Influence of Effective Microorganisms on the Quality of Poultry Products

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Abstract

Experimental results from 4 different types of poultry were summarized to reveal the application of EM for production of quality and healthy animal products. In low production animal as Muscovy duck, a battery cage trial was conducted. Results of 16 week experiment showed that EM added in feed and/or in drinking water had no significant effect on production performance but significantly increased breast percentage, significantly reduced breast ash content and tended to increase protein content of breast meat and polyunsaturated fatty acids content in duck oil.

In high production animal, Arbor Acres broiler chickens were used to evaluate the effect of EM. Results of 7 weeks experiment showed that EM added in feed and/or in drinking water had no effect on production and carcass characteristics but tended to decrease ash content of breast meat. Other quality traits included in the study were fatty acid and cholesterol composition, E. coli and Salmonella contamination. In laying chickens, EM added in feed had no significant influence on all traits studied except for such an important quality trait as yolk colour. Layers received EM in feed gave darker colour of yolk. Similar results were also obtained in a 12 week experiment in Japanese quail.

Introduction

Meat quality has assumed a greater significance and public attention. There is a strong demand for meat products that are high in lean content, low in fat, cholesterol and calories. Other concerns are about food safety and hygiene, especially the presence of pathogenic microorganisms and chemical residues. In poultry production, numerous reports in the literature can be found pertaining to the uses of probiotics or direct-fed microbial (DFM) bacteria, the addition of useful microorganisms cultures to diet or drinking water, to improve production performance and quality of products. Tortuero (1973) reported that cecal and intestinal microflora populations were changed when *Lactobacillus acidophilus* was fed to Leghorn and broiler chicks. Francies et al. (1978) reported that the addition of a culture of Lactobacilli or zinc bacitracin to the diet of Broad Breasted Large White Turkey poults resulted in numerical improvements in body weight and feed efficiency. Using other useful groups of microorganisms, Kobayashi and Tchan (1973) and Kobayashi and Kurata (1978) reported the successful uses of photosynthetic bacteria as probiotics. Recent studies have shown that large mixtures of microorganisms can be used as DFM with superior results to single bacteria strain or simple mixtures of few types of bacteria (Mead and Impey, 1986; Stavric et al., 1991). The success of complex mixtures appears to be due to interactive effects of anaerobes and facultative anaerobes (Mead and Impey, 1986; Stavric et al. 1991). As a mixed culture of beneficial and naturally occurring microorganisms, Effective Microorganisms (EM) contain selected species of microorganisms including predominant populations of lactic acid bacteria and yeasts and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms. The present experiments were conducted to evaluate the effects of direct fed EM on production and quality of poultry products.

Materials and Methods

Four experiments were conducted in 4 different types of poultry. The first experiment was carried out with a low productive poultry, the Muscovy ducks, the second was done with a high productive commercial broiler chickens while the third and fourth used commercial layer chickens and Japanese quail, respectively. All experiments were carried out suing the facilities available at the Animal Research Farm, Animal Science Department, Kasetsart University, Bangkok.

Experiment 1

A total of 144 day-old Muscovy ducks (72 males and 72 females) were assigned to Randomized Complete Block Design consisting of 4 treatments each with 6 replicate pens. Sexes of ducks were used as experimental blocks. The 3 ducks constituting each replicate were housed together in a battery cage of size 0.86 x 0.61 x 0.45 meters. All experimental ducks received the diet containing 19.56 percent crude protein during starting period (0-4 weeks of age) and 17.19 percent crude protein during growing-finishing period (4-16 weeks of age). Feed and water were available *ad libitum* for all ducks throughout the entire 16 week experimental period. The four experimental treatments were as follows:

Treatment 1: control treatment

Treatment 2: addition of EM in drinking water at 1:5,000 during starting period and 1:3,000 during growing-finishing period

Treatment 3: addition of EM (in form of Bokashi) in feed at 1:100

Treatment 4: addition of EM in drinking water as in Treatment 2 and addition of EM as in Treatment 3.

The ducks were individually weighed and pen consumption was measured at 2 week intervals throughout the experiments, one duck from each replicate cage was randomly slaughtered for carcass measurements.

Resulted data were analyzed using the General Lineal Models procedure of SAS software, Significant differences between treatments were detected using Duncan's multiple range test. Significance were based on the probability level of 0.05.

