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Full Length Article

Quality evaluation of egg composition and productivity of layers in EM (Effective Microorganisms) treatments: A field report



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ABSTRACT

Layers divided in to three groups; Group 1 (Control group) – The layers fed with standard commercial food masses with Biobantox, BMD and formaldehyde. Group 2 (EM Treatment 1) – The layers fed with standard commercial food masses with AEM solution (5 L/ton feed). Group 3 (EM Treatment 2) – The layers fed with AEM (5 L/ton feed) treated commercial food masses + AEM treated (2 L/1000 L) drinking water. Increased monthly average egg production, egg weight and the decreased mortality ratio and egg wastages identified with the group 3 EM treated layers. The values of egg shell weight, thickness, yolk weight, yolk index and Haugh index found higher in group 3 EM treated layers. The content of calcium, sodium, potassium, magnesium, iron, zinc and total protein identified maximum in the group 3 EM treated layers. The concentration of total cholesterol, saturated fat, trans-fat found reduced and the monounsaturated, polyunsaturated found higher in EM group 2 and 3 layers. The total cost identified as Rs. 110.80, 38.00 and 52.50 (in Indian Rupees) for group 1, group 2 and group 3 treatments respectively. It has inferred from the present findings that, the effect of EM treatments in commercial layers of the egg production showed good quality parameters and economic value.

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1. Introduction

Poultry products are good sources for human population. However, poultry industry confronted due to incidence of various infectious diseases still chaos for the poultry industries. Among these, salmonellosis is infectious and most important zoonotic disease. It transmits vertically and decreases the production in the infected flocks. Mortality in susceptible birds may reach up to 90% [1]. The use of hybrid layers over the world promoted for production of eggs and meat. The hybrid layers usually start laying about 20 weeks of age and peak egg production reached during the first production cycle. The average production rate of commercial layers usually remains close to 0.9 eggs per day [2]. For the control of several infectious diseases, the use of antibiotics is increasing day by day. This generous use of antibiotics causes not only severe problems (Bacterial resistance, Dysbiosis) in poultry but also responsible for harmful residual effects in meat and eggs thus leading to public health hazards [3]. In this connection, use of microorganisms is efficiency to reduce the effect of chemical hazards. Of these, several microbes such as *Lactobacillus plantarum*, *Lactobacillus casei* and *Streptococcus lactis* (lactic acid bacteria), *Rhodopseudomonas palustris* and *Rhodobacter spaeroides* (photosynthetic bacteria), *Saccharomyces cerevisiae* and *Candida utilis* (yeasts), *Streptomyces albus* and *Streptomyces griseus* (actinomycetes), and *Aspergillus oryzae*, *Penicillium sp.* and *Mucor hiemalis* (fermenting fungi) [4,5] previously used in several poultry farm applications such as sustainable agricultural, industrial, odor control, waste management and environmentation. But, studies related to the applications of Effective Microorganisms- (EM) in the poultry field are too limited. Therefore, the present study tried to identify the effect of EM-microbes in egg production, mortality ratio and egg quality analyses with the commercial layers.

2. Materials and methods

2.1. Preparation of AEM (Activated-EM) solution

The commercially available EM stock solution [Contains, *Lactobacilli sp.* ($>10^5$ CFU ml^{-1}), *Rhodopseudomonas sp.* ($>10^5$ CFU ml^{-1}) and *Saccharomyces sp.* ($>10^5$ CFU ml^{-1})] bought from ECO- Pro, Auroville, Pondicherry, Tamilnadu, India. The

shelf life of the EM stock solution is one year. One part of EM stock- solution and one part of organic jaggery (1:1 V/W ratio) mixed with 50 L of chlorine free ground water. The prepared solution transferred to the food grade plastic container and tightly closed with plastic lid and kept in shade at ambient room temperature (20–40 °C) for 10 days (Fig. 1). During the fermentation, the lid opened daily once to release pressure. After 10 days, the AEM solution verified by pleasant sweet sour smell and the acidic pH nature (pH 3.5–4.0).

2.2. Treatments

The field experiment carried out at PSP poultry farm, Mettupatty, Namakkal District, Tamilnadu, India for three months. 25 weeks old *Gallus domesticus* layers selected and divided into three groups.

2.2.1. Group 1 (Control group)

The layers to fed with standard commercial food masses with Biobantox, BMD (Bacitracin methylene disalicylate) and formaldehyde.

