

Full Length Research Paper

Effect of effective microorganisms on growth parameters and serum cholesterol levels in broilers

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This study was conducted to evaluate the effect of different administration methods of effective microorganisms (EM[®]) on the performance and serum cholesterol level of broilers at Debre Zeit Agricultural Research Center, Ethiopia. Uniform weight of mixed sex day-old-broilers of Cobb-500 strain (n = 240) were randomly distributed to 4 treatment groups with 3 replications of 20. They were kept under a standard management condition for 49 days being subjected to treatment rations since Day 10 on. Performance parameters were recorded and analyzed. Total blood cholesterol was analyzed with standard kit at the end. The result showed that there was no significant difference of EM administration methods ($p < 0.05$) on mortality of chickens during the starter (1 to 29 days) and finisher (30 to 49 days) phases. Feed consumption was found to be significantly higher for Treatment 4 (Bokashi in feed + EM in water) than the rest of the treatment groups. Weight gain was significantly higher ($p < 0.05$) for Treatment 4 (Bokashi +EM in water), during the entire period than the rest of the treatment groups. Birds fed with T4 (Bokashi +EM in water) required less feed for a unit increase in weight during the starter and finisher phase. Birds fed with T3 (Non Bokashi +EM in water) required the highest feed for a unit increase in weight. EM application in all forms has resulted in significantly lower ($p < 0.05$) total blood cholesterol, EM application both in feed and water combined being most effective in lowering the total blood cholesterol than the other application methods.

Key words: Bokashi, broiler, performance, application, feed conversion ratio, mortality.

INTRODUCTION

EM (Effective microorganisms) is the mixed-cell culture which is composed of photosynthetic bacteria, actinomycetes, yeast, lactobacillus and fungi (Higa, 1993). Effective microorganisms (EM) as a new technological advance were innovated in Japan. Effective microorganism (EM) is a combination of 70 to 80 different types of "good" and beneficial microorganisms contributing to the wide range of applications. Microorganisms in EM are not genetically engineered, but they are commonly found in everyday food and healthy forest soils. The microorganisms mutually co-exist to form beneficial relationships with each other in a liquid medium. Microorganisms in EM assist one another for survival in a food chain system and thereby form a synergy that fights off pathogens and rotting

microorganisms. EM is self sterilizing (pH between 3.4 to 3.7); therefore, pathogens cannot survive in EM (EMROSA, 2006). The principal organisms are usually five. They are photosynthetic bacteria (phototrophic bacteria), lactic acid bacteria, yeasts, actinomycetes and fermenting fungi. Photosynthetic bacteria are independent self supporting microorganisms. These bacteria synthesize useful substances from secretions of roots, organic matter and/or harmful gases (example, hydrogen sulfide) by using sunlight as sources of energy. The useful substances comprise of amino acids, nucleic acids, bioactive substances and sugars, all of which promote plant growth and development. These metabolites act as substrates for bacterial growth. Thus, increasing photosynthetic bacteria, which enhance other effective microorganisms (Higa, 1993). Lactic acid bacteria produce lactic acid from sugars, and other carbohydrates produced by Photosynthetic bacteria and yeast. Thus, food and drinks, such as yogurt and pickles

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have been made by using Lactic acid bacteria for a long period of time. However, lactic acid is a strong sterilizer. It suppresses harmful microorganisms and increases rapid decomposition of organic matter. Moreover; Lactic acid bacteria enhance the breakdown of organic matter such as lignin and cellulose, and ferment these materials without causing harmful influences arising from undecomposed organic matter (Higa and Parr, 1994). The yeasts that are present in effective microorganisms have a wide range of functions. They produce antimicrobial substances to kill off all harmful pathogens. In addition, they also produce beneficial substances, such as hormones, enzymes and vitamin B. Their secretions are useful substrates for effective microorganisms such as lactic acid bacteria and actinomycetes. Yeasts synthesize antimicrobial and useful substances from amino acids and sugars secreted by photosynthetic bacteria, organic matter and etc. Actinomycetes, the structure of which is intermediate to that of bacteria and fungi, produces antimicrobial substances from amino acids secreted by photosynthetic bacteria and organic matter. These antimicrobial substances suppress harmful fungi and bacteria. Actinomycetes can co-exist with photosynthetic bacteria. Thus, both species enhance the quality of the soil environment, by increasing the antimicrobial activity of the soil (Higa, 1993). Fermenting fungi such as *Aspergillus* and *Penicillium* decompose organic matter rapidly to produce alcohol, esters and antimicrobial substances. These suppress odors and prevent infestation of harmful insects and maggots. Each component of effective microorganisms (photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes and fermenting fungi) has its own important function. However, photosynthetic bacteria are the pivot of EM activity. Photosynthetic bacteria support the activities of other microorganisms. On the other hand, photosynthetic bacteria also utilize substances produced by other microorganisms. This phenomenon is termed "coexistence and co-prosperity". EM products that are of importance for poultry production are Stock EM, Multiplied EM and Bokashi (solid form of EM). Stock EM is the basic, concentrated EM solution that contains all the beneficial microorganisms. Stock EM is not a fertilizer, a chemical or a synthetic. It is also not genetically engineered. Stock EM is a basic dominant form of EM and is therefore usually used to produce multiplied EM (M-EM). Multiplied EM is the activated, secondary form of EM. It consists of S-EM (1 to 3%), molasses (3 to 5%) and water (94%). The molasses serves as a nutrient source for the microorganism, consequently leading to the growth and multiplication of the microorganisms. Therefore the name, multiplied EM. EM Bokashi is an essential supplement feed for animals and is made from 1 to 2% Multiplied EM, 1% molasses and 98% water, which is then added to organic feed materials. It has various applications, but is mostly used as a form of animal feed (APNAN, 1995).

