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Original Article

Influence of Effective Microorganism Supplementation to the Drinking Water on Performance and Some Blood Parameters of Laying Hens Exposed to A High Ambient Temperature

■Author(s)

Şahan Z[I] https://orcid.org/0000-0001-7878-5117
Kutay H[II] https://orcid.org/0000-0001-9163-4831
Çelik L[II] https://orcid.org/0000-0003-3352-9181

[I] Department of Veterinary Science, Kahta Vocational School, Adıyaman University, Adıyaman, Turkey.
[II] Department of Animal Science, Agricultural Faculty, Çukurova University, Adana, Turkey

■Mail Address

Corresponding author e-mail address
Zeynep Şahan
Department of Veterinary Sciences, Kahta Vocational School, Adiyaman University, Adiyaman, Turkey.
Phone: +90 416 725 81 50
Email: zysahan@gmail.com

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ABSTRACT

In the present study, seventy-two 30-week-old laying hens (Brown Hisex) were separated into two groups (control and treatment) and fed with a standard layer diet. In the treatment group the amount of effective microorganism (EM) added to the drinking water is equal to1000 ppm EM dose. Throughout the 8-week study period, 16 h thermoneutral (20-22 °C) and 8 h hot (35-37 °C) environmental temperature regime was applied daily. The results indicated that EM supplementation affected performance and some egg quality characteristics of layers exposed high environmental temperature. Feed intake and conversion ratio, yolk index, albumen index, yolk colour b, Haugh unit were decreased by supplemental EM. Serum glucose, cholesterol, triglycerides, calcium, total oxidant- antioxidant concentration were not affected by EM. It is concluded that EM supplementation in laying hens could have potential to improve feed intake and feed conversion ratio under high environmental temperature. However, supplemental EM with drinking water decreased some egg quality parameters.

INTRODUCTION

EMs are a mixed culture of microorganisms which could be consisted of yeasts, lactic acid bacteria, actinomycetes and photosynthetic bacteria. The EMs may show their effects via suppression of the growth of pathogenic bacteria and aid digestion (Higa & Parr, 1994). EM Technology was first used in Ryukyus University, Japan in 1968 and has been applied in various places in the world after the practices revealed positive results in the 1980s. In addition to its usage in increasing soil fertility (Higa & Parr, 1994) and plant production (Higa & Wididana, 1989), it has been commonly used in husbandry since the 1990s (Szymanski & Patterson, 2003). Using EM in poultry feed helps equilibrate microbial flora in the intestines, prevent intestinal pathogens to bind to epithelium and improve weight gain. EM usage in poultry feed resulted in improvement of meat and faeces quality, improvement of animal welfare, elimination of undesirable odour and reduction of toxic effects (Philips & Philips 1996; Sritoomma 1994; Sugiharto, 2018). Jwher *et al.* (2013) stated that EM application in broilers could have positive effects on performance, immunity and intestinal parameters, however extensive research should be carried out in order to comprehend the operating mechanism of EM. Safalaoh & Smith (1999) demonstrated that EM usage in broilers as an alternative to antibiotics displayed growth stimulant and hypocholesterolemic effect. Besides, increase in egg yield and improvement in egg weight up to 28.5% were detected. Chotisasitorn *et al.* (1997) identified that it was possible to improve egg production, specific gravity and feed conversion rate in laying hens by means of EM usage. It was found by Thach *et al.* (2014) that

egg production could be increased, and amount of abnormal eggs could be decreased at broiler breeder chickens by the use of EM. On the other hand, Botlhoko (1997) stated that EM usage in broiler feed and drink (50 ml/L) reduced the performance. In his second study, he found that addition of EM+antibiotics into feed of *Clostridium perfringes* inoculated broilers was more effective than EM application only. In addition, a study was conducted by Park *et al.* (2016) to examine the effect of probiotic (*Enterococcus faecium* DSM 7134) on production performance, feed intake, nutrient utilization, egg quality, excreta microflora and ammonia emission in laying hens. The study showed that, *E. faecium* probiotic supplementation become visible to have a useful effect in ISA brown laying hens and should be considered as a positive diet supplement to use in the poultry industry. While EM application in laying hens did not have an effect on performance, cholesterol concentration of egg yolk decreased, and egg protein percentage increased (El-Deep, 2011). One of the recent studies has shown that the dietary supplementation of *Bacillus species* has improved growth performance, composition of cecal bacterial, egg quality, and small intestinal morphology in laying hens (Yang *et al.,* 2020).

Birds are homoeothermic animals. It is known that broilers and laying hens decrease both their performance and product quality at high environmental temperature (Kutlu, 2001). This high temperature condition has been associated with the reduction of feed consumption in order to prevent body temperature increases. In egg production systems one of the major problems is severity of high environmental temperatures.