2.2.2. Group 2 (EM treatment 1)

The layers fed with standard commercial food masses with AEM solution (5 L/ton).

2.2.3. Group 3 (EM treatment 2)

The layers fed with AEM (5 L/ton) treated commercial food masses + AEM treated (2 L/1000 L) drinking water.

2.3. Data collection

After completion of 30 days AEM and chemical treatments (End of the October, 2012), data analysis such as egg production, mortality ratio and egg wastage analysis taken on the daily basis.

Egg Production = Number of eggs (in numbers)/Total number of birds \times 100

Mortality = Number of birds died/Total number of birds \times 100

Egg wastage = Number of broken and waste eggs per shed



AEM solution before fermentation

AEM solution after fermentation

Fig. 1 – Preparation of AEM solution.

2.4. Weight and allometric analysis of the egg samples

After 30 days of treatments 10 eggs selected randomly for the results analysis of the following parameters; the weight of eggs and their parts – albumen, yolk and egg shell with membranes determined with a precision electronic balances. The long and short axes of eggs measured with a technical caliper. The yolk and albumen heights determined with a tripod micrometer with a precision of 0.01 mm. The egg shell thickness with shell membrane measured with a special AMES micrometer in the equatorial region and both poles (sharp and blunt). The arithmetic mean of the three measurements kept as egg shell thickness.

2.5. Haugh units

The HU value was identified with the following formulas $HU = 100 \log(h + 7.57 - 1.7 \cdot EW^{0.37})$, where h is the height of thick albumen at the boundary with the yolk; EW – the egg weight [6].

2.6. Yolk index

Yolk quality was evaluated through the yolk index: $YI = h/D$, where h is the yolk height and D – yolk diameter [7].

2.7. Estimation of mineral content

Mineral content of the total egg content (Yolk and albumen) sample tested after dry ashing of samples at 550 °C and dissolution in hydrochloric acid. Elements, such as calcium, potassium, magnesium, iron and zinc determined with the atomic absorption spectrophotometer Analist 800, Perkin Elmer at Omega laboratories, Namakkal.

2.8. Estimation of the fatty acid composition

The lipid and fatty acid composition of the yolk determined by fat extraction with methanol: chloroform (2:1) by the method of [8] Bligh and Dyer at Omega laboratories, Namakkal.

2.9. Estimation of biochemical analysis

The level of total carbohydrates, total protein and total cholesterol content determined standard colorimetric methods [9] at Omega laboratories, Namakkal at Tamilnadu, India.

2.10. Cost analysis

The cost analysis of antibiotics, EM stock solution and jaggery calculated for preparing 1 ton feed masses and the results were expressed as in Indian Rupees.

2.11. Statistical analysis

Statistical analysis such as \pm SEM calculated with MS office 2003.

Table 1 – Allometric analysis of AEM and chemical treatments in commercial layers.

Parameters	Egg production (%) ^a	Mortality ratio (%) ^a	Egg wastage (%) ^a	Egg weight (g)	Egg length (mm)	Egg width (mm)	Egg shell thickness (mm)	Egg shell weight (g)	Egg albumin weight (g)	Egg yolk weight (g)	Yolk index	Haugh unit (HU)
Group 1	82.81	3.86	38.31	60.4 ± 2.60	55.40 ± 0.60	45.79 ± 0.92	1.41 ± 0.004	6.1 ± 0.29	34.09 ± 2.27	15.02 ± 2.72	0.47 ± 0.03	91.67 ± 3.90
Group 2	82.92	2.48	37.09	61 ± 4.51	54.10 ± 0.51	46.10 ± 0.27	1.39 ± 0.120	5.8 ± 0.90	35.08 ± 2.07	15.83 ± 4.09	0.48 ± 0.92	92.86 ± 3.76
Group 3	83.20	2.59	37.545	61.6 ± 5.83	55.8 ± 0.83	46.01 ± 0.61	1.44 ± 0.004	6.2 ± 1.01	35.03 ± 3.91	15.95 ± 2.93	0.52 ± 0.93	92.91 ± 3.09

^a Values are average of three months data.

Table 2 – Mineral content, biochemical composition and fatty acid analysis of AEM and chemical treatments in commercial layers.