According to Chantsawang and Watcharangkul (1999), EM was first introduced and used extensively in Asia; the technology was later introduced to other various countries. The use of EM in animal husbandry nowadays is very well identified in many parts of the world. In a study conducted in Belarus by Konoplya and Higa (2000), EM was successfully used in poultry and swine units as feed constituent and sanitation spray. In South Africa EM was used to increase productivity in integrated animal units and poultry farms (Hanekon et al., 2001). Result of EM experiment conducted on 27000 Kuroki broilers in Japan is reported by Higa (1994). According to the author; EM was administered in drinking water, EM Bokashi in feed and sluicing or cleaning out the hen coops with an EM dilute solution. Of the 27000 chicks reared by this operation, survival rate for shipment was only 83% prior to the introduction of EM method; whereas, the survival rate increased to 97% for the birds raised by EM methods. In actual figure, this made an increase of 3780 fowls ready and shipped to market. Tortuero (1973) found that a lactobacillus probiotic and zinc bacitracin had similar effects in stimulating weight gain and feed efficiency of broiler chicks. An alternative is the use of probiotics, prebiotics and symbiotic (feeding probiotic microorganisms together with prebiotic substances) which might contribute due to the development of beneficial microorganisms in the gastrointestinal tract (Pelicano et al., 2004). In human health there is significant evidence that probiotics such as specific types of lactobacillus bacteria and bifido bacteria can lower the three major risk factors for coronary heart diseases and stroke: excessive cholesterol, high blood pressure and high triglyceride levels. Study in Argentina (Taranto, 1999) indicated that Lactobacillus bacteria lowered total blood cholesterol by 22% and triglycerides by 33%. However, research done by (Pelicano et al., 2004; Greany et al., 2004) has shown no benefit of using microbial preparation or probiotics. Therefore, since not all strains of bacteria such as *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* work to lower cholesterol level, note worthy care should be taken with strain selection of beneficial bacteria to get the best results. The other benefit of EM is that it eliminates odors without producing odorous gases by dominating the microbial ecology with organisms that exploit a fermentative pathway (Yongzhen and Weijiong, 1994).

The present study was therefore conducted to add information on this relatively new technology (introduced by Japanese Professor, Dr. Teuro Higa in 1980); the effect of EM on growth parameters and total serum cholesterol level of broilers.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Debre Zeit Agricultural Research

Table 1. Composition and nutrient content of starter and finisher.

Nutrient	Starter (0-29 days)	Finisher (30-49 days)
Metabolizable energy (kcal/kg)	3200	3100
Crude protein (%)	21.82	22
Calcium (%)	0.59	0.56
Phosphorus (%)	0.43	0.41
L-Lysine (%)	1.13	0.84
DL-Methionine (%)	0.39	0.32
Methionine + cysteine (%)	0.71	0.70

Center (DZARC), poultry research farm, which is located at 47 km south east of Addis Ababa. The elevation of the area is around 1920 m above sea level. The average temperature of the area is 16.6°C with the mean minimum and maximum annual temperature of 8.9 and 24.3°C respectively. The area has two rainy seasons; the minor one is between February and April and the major one is from June to September. The annual mean rainfall is about 851 mm (DZARC, 2003).