Experiment 2

Two hundred and eighty eight day-old Arbor Acres (CP707) broiler chickens of both sexes were randomly assigned to 24 litter pens (0.86 x 1.22 x 0.45 meter, 12 chickens per pen). There were 4 experimental treatments with 6 replicates (pens) each. The 4 treatments used in this experiment were as follows :

Treatment 1: control treatment

Treatment 2: addition of EM in drinking water at 1:5,000

Treatment 3: addition of EM (in form of Bokashi) in feed at 1:100

Treatment 4: addition of EM in drinking water at 1:5,000 and in feed at 1:100

Feed and water available *ad libitum* for consumption during the entire 7 week experimental period. The calculated nutrients per kilogram were 3,045 kcalME and 22 percent CP for diet used during growing period (0-4 weeks of age) and 3, 161 kcalME and 20 percent CP for diet used during finishing period (4-7 weeks of age). At the end of experiment, 2 birds per replicate pen were randomly slaughtered for carcass evaluation. Breast meat samples were taken and examined for nutritional values and microbial (*E. coli* and *Salmonella*) contamination. Fatty acid compositions and cholesterol contents were analyzed using samples of abdominal fat taken from the slaughtered chickens. Statistical analyses of data were obtained using the same procedure as in Experiment 1.

Experiment 3

Eighty four 10 month old Isa Brown layers were randomly allocated to 3 experimental treatments. Each treatment had 4 replicates of 7 birds. The birds received a commercial layer diet (protein $\geq$ 29.5 percent, fat $\geq$ 1 percent, crude fiber $\leq$ 1 percent and moisture $\leq$ 13 percent) and they were housed in individual cages. All experimental chickens were allowed to consume diets and water *ad libitum* throughout the entire three 28 day experimental periods. The 3 treatments used in the study were as follows:

Treatment 1: control treatment

Treatment 2: addition of EM (in form of Bokashi) in feed at 0.5 percent

Treatment 3: addition of (EM in form of Bokashi) in feed at 1 percent

Egg production was recorded on hen-day production basis. Two eggs from each replicate were randomly sampled on the last 3 days of each experimental period, and were used for egg quality measurements. At the second period of experiment, two eggs were randomly collected from each replicate and used in the study of *E. coli* and *Salmonella* contamination (in albumen of egg). Two eggs were randomly collected from the last period of experiment for analyzing fatty acid composition and cholesterol content of egg yolk. Statistical analyses of data were obtained using the procedure mentioned earlier.

Experiment 4

The experiment divided into 2 periods. In growing period (1 day – 4 weeks of age) 1,000 day old Japanese quail were assigned to Completely Randomized Design consisting of 4 treatments each with 5 replicate pens of 50 chicks per pen. Each pen had 90x45x30 cm^3 space. Supplemental heat was provided to each pen by 100 w electric bulb throughout the day. Feed and water were available *ad libitum* during the entire 4 week growing period. All birds received a growing diet containing 26 percent protein. In the first treatment (served as control), the birds were fed the unsupplemented growing diet. In the second, third and fourth treatments, the birds were fed the same growing diet supplemented respectively with EM in drinking water at the level of 1:5,000, with EM in feed in form of fermented compost or Bokashi at the level of 1:100 and with EM both in drinking water at 1:5,000 and in feed at 1:100. Body weight gain and feed conversion were calculated at the end of 2 and 4 weeks. Mortality was recorded daily.

In laying period (4 weeks – 12 weeks of age), 400 pullet quail randomly selected from earlier study were used applying the same design and treatments. The diet used in this period was laying diet containing 24 percent protein. The birds were maintained in 90x45x25 cm^3 pens each containing 20 pullet quail. Feed and water were supplied *ad libitum*. Body weight, feed consumption, mortality, egg weight and hen-day egg production including number of days to first egg, 50 percent and 80 percent egg production were recorded. For egg quality traits, the eggs were collected on the last 3 days of the 8 and 12 weeks of the experiment, and 5 from each group were measured for eggshell thickness and were graded for yolk color using a ROCHE Yolk Color Fan (numbered from 1 to 15 with increasing degrees of color from light yellow to orange).