This study aims at reducing the negative effects of high ambient temperature on performance, egg yield, egg weight, egg quality of laying hens together with immune system problems, by means of EM application.

MATERIALS AND METHOD

The animal material of the study consisted of 72, 30-week-old brown laying hens. Before the trial period; the animals were fed ad libitum with a standard layer diet for a week called training period. During the period, daily egg yield and egg weight were recorded. Experimental animals were divided into two groups, 36 in each group (control and EM treatment), according to similar mean body weight and egg yield level. The experimental animals were held in individual layer cages of two-tier wire blocks in a complete randomized design. The diet in the experiment do not contain any additives and are basal feeds (Table 1-2) obtained from a commercial feed company. The treatment group received drinking water included (EM a solution form, 3×10^3 cfu; EM Agriton Ltd. İzmir, Turkey) at the dose of 1000 ml EM/ton water. Water containing EM to the EM group was performed fresh daily. Feed and water were given ad libitum during the 8-week experiment. The conventional ambient temperature (20-22 °C) with a relative humidity of 60-70%, except during heating period when the environmental temperature rise and fall from 35 to 37 °C with a relative humidity of 40-50% for 8 h per day (09:00-17:00 hours). During the experiment daylight was supplied for 16 hours (05:00 to 21:00 hours). The animal performance was calculated by daily measuring the feed intake, egg mass, feed conversion, pretrial and post-trial live weight. For estimate egg quality, egg-shape index (width/length), shell weight, shell thickness, shell breaking strength, egg weight, yolk weight, albumen weight, albumen height, yolk colour score (Hunter Lab) were measured weekly. Also (1), albumen index (2) and haugh unit (3) were calculated as follows.

Calculations:

yolk index = yolk height / yolk width ‘100

albumen index = height of the thick albumen / (length + width / 2) × 100

haugh unit = 100 log (thick albumen height + 7.57 - 1.7 egg weight 0.37

Shell samples were measured from the top, middle and bottom of the egg using a micrometer to evaluate egg thickness. Feed nutrient ingredients (dry matter, crude fat, crude ash, crude protein) analysis were made according to the Association of Official Analytical Chemists (AOAC 1998).

Table 1 – Contents of the diet.

Raw Material Composition	(%)
Maize	59.3
Soybean Meal (%48)	19.3
Sunflower Meal (%36)	8.2
Vegetable Oil	2.7
Salt	0.4
DCP	1.4
Marble Powder	8.3
DL-Methionine	0.1
Vitamin Premix [a]	0.2
Mineral Premix [b]	0.1
Total	100.0

[a] 2 kg of vitamin premix include: 6 10^6 IU vit A, 8 10^5 IU vit D3, 14 10^3 mg vit E, 1600 mg vit K3, 1250 mg vit B1, 2800 mg vit B2, 810^3 mg niacin, 410^3 mg Ca-D--pantothenate, 2000 mg vit B6, 6 mg vit B12, 400 mg folic acid, 18 mg d-biotin, 2 10^5 mg vit C, 510^4 mg choline chloride.

[b] Each kg of mineral premix include 8 10^4 mg Mn, 6 10^4 mg Fe, 610^4 mg Zn, 510^3 mg Cu, 200 mg Co, 10^3 mg I, 150 mg Se.

Table 2 – Nutrient contents (g/kg) and ME of the diet.

Nutrient Ingredients (%)	
Crude protein	17.00
Crude fat	5.10
Crude cellulose	3.70
Crude ash	12.00
Lysine	0.80
Methionine	0.45
Methionine + Cystine	0.75
Calcium	3.50
Available P	0.40
Sodium	0.18
ME (kcal/kg)	2750

Ten animals from each group were chosen at random and samples of blood were taken via vena brachialis at the beginning and at the end of experimental periods. The blood samples were centrifuged (Universal 320R, Hettich, Germany) at 4 °C and 5000 rpm for 10 minutes to obtain serum. Then obtained serums were kept at 20 °C for analysis of glucose, total cholesterol, triglycerides, calcium, Total Antioxidant Status (TAS), Total Oxidant Status (TOS). The serum samples were analysed in a private Science Veterinary Diagnostic and Analysis Laboratory (Istanbul-Turkey). Oxidative Stress Index (OSI) was calculated by dividing the value of TOS by TAS.

The data obtained from the experiment were subjected to t-test using SAS (1996).

RESULTS

The influence of effective microorganisms added into the drinking water of the laying hens raised under high temperature upon the performance is showed in Table 3.

Table 3 – Influence of effective microorganisms supplementation to the drinking water on performance of laying hens exposed to a high ambient temperature.

