Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Impact of DL-methionine and lactobacillus as dietary supplements on growth performance, nutrients digestibility and some blood constituents of chicken

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Received in 3/11/2024
Received in revised from 24/11/2025
Accepted in 25/12/2024

Keywords:
Lactobacillus,
Hubbard chicks
DL-methionine
Gamma globulin
Glutathione

ABSTRACT:

Enhancing health and production efficiency of poultry industry using natural products is now being applied as an alternative for synthetic compounds and antibiotics. Lactobacillus recognized for their positive impact on gut health and immunity due to their direct interaction with the gut microbiota, which is essential for a robust immune system. On the other hand, methionine is an essential amino acid in poultry nutrition, playing a crucial role in protein synthesis, growth, and overall health. The present study was designed to present and evaluate the effects of DL-methionine. and lactobacillus as dietary supplements on the growth performance, nutrients digestibility and blood constituents of poultry. The study was conducted on 50 one-day old Hubbard chicks. The chicks were assigned to five groups. 1st group (control negative) was fed on a basal diet for 4 successive weeks. 2nd group was fed on a basal diet mixed with DL-methionine at a dose of (1 g/ kg diet). 3rd group was fed on a basal diet mixed with lactobacillus at a dose of (1 ml / liter of water). 4th group was fed on a basal diet mixed with DL-methionine at a dose of (2 g/ kg diet). 5rd group was fed on a basal diet mixed with lactobacillus at a dose of (2 ml / liter of water). The results showed that DL-methionine (specifically the higher dose 2 g/ Kg feed) induced a significant decrease in feed conversion, while total proteins and albumin levels were increased in both groups that consumed 1 and 2 ml lactobacillus /liter of water. Gamma globulin was increased significantly in the group that was supplied with 1 ml lactobacillus/liter of water. Superoxide dismutase showed significant increase in groups given DL-methionine with dose 2 g/kg diet and lactobacillus with dose 2ml /liter of water compared to control group. Glutathione showed significant elevation in the group given DL-methionine with dose 2 g/kg diet.

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DOI: 10.21608/ejah.2025.411344

INTRODUCTION:

Feed additives are minor components in animal feed that are used for enhancing the nutrition, performance and health. Some of the most commonly used feed additives in animal diet include enzymes, pre- and probiotics and antibiotic growth promoters **(Jha et al. 2020).**

Methionine (MET) plays a crucial role in poultry nutrition, acting as a building block for proteins and as a methyl donor in various metabolic processes. Its importance is underscored by the fact that poultry cannot synthesize it in adequate amounts, necessitating its inclusion in diets to maintain optimal health and productivity **(Burley et al. 2016 a).** The supplementation of MET in broiler diets is a well-established practice that has been shown to enhance growth parameters such as body weight, feed intake, and feed conversion ratio **(Majdeddin et al. 2019).** These improvements are not just beneficial for the birds' well-being but also translate into economic advantages for poultry producers. The precise requirements for MET vary depending on several factors, including the age, breed, and intended production outcomes of the broilers. For instance, younger broilers have higher MET requirements to support their rapid growth phases **(Burley et al. 2016 b).** As research continues to evolve, the understanding of MET's role in poultry diets will further refine these dietary recommendations, ensuring that the health of the birds and the efficiency of poultry production continue to improve.

The use of probiotics in poultry farming has indeed revolutionized the approach to animal health and nutrition. These live microorganisms, which are beneficial to the host, have been increasingly adopted due to a shift towards antibiotic-free poultry production, driven by consumer awareness and regulatory changes. Probiotics, comprising mainly of bacteria like Lactobacillus as well as beneficial yeasts, have shown to significantly improve the intestinal microbial balance in poultry. This leads to enhanced gut health, which is a critical factor in boosting the birds' immune system, thereby reducing the need for antibiotics. Moreover, the enzymes produced by probiotics facilitate the breakdown of complex feed molecules, enhancing nutrient absorption and feed conversion ratio, which contributes to better growth performance and overall poultry health **(Jha et al. 2020; Park et al. 2016; Hargis et al. 2018).** The administration of probiotics through feed is the most widespread method, but other approaches such as water-soluble formulations are gaining traction. This versatility in administration ensures that probiotics can be administrated efficiently to flocks of all sizes, promoting a sustainable and health-focused approach to poultry farming **(Hargis et al. 2018; Jiang et al. 2017).**

One of the main challenges that face the poultry industry is how to improve their health and maximize their production. Therefore, the aim of the present study is to assess the effect of supplementing lactobacillus and DL-methionine with different doses on weight and general health of chicken, and also to recommend the optimum dose which when used will result in increasing in production and enhancing general health.

2. MATERIALS AND METHODS:

2.1. Ethical statement:

This study was approved by the institutional Animal Care and Use Committee (ARC-IACUC) (approval no. ARC/AHRI/131/24).