Results and Discussion

Experiment 1

Summary of production performance and carcass quality of Muscovy ducks received control and EM supplemented groups are presented in Table 1 and Table 2, respectively. Experimental results related to the effects of supplementation of EM in drinking water and/or in feed on nutritional values of duck meat and fatty acid composition including cholesterol content of duck oil are summarized in Table 3 and Table 4, respectively.

Results of the experiment with reference to duck production performance and carcass quality (as shown in Tables 1 and 2) indicated that direct-fed EM (by adding in drinking water and/or in feed) had no significant effects on growth, feed consumed, feed conversion and mortality rate and all carcass quality traits studied expect breast percentage. Ducks received EM supplemented in drinking water and feed yielded higher percentage of breast than of the control group (the values for the 3 EM supplemented groups were 24.08, 24.65 and 24.13 percent compared to 22.46 percent of the control group). Sexes were found to influence significantly on growth, feed consumed and feed conversion of Muscovy ducks aged 4 weeks up to 16 weeks. For carcass characteristics, sexes had significant effects on eviscerate weight, liver, gizzard, breast and abdominal fat percentage.

In relation to the nutritional qualities of duck meat and oil, it was found in this study that EM supplemented in drinking water and/or in feed significantly decreased ash content of breast meat (the values of the 3 EM supplemented groups were 4.65, 4.69 and 4.51 percent compared to 5.03 percent of the control group) and tended to increase the content of crude protein in breast meat (the values for the 3 EM supplemented groups were 80.45, 83.42 and 82.87 percent compared to 79.62 percent of the control group, the differences were not significant statistically).

For fatty acid composition and cholesterol content in duck oil, test results of statistical analyses showed that the effect of EM supplementation were not significant on content of saturated fatty acids and were not consistent on contents of monounsaturated cholesterol, but tended to increase polyunsaturated fatty acid content.

Table 1.Effects of Sexes and EM Supplementation on Duck Performance During 0-4, 0-8, 0-12 and 0-16 Weeks of Age

	Control	EM Supplementation			
		Water	Feed	Water & Feed	Mean
Initial weight (kg)					
Male	0.066	0.069	0.067	0.068	0.068
Female	0.065	0.066	0.066	0.064	0.065
Mean	0.065	0.068	0.067	0.066	
0-4 weeks of age					
Weight gain (kg)					
Male	0.73	0.75	0.75	0.73	0.74*
Female	0.59	0.65	0.60	0.64	0.62
Mean	0.66	0.70	0.67	0.68	
Feed Conversion					
Male	2.11	2.03	2.07	2.16	2.09*
Female	2.17	2.14	2.28	2.14	2.18
Mean	2.14	2.08	2.18	2.15	
Mortality (%)					
Male	0.00	0.00	0.00	0.00	0.00
Female	5.56	0.00	0.00	0.00	2.78
Mean	2.78	0.00	2.78	0.00	
0-8 weeks of age					
Weight gain (kg)					
Male	2.17	2.19	2.18	2.16	2.17*
Female	1.60	1.63	1.54	1.58	1.59
Mean	1.88	1.91	1.86	1.87	
Feed Conversion					
Male	2.42	2.39	2.43	2.45	2.43
Female	2.74	2.85	2.92	2.78	2.82*
Mean	2.58	2.62	2.68	2.62	
Mortality (%)					
Male	0.00	0.00	0.00	0.00	0.00
Female	5.56	0.00	5.56	0.00	2.78
Mean	2.78	0.00	2.78	0.00	
0.12 weeks of age					
Weight gain (kg)					
Male	2.91	2.91	2.85	2.84	2.88*
Female	1.70	1.79	1.75	1.71	1.74
Mean	2.31	2.35	2.30	2.27	
Feed conversion					
Male	3.23	3.15	3.22	3.23	3.21
Female	4.11	4.23	4.15	4.13	4.16*
Mean	3.67	3.69	3.68	3.68	
Mortality					
Male	0.00	0.00	0.00	0.00	0.00
Female	5.56	0.00	5.56	0.00	2.78
Mean	2.78	0.00	2.78	0.00	
0-16 weeks of age					
Weight gain (Kg)					
Male	3.00	3.05	3.00	2.95	3.00*
Female	1.86	1.94	1.74	1.88	1.86
Mean	2.43	2.49	2.37	2.42	
Feed Conversion					
Male	3.94	3.82	4.05	4.11	3.98
Female	4.96	5.04	5.08	5.07	5.04*
Mean	4.45	4.43	4.56	4.59	
Mortality (%)					
Male	0.00	0.00	0.00	0.00	0.00
Female	5.56	5.50	5.56	0.00	4.15*
Mean	2.78	2.75	2.78	0.00	

