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INFLUENCE OF EFFECTIVE MICROORGANISMS ON PERFORMANCES, BLOOD BIOCHEMISTRY, DIGESTIVE ENZYMES, IMMUNITY, ANTIOXIDANT INDICES, INTESTINAL MORPHOMETRIC AND MICROBIAL POPULATION OF GROWING JAPANESE QUAILS

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ABSTRACT: The aim of this research was to investigate the influence of effective microorganisms (EM) on growth performance, microbial count, gut structure and some blood indices of Japanese quail. For a feeding study that lasted 38 days, 240 Japanese quail that were 10 days old were randomly assigned to three treatment groups, each consisted of four replicates of 20 birds. The experimental diets included a basal diet (first group), a basal diet supplemented with 10 and 20 ml EM /kg feed (2nd and 3rd groups, respectively). Quails receiving diets supplemented with 20 ml EM /kg feed exhibited significantly ($P \leq 0.01$) best live body weight, body weight gain, growth rate, performance index, and feed conversion ratio, with the least amount of feed during the study period. Quails fed dietary 20 ml/kg feed of EM had the lowest levels of total cholesterol, triglycerides, alanine transaminase, and aspartate transaminase, and the highest levels of hepatosomatic index, amylase, lipase, and trypsin. Morphological intestine length, intestine weight, cecum length, cecum weight, liver weight, gizzard weight, and glandular weight were higher in the group fed dietary 20 ml EM /kg feed, and this group also had lower abdominal fat. Furthermore, the group received 20 ml EM /kg feed had the lowest number of *Salmonella and Escherichia coli*, as well as the best antioxidant parameters, immune responses, and *Lactobacilli* number, when compared to the control group ($p < 0.05$). In conclusion, the inclusion of EM at a rate of 20 ml/kg feed led to improvements in performance, antioxidant, blood biochemistry, gut structure, immunological indices, and intestinal microbiota in growing Japanese quails.

Keywords: Effective microorganisms; growth; antioxidant; intestinal morphometric, immunity; quail

INTRODUCTION

Excessive utilization of antibiotics gives rise to various issues, such as the development of drug resistance in animals and the presence of drug residues in livestock products. These problems pose a threat to both the sustainable development of human and environmental systems, thereby emerging as a severe food security concern (Rana, et al., 2019). In order to tackle this problem, numerous countries have implemented legislation and banned the use of antibiotics as growth promoters in animal feed (Vieco-Saiz, et al., 2019). According to Park, et al., (2016) probiotics are defined as "living microorganisms that, when administered in sufficient quantities, confer health benefits on the host" by the United Nations Food and Agriculture Organization and the World Health Organization (WHO). Antibiotic-resistant bacteria are regrettably a reality due to the overuse of antibiotics in veterinary medicine. As a result, it is now difficult to control harmful bacteria without using antibiotics (Ohimain and Ofongo, 2012; Wallace et al., 2010). Given the escalating concerns regarding the high mortality rate caused by gastrointestinal problems and the limitations on antibiotic usage, it is crucial to explore alternative methods for enhancing gut health and reducing productivity losses. probiotics can lessen the negative effects on consumers, where present a promising alternative to antibiotics in poultry diets, where probiotics have a group of beneficial live microorganisms that reduce intestinal pathogens, thereby improving performance, FCR, health and immunity of poultry (Getachew, 2016 and Sethiya, 2016). The major bacteria found in probiotics consist of lactic acid bacteria categorized under the genus Lactobacillus and Bifidobacterium, with some useful bacterial strains and yeasts (Jadhav et al. 2015 and Jeong and Kim, 2014). In poultry feeding, *Lactobacillus* have various benefits effects such resilience to stomach acidity, prolonged feed storage; enhance immunity and antimicrobial properties (Cutting, 2011 and Lee et al., 2010). Probiotics can be added to feed production as additives; they are primarily made in the feed industry by the processes of isolation, cultivation, and fermentation (Attia, et al., 2017 and Qamar Memon, et al., 2020). Numerous studies have demonstrated the potential benefits of probiotic supplementation for commercial poultry, including enhancements in feed conversion ratio (FCR), weight gain (WG), immune response, antioxidant activity, gastrointestinal microecological balance, egg production, and reduction in feed intake (FI), pathogenic microorganisms, and mortality rates (Abdel Baset, et al., 2020, Zhao, et al., 2020 and Rashidi, et al., 2020). The mode of action for probiotic microorganisms involves the generation of antimicrobial conditions like low pH and competition for attachment sites on the intestinal, with activation to the immune system (Rolfe, 2000). The acidic environment and high bile concentration in the gastrointestinal tract contribute to the efficient functioning of probiotics by working in conjunction with the primary gut barrier. Probiotics are commonly used as feed supplements or additives in the industry (Cromwell, 2002). In order to effectively administer probiotics to animals, specific probiotic species and correct dosages must be employed. Probiotics released as feed additives in the market require specific strains, standardized dosages, viability, and biosafety (Vohra and Satyanarayana, 2012). Japanese quails (Coturnix japonica) are currently being used as a food source for humans and can be found in various regions such as Asia, Europe, and Africa (Kayang et al., 2004). Limited studies have been conducted to investigate the effects of EM as a feed supplement in Japanese quails compared to other medicinal plant constituents with anti-inflammatory, antimicrobial, and antioxidant properties. Therefore, the objective of this study was to assess the impact of using graded levels of EM on the growth performance, blood biochemistry, digestive enzymes, gut ecology, antioxidant indices, and immune response of growing quail.

MATERIALS AND METHODS

The investigation was carried out at the Poultry Research Center, situated within the Faculty of Agriculture at Fayoum University in Egypt.

The examination involving live animals adhered to the regulations sanctioned by the Fayoum University Institutional Animal Care and Use Committee in Egypt (Code No. of the research proposal: **AEC 2359**).

