016), the weight of the egg shell increases steadily as the hen's age increases. Supporting this, Minelli et al. (2007) reported egg shell weights of 6.00 g at 28-32 weeks, 6.16 g at 47-50 weeks, and 6.29 g at 70-73 weeks. Rath et al. (2015) reported an egg shell weight of 6.00 g at 50 weeks, while Tumova et al. (2011) observed a range of 6.91-7.81 g in the New Black breed from 28 to 60 weeks of age, and 6.50-6.91 g for litter-raised Hisex Brown at 60 weeks. Ewa et al. (2005) obtained lower values of 5.05 g for the Black Olympia breed and 5.34 g for the H and N Brown Nick breed at 36 to 46 weeks of age. Additionally, Begli et al. (2010) reported an egg shell weight of 4.45 g for Iranian fowl at 30 weeks of age. Sreenivas et al. (2013) measured egg shell weights ranging from 4.32-5.12 g for four genetic groups in the White Leghorn breed at 40 weeks. According to John-Jaja et al. (2016), the average values of egg quality traits indicate an increase in egg weight from 55 to 63 g, and egg shell weight increased from 6 to 7 g. The observed variations in these measurements may be attributed to breed differences, the age of the layers, environmental temperature, and management practices, especially the type of feed used (FAO, 1998). In the present experiment, the shell percentage fell within the range of 9.1%. Gupta (2008) suggested that the average egg shell weighs 5-6 g, constituting 10-11% of the total egg weight. John-Jaja et al. (2016) reported egg shell percentages of 8.39% at 25 weeks, 10.05% at 51 weeks, and 10.18% at 72 weeks of the hen. Other studies by Begli et al. (2010), Mube et al. (2014), and Zhang et al. (2010) reported egg shell percentages of 10.6%, 12.1%, and 10.90%, respectively. According to Mohammed and Eva (2018), the egg shell percentage ranged from 10.07 to 12.21%.

In the current study, the egg shell thickness ranged from 0.37 to 0.39 mm, a finding consistent with Atsbeha and Hailu (2021), who reported egg shell thickness of 0.35 to 0.40 mm for eggs supplemented with EM. On average, egg shell thickness is approximately 0.30 mm (Yalcinalp, 2018), while Ketta and Tůmová (2018) reported a range of 0.28 to 0.41 mm. Gnanadesigan et al. (2014) reported higher shell thicknesses ranging from 1.39 to 1.44 mm. Factors influencing shell thickness include the duration of the egg's stay in the uterus/shell gland and the rate of calcium deposition during shell formation. A shorter stay in the shell gland may result in less thickness. The time of day when the egg is laid also plays a role, with thicker shells occurring earlier in the day or during the light part of the photoperiod (Gupta, 2008). An egg shell, with a thickness of 0.03 millimeters, is composed of 7,000 to 17,000 tiny pores, ensuring strength while allowing the passage of oxygen, carbon dioxide, and moisture (Biggs, 2017). Larger pores and thinner shells diminish the protective capacity of eggs. The functional quality of the egg shell is influenced by various pre-laying factors, including strain, disease, management practices, moulting, age of the bird, medications, stress, environmental temperature, and nutrition (Gupta, 2008).

Shape index, egg mass, egg volume, and egg specific gravity

An important factor in assessing egg quality is the egg shape index, which is defined as the proportion of width to length of the egg (Narushin and Romanov, 2002). The shape index of the egg ranged from 76.81 to 79.11% in the current study. Similarly, Şahan et al. (2020) reported that the shape index of the control (80.63) and EM supplemented (80.09) group did not show any significant difference. According to Rath et al. (2015), the shape index of an egg was 73.53%. The shape index classifies eggs as sharp (<72), normal (standard, 72-76), and round >76 (Sarica and Erensayin, 2004). Normal chicken eggs have an oval shape. The hen eggs with unusual shapes, such as long and narrow, round, or flat-sided, cannot be graded AA (almost perfect) or A (slightly worse than AA), as eggs are typically oval in shape (72-76). When eggs are being packaged and transported, trays easily accommodate standard eggs. According to Sarica and Erensayin (2004), round eggs and eggs that are unusually long may not have an appealing appearance and may not fit well in egg cartons, making them more prone to breakage during shipping compared to eggs with a normal shape. According to Anderson et al. (2004), the shape index significantly influences the percentage of breakage strength variation. The risk of cracked eggs is influenced by egg characteristics such as shape index and shell thickness. As a result, the way the egg is handled after it is laid is also crucial, taking shape index and shell thickness into account (Galic et al., 2019).