* Significant difference at 5%

Table 2. Effect of Sexes and EM Supplementation on Duck Carcass Quality

	Control	EM Supplementation			
		Water	Feed	Water & Feed	Mean
Number of ducks					
Male	6	6	6	6	
Female	6	6	6	6	
Live weight (gm)					
Male	2,962.50	3,069.50	2,912.50	3,114.67	3,014.79*
Female	1,855.67	1,945.67	1,851.50	1,960.50	1,903.33
Mean	2,409.08[bc]	2,507.58[ab]	2,382.00[c]	2,537.58[a]	
Carcass weight (gm)					
Male	2,420.17	2,485.17	2,374.83	2,512.08	2,448.06*
Female	1,495.92	1,590.50	1,476.33	1,569.75	1,533.13
Mean	1,958.04[ab]	2,037.83[a]	1,925.58[b]	2,040.92[a]	
Dressed weight (gm)[1]					
Male	91.83	91.15	91.80	91.81	91.40
Female	92.39	91.95	91.90	92.27	92.12
Mean	92.11	91.55	91.85	91.54	
Eviscerated weight (%)[2]					
Male	88.98	88.79	88.80	88.83	88.85*
Female	87.36	89.00	86.81	87.08	87.56
Mean	88.17	88.89	87.95	87.80	
Liver (%)[2]					
Male	2.10	2.02	2.09	1.99	2.05
Female	2.33	2.18	2.14	2.17	2.20*
Mean	2.21	2.10	2.12	2.08	
Gizzard (%)[2]					
Male	1.92	2.00	1.96	1.97	1.96
Female	2.45	2.40	2.58	2.47	2.48*
Mean	2.18	2.20	2.27	2.22	
Breast (%)[3]					
Male	22.18	23.51	23.77	23.39	23.21
Female	22.14	24.65	25.53	24.86	24.45*
Mean	22.46[b]	24.08[a]	24.65[a]	24.13[a]	
Thigh+Drumstick (%)[3]					
Male	20.55	21.22	19.89	20.33	20.50
Female	20.72	21.33	20.09	19.76	20.47
Mean	20.63[ab]	21.28[a]	19.99[b]	20.05[b]	
Abdomen fat (%)[3]					
Male	1.60	2.02	1.30	1.80	1.68
Female	2.81	3.48	2.29	2.67	2.81*
Mean	2.20[ab]	2.75[a]	1.80[b]	2.23[ab]	

[1] % of live weight
[2] % of dressed weight
[3] % of carcass weight
Means in the same row with different superscripts were significantly different at P<0.05

Table 3. Effect of Sexes and EM Supplementation on Nutritive Values of Duck Meat (breast)

	Control	EM Supplementation		
		Water	Feed	Feed & Water
Number of Ducks	4	4	4	4
Dry matter (%)	26.23	23.36	26.83	27.45
Crude protein (%)	79.62	80.45	83.42	82.17
Crude fat (%)	9.32	9.67	8.16	12.71
Ash (%)	5.03a	4.65b	4.69b	4.51b

Means in the same row with different superscripts were significantly different at P<0.05

Table 4. Effect of EM Supplementation on Fatty Acid Composition and Cholesterol Content of Duck Oil

	Control	EM Supplementation		
		Water	Feed	Feed & Water
Saturated fatty acid				
Myristic (C14:0)	0.67	0.68	0.67	0.75
Palmitic (C16:0)	23.92	24.53	22.91	24.54
Stearic (C18:0)	4.29	4.40	4.55	4.56
Arachidic (C20:0)	0.55	0.51	0.55	0.51
Total	29.42	30.12	28.68	30.35
Monounsaturated fatty acid				
Palmitoleic (C16:1)	3.88^{ab}	3.91^{ab}	3.16^{b}	4.03^{a}
Oleic (C18:1)	50.57	49.13	49.32	49.10
Total	54.45	53.04	52.48	53.13
Polyunsaturated fatty acid				
Linoleic (C18:2)	15.56^{b}	16.18^{b}	18.21^{a}	18.56^{b}
Linolenic (C18:3)	0.57	0.66	0.63	0.65
Total	16.13^{b}	16.84^{b}	18.84^{b}	16.52^{b}
Cholesterol (mg/100g)	61.56^{b}	50.96^{a}	82.24^{b}	55.22^{ab}