Experimental design and treatments

The experiment was carried out from September 4 to October 23, 2009. Mixed sex day-old-broiler chickens of Cobb-500, bought from local hatchery. Uniform weight of mixed sex day-old-broilers of Cobb-500 strain (n = 240) were randomly assigned to 4 treatment groups with 3 replications of 20. EM was made and mixed with compounded standard feed to make EM in feed and with water to make EM treatment in a solution form. The Bokashi (EM in a solid form) and the EM activated solution (EM a solution form) were prepared following the procedures of Asia Pacific natural agriculture network (APNAN, 1995). EM in a feed was prepared by adding 3% of Bokashi (EM in a solid form) mixed to the feeds to make up Bokashi added feed and 2 ml of activated EM solution was added in a liter of drinking water. Using the Bokashi and activated EM solution, 4 feed types (treatments) were prepared. Treatment 1 control: a diet with no EM in feed + EM in water, Treatment 2: EM in drinking water only, Treatment 3: EM in feed only and Treatment 4: EM in feed and EM in drinking water.

Management of experimental animals

Standard commercial management was followed during the trial. The chicks were housed in an open house, concrete floor, in 12 pens with 20 chicks per pen with a density of 10 birds per square meter. Saw dust sprayed with standard disinfectant was used as litter material with about 7 mm thickness. Round feeders and drinkers of small sizes were used during the starter phase (0 to 29 days) throughout the trial. Infrared lamp of 250 W was used for the first week. Bigger size drinker of 10 L and round hanging type feeder of 12 kg were used during the finisher phase. (30 to 49 days). Internal temperature and humidity was controlled by opening and closing the curtains to keep the chickens comfortable. The chickens were vaccinated with Newcastle (HB1 at Day 7 and Lasota at Day 21) and Gumboro at Day 10. Treatment feeds and water were given *ad-libitum* to the chickens throughout the experiment period starting Day 10 onwards.

Experimental diets

Experimental diets were formulated to contain, metabolizable energy

(ME) 3200 kcal/kg and 21% crude protein (CP) during the starter phase (1 to 29 days); and ME 3100 kcal/kg and 22% CP for finishers (30 to 49 days) using trial and error feed formulation software. The nutritive content as per the analysis of the sample is presented in (Table 1).

Parameters studied

Data were recorded during the periods from 1 to 29 days and 30 to 49 days. Feed intake was determined by subtracting the quantity leftover from the quantity offered every day. Weight gain was measured every day and gain was calculated every week as a difference between final and initial weight. Feed conversion ratio was determined by calculating the ratio of feed consumed per a unit of growth. Mortality was calculated as the ratio of death occurrences per the remaining animals multiplied by 100. Serum cholesterol was also analyzed.

Sample collection

At the end of the experiment 3 ml of blood sample from each slaughtered broiler chickens (which were fed EM with feed, EM with water, EM with feed and water, and control groups) was collected from the jugular vein by using plain vacutainer tube for total cholesterol analysis. The collected blood was allowed to clot over night (12 h) in a vertical position to separate the serum. The collected sera were stored at -20°C until analysis. Total cholesterol assay was done using cholesterol liquicolour commercial kit (Human Diagnostics Worldwide) based on CHOD-PAP method according to the manufacturer's instruction at faculty of veterinary medicine, Debre Zeit laboratory.

Statistical analysis

Statistical software (SAS, 2000) was used to analyze the data. Differences between treatment means were evaluated by Duncan's multiple range tests at a significance level of 5%.

RESULTS

Feed intake and weight gain

Feed intake was significantly higher ($p < 0.05$) for broilers fed EM in feed and water followed by those fed with control diet during the starter phase (Table 2). During the finisher phase, the same parameter was found to be significantly higher ($p < 0.05$) for the birds supplemented

Table 2. Production performance of broilers as affected by EM supplementation in drinking water and feed: Starting phase (0 to 29 days) (n = 200, x ± SD).

Variable	Control	EM application		
		EM in drinking water	EM in feed	EM in feed and EM drinking water
Weight gain (g)	744 ^{ab}	724 ^{ab}	706 ^{ab}	780 ^a
Feed intake (g)	1391.57 ^b	1347.83 ^C	1334.93 ^C	1513.2 ^a
FCR(amount of feed required/a unit gain in weight in Kg)	1.87 ^a	1.86 ^a	1.89 ^a	1.94 ^a
Mortality (%)	5.0 ^a	6.0 ^a	4.0 ^a	5.0 ^a

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

Table 3. Production performance of broilers as affected by EM supplementation in drinking water and Feed: Finisher phase (30 to 49 days) (n = 200, x ± SD).

Variable	Control	EM application		
		EM in drinking water	EM in feed	EM in feed and EM in drinking water
Weight gain (g)	1789 ^{bc}	1681 ^{bc}	1724 ^{bc}	2143 ^a
Feed Intake (g)	4151.73 ^b	4068.3 ^c	4016.57 ^c	4306.87 ^a
FCR(amount of feed required/a unit gain in weight in kg)	2.32 ^b	2.42 ^c	2.33 ^b	2.01 ^a
Mortality (%)	2.0 ^a	2.0 ^a	4.0 ^a	2.0 ^a

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

with EM in feed and water, followed by those fed control diet (Table 3). Weight gain of the broilers fed with EM in feed and water was significantly higher (p < 0.05), followed by those fed with EM in feed alone during the starter phase (Table 2). But during finisher phase, weight gain of the birds fed with EM in feed and water was found to be significantly higher (p < 0.05) than the rest of the treatment groups including the control diet.