Effective micro rganisms (EM):

The EM were purchased from the Agricultural Research Center, Ministry of Agriculture, Giza, Cairo, in the form of liquid containers and were prepared according to the processes of the Asia Pacific natural agriculture and were mixed with the basal diets and left for seven days before use to form the soiled fermented feed (bokashi). According to EMRO (2010), EM culture is a high cocktail of live beneficial microorganisms including high populations of Lactic acid bacteria (Lactobacillus plantarum, Lactobacillus casie and Streptococcus lactis), yeast (Saccharomyces cervisiae and Candida utlitis) and fewer amounts of photosynthetic bacteria (Rhodopseudomonus palostris and Rhodobactor Spaeroids) Ray fungi (Streptomyces albs and Streptomyces griseus) and fungi (Aspergillus oryza and Mucor hiemalis).

Experimental design and diets:

A total of 240 one-day-old unsexed quails were procured from the market and acclimatized for a period of 10 days. These quails were allocated randomly into three groups: basal diet (control without EM), basal plus10 ml EM/kg diet, and basal plus 20 ml EM/kg diet. Each group was consist of four replicates with 20 chicks per replicate. Chicks were housed in a six decks, two sections quail cages with stand and dropping pans with automatic watering. The control diet was formulated to meet the nutrient requirements of the quails during the experiment period from zero - 38 days according to National Research Council (NRC, 1994). Composition and analysis of basal diet of growing Japanese quail is presented in Table 1. Chicks were exposed to 23-24 hour lighting and were fed and watered *ad libitum*.

Growth performance:

Live body weights of chicks (LBW) were individually measured and feed consumptions per pen were recorded on a weekly basis (FI). The uneaten feed was discarded, body weight gain (BWG) was determined as the difference between final and initial body weights, and feed conversion ratio (FCR) was also calculated. In addition, the performance index (PI) was determined according to North (1981) using the formula: PI = BWkg/FCR. Furthermore, the growth rate was calculated based on the method proposed by Brody (1945) as follows: GR = (LBW38 - LBW10) / 0.5 (LBW10 + LBW38).

Morphometric characteristics of the digestive system:

At the end of the experiment (38 of age), 8 birds (4 males and 4 females) from each treatment were reweighed and slaughtered by cutting the Jugular vein, defeathered and eviscerated liver, gizzard , proventicular and abdominal fat individually weighed.

Hepatosomatic Index was calculated according to Nur-Azri *et al.*, (2018) using the following equation

Hepatosomatic Index (HSI) = $weight\ of the\ liver\ (g)$/final body $weight\ (g)$ x 100.

Immediately after slaughtered, the abdomen was opened and the intestinal tract exposed the length of the small intestine is measured from the base of the gizzard to the bifurcated cecum (ileo-caecal junction) and the length of the cecum from the bifurcated cecum (ileo-caecal junction) to the end of every caecal appendage multiplied in two. After ligation of the small intestine, to prevent post-mortem movements of digesta, the total intestinal content was collected then the small intestine and the two caecal appendage weighed empty. Digesta content collected from the quails in each pen was pooled, representing one replicate, and placed directly in a centrifuge tube (10 ml). The centrifuge tubes with fresh digesta were immediately placed on ice in an isolated box until viscosity measurements were done within 1 h following killing.

Measurements of digesta pH and viscosity

The pH of digesta from each bird was measured using a digital pH-meter (pH 315i, WTW Wissenschaftlich- TechnischeWerkstätten, Weilheim, Germany). The viscosity of intestinal digesta was determined according to Tsiouris et al., (2013) using a Brookfield DV-II + PRO Digital Viscometer (Brookfield

Engineering Laboratories, Stoughton, MA, USA). Two readings were taken from each sample and were represented in units of centipoise (CP).

Microbial analysis:

After slaughter, intestinal content was immediately collected in sterile glass containers, digesta was evacuated and mixed. At 4°C, the sealed containers were kept in the laboratory till enumeration of microbial population. Samples (1g of the mixed fresh mass) were taken into sterile test tubes, diluted 1:10 in sterile 0.1% peptone solution and homogenized for 3 min in a Stomacher homogenizer. Ten fold serial dilutions up to 10^{-7} of each sample were prepared in nine ml of 0.1% sterile peptone solution. Viable counts of *Salmonella spp, Escherichia coli (E.coli)* and *Lactobacilli spp* were performed. One milliliter of the serial dilution was incubated into sterile Petri dishes and sealed with an appropriate medium. *Lactobacillus spp*. colony count was determined using MRS agar (Biokar Diagnostic, France) after incubation in an anaerobic chamber at 37 °C for 72 h. *Salmonella and E. coli* colonies were counted on brilliant green agar plate and incubated at 37°C for 24 h). After cultivation in Petri dishes, the total colony count for *Lactobacilli*, *Salmonella* and *E. coli* was then calculated as the number of colonies by reciprocal of the dilution. The microbial counts were determined as colony forming units (cfu) per gram of sample.

Blood biochemical, anti-oxidant and immunity:

At slaughter, individual 24 blood samples (3 treatment x 4samples x 2sex) were collected in dry clean centrifuge tubes and serum was separated through centrifugation at 3000 rpm for 15 minutes and assigned for subsequent determination. Quantitative determination was done for the following: total cholesterol (TC), triglycerides (TG), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). All blood biochemical parameters were calorimetrically determined using commercial diagnosing kits (produced by Spectrum Diagnostics Company, Egypt). Amylase and lipase enzymes were assayed by Friedman and Young (2005) and according to Bovine Trypsin ELISA Kit MBS706461 trypsin enzyme was determined. The glutathione peroxidase (GPx, EC 1.11.1.9) determined calorimetrically according to Paglia and Valentine (1967) and thiobarbaturic acid- reactive substances' (TBARS) were performed according to Yagi (1998) using commercial diagnosing kits produced by Cayman Chemical Company (USA). The method used for the assay of chicken Immunoglobulins Isotypes IgG, IgM, and IgA in Sandwich ELISA described by Erhard et al. (1992) the absorbance measured on an ELISA plate reader set at 450 nm.

Statistical analysis

The results obtained studied using the statistics tools (analysis of variance) of Infostat (Di Rienzo, 2017). As the following model:

$Yij = \mu + Ti + eij$

Where: Yij: observation of traits, μ: overall mean, Ti: treatment effect, eij: random error. All means were compared using multiple range test (Duncan, 1955). At significance level of 0.05.

