JWPR
Journal of World's Poultry Research

2021, Scienceline Publication

J. World Poult. Res. 11(3): 359-367, September 25, 2021

Research Paper, PII: S2322455X2100043-11

DOI: https://dx.doi.org/10.36380/jwpr.2021.43

Effect of Beneficial Microorganisms, Turmeric (*Curcuma Longa*), and Their Combination as Feed Additives on Fertility, Hatchability, and Chick Quality Parameters of White Leghorn Layers

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Received: 13 June 2021
Accepted: 04 August 2021

ABSTRACT

The use of probiotics, yeast, and other natural feed additives in poultry feeds has received a lot of attention in recent years. The increased public awareness and opposition to the use of antibiotics as a growth promoter has sparked a lot of interest. Therefore, this study was conducted to evaluate the effect of multi-strain effective microorganisms (EM), turmeric powder (TP), and their combination (EM-TP) on fertility, hatchability, and chick quality of White Leghorn layer chickens. A total of 144 White Leghorn hens aged 26 weeks were assigned into four treatments with three replications for each treatment (12 layer chickens and 2 cocks per replications). The treatments consisted of no additive or control (CTL), control + 0.5 ml/lit EM, control + 0.5% TP, and control + 0.25 ml/lit EM + 0.25% TP (EM-TP) which were arranged in a complete randomized design. There was no significant difference in embryonic mortality at different growth stages among treatments while the highest fertility was for EM. The lowest hatchability on fertile egg and total egg basis was observed in hens fed the control diet. Hatchability on the total egg basis for TP was lower than that of EM. The lowest average chick weight and length values were for the control treatment. The yield percentage for the control was lower than those fed a diet containing EM and a combination of EM and TP. There were no significant differences in the visual score of chick quality measurement among treatments. In conclusion, the use of EM and TP alone and its combination as an additive to the diet of White Leghorn layer chickens improved hatchability percentage, chick weight at hatch, and chick length. Further study is suggested to determine the optimum level of EM and TP inclusion in layer breeder diet to achieve the desired beneficial outcome on fertility, hatchability, and chick quality traits.

Keywords: Chick quality, Effective microorganism, Fertility, Hatchability, Turmeric

INTRODUCTION

In poultry production, a healthy and viable chick is not only an important welfare implication but also of economic importance for both hatcheries and poultry farmers (Cecilia, 2018). The value of quality chick is, therefore, of the worry for both hatcheries and producers. During incubation, maternal antibodies are given from the mother hen to the chick, and these antibodies protect the chick against infections during its early weeks of life. Then, anti-body is started to be produced by the layer breeds (Lawrence et al., 1981). The embryo can acquire the antibody of the mother through the egg. In the opposite of mammals, where antibodies are acquired directly from the milk of the mother, but in poultry, it has a two-step process of antibody transfer which is from the hen to the egg and from the egg to the embryo (Patterson et al., 1962). Antibodies found in the hen's serum and egg may predict how well the chick survives its first week of life. In order to improve the health of the chicks, researchers have focused on feed additives to replace antibiotics which could have a negative effect on animal's health and production, such as residue in the final products, development of bacterial resistance, and accumulation in poultry excretion with consequent environmental pollution (Edens, 2003).

Feed additives like prebiotics, probiotics, synbiotics, herbs, spices, and essential oils have been investigated as an alternative to antibiotics because of their antibacterial, antioxidant, digestive, and metabolic enhancing effects (Prakasita et al., 2019; Yuanita et al., 2019; Hussein et al.,

To cite this paper: Wakjira ChK, Zeleke NA, Abebe MG, and Abeshu AN (2021). Effect of Beneficial Microorganisms, Turmeric (*Curcuma Longa*), and Their Combination as Feed Additives on Fertility, Hatchability, and Chick Quality Parameters of White Leghorn Layers. *J. World Poult. Res.*, 11 (3): 359-367. DOI: https://dx.doi.org/10.36380/jwpr.2021.43

2020). These additives could improve the balance of intestinal microbial flora, reduce the population of pathogenic microorganisms, stimulate the immune system, enhance nutrient availability to the host, and reduce losses and poor performance due to stress (Toms and Powrie, 2001; Khan and Naz, 2013).