Feed conversion ratio and mortality

There was no significant difference among the treatment groups and mortality during the starter phase (Table 2). Birds fed with EM in feed and water required lower feed for a unit increase in weight (p < 0.05), while no significant difference

was observed with respect to mortality during the finisher phase (Table 3).

Total serum cholesterol level

Total cholesterol level of blood taken from broilers fed with control diet (no EM supplemented) showed significantly higher than groups fed with EM in different forms at (p < 0.05) (Table 4).

DISCUSSION

Feed intake and weight gain

Feed intake was higher for boilers fed EM in feed and water followed by those fed with control diet

during the starter phase. And during the finisher phase, the same was found to be true for the birds supplemented with EM in feed and water. However, in similar study by Chatsavang and Watchangkul (1999) showed no significant differences in the feed intake among the treatment groups during the starter and finisher phases. In this study weight gain of the broilers fed with EM in feed and water was significantly higher, followed by those fed with EM in feed alone during the starter phase. But during finisher phase, significant weight gain was observed on those birds which were fed with EM in feed and water. However, in similar work done by Chatsavang and Watchangkul (1999), EM supplementation in feed only resulted in significantly higher body weight. (p < 0.05), but during the finisher phase all treatments did not reveal any significant difference

Table 4. Total serum cholesterol level (n = 80, x ± SD).

Variable	Control	EM application		
		EM in drinking water	EM in feed	EM in feed and EM in drinking water
Serum cholesterol(mg/dl)	131.33 ± 3.17 ^a	102.94 ± 8.7 ^b	103.81 ± 13.9 ^b	103.51 ± 14.7 ^b

Means in the same row with different superscripts were significantly different.

among themselves. In addition, study by ZuAnon et al. (1998), Patidar and Prajapati (1999), Ergun et al. (2000) and Kumprechtova et al. (2000) stated that supplementation of probiotics had no effect on the performance of broiler chicks. On the other hand, there are reports which state that EM fed birds had significantly higher weight gain and lower FCR than Non-EM groups (Safalaoh, 2006; Jagdish and Sen, 1993; Alvarez et al., 1994; Hamid et al., 1994).

Feed conversion ratio and mortality

In terms of mortality, no significant difference was seen in the starter phase and finisher phase among the treatment groups. This is same in line with the finding by Yoruk et al. (2004) that mortality of hens fed with control diet was not different from those fed with probiotic diets. On the contrary, other studies have shown that probiotics significantly reduced mortality in chickens (Vicente et al., 2007). Several previous works suggested that supplementation of probiotics does not influence feed conversion ratio significantly or no such effect on FCR (Samanta and Biswas, 1995; Gohain and Sapkota, 1998; Panda et al., 2000; Ergun et al., 2000; Mohit et al., 2007; Ahmad, 2004). In addition, study by Chatsavang and Watchangkul (1999) did not reveal any significant difference among the treatment groups.

Total serum cholesterol level

Total cholesterol level of blood taken from broilers fed with control diet (no EM supplemented) showed significantly higher than groups fed with EM in different forms. This is of course in line with a result reported somewhere else by Taranto (1999) that EM application has resulted in lowering excessive cholesterol, high blood pressure and high triglyceride levels in humans. The ability of probiotics to depress serum cholesterol content has been reported in broilers and rats (Mohan et al., 1996; Grunewald, 1982). Similarly, though not significantly different ($P < 0.05$), the level of serum cholesterol at 42 days was lower for the EM supplemented birds than for the control (Safalaoh, 2006). However, the results of current and previous studies are different from that of other studies that showed that probiotic supplementation had no beneficial effects on broiler performance and did not lower cholesterol levels in post-menopausal women (Pelicano et al., 2004; Greany et al., 2004).

CONCLUSION AND RECOMMENDATION

According to the result of the current study, EM application in various forms can positively affect weight gain, feed intake and lowers FCR. It has been shown that EM application can also lower cholesterol level in Broilers. The results of these three experiments show that EM has positive

effect on broilers in terms of body weight gain. This study has demonstrated that supplementation of broiler diets with a microbial preparation, such as EM, may offer potential benefits to the poultry industry, such as improvements in body weight gain and feed utilization efficiency. Due to variations among previous studies conducted elsewhere, further research is required to clearly indicate the effect of EM supplementations in poultry diets.

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