RESULTS

3.1 | Growth Performance

The results presented in Table 2 illustrate that the growth performance of the quails was significantly affected by the different treatments administered during the study period from 10 to 38 days of age ($P < 0.001$). Quails fed diets supplemented with 20 ppm EM displayed the most favorable LBW, BWG, FCR, PI, and GR values, which were measured at 250.87, 199.18, 2.97, 8.49, and 1.32, respectively. In comparison, the control group exhibited significantly ($P < 0.001$) poorer LBW, BWG, FCR, PI, and GR values, which were recorded at 230.86, 179.34, 3.39, 6.85, and 1.27, respectively. Moreover, the birds received diet treated with 10 and 20 ppm EM demonstrated significantly ($P < 0.001$) lower feed intake values compared to control group, with values measured at 588.69, 589.35, and 605.62, respectively.

3.2 | Serum Biochemistry

The findings presented in Table 3 show that the inclusion of EM in the diet had a significant

effect ($P < 0.001$) on various serum biochemistry parameters, including hepatosomatic index (HSI) (g liver/ g final body *weight*), lipids (total cholesterol, TG), liver enzymes (ALT and AST), and digestive enzymes (Amylase, Lipase and Trypsin), when compared to the control group. Quails that were fed a diet supplemented with 20 ppm EM exhibited significantly ($P < 0.001$) lower levels of cholesterol, TG, ALT, and AST, with values recorded at 158.77 mg/dl, 112.94 mg/dl, 3.15 IU/L, and 280.86 IU/L, respectively, in comparison to the control group, which had values of 178.14, 123.00, 4.00, and 302.14, respectively. Additionally, quail fed dietary 20 ppm EM prevented higher HSI, Amylase, Lipase, and Trypsin values, measured at 1.75, 721.98 U/L, 99.99 U/L, and 99.04

U/L, respectively, compared to the values of the control group, which were 1.45, 593.50, 88.99, and 76.88, respectively. Lastly, the addition of varying levels of EM to the diets improved the lipid profile, hepatic enzymes, and digestive enzymes.

3.3 | Intestinal Morphology Traits

Table 4 summarizes the impact of EM on intestinal morphology traits in quails. The data reveals that quails fed diets treated with EM exhibited significant effects ($P<0.001$) on various parameters including small intestine length and weight, cecum length and weight, liver weight, gizzard weight, glandular stomach weight, and abdominal fat. Among the different diets, the highest values for small intestine length and weight, cecum length and weight,

liver weight, gizzard weight, glandular stomach weight were observed in the group receiving a diet supplemented with 20 ppm EM. Conversely, the control group exhibited lower values for these parameters.

3.4 | Antioxidant Capacity and Immunity

Table 5 presents the effects of EM therapies on antioxidant characteristics (GPx and TBAR) and immune response index. The data demonstrates that EM supplementation in quails diet significantly ($P \leq 0.001$) influenced the levels of these parameters compared to the control treatment. Quails fed diets supplemented with EM improved levels of IgG, IgA, IgM, and glutathione peroxidase, along with reduced levels of thiobarbaturic acid-reactive compounds. Notably, the group receiving EM at 20 ppm showed the most favorable impact on GPx, TBAR, IgG, IgA, and IgM levels. Specifically, the values for these parameters were 8.24, 1.15, 72.17, 39.79, and 43.23, respectively. The TBAR level was lowest in the group fed with EM at 20 ppm (1.15), while the control group exhibited the highest TBAR level (1.66). Overall, increasing the dietary levels of EM positively influenced the immune index values (IgM, IgA, and IgG) and GPx levels.

3.5 | Gut Ecology

Table 6 presents the impact of dietary EM on gut ecology in Japanese quail during the growing period. The results of our study indicate that EM supplementation in diets enhanced the population of healthy gut bacteria (*Lactobacillus population*), while significantly reducing ($P > 0.001$) the population of harmful diverse microbes (*Salmonella and E. coli population*). Moreover, addition of EM to quails diet at all levels increased the overall population of beneficial intestinal *Lactobacilli*, while decreasing the population count of *E. coli and Salmonella* in the small intestine. These effects were particularly pronounced in the group receiving a diet supplemented with 20 ppm EM, which exhibited the lowest levels of *E. coli and Salmonella* (6.56 and 6.30, respectively), and the highest *Lactobacilli* population (7.67). Additionally, the group receiving the 20 ppm EM diet had the highest viscosity and lowest intestinal pH levels (2.58 and 6.33, respectively) compared to the control treatment (2.26 and 6.71, respectively).

DISCUSSION

Quails feeding with diets including 20 ppm of EM have the best LBW, BWG, FCR, PI, and faster GR in comparison to untreated group (control), which exhibited significantly ($P < 0.001$) inferior LBW, BWG, FCR, PI, and GR. Additionally, the avian subjected to 20 ppm EM exhibited the lowest feed intake value, followed by those receiving 10 ppm EM, as opposed to the control cohort, which demonstrated the highest value. The noted improvement in growth performance among