S. no	Test parameters (mg/100 gms)	Group 1	Group 2	Group 3
1.	Calcium	44.5 ± 0.50	45.5 ± 0.5	64.5 ± 0.50
2.	Phosphorus	124.0 ± 1.00	125.0 ± 5	122.0 ± 2.00
3.	Sodium	15.0 ± 0.00	15.5 ± 0.5	21.0 ± 1.00
4.	Potassium	41.0 ± 1.00	42 ± 2.00	43.0 ± 1.00
5.	Magnesium	02.0 ± 0.00	2.0 ± 0.00	2.5 ± 0.00
6.	Iron	1.05 ± 0.05	1.25 ± 0.05	1.50 ± 0.00
7.	Zinc	0.75 ± 0.05	0.82 ± 0.025	0.95 ± 0.05
8.	Total protein	5.43 ± 0.03	5.51 ± 0.24	6.44 ± 0.06
9.	Total carbohydrate	1.27 ± 0.025	1.40 ± 0.235	1.10 ± 0.025
10.	Total cholesterol	363.00 ± 11.50	355.00 ± 0.03	320.00 ± 20.00
11.	Calories (K.cal)	108.00 ± 2.00	112.50 ± 0.03	127.50 ± 7.5
12.	Total fat	9.3 ± 0.10	9.25 ± 0.98	10.3 ± 0.15
13.	Monounsaturated fat	4.0 ± 0.0	4.24 ± 0.04	5.55 ± 0.05
14.	Polyunsaturated fat	1.42 ± 0.02	3.0 ± 0.00	2.9 ± 0.10
15.	Saturated fat	3.05 ± 0.05	2.50 ± 0.00	2.55 ± 0.05
16.	Trans fat	1.54 ± 0.005	1.01 ± 0.01	0.75 ± 0.05

3. Results

The percentage occurrence of maximum monthly average egg production (88.28%, 84.28% and 83.20%) identified with the group 3 EM treated layers in all the three consecutive months over the group 1 and group 2 treated layers. Further, the layer mortality ratio and egg wastages found decreased when compared with the group 1 and group 2 treated layers. The range of maximum egg weight (61.6 ± 5.83 g) identified with the group 3 EM treated layers. Further, the results of the egg height and width not showed and major variations between the groups. Further, the maximum range of egg shell weight (6.2 ± 1.01 g) and shell thickness (1.44 ± 0.004 mm) identified with the group 3 EM treated layers. Additionally, the results of the albumin weight not showed any major differences between the chemical and EM group treated layers. Further the value of yolk weight (15.95 ± 2.93 g), yolk index (0.52 ± 0.93) and Haugh index (92.91 ± 3.09) found higher in group 3 EM treated layers when compared with the control layers (Table 1). The maximum concentration of calcium (64.5 ± 0.50 mg/100 g of egg), sodium (21.0 ± 1.00 mg/100 g of egg), potassium (43.0 ± 1.00 mg/100 g of egg), magnesium (2.5 ± 0.00 mg/100 g of egg), iron (1.50 ± 0.00 mg/100 g of egg) and zinc (0.95 ± 0.05 mg/100 g of egg) identified in the group 3 EM treated layers when compared with the control and group 1 treatments. Similarly, the maximum (125.0 ± 5 mg/100 g of egg) concentration of the

phosphorous identified with the group 2 EM treated layers. The maximum concentration of the total protein (6.44 ± 0.06 mg/100 g of egg) identified with the group 3 EM treated layers. Further, the concentration of total cholesterol reduced (320.00 ± 20.00 mg/100 g of egg) when compared with the group 2 and control (Group 1) layers. In addition, the maximum range of energy value (127.50 ± 7.5 Kcal) identified with the group 2 EM treated layers. Further, the maximum concentration (1.40 ± 0.235 mg/100 g of egg) of total carbohydrate identified with the group 1 EM treated layers. The monounsaturated (5.55 ± 0.05 mg/100 gms), polyunsaturated (3.0 ± 0.00 mg/100 gms) found higher in EM group 3 and group 2 layers than the chemical (Group 1) treated layers. Further, the saturated (2.55 ± 0.05 mg/100 gms) and trans fat (0.75 ± 0.05 mg/100 gms) concentrations found decreased than the chemical group treated layers (Group 1) (Table 2). The total cost identified as Rs.110.80, 38.00 and 52.50 (Indian Rupee) for group 1, group 2 and group 3 respectively (Table 3).