Means in the same row with different superscripts were significantly different at P<0.05

Experiment 2

Production performance and carcass characteristics of broiler chickens as affected by EM supplementation are summarized in Tables 5 and 6, respectively. Experimental results pertaining to the effects of EM supplementation are presented in Table 7 for meat quality traits, Table 8 for fatty acid composition and cholesterol content and Table 9 for microbial quality of meat.

Results of the present 7 week experiment revealed that there were no significant effects of EM supplemented in drinking water and/or feed on all of the studied production performance and carcass characteristics of broiler chickens. For the qualities of broiler meat and fat, the effects of direct-fed EM were not significant on crude fat percentage and content of saturated fatty acids and were not consistent on crude protein percentage, monosaturated and polyunsaturated fatty acids (Tables 7 and 8). Ash content in breast meat, on the other hand, the values of the 3 EM supplemented groups were consistently lower than that of the control group (4.26, 4.24 and 4.19 percent compared to 4.35 percent). However, the only significant difference was found between EM supplemented both in drinking water and feed group and the control group. In the study of microbial quality of broiler meat, contaminations of *E. coli* were observed in all treatment groups while the contaminations of *Salmonella* were observed in 2 EM supplemented groups.

Table 5. Production Performance of Broilers as Affected by EM Supplementation in Drinking Water and Feed

	Control	EM Supplementation		
		Water	Feed	Feed & Water
Initial weight (kg)	0.04	0.04	0.0	0.04
0-2 weeks of age				
Weight gain (kg)	0.36	0.36	0.37	0.35
Feed intake (kg)	0.52	0.53	0.54	0.55
Feed conversion	1.45^{b}	1.45^{b}	1.47^{ab}	1.55^{a}
Mortality (%)	0.00	2.78	1.39	0.00
0-4 weeks of age				
Weight gain (kg)	0.94^{ab}	0.91^{b}	0.95^{a}	0.93^{ab}
Feed intake (kg)	1.73	1.77	1.79	1.77
Feed conversion	1.85^{b}	1.96^{a}	1.88^{b}	1.90^{ab}
Mortality (%)	0.00	2.78	2.78	0.00
0-6 weeks of age				
Weight gain (kg)	1.58	1.55	1.58	1.58
Feed intake (kg)	3.09	3.18	3.18	3.20
Feed conversion	1.96	2.05	2.02	2.03
Mortality (%)	1.39	3.94	5.55	1.39
0-7 weeks of age				
Weight gain (kg)	1.83	1.73	1.86	1.74
Feed intake (kg)	3.80	3.71	3.92	3.88
Feed conversion	2.07^{b}	2.21^{ab}	2.10^{ab}	2.24^{a}
Mortality (%)	5.55^{b}	8.33^{ab}	13.89^{a}	2.78^{b}

Means in the same row with different superscripts were significantly different at $P<0.05$

Table 6. Carcass Characteristics of Broilers as Affected by EM Supplementation in Drinking Water and Feed

	Control	EM Supplementation		
		Water	Feed	Feed & Water
Number of chickens	12	12	12	12
Live weight (gm)	1,909.17	1,952.50	2,021.67	1,955.00
Carcass weight (gm)	1,569.25	1,606.79	1,650.13	1,595.96
Dressed weight (%)[1]	87.83	87.07	87.17	86.77
Eviscerated weight (%)[2]	93.59	94.44	93.63	94.07
Liver (%)[2]	2.74	2.39	2.65	2.67
Gizzards (%)[2]	1.87	1.99	2.03	2.15
Breast (%)[3]	14.76	14.63	14.35	13.81
Thigh + Drumstick (%)[3]	29.86	30.87	29.83	29.89
Abdomen fat (%)[3]	1.88	1.98	2.38	2.02

[1] % of live weight
[2] % of dressed weight
[3] % of Carcass weight

Table 7. Nutritive Values of Broilers Meat (breast) as Affected by EM Supplementation in Drinking Water and Feed