EM-treated quails could be ascribed to the decrease in pathogenic bacteria (*E. coli*) with the rise in beneficial bacteria (*Lactobacillus Spp.*), Also, EM can enhanced digestive enzymes activity and intestinal pH (Chen et al., 2013). Probiotic bacteria adhere to the intestinal mucosa, creating a protective layer that impedes the proliferation of pathogenic bacteria, thereby boosting digestive tract function, intestinal health, and enhancing intestinal microflora that enhance growth performance (Mountzouris et al., 2010). Additionally, probiotics can caused an optimal environment in the digestive tract facilitates efficient nutrient absorption by the small intestine villi with improving in intestinal structure (length, width, and depth of intestinal villi) (Hidayat et al., 2016). Moreover, the impact of microbial activity in probiotics on digestive tract weight surpasses that of other factors. Where, Lactic acid-producing bacteria, such as *Lactobacillus,* generate acids that lower pH levels, establishing an environment that impedes the proliferation of pathogenic bacteria (Hatmanti, 2000). Consequently, optimal nutrient absorption with significantly higher growth performance for birds (Susanti and Tugiyanti, 2020). Our findings align with those of Abdelkader et al. (2023), who reported that groups treated with higher levels of effective microorganisms (EM10) exhibited significantly better values for the studied growth parameters LBW, BWG, FCR, GR, and PI compared to the control group ($P < 0.01$). This improvement in broiler performance associated with EM may be attributed to the enhancement of crude protein digestibility and crude fiber digestibility (Kierhan, 2010). Similar to this, Rehman et al. (2020) found that broiler chicken treated with probiotics at (0, 1, and 2 g/kg) and prebiotics at (0, 1, and 1.5 g/kg) recorded the best-feed conversion ratio and body weight gain. Also, Forte et al., (2018) showed that broiler chicken fed with Lactobacillus acidophilus at 2 g/100 g have positive effects on LBW, BWG, and FCR when compared to the control group. Additionally, Kaiwen et al., (2017) reported that BWG and FCR were significantly improved in broiler chickens fed diets supplemented with Bacillus subtilis. Li et al., (2008) and Sharef and Dabbagh, (2009) showed that broilers supplemented with probiotics at 1%, 1.5%, and 2% have the good BWG and FCR. In addition, Jayathilaka et al., (2017) observed that broiler chickens treated with EM exhibited greater BWG compared to the control group. Simeamelak et al., (2012) demonstrated that the adding of EM in drinking water resulted in enhanced growth performance. Furthermore, Zhang et al., (2021) confirmed these findings by showing that broiler chickens treated with EM throughout the entire rearing period exhibited the highest LBW, BWG, and best FCR. Similarly, other researchers such as Wondmeneh et al., (2011), Jwher et al., (2013), Hatab et al., (2016), Dorra et al., (2016), Nur-Azri et al., (2018), and Ye et al., (2021) have reported similar results in broilers, turkeys, quails, and partridges, respectively. However, Nuengjamnong & Luangtongkum, (2014) found that the inclusion of microbial additives in broiler feed or drinking water did not have ~~any~~ significant effects on growth performance traits. Similarly, Huang et al. (2004) found no discernible rise in BWG in broilers fed diets supplemented with Lactobacillus casei or L. acidophilus. In a similar vein, Ramaro et al. (2004) observed that adding different dosages of probiotics to the diets of broilers had no significant effect on BWG. Furthermore, Panda et al. (2000) discovered no discernible differences in BWG between chickens fed non-supplemented diets and those supplemented with EM.

Numerous research efforts have demonstrated that incorporating probiotics into bird's diet has the potential to enhance feed conversion rate (FCR) (Silva et al. in 2000). The use of effective microorganisms (EM) can also enhance feed utilization by improving digestibility, reducing feed consumption, and enhancing animal growth performance (Nur-Azri et al., 2018, Ye et al., 2021, and Zhang et al., 2021). Research conducted by Kompiang (2002) has demonstrated that probiotics play a significant role in enhancing the functionality of the digestive system, leading to improved feed digestibility. This is achieved through the

inhibition of harmful bacteria and the facilitation of the proliferation of advantageous bacteria, which contribute to the absorption of nutrients. Chickens supplemented with probiotics Bacillus sp. have a larger surface area in the intestine for nutrient absorption compared to control chickens (Sjofjan, 2003). Probiotics have the capability to augment mucin transport and microbial communities within the small intestine of poultry, thereby resulting in enhanced intestinal functionality, microflora composition in the cecum, and absorption of nutrients (Mountsouris et al., 2010). EM has been reported to increase beneficial microbial populations in the intestine, enhance nutrient utilization, improve intestinal enzyme activities, and compete pathogenic microorganisms in the gastrointestinal tract (Nahashon et al., 1994 and Wood and Abuchar, 1998). Therefore, the supplementation of EM in poultry diets can enhance nutrition digestibility (Gao et al., 2008 and Li et al., 2008). Probiotic supplementation has been shown to improve digestibility, animal growth performance, and reduce feed consumption (Bedford and Schulze, 1998). However, some previous studies have suggested that probiotic supplementation may not significantly influence FCR (Panda et al., 2000 and Mohit et al., 2007). Furthermore, no considerable disparity in feed conversion ratio (FCR) was observed between the control and treatment cohorts, suggesting that the addition of EM did not yield a noteworthy impact on FCR (Ahmad, 2004). Another study by Chatsavang and Watchangkul (1999) also found no significant difference in FCR between treatment groups.

Quails that were given a diet enriched with 20 ppm EM exhibited a noteworthy ($P < 0.001$) reduction in cholesterol, TG, ALT, and AST in comparison to the control treatment. Also, the treatment supplemented with 20 ppm EM resulted in increased HSI, Amylase, Lipase, and Trypsin levels when compared to the control group. Furthermore, the inclusion of varying levels of EM in the diets improved the lipid profile, hepatic, and digestive enzymes. In terms of liver enzymes (ALT and AST), our findings are consistent with those of Abdelkader et al. (2023), who demonstrated a significant decrease in ALT and AST levels in broilers fed diets supplemented with EM compared to the control group. Specifically, broilers that received diets containing EM exhibited a significant reduction in liver enzymes (ALT and AST), particularly with the highest level of EM (10 ppm EM). Similarly, Abdel-Aziz et al. (2020) reported similar findings. Moreover, Hatab et al. (2016) found that broilers receiving diets supplemented with EM exhibited a significant decrease in serum AST, ALT, cholesterol, and triglyceride concentrations across all tested groups compared to the control group. In contrast, Abd (2014) reported that the EM feeding groups had higher liver enzyme levels compared to the control group. Additionally, Ladine et al. (2014) indicated that EM had no impact on the serum ALT and AST concentrations.