Egg mass refers to the correlation between egg weight and production (Singh, 2000). In the present study, the egg mass of the treatments ranged from 42.67 g to 50 g, with the EM-supplemented groups in drinking water and feed exhibiting the highest values. Desirable egg masses are considered to be 50 g and higher. To attain the desired output for the farm, it is crucial to supplement EM in drinking water and include Bokashi for Bovans Brown laying hens (Kocevski et al., 2015).

Two crucial geometrical calculations, namely egg volume (V) and surface area (S), are required to predict chick weight, egg hatchability, shell quality traits, and egg interior parameters. These calculations are used in the poultry industry and biological studies. The volume of an egg can be assimilated into an ellipsoid (Stelzer, 2001). The volume of the eggs in this experiment ranged from 54.19 to 57.25 cm^3. The findings of the current study align with Narushin (2005), who reported minimum, maximum, and average chicken egg mass of 52 cm^3, 70.4 $cm^{3,}$ and 60.19 $cm^{3,}$ respectively. The current result is less than the egg volume reported as 63.0 cm^3 for a standard chicken egg (Zeidler, 2002).

By weighing an egg and dividing its weight by its volume, the specific gravity of an egg can be calculated (Iqbal et al., 2017). Egg shell thickness has a significant impact on specific gravity. The prevalence of cracks typically rises as specific gravity decreases. With increasing hen age, egg specific gravity typically decreases (Roberts, 2004). There was no significant difference between the treatment groups in the current study despite the specific gravity of an egg ranging from 1.06 to 1.08 g/cm3 at 67 weeks of age in the treatment groups ($p > 0.05$). In most cases, 85-90% of the eggs fall into the 1.075, 1.080, and 1.085 categories (Gualhanone et al., 2012). According to Krist (2011), the specific gravity of sharp, standard, and round eggs was 1.088, 1.087, and 1.086, respectively. The specific gravity of the best-quality eggs is greater than 1.08 g/cm3, while normal eggs fall between 1.06 and 1.10 g/cm3 (Inoti, 2020). In a study comparing deep litter, legume pasture, and grass pasture production systems, the specific gravity of eggs was reported as 1.12, 1.21, and 1.12 g/ cm3, respectively, at 24 weeks old, 1.16, 1.13, and 1.10 g cm-3, respectively at 38 weeks old and 1.17, 1.12, and 1.25 g/ cm3, respectively at 60 weeks old (Oke et al., 2014). Sarica et al. (2012) noted that egg shape index and specific gravity had a positive non-significant correlation. Ozelik (2002) found a non-significant tendency to a negative correlation between egg shape index and specific gravity.

Internal egg quality characteristics

Albumen weight and albumen height

The egg white, or albumen, makes up two-thirds of an egg's weight (Sugino et al., 2018). In this study, the egg's albumen weight ranged from 34.87 to 38.04 g. Compared to the control group, layers that received EM in water and/or bokashi supplements in feed had higher albumin weights. The current result is better than the report of Gnanadesigan et al. (2014), indicating that the albumen weight of layers fed with standard commercial food masses with Activated Effective Microorganisms (AEM) solution (5 L/ton), and the layers fed with AEM (5 L/ton) treated commercial feed masses and AEM treated (2 L/1000 L) drinking water were 35.08 and 35.03 g, respectively. The study by Ahan et al. (2020) reported that the egg supplemented with EM had an albumen weight of 40.01g, which was higher than the results of the present investigation. In another study, layers supplemented with EM in drinking water, feed, and both drinking water and feed had albumen weights of 37.58 g, 37.97 g, and 38.53 g, respectively (Atsbeha and Hailu, 2021). In a different production system comparison, the albumen weight of eggs in deep litter, legume pasture, and grass pasture production systems were reported as 33.19 g, 32.20 g, and 33.98 g, respectively, at 24 weeks old; 36.81 g, 37.62 g, and 39.74 g, respectively, at 38 weeks old; and 39.36 g, 40.88 g, and 38.80 g, respectively, at 60 weeks old (Oke et al., 2014). According to Dottavio et al. (2005), the albumen weight of the egg for Fayoumi, White Leghorn, and Rhode Island Red layer chickens was 30.08, 35.07, and 40.01 g, respectively.