	Control	EM Supplementation		
		Water	Feed	Feed & Water
Number of Chickens	4	4	4	4
Moisture (%)	74.29	73.92	74.13	74.00
Crude protein (%)	52.31[ab]	53.24[a]	52.44[ab]	51.32[b]
Crude fat (%)	2.90	2.75	2.58	2.06
Ash (%)	4.35[a]	4.26[ab]	4.24[ab]	4.19[b]

Means in the same row with different superscripts were significantly different at $P<0.05$

Table 8. Effects of EM supplementation on Fatty Acid Composition and Cholesterol Content of Broiler Adipose Tissue

	Control	EM Supplementation		
		Water	Feed	Feed & Water
Saturated fatty acid				
Lauric (C12:0)	0.248	0.223	0.223	0.223
Myristic (C14:0)	0.905	0.933	0.973	0.938
Palmitic (C16:0)	27.910[a]	26.437[b]	27.612[ab]	27.595[ab]
Stearic (C18:0)	5.415	4.980	5.220	5.305
Total	34.478	32.575	34.030	31.315
Monounsaturated fatty acid				
Palmitoleic (C16:1)	6.622	6.812	6.867	6.838
Oleic (C18:1)	42.830[b]	45.443[a]	41.273[b]	42.578[b]
Eicosamonoenoic (C20:1)	0.350	0.308	0.408	0.413
Total	49.802[ab]	52.560[a]	48.548b	49.828[ab]
Polyunsaturated fatty acid				
Linoleic (C18:2)	14.945[ab]	14.077[b]	16.300[a]	15.053[ab]
Linolenic (C18:3)	0.593[b]	0.558[b]	0.798[a]	0.720[ab]
Arachiconic (C20:4)	0.177	0.228	0.325	0.335
Total	15.715[ab]	14.863[b]	17.422[a]	16.107[ab]
Cholesterol (mg/100 g)	46.480	50.703	49.908	45.295

Means in the same row with different superscripts were significantly different at $P<0.05$

Table 9. Contamination of *E. coli* and *Salmonella* in Broilers Meat (breast) as Affected by EM Supplementation in Drinking Water and feed

	Control	EM Supplementation		
		Water	Feed	Feed & Water
Number of samples	8	8	8	8
E coli (MPN/gm)	183.50	235.80	22.35	91.81
Salmonella (in 25 gm)	Negative	12.5% Positive	Negative	12.5% Positive

Experiment 3

Experimental results with reference to the effects of direct-fed EM (by adding in feed) are summarized in Table 10 for production performance, Table 11 for egg quality trait, Table 12 for fatty acid profile and cholesterol content of egg yolk and Table 13 for microbial quality of egg.

In production performance, no significant differences statistically among the treatment means were found in egg production, feed per dozen eggs and mortality. However, consistently higher in egg production means were observed in the three 28 day periods of study for layers that received EM supplemented in feed. In egg quality traits, no significant differences wee found in egg weight, percent yolk, Haugh unit, percent albumen, shell weight, shell thickness and specific gravity. However, consistently higher in egg weight and lower in shell weight were also observed in the three 28-day studied periods for the 2 EM supplemented groups. Significant effect of EM was found in yolk color trait. Layers received EM supplemented in feed gave darker color of yolk (the mean values were 11.07 and 11.27 for the 2 EM supplemented groups compared to 9.56 of the control group). In relation to the nutritional qualities of egg, the effects of EM supplemented in feed were not significant on saturated fatty acids, cholesterol contents and total content of unsaturated fatty acids. When the content of unsaturated fatty acids was subdivided, effects of EM supplementation were found to decrease polyunsaturated fatty acids but increase monosaturated fatty acids.

Table 10. Layer Production Performance as Affected by EM Supplementation in Feed

	Control	**0.5% EM**	**1 % EM**
Egg production (%)			
Period 1	65.92	74.36	70.66
Period 2	71.81	76.53	76.43
Period 3	61.86	76.61	68.62
Mean	67.73	75.83	71.91
Feed intake (gm/bird/day)			
Period 1	90.12	90.71	91.01
Period 2	103.19^{b}	111.74^{a}	$108/29^{ab}$
Period 3	96.30^{b}	112.68^{a}	98.34^{b}
Mean	96.54^{b}	105.04^{a}	99.21^{b}
Feed /12 eggs (kg)			
Period 1	1.56	1.47	1.58
Period 2	1.75	1.76	1.72
Period 3	2.11	1.83	1.73
Mean	1.81	1.69	1.68
Mortality (%)			
Period 1	0.00	0.00	0.00
Period 2	0.00	0.00	0.00
Period 3	0.00	7.14	0.00
Mean	0.00	2.38	0.00