Regarding the lipid profiles of quails, it was observed that diets supplemented with 20 ppm EM resulted in the lowest levels of cholesterol (chol) and triglycerides (TG) compared to the control and other treatments. The decline in overall serum cholesterol levels can be attributed to the biosynthesis of hepatic cholesterol, a process that is tightly regulated and transformed into bile acids. The elimination of these acids is facilitated by the adherence of EM. As a result, EM might have a role in the reduction of serum cholesterol levels. Moreover, probiotics could impede the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase and influence the reutilization of bile salts in the intestinal tract. Additionally, specific probiotic strains of lactic acid bacteria, like *Lactobacillus acidophilus*, have demonstrated interference with the transportation and assimilation of intestinal bile acids, ultimately leading to an increased excretion of these acids. Consequently, this process contributes to a hypocholesterolemic impact (Esatu et al., 2011, Sjofjan et al., 2021 and Şahan et al., 2021). Esatu et al. (2011) further documented that supplementation with EM led to a reduction in serum cholesterol levels in poultry. Bile acids, which are products

of cholesterol synthesis within the liver cells, which bind with EM and facilitate their elimination from the body. This process contributes to the regulation of hepatic cholesterol production and its conversion into bile acids, potentially elucidating the observed decline in serum cholesterol concentrations. Another pathway involves the transformation of cholesterol into copresterol, subsequently being expelled through fecal matter. Lye et al. (2010) identified Lactobacillus acidophilus as participating in this conversion of cholesterol to copresterol. Probiotics can metabolize substrates in the gut, resulting in the generation of short-chain fatty acids like propionate. Propionate functions as a potent suppressor of fatty acid biosynthesis and governs cholesterol formation in the liver, ultimately culminating in a reduction of circulating cholesterol levels. These findings are consistent with Abdelkader et al. (2023), who reported that broilers fed diets treated with EM showed significantly lower levels of total cholesterol, LDL, and TG, along with the highest HDL value compared to the control group. Additionally, broilers treated with EM at any level exhibited a significantly lower abdominal fat percentage compared to the untreated group. Abd (2014) further identified that the incorporation of EM could potentially yield a beneficial impact through the reduction of serum cholesterol and triglyceride levels in broiler chickens. Significantly elevated total cholesterol levels were observed in the bloodstream of broilers that were provided with a control diet in contrast to those groups receiving EM in various levels. However, some studies, such as those by Greany et al. (2004) and Pelicano et al. (2004), have reported that probiotics did not have a significant effect on total cholesterol levels. Chatsawang and Watchangkul (1997) did not observe—significant enhancements in serum cholesterol levels when subjected to additional dietary EM. Moreover, previous studies conducted by Pelicano et al. (2004) and Greany et al. (2004) revealed that probiotic supplementation did not yield any beneficial effects on broiler performance or reduce cholesterol levels.

The hepatosomatic index (HSI) is a methodological approach utilized to evaluate the proportion of liver mass in relation to the overall carcass mass of animals. HSI functions as a dependable metric for assessing the growth and maturation of the liver, considering variables such as age and the physiological or physiochemical condition of this organ. The significance of HSI lies in its ability to elucidate the effects of dietary additives and act as a trustworthy gauge of the feeding behavior of animals. In this specific scenario, quails subjected to 20 ppm EM treatment demonstrated an elevation in HSI values. Our findings in the same line with Abdelkader et al., (2023), who demonstrated that chicks fed diets with 5ppm EM, have the highest HSI value. Additionally, the other groups (EM7.5 and EM10) exhibited higher HSI values without ~~any~~ significant differences compared to untreated group. Broilers that received higher doses of EM in their feed experienced positive effects on their health. This outcome aligns with the results of Nur-Azri et al., (2018), who demonstrated that higher amounts of probiotics administered to quails through feed have a useful effect on quail health. This indicates that the liver maintains its normal size without affecting amino acid catabolism. In contrast, Chen et al., (2013) reported that liver weight was unaffected by the treatments. Furthermore, Awad et al., (2009) found no significant differences in liver weights resulting from the addition of probiotics to the feed of broiler chickens.

All quails that were provided with diets supplemented with EM exhibited the highest values of digestive enzymes, namely Amylase, Lipase, and Trypsin. This observation agree with Abdelkader et al., (2023), who investigated the influence of different levels of EM supplementation on digestive enzymes (Amylase, Lipase, and Trypsin). Their study revealed that the birds that consumed diets supplemented with EM displayed the highest values for all three enzymes in comparison with control, which recorded the lowest values for previous enzymes. The findings align with the results presented in the study conducted by

Zhang, et al., (2021) illustrating the positive impact of supplementing probiotics in the diet of broiler chickens on the enhancement of their digestive enzymes secretion. Moreover, recent investigations conducted by Abd El-Hack et al. (2020) and Ye et al. (2021) found that broiler chickens receiving probiotics exhibited higher amylase and protease activity.

Chicks that were provided with a diet supplemented with 20 ppm exhibited the highest values for gut morphology (small intestine and cecum), gizzard, glandular stomach, liver and the lowest value for abdominal fat. In this respect, EM play a crucial role in maintaining gut health in poultry by promoting a balanced gut microbiome (Mountsouris et al., 2010). These beneficial bacteria, such as *Bifidobacterium and Lactobacillus*, help restore and maintain the normal population of good gut bacteria in chickens. Moreover, another crucial indicator of gut health is the quantity of goblet cells present in intestinal villi, since these cells promote the production of mucin and lessen the possibility that harmful bacteria will adhere to the intestinal epithelium. Probiotics can serve as a potential alternative to antibiotic growth promoters in poultry production, contributing to improved nutrient utilization, growth, laying performance, and overall gut health of poultry birds (Shroyer and Kocoshis, 2011, Alagawany et al., 2018 and Michalska, et al., 2021). According to several studies (Chichlowski et al., 2007; Messaoudi et al., 2013; Martínez et al., 2016; Jazi et al., 2018; Zhen et al., 2018 and He et al., 2019) adding various levels of EM in broiler diets caused positive impactes on gut histomorphology. Where increased length and depth of villus. Furthermore, *Lactobacilli* have a beneficial effect on gut health, with improvements in ileum histologic (Hayashi et al., 2018). Recently, Hazrati et al. (2020) demonstrated that intestine and caeca length was enhanced for quails treated by EM. In addition, Abd El-Moneim et al., (2019 and 2020) and Olnood et al., (2015) showed the same findings, which adding probiotic in bird's diet caused a significant increase in ileum villus height. The enhancement in gut morphology for birds treated by EM may be due to the capability to stimulate mitotic cell division and trigger the expansion of intestinal epithelial cells (Bai et al., 2013; Samanya & Yamauchi, 2002). Pelicano et al., (2005) found that broilers fed a diet treated with *Lactobacillus* could enhance the zigzag form of villi in jejunum. Similarly, an elevated proportion of villus length to crypt depth and villus height enhanced absorption surface and nutrient uptake, which enhance poultry performance and health (Shroyer and Kocoshis, 2011). Manafi et al. (2017) found that broilers fed diets with EM have positive impacts on intestinal morphology. It is worth noting that the surface area of the intestine, which facilitates nutrient absorption, is greater in chickens that receive supplementation with EM than control treatment (Sjofjan, 2003). In contrast, the study conducted by Rezaeipour et al. (2015) indicated that the administration of EM to quail did not result in any significant effects on the relative weight of their internal organs. Likewise, Salehimanesh et al. (2016) reported the same outcomes in broilers. Recently, Susanti and Tugiyanti (2020) discovered that the weight of the gizzard and liver, and the length of gastrointestinal tract and caeca, were not significantly influenced by the addition of EM in drinking water. In terms of abdominal fat, Zhang et al. (2021) showed that the inclusion of EM in broiler diets caused increasing in eviscerated yield with decreasing in abdominal fat. Thus, it appears that the utilization of EM enhances digestion and absorption by increasing beneficial bacteria in the intestine, which improving gut health, and enhancing absorption of nutrients, ultimately leading to a reduction in abdominal fat (Zhang et al., 2021).