Another additive which could be used in poultry is beneficial microorganisms. The effective microorganism (EM) solution consists of a wide variety of effective, beneficial, and non-pathogenic micro-organisms of both aerobic and anaerobic types co-existing, having predominant populations of lactic acid bacteria and yeasts, and smaller numbers of photosynthetic bacteria, actinomycetes, and other types of organisms (Higa and Parr, 1994; Naqvi et al., 2000). It has been reported that multi-strain probiotics enhance performance more than single strain products (Balevi et al., 2001; Gardiner et al., 2004; Timmerman et al., 2004). Dietary supplementation of *Bacillus subtilis* (a single strain probiotic) exerts positive effects on production performance, improving intestinal health and systemic immunity in poultry (Lee et al., 2014; Hatab et al., 2016).

Turmeric (*Curcuma longa*) is also another feed additive which has nutritional and medical effects, such as anti-inflammatory, anti-microbial, antiprotozoal, antioxidant, and anti-aging in poultry (Amalraj et al., 2017). Studies have indicated that curcumin or turmeric supplementation improves meat quality and stability, liver enzyme activity, and immunological response (Daneshyar et al., 2011; Zhang et al., 2015), and semen quality (Yan et al., 2017) in broiler chickens. Moreover, Shashidhara and Devegowda (2003) found an increase in the percentage of fertile eggs and hatchability in broiler breeders with the feeding of 0.5 kg/ton mannan oligosaccharide, a prebiotic agent. On the other hand, Hidir et al. (2018) reported that the addition of turmeric at the level of 0.5% to laying hen diets has no change on final body weight, egg production, egg weight, and feed intake, compared to the control.

Regarding the combination of EM and TP, Moorthy et al. (2009) found high feed intake in broiler chickens fed probiotics and turmeric at a 1% inclusion level than the control. This was contrary to the findings of Al-Sultan (2003) and Durrani et al. (2006) who observed reduced feed intake when turmeric and probiotics were added to the diet of layer chickens. There are limited studies which investigated the effects of beneficial microorganisms and turmeric as the only additive or in combination on fertility, hatchability, and chick quality of layer chickens. Hence, the current investigation was performed to assess the impacts of EM, turmeric, and their combination as feed additives on fertility, hatchability, and chick quality of White Leghorn layer chicken breeds.

MATERIALS AND METHODS

Ethical approval

The layer hens were handled with respect to animal rights. The present study did not involve feeding of White Leghorn chicken breed with pathogenic microorganisms, introduction of any intervention in/on chickens, or direct collection of cells, tissues, or any material from chickens.

Study area

The experiment was conducted at Haramaya University poultry farm, which is located 515 km east of the capital, Addis Ababa, Ethiopia. The site is situated at an altitude of 2006 meters above sea level, 9^{o} 41' N latitude, and 42^{o} 4' E longitude (Kebede et al., 2015). The mean annual rainfall of the area is 790 mm and the annual mean temperature of 17^{o}C with mean minimum and maximum temperatures of 14 and 23.4^{o}C, respectively (Ambachew et al., 2016).

Treatments and ingredients used in diet formulation

Maize grain, wheat short, soybean meal, noug seed cake, turmeric, and salt were among the feed items used to make the diet in the current study. Vitamin premix, methionine, limestone, and dicalcium phosphate were also included in the diet (Table 1). Activated EM1 packed in a plastic jar was obtained from Weljijie PLC located in Bishoftu, Ethiopia. The EM preparations used in this study were made following the guidelines prepared by the EM research organization (Lindani and Brutsch, 2012)**.** This EM consists of high populations of lactic acid bacteria (*Lactobacillus* and *Pedicoccus*) at 1 × 10^{5} CFU/ml suspensions, yeast (Sacharomyces) at 2 × 10^{6} CFU/ml suspension, and fewer amounts of photosynthetic bacteria, actinomyces, and other organisms (Wood, 2002). The proposed amount of activated EM1 was added directly into chlorine-free clean drinking water. Turmeric was purchased from the local market and ground in the size of 5 mm by hammer mill and was mixed with the total ration. The treatments were no additive or control (CTL), control + 0.5 ml/lit effective microorganisms (EM), control + 0.5% turmeric powder (TP), and control + 0.25 ml/lit EM + 0.25% turmeric powder (EM-TP) which was arranged in a complete randomized design. The diet was formulated to be isocaloric (2800-2900 KCal/ME per kg DM) and

isonitrogenous (16-17% CP) to meet the nutrient requirements of the layer hen (NRC, 1994).