Means in the same row with different superscripts were significantly different at $P<0.05$

Table 11. Egg Qualities as Affected by EM Supplementation in Feed

	Control	0.5% EM	1 % EM
Egg weight (gm)			
Period 1	56.54	57.85	60.00
Period 2	58.55	59.17	59.76
Period 3	59.55	59.93	61.00
Mean	58.21	58.98	60.25
% Yolk			
Period 1	24.73	24.39	25.10
Period 2	25.15	25.63	25.61
Period 3	24.98	24.93	25.13
Mean	24.95	24.98	25.13
Yolk colour			
Period 1	9.50 [b]	11.28[a]	11.06 [a]
Period 2	6.69 [b]	11.06 [a]	11.38 [a]
Period 3	9.50 [b]	10.88 [a]	11.38 [a]
Mean	9.56 [b]	11.07 [a]	11.27 [a]
Haugh unit			
Period 1	86.75	82.94	82.00
Period 2	83.25	79.19	81.19
Period 3	73.81	75.31	68.81
Mean	81.27	79.15	77.33
% Albumen			
Period 1	65.03	65.07	65.30
Period 2	64.44	64.29	64.58
Period 3	62.02	65.04	65.60
Mean	64.83	64.80	65.16
Shell weight (gm)			
Period 1	10.14	10.03	10.11
Period 2	10.42	10.08	9.97
Period 3	10.31	10.03	9.83
Mean	10.29	10.05	9.97
Shell thickness (mm)			
Period 1	0.43	0.42	0.42
Period 2	0.41	0.40	0.41
Period 3	0.40	0.40	0.40
Mean	0.41	0.41	0.41
Specific gravity			
Period 1	1.09	1.09	1.09
Period 2	1.09	1.09	1.09
Period 3	1.09	1.09	1.09
Mean	1.09	1.09	1.09

Means in the same row with different superscripts were significantly different at $P<0.05$

Table 12. Effects of EM Supplementation on Fatty Acid Composition and Cholesterol Content of Egg Yolk

	Control	EM Supplementation in Feed	
		0.5%	1 %
Saturated fatty acid			
Myristic (C14:0)	0.335[b]	0.367[ab]	0.375[a]
Palmitic (C16:0)	23.900	24.520	24.723
Stearic (C18:0)	7.533	7.355	7.567
Total	31.767	32.240	32.660
Monounsaturated fatty acid			
Palmitoleic (C16:1)	3.125[b]	3.468[ab]	3.620[a]
Oleic (C18:1)	41.420[b]	43.612	43.078[ab]
Eicosamonoenoic (C20:1)	0.450	0.388	0.330
Total	44.995[b]	47.468[a]	47.028[ab]
Polyunsaturated fatty acid	19.163[b]	16.260[b]	16.347[b]
Linoleic (C18:2)	0.343	0.293	0.268
Linolenic (C18:3)	1.973	1.913	1.860
Docosahexaenoic (C22:6)	1.738	1.830	1.840
Total	23.237[a]	20.295[b]	20.315[b]
Cholesterol (mg/100 g)	1248.100	1242.100	1274.160

Means in the same row with different superscripts were significantly different at $P<0.05$

Table 13. Contamination of *E coli* and *Salmonella* in Eggs as Affected by EM Supplementation in Feed

	Control	0.5 % EM	1 % EM
Number of Samples	4	4	4
E. coli (MPN/gm)	<3	<3	<3
Salmonella (in 25 gm)	negative	negative	negative

Experiment 4

Effects of EM on growth, hen-day egg production including ages to produce first egg, 50 percent and 80 percent egg production and livability of laying Japanese quail are summarized in Table 14. Experimental data for egg weight, egg quality and feed efficiency of the control and EM supplemented groups are presented in Table 15.