4. Discussion

Egg is a biological structure intended by nature for reproduction. It protects and provides complete diets for the developing embryo and serves as the principle source of food for the first few days of the chick's life. Eggs are special cells found

Table 3 – Cost analysis of EM and chemical treatments in commercial layers.

Parameters	Ingredients	Cost of ingredients/ton/L of feed production (in Indian Rupees)	Total grand (in Indian Rupees)
Group 1- layers treated with chemicals	I) Biobandox	62.00/-	110.80/-
	II) BMD	9.80/-	
	III) Formaldehyde	39.00/-	
Group 2- layers treated with AEM in food masses	AEM solution (5 L of AEM/ton of feed masses)	38.00/-	38.00/-
Group 3- layers treated with AEM in drinking water and food masses	AEM solution (5 L of AEM solution/ton of feed masses + 2 ml of AEM/L of drinking water)	52.50/-	52.50/-

in female animals and nearly all animals produce them, some animals including birds lay their eggs external to their body and it is unquestionably one of the most nutritionally balanced foods for man. In poultry product egg is a major product composed of albumin (58%), egg yolk (31%) and egg shell (11%). In addition, albumen contains half their contents of the egg portion and the yolk contains the major portion of the vitamins and lipids [10,11]. In general, the quality of the egg started with the consumer's needs and the treatment methods which give general characteristics of the egg with intact shell, shape size, appearance and the internal quality of the egg parts. In addition, the several antibiotics also used as potential agents to cure several diseases including the mortality decline in the livestock's [12]. But, the efficiency of antibiotics is good in poultry products. Unfortunately, the consumer's views are that edible poultry tissues contaminated with harmful concentrations of drug residues. Because of this, the present study tried to identify the effect of effective microorganisms in the egg quality, mortality and production ratio in the commercial layers. The results of the present study suggested that, the average percentage production of eggs found higher in EM treated layers than the chemical treated layers; this might be due to the excessive beneficial EM- microbes in the gut motility region, thereby increasing the uptake and absorption of the nutrients [13]. Similar reports are also identified with the *Bacillus subtilis* treated layers [14]. Further, the results of the mortality ratio also found decreased when compared with the chemical treated layers this might be due to the increased survival nature of the effective microorganisms by altering the intestinal flora [15] and the suppressing nature of the pathogenic bacteria and improving the immune potency [16,17]. Further, the results of the external parameters such as egg weight, height, width, shell weight, yolk weight and Haugh units anonymously varied between the chemical and EM treated layers. This agrees with the earlier reports [18]. The results of the egg shell thickness showed the increased range values than the normal chemical control layers this might be due to the increased assimilation of the calcium in the intestinal tract [14]. This may also responsible for reducing the egg wastages in the EM treated layers. Similarly Mahdavi et al. [19] reported the improved egg shell thickness in hens supplemented with the probiotics. The results of the yolk index found higher in the EM treated layers than the chemical treated layers. Enhancing the yolk index related to the stability of the yellow pigments in the membrane of the yolk between the lipid molecules and this might be due to the increased uptake of xanthophylls from the corn [20]. The results of the mineral intakes found higher calcium, magnesium, iron, zinc and potassium and this might be due to the acidic nature environment nature of the microbial flora in the intestinal tract this may increase the mineral ionization and thus increase the mineral absorption ratio [14]. The results of the cholesterol concentration decreased than the other biochemical constituents and this might be due to assimilating the cholesterol in the gastrointestinal tract for their own cellular metabolism thus reducing the level of cholesterol absorption [21]. Similarly, Kalavathy et al. [22] showed that lactic acid bacterial strains may alter the entero hepatic cycle and reduce cholesterol through assimilating dietary cholesterol into the bacterial cells and the bile salt

hydrolyzes in the intestine. Further, the saturated and trans fat found decreased in the EM treated layers this might be due to the inhibit hydroxymethyl-glutaryl-coenzyme A, an enzyme involved in the gastrointestinal tract [23]. The cost analysis of chemical and EM treatments showed tremendous gain in the reduction of the chemical usages.

5. Conclusion

It has concluded that, the effect of EM technology in poultry applications are helpful to improve the cost value and the quality parameters of the egg compositions.