Table 1. The proportion of ingredients used in formulating experimental diets (DM basis)

Ingredients	Treatments			
	CTL	EM	TP	EM-TP
Maize (%)	46	46	46	46
Wheat bran (%)	15.5	15.5	15.5	15.5
DL-methionine (%)	0.01	0.01	0.01	0.01
Soybean meal (%)	13.39	13.39	13.39	13.39
Noug seed cake (%)	15	15	15	15
Vitamin premix (%)	1	1	1	1
Salt (%)	1	1	1	1
Limestone (%)	7	7	7	7
L-Lysine (%)	0.1	0.1	0.1	0.1
Dicalcium phosphate (%)	1	1	1	1
Total	100	100	100	100
Turmeric (%)	0	0	0.5	0.25
EM (ml/L)	0	0.5	0	0.25

CTL: Control, EM: Control + 0.5 ml/lit EM, TP: Control + 0.5% turmuric powder, EM-TP: Control + 0.25 ml/lit EM + 0.25% turmuric powder

Experimental animals and management

Before the commencement of the actual experiment, watering, feeding troughs, experimental house, and laying nests were thoroughly cleaned and disinfected with 25% hydrogen peroxide. The experimental pen was sprayed with hydrogen peroxide using Knapsack Sprayer against external parasites. A total of 168 White Leghorn layer chickens with a body weight of 1120 ± 62.30 gram at the age of 26 weeks was taken from Haramaya University Poultry Farm and randomly distributed to the four experimental diets replicated three times with12 hens and 2 cocks in each replication. The experiment lasted for 90 days with 7 days of adaptation to the experimental diet and house. The chickens were kept on deep litter floor housing, which was covered with sawdust litter of about 7 cm depth. Throughout the experiment, the house had typical daylighting (12L:12D). The chickens were fed twice a day, at 8:00 AM and 4:00 PM *ad libitum* (with ~20% refusal). Throughout the study, regular bio-security procedures were followed.

Data collection and analysis

Fertility and hatchability of eggs

Before incubation, the eggs for incubation were collected and held at 140°C for 5 days. Medium-sized eggs were selected by visual inspection and 30 eggs from each replication were set for incubation at the peak egg production period. Candling the incubated eggs on days 9, 14, and 18 of incubation assessed fertility (Bonnier and Kasper, 1990). The total number of fertile eggs detected during candling was divided by the total number of eggs laid multiplied by 100 to get the average percentage fertility.

$$\text{Fertility (\%)} = \frac{\text{Total fertile eggs}}{\text{Total eggs set}} \times 100$$

The average percentage hatchability of the fertile eggs was computed by dividing the number of chicks hatched by the number of fertile eggs set multiplied by 100 (Rashed, 2004; Fayeye et al., 2005).

$$\text{Hatchability as a percentage of fertile eggs set} = \frac{\text{Number of chicks hatched}}{\text{Total fertile eggs}} \times 100$$

$$\text{Hatchability as a percentage of total egg set} = \frac{\text{Number of chicks hatched}}{\text{Total eggs set}} \times 100$$