Parameters	Control Group	EM Group	*p*
Initial body weight (g/bird)	1712.00±25.55	1715.80±23.20	0.9127
Final body weight (g/bird)	1724.20±46.18	1753.94±49.72	0.6671
Egg yield (g/day)	60.72±0.51	60.55±0.57	0.8324
Average feed intake (gr/hen/56 day)	115.61±1.28	101.57±0.90	0.0001
Feed conversion ratio (FCR) (56 day)	1.91±0.02	1.68±0.02	0.0001

The results obtained in the experiment showed that adding in water EM had significant effects on feed intake and feed conversion ratio in laying hens. (p<0.001). The average feed intake and FCR values were 13.82% and 13.69% lower in EM group than in the control group. On the other hand, all other parameters of performance parameters were not affected (p>0.05) by added EM.

The influence of effective microorganisms (EM) added into drinking water of laying hens raised under high temperature upon egg yield and egg quality are shown in the Table 4-5.

As indicated in Table 4-5, EM supplementation affected egg yolk height, albumen height, yolk colour b*, yolk index, albumen index, Haugh unit of laying hens throughout this study. However, the other egg

Table 4 – The effect of EM added to the drinking water of laying hens exposed to high ambient temperature on egg quality.

Parameters	Control Group	EM Group	*p*
Egg weight (g)	61.34±0.62	60.72±0.64	0.4917
Egg width	44.37±0.17	44.22±0.19	0.5665
Egg length	55.05±0.32	55.22±0.24	0.6626
Egg yolk height	19.74±0.37	18.85±0.13	0.0259
Albumen height	10.55±0.24	9.60±0.24	0.0093
Egg yolk width	38.62±0.26	38.86±0.36	0.5958
Albumen width	64.33±0.38	65.40±0.95	0.3741
Albumen length	77.77±0.56	78.84±1.02	0.3771
Yolk weight	13.84±0.20	13.77±0.21	0.8232
Yolk index	51.14±0.95	48.54±0.42	0.0164
Yolk weight (%)	22.56±0.29	22.68±0.30	0.7754
Albumen weight	40.55±0.49	40.01±0.53	0.4649
Albumen weight (%)	66.09±0.29	65.87±0.35	0.6427
Albumen index	14.89±0.41	13.37±0.44	0.0173
Haugh unit	101.22±1.08	97.10±1.13	0.0135

Table 5 – The effect of EM added to the drinking water of laying hens exposed to high ambient temperature on egg quality.

Parameters	Control Group	EM Group	*p*
Shell weight	6.96±0.12	6.94±0.08	0.8915
Colour L	54.05±0.44	53.74±0.34	0.5703
Colour a	17.73±0.53	16.64±0.19	0.0563
Colour b	60.73±0.52	58.55±0.52	0.006
Shell thickness (%)	11.35±0.16	11.45±0.14	0.6479
Average Shell thickness	379.67±6.70	386.73±7.29	0.4828
Shape index	80.63±0.49	80.09±0.37	0.3656

quality parameters of laying hens were not affected by EM supplementation. Albumen height, albumen index and haugh unit values were 0.95%, 10.2% and 4.07% lower in EM group than in the control group.

The results regarding blood parameters (cholesterol, triglyceride glucose and total oxidant-antioxidant) of laying hens receiving EM with drinking water are summarized in Table 6.

Table 6 – The effect of EM added to the drinking water of laying hens exposed to high temperature on blood parameters.

Parameters	Control Group	EM Group	*p*
Initial Serum Glucose	116.50±15.18	120.20±13.03	0.8553
Final Serum Glucose	94.60±24.69	109.60±14.05	0.6011
Initial Serum Cholesterol	107.80±10.36	120.20±14.89	0.5028
Final Serum Cholesterol	88.20±14.81	103.13±9.76	0.4423
Initial Serum Triglycerides	914.00±59.50	966.50±68.22	0.5691
Final Serum Triglycerides	799.40±70.87	861.00±38.79	0.4431
Initial Serum Calcium	41.42±4.42	42.62±4.70	0.8545
Final Serum Calcium	38.58±4.02	38.37±3.79	0.9769
Initial Serum TAS [a]	79.53±15.12	49.68±12.25	0.1462
Final Serum TAS [a]	38.86±3.43	55.02±8.68	0.3088
Initial Serum TOS [b]	10.54±1.80	7.55±1.24	0.1878
Final Serum TOS [b]	5.44±0.64	8.76±1.68	0.2794
Initial Serum OSI [c]	0.15±0.01	0.19±0.03	0.1581
Final Serum OSI [c]	0.14±0.01	0.17±0.02	0.3623

[a] TAS: Total Antioxidant Status

[b] TOS: Total Oxidant Status

[c] OSI: Oxidative Stress Index

As shown in Table 6, it can be seen that there is no statistical difference between the groups in terms of blood parameters (glucose, total cholesterol, triglycerides, calcium, TAS, TOS).