At the end of this experiment (12 weeks of age), laying quail received control, EM supplemented in water, in feed and both in water feed groups had the average body weights of 159.22, 158.80, 160.63 and 158.72 grams, respectively and had the average egg production values for the four respective groups of 87.09, 79.36, 86.96 and 81.09 percent. Birds in the control group laid the first egg at the average age of 36.80 days compared to those in the EM supplemented groups which laid the first egg at the average age of 37.40, 35.40 and 36.80 days. Livability percentages of birds were similar among the treatment groups, the values were 94, 99, 94 and 98 percent for birds in control, EM supplemented in water, in feed and both in water and feed, respectively during 8-12 week experimental period.

The birds fed control diet laid average egg weight of 10.67 grams while those being fed EM supplemented diets laid an average egg weights of 10.02, 10.47 and 10.64 grams, respectively (Table 4). Feed intake and feed utilization were found to be similar among the treatment groups, birds in control group consumed 3.24 kg of feed to produce 100 eggs compared to those fed diets supplementation with EM culture which consumed 3.36, 3.35 and 3.39 kg of feed, respectively to produce 100 eggs.

Results of statistical analyses show that there were no significant differences among treatment groups for the production characteristics mentioned above. Significant influence of EM supplementation was found in egg quality trait. Pullet quail that received EM supplementation gave darker color of yolk. At 8 weeks of age, egg yolk color score was 4.14 for control group compared to those of 5.80, 6.40 and 6.84 for groups supplemented with EM in water, in feed and both in water and feed, respectively. At the end of this experiment, egg yolk colour score for control group was 5.52 while scores for the EM supplemented groups were 6.32, 6.72 and 6.96. Eggshell thickness increased slightly at 8 weeks and 12 weeks of age as EM was supplemented in water and/or in feed (Table 15).

Table 14. Performance of Laying Japanese Quail

	Control	**EM added in**		
		Water	**Feed**	**Feed & Water**
Body weight (gm)				
4 week	99.50	98.50	99.80	101.00
6 week	136.50	138.60	139.02	139.34
12 week	159.22	158.80	160.63	158.72
Weight gain (gm)	59.72	60.30	60.83	57.72
Age to produce (days)				
First egg	36.80	37.40	35.40	36.80
50% egg production	47.60	45.00	45.40	46.40
80% egg production	52.80	53.80	53.70	53.00
Hen-day egg production				
8 week	33.81	32.11	34.25	31.65
12 week	87.09	79.36	86.96	81.09
Livability (%)				
4-8 week	100.00	100.00	98.00	99.50
8-12 week	94.00	99.00	94.00	98.00

No significant difference due to treatment was found

Table 15. Egg Weight, Quality of Egg and Feed Efficiency of Laying Japanese Quail as Affected by EM Supplementation in Water and Feed

	Control	**EM added in**		
		Water	**Feed**	**Feed & Water**
Egg weight (gm)				
At 8 week	9.75	9.93	9.65	9.66
At 12 week	10.67	10.02	10.47	10.64
Egg yolk colour				
At 8 week	4.14^{c}	5.80^{b}	6.40^{ab}	6.84^{a}
At 12 week	5.52^{c}	6.32^{b}	6.72^{ab}	6.96^{a}
Egg shell thickness (mm)				
At 8 week	0.220	0.220	0.230	0.230
At 12 week	0.231	0.234	0.234	0.233
Feed intake (gm)				
At 8 week	22.53	22.39	23.04	22.72
At 12 week	28.16	26.52	29.04	27.01
Feed/100 eggs (kg)				
At 8 week	6.33	6.21	6.32	6.38
At 12 week	3.24	3.36	3.35	3.39

Means in the same row with different superscripts were significantly different at P<0.05

Conclusion

Under the conditions of the present experiments, there were no significant effects of EM supplemented in drinking water or feed on production performance of Muscovy ducks, broiler chickens, laying chickens and Japanese quail. For carcass characteristics and nutritional values of meat, the effects of EM supplementation were different in poultry types of different production capacities. In Muscovy ducks, considered as a low productive poultry, effects of EM supplementation were found to significantly increase breast percentage, significantly reduce breast ash content and tended to increase protein content of breast meat and polyunsaturated fatty acids content in duck oil.

In commercial broiler chickens, considered as a high productive poultry, EM supplemented in drinking water and/or feed had no significant effect on carcass characteristics including breast percentage, but tended to decrease ash content of breast meat. In laying poultry, EM added in drinking water and or feed found to improve the yellow colour of egg yolk in laying chickens and Japanese quail.

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