Embryonic mortality

Embryonic mortality was determined by breaking eggs that seemed to be mortal on the days of candling eggs at 9th, 14th, and 18th days of incubation and the last three days of hatching to determine early, mid, late, and piped embryonic mortalities, respectively (Bonnier and Kasper, 1990). The eggs that did not hatch were opened for visual observation and classified according to the time of embryonic mortality. The embryonic mortality was computed by dividing the number of dead embryos by the number of fertile eggs set and multiplied by 100 (Rashed, 2004). The formulas are given below:

$$\text{Mid mortality (\%)} = \frac{\text{Total number of an early dead embryo}}{\text{Total number of fertile eggs}} \times 100$$

$$\text{Mid mortality (\%)} = \frac{\text{Total number of a mid dead embryo}}{\text{Total number of fertile eggs}} \times 100$$

$$\text{Late mortality (\%)} = \frac{\text{Total number of a late dead embryo}}{\text{Total number of fertile eggs}} \times 100$$

$$\text{Pip mortality (\%)} = \frac{\text{Total number of pip dead embryo}}{\text{Total number of fertile eggs}} \times 100$$

Chick quality measurement

Chick quality is defined as chicks that have developed appropriately throughout incubation and have demonstrated good performance (Molenaar et al., 2008). Chick quality assessment was performed by employing the commonly used methods for chick quality assessment such as visual scoring, Tona or Pasgar scoring, chick length, yield percentage, and day-old chick weight. For visual

scoring chick's cleanness (free from adhering dried yolk, shell, and membrane), dryness with a completely sealed novel, no deformities (straight feet and legs with no lesion or swelling), and alertness was observed (Meijerhof, 2009). The percentage of quality chicks was calculated by expressing the number of quality chicks as a percentage of the total number of chicks hatched.

$$\text{Quality chick of the visual score (\%)} = \frac{\text{Total number of quality chicks}}{\text{Total number of hatched chicks}} \times 100$$

Tona or Pasgar scoring was done according to Molenaar et al. (2008) following a series of observations including good activity, clean and dry appearance, open and bright eyes, normal legs and toes, completely closed and clean novel, no remaining yolk and membrane. The length of a chick was measured by stretching the chick along a ruler from the beak to the end of the middle toe (Molenaar et al., 2008). Yield percentage was calculated as the percentage of chick weight to the initial egg weight x 100 (Tona et al., 2001). Moreover, chick weight was measured by weighing the whole day-old chick.

Statistical analysis

The data were analyzed with statistical analysis systems software using the general linear model approach (SAS, 2009). Differences between treatment means were separated using Duncan's multiple range tests. P value less than 0.05 was considered statistically significant. The model of $Y_{ijk} = \mu + T_i + E_{ij}$ was used. Where, Y_{ij} represents the j^{th} observation in the i^{th} treatment level, μ denotes the overall mean of a response variable T_i refers to the effect of i^{th} treatment in the response variable, and E_{ij} is error term.

RESULTS

Chemical composition of feeds

The chemical composition of feed ingredients used and the treatment diets are given in Table 2. The CP content of turmeric (8.63%) was lower than the other feed ingredient used except maize (8.45%) while ME (3852.38 kcal/kg) content was higher than the other feed ingredients.

Fertility, hatchability, and embryonic mortality

There was no significant difference ($p > 0.05$) among treatments in embryonic mortality at different growth stages (Table 3). The highest significant fertility was for EM ($p < 0.05$). The lowest) hatchability on fertile egg and total egg basis was observed in hens fed the control diet ($p < 0.05$. Hatchability on the total egg basis for TP was lower than that of EM ($p < 0.05$).

Chick quality measurement

The lowest average chick weight and length were for the control treatment ($p < 0.05$). The yield percentage for the control chicks was lower ($p < 0.05$) than those fed a diet containing EM and a combination of EM and TP. There were no significant differences in the visual score of chick quality measurement among treatments (Table 4).

Table 2. Chemical composition of feed ingredients and experimental diets for White Leghorn layers

Feed ingredients and treatment diets	Chemical composition							
	DM (%)	CP (% DM)	EE (% DM)	Ash (% DM)	CF (% DM)	Ca (% DM)	P (% DM)	ME (kcal/kg DM)
Feed ingredients								
Maize	90.5	8.45	4.28	4.73	2.97	0.03	0.83	3736
Wheat short	91	15	3.84	5.02	9.87	0.19	0.78	2980
Soybean meal	93.75	39.68	8.53	6.37	6.04	0.34	0.66	3617
Noug seedcake	93	30.8	7.84	9.38	18.5	0.33	0.32	2314
TP	89.37	8.63	3.99	4.15	1.65	0.28	0.15	3852
Treatments								
CTL	89.41	18.08	4.42	11.48	3.31	3.23	0.42	3429.47
EM	89.41	18.08	4.42	11.48	3.31	3.23	0.42	3429.47
TP	90.27	18.43	4.70	13.37	3.17	3.79	0.65	3380.00
EM-TP	89.46	18.65	4.46	13.36	3.2	3.02	0.17	3364.69

DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, Ca: Calcium, P: Phosphorus, ME: Metabolizable energy, EM: Effective microorganisms, TP: Turmeric powder, CTL: Control, EM: Control + 0.5 ml/lit EM, TP: Control + 0.5% TP, EM-TP: Control + 0.25 ml/lit EM + 0.25% TP

Table 3. Fertility, hatchability, and embryonic mortality of White Leghorn layer eggs fed diets containing effective microorganisms, turmeric powder and a combination of effective microorganisms and turmeric powder

Parameters	Treatments				SEM	SL
	CTL	EM	TP	EM-TP		
Fertility	91.67[b]	100[a]	86.67[b]	90.00[b]	1.86	0.006
Hatchability on fertile egg base	70.96[b]	93.33[a]	96.08[a]	94.43[a]	2.15	0.0001
Hatchability on total egg base	65.00[c]	93.33[a]	83.33[b]	85.00[ab]	2.76	0.0006
Embryonic mortality						
Early	1.67	-	3.33	1.67	0.71	0.49
Mid	-	-	1.67	-	0.42	0.44
Late	-	-	-	-	-	
Pip	1.67	-	-	-	0.42	0.44

[abc] Means within a row with different superscript letters differ significantly ($p < 0.05$). SEM: Standard error of mean, SL: Significance level, CTL: Control, EM: Control + 0.5 ml/lit EM, TP: Control + 0.5% TP; and EM-TP, Control + 0.25 ml/lit EM + 0.25% TP.

Table 4. Chick quality of White Leghorn chicken fed on diets containing effective microorganisms, turmeric powder, and a combination of effective microorganisms and turmeric powder

Parameters	Treatments				SEM	SL
	CTL	EM	TP	EM-TP		
Average chick weight (g)	31.59[b]	34.34[a]	34.16[a]	35.26[a]	0.48	0.004
Average chick length (cm)	15.17[b]	15.89[a]	16.01[a]	16.12[a]	0.18	0.021
Yield percentage	63.28[b]	67.83[a]	65.60[ab]	67.03[a]	0.92	0.035
Chick visual score (%)	94.87	93.75	100	96.30	1.52	0.56

[ab] Means within a row with different superscripts letters differ significantly ($p < 0.05$). SEM: Standard error of mean, SL: Significance level, CTL: Control, EM: Control + 0.5 ml/lit EM, TP: Control + 0.5% TP, EM-TP: Control +0.25 ml/lit EM + 0.25% TP

DISCUSSION

Fertility hatchability and embryonic mortality

The findings addressing fertility percentage in the current experiment for EM were in accordance with the finding of Shashidhara and Devegowda (2003) who reported an increase in the percentage of fertile egg and hatchability in broiler breeders with 0.5 kg/ton MOS, compared to the control. Similarly, the study of Liu et al. (2019) indicated a linear increase in fertility and hatchability of laying breeders with increasing levels of *Bacillus subtilis* C-3102 supplementation which was similar to the current EM group. Mazanko et al. (2018) reported that the hatchability of eggs was significantly improved by supplementation of diets with *Bacillus species* which is similar to the current finding. Wang et al. (2017) also reported that dietary supplementation with *Bacillus subtilis* (*B. subtilis*) has significantly increased gonadotropin-releasing hormone levels that induce the fertility of the male chickens. Also, Jeong and Kim (2014) reported that supplementation with 300 and 600 mg/kg *B. subtilis* C-3102 has improved growth performance and nutrient digestibility in broilers.