Birds that were provided with diets supplemented with EM exhibited the highest levels of IgG, IgA, IgM, and glutathione peroxidase, while also experiencing a decrease in thiobarbaturic acid-reactive compounds. Generally, the augmentation of EM levels in the diet resulted in improved values of immune index (IgM, IgA, and IgG) and GPx. Antioxidant enzyme activity, such as that of

MDA and GSH-Px, can be used to measure the human body's ability to eliminate free radicals (Rehman et al., 2018). Therefore, it is possible to ascertain the quantity and activity of antioxidant enzymes and lipid peroxidation products present in the serum by thoroughly assessing the capacity of additive antioxidants (Attia, et al., 2020). Several studies have illustrated the capacity of EM to affect the functionality and composition of antioxidant a conspicuous antioxidant impact on broilers. Recently, Zhao et al. (2020) and Peng, et al., (2021) revealed that diets supplemented with EM significantly enhanced GSH-Px activities with reduction in MDA levels. Moreover, Sun, et al., (2022) demonstrated that the serum GSH-Px contents of the group that received EM were higher compared to the control group, thus the inclusion of EM in the diet can potentially impact the composition of intestinal flora and enhance the antioxidant capacity of the body.

In terms of immunity, EM has a significant impact on poultry's immune system's defense against bacteria and other pathogens. Where it could stimulate the production of cytokines and the secretion of immunoglobulins (IgM, IgG, and IgA), as well as activate dendritic cells and natural killer cells and improve T-cell responses (Alkhalf et al., 2010 and Tsai et al., 2012). The presence of EM leads to an increase in the lymphocyte population in gut-associated lymphoid tissues, which enhance immunity through the secretion of IgA (Haghighi et al., 2006). According to Paszti-Gere et al., (2012) who showed that EM may be enhance the immune system by enhancing the production of anti-inflammatory cytokines, decreasing oxidative stress and intestinal permeability. Recently, Abdelkader et al. (2023) demonstrated that broilers treated with EM exhibited significantly higher levels of IgG and IgA in compare to untreated treatment. According to Stefaniak et al. (2020), EM can promote immunoglobulin secretion, immune cell proliferation, and nonspecific immunity by increasing macrophage phagocytic activity. Similarly, Zhang et al. (2021) observed that EM supplementation led to a significant increase in the weight of spleen, bursa of Fabricius, IgG enzymes in the body, leading to a decrease in oxidative stress-induced harm in the gastrointestinal tract (Inatomi and Otomaru, 2018, Bai, et al., 2018 and Yu, et al., 2019). In this regard, Attia et al., (2013, 2017, and 2018) and Zhang et al. (2021) detected substantial elevations in the concentrations of T-AOC and SOD, accompanied by a marked reduction in MDA concentration, suggesting that EM exhibit

and IgA levels in broilers. Moreover, specific probiotic strains like *Lactobacillus and Bifidobacterium* have been shown to elevate the levels of immunoglobulins (IgA, IgG, and IgM) in broilers and turkeys, thereby enhancing growth performance and disease resistance (Abdel-Moneim et al., 2019). Moreover, EM can also improve immunity from encouraging macrophage phagocytic activity, inducing immunoglobulin secretion, and boosting immune cell proliferation (Kaur et al., 2009). Previous study by Ahmed (2006) who indicated that the addition of EM in broiler diets could increase production of serum antibodies, especially IgM and IgG. With respect to gut ecology, chicks consuming diets supplemented with EM have shown a significant increase in the population of beneficial *Lactobacilli* in the intestines. This increase is accompanied by a decrease in the population of harmful E. coli and Salmonella, especially in chicks fed a diet with 20 ppm EM supplementation. This particular group exhibited the lowest levels of *E. coli and Salmonella*, along with the highest number of *Lactobacilli*. Moreover, chicks fed the 20 ppm EM diet displayed higher viscosity and lower pH levels in the intestines compared to the control group. Various primary and secondary metabolites, including volatile fatty acids, organic acids, and lactic acid, contribute to lowering the intestinal pH, thereby hindering the growth of pathogens like *Salmonella and E. coli* (Marteau et al., 2004). For instance, *Lactobacilli* produce lactic acid, indirectly increasing the concentration of butyric acid in the gut. This increase prompts the growth and proliferation of butyric acid-producing bacteria through cross-feeding interactions (Van