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REFERENCES

- [1] Ashraf M, Siddique M, Rahman SU, Arshad M, Khan HA. Effect of various microorganisms culture feeding against *salmonella* infection in broiler chicks. *J Agric Soc Sci* 2005;1(1):29–31.
- [2] Kekeocha CC. *Poultry production handbook*. London; UK: Macmillan Publishers Ltd.; 1985.
- [3] Medina R, Katz M, Gonzalez S, Oliver G. Characterization of the lactic acid bacteria in ewe's milk and cheese from Northwest Argentina. *J Food Prot* 2001;64:559–63.
- [4] Diver S. Nature farming and effective microorganisms. Rhizosphere II: Publications; 2001. Resource Lists and Web Links from Steve Diver, <http://ncatark.uark.edu/~steved/Nature-Farm-EM.html>.
- [5] Simeamelak M, Solomon D, Taye T. The effect of effective microorganisms on production and quality performance of Rhode Island red layers. *Int J Livest Prod* 2013;4(2):22–9.
- [6] Haugh RR. The haugh unit for measuring egg quality. *U.S. Egg Poultry Magazine* 1937;43. 552–555 and 572–573, http://sizes.com/units/haugh_unit.htm. Accessed 14 November 2009.
- [7] Romanoff AL, Romanoff AJ. *The avian egg*. Moscow: Pishtepromizdat; 1959.
- [8] Bligh EG, Dyer W. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911.
- [9] Sperry W, Webb MA. A revision of the Schoenheimer-Sperry method for cholesterol determination. *J Biol Chem* 1950;187:97–101.
- [10] Khurshid A, Farooq M, Durrani FR, Sarbilan K, Chand N. Predicting egg weight shell weight, shell thickness and hatching chick weight of Japanese quails using various egg traits as regressors. *Int J Poult Sci* 2005;2:164–7.
- [11] Orji BI, Igodi C, Oyke PJ. The effects of pre-incubation storage embryonic growth of rate mortality, hatchability and total incubation period of fowl egg. *Niger J Agri Sci* 1998;3:99–103.
- [12] Donoghue DJ. Antibiotic residues in poultry tissues and eggs: human health concerns?. Symposium: use of antimicrobials in production; 2002. pp. 618–21.

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- [13] Francis C, Janky DM, Arafa AS, Harms RH. Inter relationship of *Lactobacillus* and zinc bacitracin in the diets of turkey poults. *Poult Sci* 1978;57:1687–9.
- [14] Xu CL, Ji C, Ma Q, Hao K, Jin Y, Li K. Effects of a dried *Bacillus subtilis* culture on egg quality. *Poult Sci Assoc* 2005;364–8.
- [15] Netherwood T, Gilbert HJ, Parker DS, Gilbert HJ. Probiotics shown to change bacterial community structure in the avian gastrointestinal tract. *Appl Environ Microbiol* 1999;65:5134–8.
- [16] Ehrmann MA, Kurzak P, Bauer J, Vogel RF. Characterization of *lactobacilli* towards their use as probiotic adjuncts in poultry. *J Appl Microbiol* 2002;92:966–75.
- [17] Balevi T, Ucan US, Coskun B, Kurtoglu V, Cetingul IS. Effect of dietary probiotic on performance and humoral immune response in layer hens. *Br Poult Sci* 2001;42:456–61.
- [18] Nahashon SN, Nakaue HS, Mirosh LW. Production variables and nutrient retention in single comb white leghorn laying pullets fed diets supplemented with direct fed microbials. *Poult Sci* 1994;73:1699–711.
- [19] Mahdavi AH, Rahmani HR, Pourreza J. Effect of probiotic supplements on egg quality and laying hen's performance. *Int J Poult Sci* 2005;4(7):488–92.
- [20] Mansoub NH. Evaluation of herbal plant on different parameters of laying hens. *Ann Biol Res* 2011;2(5):510–5.
- [21] Gilliland SE, Nelson CR, Maxwell C. Assimilation of cholesterol by *Lactobacillus acidophilus* bacteria. *Appl Environ Microbiol* 1985;49:377–81.
- [22] Kalavathy R, Abdullah N, Jalaludin S, Ho YW. Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *Br Poult Sci* 2003;49:139–44.
- [23] Fukushima M, Nakano M. The effect of a probiotics on faecal and liver lipid classes in rats. *Br J Nutr* 1995;73:701–10.