Radwan et al. (2008) suggested that turmeric powder has been shown to improve the uterine environment (particularly the location of calcium deposition) and, as a result, increase shell weight and thickness. Moreover, The addition of 0.5 or 1.0 percent turmeric to eggs boosted egg weight, egg mass, and egg production according to studies by Riasi (2012). In the current study, the improvement in hatching performance might be due to the use of effective microorganisms and turmeric that increase the secretion of reproductive hormones and enhancement of nutrient availability to the laying chickens as suggested by Lei et al. (2013) and Wang et al. (2017). Kinati et al. (2021) observed improvement in egg size due to the use of EM and a combination of EM and TP in White Leghorn layer chickens' diets. Since EM and TP increase layer chickens' digestion and nutrient absorption via the intestinal villi, they may result in higher nutritional deposition to the egg content, and consequently improved embryo development and health, compared to the control group.

Chick quality

The improvement in chick weight and length due to the feeding of EM, TP, and its combination as additive agree with the findings of Beyene et al. (2015) and Alemayehu (2012) who reported that chick length is directly correlated with chick weight. Similarly, other researchers have shown that egg weight is a dominant factor affecting chick weight at hatch (Bray and Iton, 1999; Silversides and Scott, 2001; Tona et al., 2003). Chicks with better yolk utilization develop more body mass during the incubation period, and therefore grew longer (Meijerhof, 2006). Petek et al. (2008) classified length intervals into short, middle, and long for day-old chicks.

According to Petek et al. (2008), layer chicks with a length of < 17.8, 17.8 - 18.2, and > 18.2, are grouped as short, medium, and long chicks, respectively. Based on this classification, the length of chicks in all treatments falls within the short category which might be associated with breed type (Wilson, 1991). Although the length of the chick was in a short category those which were fed with additives were longer and weighed more than the control which shows that EM and TP have a positive effect on the growth performance of chicks. It is reported that EM improves digestion, absorption, and availability of nutrition accompanied by positive effects on intestine activity and increasing digestive enzymes (Gilliland and Kim, 1984; Saarela et al., 2000). In contrary to the current result Kassu et al. (2017) indicated that when compared to the control, adding black cumin, fenugreek, and turmeric to the broiler has no significant influence on BW and BWG ($P > 0.05$).

Hatchability and chick quality at hatching are directly related to quality parameters of eggs, the better egg size, the better yolk, the better albumen, and better shell thickness resulting in best hatchability with best chick quality (King'ori, 2011). Yadgary and Uni (2012) noted that the developing embryo and the hatched chick are completely dependent on their growth and development on nutrients deposited in the egg. Berrin (2011) indicated that effective microorganism preparations, which are mono or mixed cultures of live, protective microorganisms beneficially affect the host animal by competing with other microorganisms for the adhesive site. Effective microorganisms stimulate appetite, improve the host's intestinal microbial balance and intestinal environment for processes of the digestion and absorption of nutrients (Fuller, 1989). Therefore, the use of EM and EM-TP resulted in better chick yield percentages compared with the control which might be associated with improvement in digestion, absorption, and availability of nutrients accompanied by positive effects on intestine activity and increasing digestive enzymes that increase the yield percentage (Gilliland and Kim, 1984; Saarela et al., 2000).

CONCLUSION

In conclusion, the use of EM and TP or a combination of EM and TP as an additive resulted in better hatchability, chick weight, chick length, and yield percentage, compared to the control. Further studies are suggested on EM and TP inclusion levels in the diet of layer breeders to achieve the desired outcome in fertility, hatchability, and chick quality traits.

DECLARATIONS

Authors' contribution

Chala Kinati Wakjira conceptualized and wrote the manuscript. Negasi Ameha Zeleke, Meseret Girma Abebe, and Ajebu Nurfeta Abeshu have critically revised the manuscript for important intellectual content and approved the final version of the manuscript for publication.

Competing interests

The authors have not declared any conflict of interest in the current research work.

Acknowledgments

The authors would like to thank the Ethiopian Ministry of Education, Ambo University, and Haramaya University Research Affairs Office for facilitating the working area to implement the research project. And much gratitude to the Ethiopian Institute of Agricultural Research for partial funding of the research. Special appreciation is to Haramaya University poultry farm and Animal nutrition laboratory workers for their kind support.

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 authors.

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