Immerseel et al., 2009). *Lactobacillus* is beneficial for domestic animals as it can inhibit the growth of pathogenic bacteria and stimulate the growth of nonpathogenic bacteria by producing various metabolites, thus enhancing the intestinal microecological environment (Attia et al., 2013, 2017, 2018). Maintaining a healthy gut flora requires improving the *Lactobacillus plantarum*, for example, competes with *E. coli* for adhesion sites by inducing MUC3 mucins (Mack et al., 1999). The concept of competitive exclusion involves beneficial microorganisms like *Lactobacilli* outcompeting pathogens, preventing their adhesion to the gut surface, and ultimately expelling them from the intestines through ingestion. This principle has been crucial in the use of probiotics in poultry and livestock production systems to counteract the harmful effects of pathogens such as *E. coli, Salmonella, Clostridia, and Campylobacter* (Jin et al., 1997). Research by Mulder (1991) demonstrated that oral administration of probiotics reduces the incidence of *Salmonella* infection in chicks. *Lactobacilli and Bifidobacteria* have exhibited potent antimicrobial activity against various pathogens such as *E. coli, Salmonella, Listeria monocytogenes, Campylobacter pylori, and Rotavirus* (Bujalance et al., 2014). Probiotics can deactivate toxin receptors on the intestinal mucosa through enzymatic mechanisms; for instance, Saccharomyces boulardii protects against *Clostridium* difficile infection by suppressing toxin production in the ileum of rabbits and can produce polyamines that inhibit the secretion of cholera toxins in the jejunum of rats (Valdes-Varela et al., 2018). A recent study by Abdelkader et al. (2023) revealed that broilers fed diets supplemented with 10 ppm EM had lower levels of E. coli and Salmonella compared to the control group, with higher Lactobacilli counts. These findings are consistent with those of Hamad et al. (2020), who found that 1% EM had the most significant impact on reducing the growth of pathogenic bacteria such as *E. coli and S. aureus.* The use of effective microorganisms, particularly *Lactobacillus*, is highly suitable for poultry and animals, as it suppresses the proliferation of pathogenic microorganisms while promoting non-pathogenic ones through the production of various metabolites, thereby enhancing the intestinal microecological environment (Attia *et al.,* 2018). The broad spared encountered pathogenic microorganisms in poultry farming include *Salmonella and E. coli enterica* (Vieco et al., 2019). The supplementation of effective microorganisms, such as *Lactobacillus plantarum and Lactobacillus casie*, has been shown to reduce *E. coli* levels and enhance *Lactobacillus counts* (Zhang et al., 2021). Similarly, Pereira et al. (2019) and Chang et al. (2020) observed a similar pattern in the gut microbial population of broilers when fed probiotics. Overall, the addition of probiotics in water has the potential to improve the composition of the gut microflora.

microbial environment by replacing pathogenic bacteria. Probiotics can adhere to and colonize the gut epithelial surface, competing with pathogens for adhesion sites, enhancing the complexity of enterocytes, and facilitating interactions among different cell types, thereby boosting phagocytosis (Bene et al., 2017).

CONCLUSION

It can be inferred that incorporating effective microorganisms up to20 ml EM /kg feed in quail diets has the ability to enhance various aspects of productivity, physiological functions, liver health, lipid profiles, enzymatic activity, antioxidant capacity, intestinal morphology, gut ecology, and immune response. Therefore, effective microorganisms have the potential to serve as a growth promoter for growing quail.

Table (1): Composition and analysis of basal diet of growing Japanese quail.

Ingredients	**%**
Maize	56.00
Soybean meal (44 CP%)	32.00
Plant concentrate meal (50 CP%)[1]	9.00
Ground lime stone	1.30
Dicalcium phosphate	0.50
Vegetable oil	0.50
DL-methionine	0.10
Salt(NaCl)	0.30
Vitamin and mineral premix[2]	0.30
Calculated analysis[3]	
Metabolisable energy (kcal/kg	2919
Crude protein	24.00
Crude fiber	3.5
Calcium	0.8
Available phosphorus	0.5

1-Plant concentrate contains (%): CP 50, CF 1.3, Ca4.72, Av P 3.1, lysine 6, methionine 2 and ME 2650 kcal/kg.

2-Premix provided per kg of diet: vitamin A, 12.000 IU; vitamin D3, 2.400 IU; vitamin E, 30 mg; vitamin K3, 4 mg; vitamin B1, 3 mg; vitamin B2, 7 mg; vitamin B6, 5 mg; vitamin B12, 15 µg; niacin, 25 mg,Fe, 80 mg; folic acid, 1 mg; pantothenic acid, 10 mg; biotin, 45 mg; choline, 125,000 mg; Cu, 5 mg; Mn, 80 mg; Zn, 60 mg; Se, 150 µg.

3-According NRC, 1994**.**

Table (2): Effect of dietary effective microorganisms on growth performance in growing Japanese quail.

Items / **Treat.**	**Control**	**EM 10 ml/kg**	**EM 20 ml/kg**	**SE**	**P-value**
Initial LBW(g)	51.52	51.52	51.68	0.60	0.9768
LBW38d (g)	230.86^{c}	245.78^{b}	250.87^{a}	1.16	0.0001
BWG10-38 (g)	179.34^{c}	194.26^{b}	199.18^{a}	1.10	0.0001
FI 10-38 (g)	605.62^{a}	588.69^{b}	589.35^{b}	0.48	0.0001
FC 10-38 (g/g)	3.39^{a}	3.04^{b}	2.97^{c}	0.02	0.0001
GR_{10-38}	1.27^{b}	1.31^{a}	1.32^{a}	0.01	0.0001
PI_{10-38}	6.85^{c}	8.12^{b}	8.49^{a}	0.08	0.0001

Abbreviations: LBW: Live Body Weight, BWG: Body Weight Gain, FI: Feed Intake, FC: feed conversion, GR: Growth rate, PI: Performance index, SE: Standard Error, EM: Effective Microorganisms, $^{a-c}$: Means within the same row with different superscript.

Table (3): Effect of dietary effective microorganisms on lipid profile, liver functions and digestive enzymes in growing Japanese quail.

Items / Treat.	Control	EM 10 ml/kg	EM 20 ml/kg	SE	P-value
lipids profile					
Total chol. mg/dL	178.14[a]	162.17[b]	158.77[b]	1.21	0.0001
TG , mg/dL	123.00[a]	119.59[a]	112.94[b]	1.58	0.0007
liver functions					
ALT, IU/L	4.00[a]	3.34[b]	3.15[b]	0.09	0.0001
AST, IU/L	302.14[a]	286.50[b]	280.86[b]	3.54	0.0011
HIS	1.45[b]	1.75[a]	1.75[a]	0.06	0.0017
Digestive Enzymes					
Amylase U/L	593.50[b]	617.85[b]	721.98[a]	17.44	0.0001
Lipase U/L	88.99[b]	87.80[b]	99.99[a]	3.18	0.0242
Trypsin U/L	76.88[b]	98.29[a]	99.04[a]	1.77	0.0001

Abbreviations: Total Chol: Total Cholesterol, TG: triglycerides, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, HIS: Hepatosomatic Index (HSI) (g liver/ g final body *weight*), SE: Standard Error, EM: Effective Microorganisms, [a-b]: Means within the same row with different superscript**.**

Table (4): Effect of dietary effective microorganisms on intestinal morphometric traits in growing Japanese quail.