Albumen height indicates the sign of freshness of the egg. When all eggs have the same age, they can have the same number of albumen heights (Silversides and Budgell, 2004). Internal egg quality is determined by measuring albumen height and Haugh units (Roberts, 2004). However, because albumen height is a factor in determining Haugh units (Silversides and Villeneuve, 1994), thick albumen height is considered a more useful tool for gauging eggs' freshness level. The albumen heights in this study ranged from 6 mm to 8 mm on average. Atsbeha and Hailu (2021) found significant differences in albumen height when layer hens were supplemented with EM in drinking water (5.65 mm) and in feed and drinking water (6.15 mm). In contrast, Sahan et al. (2020) reported lower albumen height (9.6 mm) for laying hens with EM added to drinking water compared to the control group (10.55 mm). Kinati et al. (2021) found no statistically significant difference in albumen height between the EM-supplemented group (7.28 mm) and the control group (6.49 mm). Rath et al. (2015) reported an albumen height of 8.41 mm. The albumen height in this study falls within the acceptable range for superior quality, as mentioned by Zeidler (2002). The higher albumen height may be attributed to the freshness of eggs and the young age of the hens.

Albumen length, albumen percentage, and Hough unit

In this study, the albumen length for treatment groups ranged from 60.66 to 63.45 mm, with the control group having the lowest albumen length compared to other supplemented groups. This finding contrasts with the results reported by Sahan et al. (2020), where albumen length did not significantly differ between the control group (77.77 mm) and the treatment group (78.84 mm) receiving drinking water with a dose of 1000 ml EM/ton water. Rath et al. (2015) reported a higher albumen length of 92.37 mm. The albumen percentage of the egg in this study ranged from 60.5% to 60.9%. Sahan et al. (2020) reported no significant difference between the control group (65.87%) and the EM-supplemented group (66.09%) in terms of albumen percentage. Sapkota et al. (2020) reported that the average albumen percentage of Sakini chicken was 59.84%. Hanusova et al. (2015) reported lower albumen percentages for Oravka and Rhode Island Red chicken, at 57.26% and 56.74%, respectively. For Isa Brown laying hens, the albumen percentage ranged from 64.14% to 65.93% (Koja Abbas et al., 2020).

Simeamelak et al. (2013) noted that using 4 to 12 ml of EM/liter of drinking water for laying hens had a significant improvement in egg quality (Haugh unit, yolk, and albumen height). Gnanadesigan et al. (2014) reported Haugh unit values for EM-supplemented groups ranging from 92.86 to 92.91, which were higher than the values observed in the current study. Atsbeha and Hailu (2021) noted that EM-supplemented groups had higher Haugh units in their drinking water (74.03) and feed and drinking water (77.25) compared to the control group (69.43). Koja Abbas et al. (2020) reported a range of Haugh unit values for Isa Brown laying hen eggs from 76.14 to 85.29. Kumar et al. (2014) also noted Haugh unit values of 82.15 for Bovans Brown breed and 83.67 for Rhode Island Red, both comparable to the results in the current study. The higher Haugh unit values in the current study indicate the freshness of the eggs. Assefa et al. (2019) highlighted that albumen height and egg weight have the greatest impact on the Haugh unit. The observed differences in EM supplementation amounts may be related to breed type, environment, diet composition, and other management techniques.

Yolk weight, yolk height, and yolk length

The yolk weight in this study was within the range of 17.51-18.81 g, and Bovans Brown laying hens supplemented with EM in drinking water and feed had the highest egg yolk weight among other treatment groups. According to Atsbeha and Hailu (2021), the yolk weight of the eggs ranged from 15.13 to 16.29 g and did not show any significant difference among the control group and EM-supplemented groups. Gnanadesigan et al. (2014) found that layers fed with commercial food masses treated with AEM (5 L/ton) had egg yolk weights of 15.83 g while layers fed with commercial food masses treated with AEM (5 L/ton) and AEM-treated drinking water (2 L/1000 L) had egg yolk weights of 15.95 g. According to Sahan et al. (2020), the yolk weight of the control group (13.84 g) and the EM-supplemented group (13.77 g) did not differ in any way, which was statistically significant. The higher yolk weight in this study could be attributed to various factors, including the breed of laying hen, the doses of EM supplementation, nutrient composition of the feed, environmental conditions, and other management factors. These variables can influence the overall egg quality, and understanding their interactions is essential for interpreting the study's results accurately.