DISCUSSION

In our study, it is convenient to explain the lower feed intake and feed conservation values of EM group in contrast to the control group by loss of appetite. It is also considered that EM may have affected the digestive system and the interaction of microorganisms in the EM group. Indeed, contrary to the results of this study, Gnanadesigan *et al.* (2014) in their study investigating the effect of EM on the performance and egg quality of laying hens, observed that all of the values were better in the EM group with regards to the control group. It is possible to see clearly by observing the difference of results in various studies of different methods and amounts that the method and amount of EM addition affect all of the parameters. Miles *et al.* (1981) explain this difference in EM studies based on two reasons. The first one, being of an extreme number of organisms in the digestive system can increase intestinal motility then altering nutrient availability for absorption at desired dots. The second one, population of other beneficial bacteria can be changed, thereby the coexistence of established microflora is disturbed. Regardless of the possible cause, birds fed with higher probiotic levels have a parabolic dose relationship (Francis *et al.* 1978).

Haugh unit is commonly used in measuring the albumen quality and an important criterion in identifying the freshness of the egg. Higher Haugh unit value (90 and above: excellent) means higher egg quality; therefore, in the study, a decrease in Haugh unit in the group that added EM to the drinking water means a decrease in egg quality.

This is probably the result of not being able to form a synergy between the microorganisms in EM used and the intestinal flora system of the hens, which, in turn, is related to the type of microorganisms used both in terms of amount and probiotics. Studies with positive as well as negative results in EM studies also support this explanation. (Francis *et al.,* 1978; Naqvi *et al.,* 2000; Xu *et al.,* 2006; Yousefi & Karkoodi, 2007). Contrary to our study Simeamelak *et al.* (2013) reported using 4 to 12 ml of EM/liter of drinking water in laying hens and obtained significant development in egg quality (Haugh unit, yolk and albumen height).

Blood glucose concentration was used as a measure of the nutritional status of laying hens. In this study blood glucose concentration did not affect the hens that received 1000 ppm EM with drinking water. Our serum glucose results disagree with (Abd 2014) who indicated that administration of EM produced a significant increase in glucose levels of broilers. He suggests that the increase of glucose levels may be due to the effect of EM on gluconeogenesis and may be due to the lowering effect of EM on insulin secretions from the pancreas.

The Haugh unit, egg yolk and egg albumin height values obtained from the control group were significantly lower than the EM group ($p<0.05$). Daniele *et al.* (2008) reported that the use of probiotics in laying hens causes increase in the Haugh units ($p<0.05$) which is consistent with our results.

There was no significant difference between groups in serum calcium levels. The results of this study are consistent with the study reported that EM did not affect calcium utilization in feed for production (Chotisasitorn, 1997) Whereas Nagvi *et al.* (2000) reported a significant decrease in serum calcium concentration with addition of EM in layer feeds.

EM is live microbial feed supplement which have beneficial influence on serum total cholesterol and triglycerides. Abd (2014) found that EM addition may have some useful effect by lowering serum cholesterol and triglycerides concentrations in broiler. Esatu *et al.* (2011) reported that EM supplementation resulted in birds with low serum cholesterol levels. Bile acids are derivatives of cholesterol synthesized in the hepatocyte. Adherence of deconjugated free bile acids to EM enhances excretion of the bile acids. This mechanism has played a role in regulating liver cholesterol synthesis and subsequent conversion to bile acids, which may be responsible for lowering serum cholesterol levels (Safalaoh & Smith, 1999). However, Chatsawang & Watchangkul (1997) observed no significant improvements on serum cholesterol in response to the supplemental dietary EM as in the current study.

Effective Microorganisms produce polysaccharides, chelated minerals with catalytic activity as well as limited quantities of vitamins C and E. Parameters of TAS and TOS has been displayed as a tendency to increase and that of OSI as a tendency to decreased compared to their initial value in EM supplemented group compared with the control group. The beneficial effects of EM in increasing antioxidant system in poultry have been reported. Moon *et al.* (2010) reported that EM are capable of producing the antioxidant activity. In the current study, although not statistically significant, EM tended to show antioxidant activity. However, there aren't enough studies available for comparison.

CONCLUSIONS

It can be concluded that the additional EM provided in drinking water may have the potential to improve feed intake and feed conversion rate in laying hens exposed to high ambient temperature. However, additional EM to drinking water has reduced some egg quality parameters. Therefore, before the use for poultry industry, more investigations are required to determine the effect of EM on animal performance and economic aspects of the supplementation

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