Items / Treat.	Control	EM 10 ml/kg	EM 20 ml/kg	SE	P-value
Small intestine length cm	54.75[b]	61.01[a]	63.09[a]	0.94	0.0001
Small intestine weight g	4.65[c]	5.61[b]	6.73[a]	0.13	0.0001
Cecum length cm	16.33[c]	17.53[b]	17.99[a]	0.12	0.0001
Cecum weight g	1.08[c]	1.14[b]	1.31[a]	0.02	0.0001
Liver weight g	3.61[b]	4.23[a]	4.32[a]	0.16	0.0118
Gizzard weight g	4.05[c]	5.07[b]	5.31[a]	0.04	0.0001
Glandular stomach weight g	0.74[b]	0.81[a]	0.83[a]	0.01	0.0001
Abdominal fat g	0.96[a]	0.44[b]	0.38[b]	0.06	0.0001

Abbreviations: SE: Standard Error, EM: Effective Microorganisms, [a-c]: Means within the same row with different superscript

Table (5): Effect of dietary effective microorganisms on antioxidant parameters and immune response in growing Japanese quail.

Items / Treat.	Control	EM 10 ml/kg	EM 20 ml/kg	SE	P-value
Antioxidant Parameters					
GSH-PX (nmol/min/ml)	6.29[c]	7.65[b]	8.24[a]	0.17	0.0001
TBARS (nmol /ml)	1.66[a]	1.39[b]	1.15[c]	0.05	0.0001
Immune Indices					
IgG (mg/dl)	62.25[b]	69.90[a]	72.17[a]	2.06	0.0070
IgA (mg/dl)	34.37[b]	40.70[a]	39.79[a]	1.04	0.0006
IgM (mg/dl)	39.12[b]	42.68[a]	43.23[a]	0.80	0.0030

Abbreviations: GSH-PX: Glutathione Peroxidase TBARS: Thiobarbaturic Acid- Reactive Substances, IgG: Immunglobin G, IgA: Immunglobin A, IgM: Immunglobin M, SE: Standard Error, EM: Effective Microorganisms,
[a-c]: Means within the same row with different superscript

Table (6): Effect of dietary effective microorganisms on intestinal bacteria and intestinal environment in growing Japanese quail.

Items / Treat.	Control	EM 10 ml/kg	EM 20 ml/kg	SE	P-value
Gut Ecology					
E.coli log 10 cfug	7.82[a]	7.03[b]	6.56[c]	0.08	0.0001
Salamonella log 10 cfug	7.40[a]	6.62[b]	6.30[c]	0.09	0.0001
Lactobacillus log 10 cfug	6.83[b]	7.67[a]	7.67[a]	0.13	0.0001
Viscosity	2.26[c]	2.47[b]	2.58[a]	0.03	0.0001
Intestines PH	6.71[a]	6.43[b]	6.33[b]	0.06	0.0011

Abbreviations: SE: Standard Error, EM: Effective Microorganisms,
[a-c]: Means within the same row with different superscript,

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الملخص العربي

تأثير الكائنات الحية الدقيقة الفعالة على الأداء، والكيمياء الحيوية للدم، والإنزيمات الهضمية، والمناعة ومؤشرات مضادات الأكسدة، والمقاييس المظهرية للأمعاء والمجموعات الميكروبية في السمان الياباني النامى

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كان الهدف من هذا البحث هو دراسة تأثير الكائنات الحية الدقيقة الفعالة ((EM على أداء نمو السمان الياباني وتعداد الميكروبات وبنية الأمعاء وبعض مؤشرات الدم. في دراسة غذائية استمرت ٣٨ يوماً، تم توزيع ٢٤٠ طائر سمان ياباني بعمر ١٠ أيام عشوائياً على ثلاث معاملات تجريبية تكونت كل منها من أربع مكررات بكل مكرر ٢٠ طائر. اشتملت العلائق الغذائية التجريبية على نظام غذائي قاعدي (مجموعة اولى)، ونظام غذائي قاعدي مكمل بـ ١٠ و٢٠ مل EM/ كجم من العلف (المجموعة الثانية والثالثة). أظهرت طيور السمان التى غذيت على العلائق المحتويه على ٢٠ مل من EM/ كجم من العلف أفضل وزن للجسم الحي، وزيادة وزن الجسم، ومعدل النمو، ومؤشر الأداء، ونسبة تحويل العلف، مع أقل كمية من العلف المستهلك خلال فترة الدراسة. أيضا سجلت الطيور التي غذيت على علائق مضاف إليها ٢٠ مل من ME/ كجم من العلف أقل مستويات من الكوليسترول الكلي والدهون الثلاثية والألانين ترانساميناز والأسبارتات ترانساميناز، وأعلى مستويات HSI والأميلاز والليباز والتربسين. الخصائص المورفولوجية مثل طول الأمعاء ووزن الأمعاء وطول الأعور ووزن الأعور ووزن الكبد ووزن القانصة ووزن المعدة الغدية أعلى في المجموعة التي تم تغذيتها بعليقة احتوت على ٢٠ مل من EM/ كجم من العلف، كما كانت هذه المجموعة أقل في دهون البطن. علاوةً على ذلك، كان لدى المجموعة التي تم تغذيتها ب ٢٠ مل من EM/ كجم من العلف أقل عدد من السالمونيلا والإشريكية القولونية، بالإضافة إلى أفضل معايير مضادات الأكسدة والاستجابات المناعية وعدد العصيات اللبنية (بكتيريا حامض الاكتيك) مقارنةً بالمجموعة الضابطة . من نتائج هذه الدراسة، أدى إضافة EM بمعدل ٢٠ مل/كجم من العلف إلى تحسينات في الأداء، ومضادات الأكسدة، والكيمياء الحيوية للدم، وبنية الأمعاء، والمؤشرات المناعية، والميكروبات المعوية في طيور السمان الياباني النامى