The yolk height of the EM-supplemented groups with drinking water and in feed (19.13 mm) was higher than the control group (18.13mm). The findings in this report were different from those by Şahan et al. (2020), where the EM-supplemented groups exhibited lower yolk height (18.85 mm) than the control group (19.74 mm). Commercially available brown table eggs were reported to have a yolk height ranging from 20.1 to 24.5 mm (Hisasaga et al., 2020). In the current study, yolk length ranged from 40.04 to 41.16 mm, with no significant difference observed among the treatment groups ($p > 0.05$). Rath et al. (2015) reported a higher yolk length of 45.98 mm, which surpasses the findings in the current study. The discrepancies may arise from various factors, including differences in experimental conditions, breeds, and management practices across studies.

Yolk percentage, yolk index and yolk color

The yolk percentage of the eggs ranged from 30.1% to 30.4% for Bovans Brown egg layers, both in the control and EM-supplemented groups. In contrast, Sahan et al. (2020) observed that the yolk percentage between the control (22.56%) and EM-supplemented groups (22.68%) did not differ significantly. In a study involving Fayoumi, White Leghorn, and Rhode Island Red chickens in their last egg production cycle, the yolk percentages were reported as 33.3%, 30.2%, and 28.6%, respectively (Dottavio et al., 2005). Leeson (2006) stated that the components of a fresh egg are approximately 32% yolk, 58% albumen, and 10% shell. Zaheer (2015) noted that the egg's composition includes approximately 9-12% shell, 60% albumen, and 30%-32% yolk of the total volume. These variations in yolk percentage may be influenced by factors such as breed, nutritional conditions, and other environmental factors. The yolk index, calculated as the ratio of yolk height to yolk diameter, provides an indication of how recently an egg was laid. Eggs are considered extra fresh if their yolk index is higher than 0.38 (Yuceer and Caner, 2014). The yolk index for fresh eggs typically varies between 0.30 and 0.50, with a mean value of 0.42 (Ihekoronye and Ngoddy, 1985). In this study, the yolk index ranged from 0.45 to 0.49, which is higher than 0.38 and considered extra fresh; however, there was no significant difference among the treatment groups. Similarly, Gnanadesigan et al. (2014) reported a yolk index of 0.48 for layers fed with standard commercial ration and activated effective microorganisms (AEM) solution (5 L/ton), while layers fed with AEM-treated commercial ration and AEM-treated drinking water (2 L/1000 L) had a yolk index of 0.52. In contrast, Sahan et al. (2020) reported a higher yolk index for the control group (0.51), compared to the EM-supplemented group (0.48).

A hen's diet impacts the color of the yolk in her eggs, but the yolk color does not indicate the freshness of the egg. Egg yolks come in various colors, from pale yellow to deep orange. Carotenoids, organic pigments in some plants, lead to darker in color eggs (Zia-Ul-Haq, 2021). The yolk color of an egg in the treatment groups ranged from 5.27 to 7.33. The EM-supplemented groups had the highest yolk color than the control group. According to Atsbeha and Hailu (2021), the yolk color ranged from 6.2 to 6.8, which was in between the current study.

CONCLUSION

Layers supplemented with 16 ml EM in drinking water and 5% Bokashi in feed had significantly improved feed intake, hen day egg production percentage, external egg quality traits (egg weight, egg length, shell weight, and egg mass) and internal egg quality traits (albumen weight, albumen height, albumen length, Hough unit, yolk weight, yolk height, and yolk color). Therefore, it can be recommended that small-scale, medium-scale, and large-scall producers can supplement 16 ml EM per liter of drinking water and 5% Bokashi in feed to improve egg production and egg quality without any deleterious effect. Further investigation beyond the recommended level of this study is crucial.

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Authors' contributions

Melkamu Bezabih Yitbarek generated the idea, drafted the research protocol, developed the study design, analyzed the laboratory analysis, collected the data, performed the data analysis, and wrote and revised the manuscript. The author has read and approved the final data and manuscript.

Competing interests

The author declares that there is no financial or interpersonal competing interest.

Ethical considerations

This article has been checked by the author, and ethical issues such as plagiarism, publication consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy were not found.

Availability of data and materials

The prepared data of the present study will be sent by the corresponding author according to the reasonable requests.

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