2.2. Materials:

DL- Methionine feed grade 99% : produced by Evonik Antwerpen N.V. obtained as a powder.
Lactobacillus: produced by Bactizad Company and obtained as a solution containing $1X10^{12}$ CFU / liter.

2.3. Methods:

The present study was carried out at Animal Health Research Institute, Dokki. 50, one day old Hubbard chicks were used. The chicks were obtained from Pyramids Poultry company for broiler production, Giza. Egypt. All chicks were active and healthy. Chicks were allowed to acclimate for 1 week before starting the experiment. The birds were kept in clean batteries under strict hygiene measures. Each group was raised in a separate pen with an identifying data sheet to record all the required information. Chicks were vaccinated according to the schedule obtained from the company. Food and water were available and *ad libitum*.

2.4. Experimental design:

50 one-day old chicks were divided into five groups 10 chicks each. All birds in groups 2 to 5 were drinking or eating formulated diet throughout the experiment (4 successive weeks). 1st group (control negative) was fed on a basal diet for 4 successive weeks **according to NRC (1994).** 2nd group was fed on a basal diet mixed with DL-methionine at a dose of (1 g/ kg diet) **(Pokoo-Aikins et al. 2021).** 3rd group was fed on a basal diet mixed with lactobacillus at a dose of (1 ml / liter of water) (as instructed by the manufacturer). 4th group was fed on a basal diet mixed with methionine at a dose of (2 g/ kg diet) **(Pokoo-Aikins et al. 2021).** 5rd group was fed on a basal diet mixed with lactobacillus at a dose of (2 ml / liter of water) (double the dose instructed by the manufacturer).

2.5.Growth performance parameters:

Over the entire period of the experiment (4 weeks) all birds were weighed weekly. The gain in the body weight was calculated as the difference between the initial and the final weight. Daily feed intake was also recorded. The data obtained were used to calculate growth performance parameters (feed conversion).

2.6. Sampling:

After the end of the experiment blood samples were collected from the birds by puncture of the tibia vein **(Davies et al. 1976).** Sera were obtained to carry out protein electrophoresis and for determination of some biochemical parameters (glutathione, superoxide dismutase).

2.7. Biochemical parameters:

Serum total protein and it's electrophoretic pattern were determined by Spectrum kit CAT. NO 310 001 and chemical preparation of polyacrylamide gel electrophoresis using the continuous buffer system of **Kaplan and Szalbo (1983) and Davis (1964)** respectively, and the calculated based on SynGene S. No. 17292*14518 smempcs program using Scie Plas TV100 Mini Vertical Gel Unit UK with Power Supply Consort EV714, Belgium.

Reduced glutathione was determined by kits obtained from biodiagnostic according to the method described by Beutler et al., 1963. Superoxide dismutase was determined by kits obtained from biodiagnistic according to the method described by (**Nishikimi et al. 1972)**

Statistical analysis:

Analysis of data obtained from both growth parameters and biochemical investigations was performed using analysis of variance (ANOVA) processed by SPSS software version 20 (Statistical Package for Social Science), followed by "less significant difference" (LSD) with p>0.05considered statistically significant.

RESULTS:

3.1.Growth performance parameters:

Feed conversion results showed significant decrease (higher weight gain with lower feed intake) in group 4 that consumed DL-methionine with dose 2 g/kg diet (Table (1)).

3.2. Biochemical parameters:

Total proteins and albumin both showed significant increase in both groups that consumed 1 and 2 ml lactobacillus /liter of water. Gamma globulin showed significant increase in the group that was supplied with 1 ml lactobacillus/liter of water (Table (2)).

Superoxide dismutase showed significant increase in groups given DL-methionine with dose 2 g/kg diet and lactobacillus with dose 2ml /liter of water compared to control group. Reduced glutathione showed significant elevation in the group given DL-methionine with dose 2 g/kg diet (Table, 3).

Table 1. The effect of DL-methionine (1 or 2g/ Kg diet) and lactobacillus (1 or 2 ml/liter of water) on growth performance (feed intake, weight gain and feed conversion) in broiler chicken during the experiment. Mean and standard error (*P<0.05*). Means with the same letter differ significantly

Performance parameters		Control	DL-methionine (1 g/ kg diet)	lactobacillus (1 ml / liter of water)	DL-methionine (2 g/ kg diet)	lactobacillus (2 ml / liter of water)
Feed intake (g)	Mean	3354	3339	3279.25	3157.75	3087.75
	St. error	128.4296	112.6913	151.9229	149.0807	125.9831
Weight gain (final weight-initial weight)	Mean	1856.5	1989	1887.25	1970.25	1814.5
	St. error	68.69437	28.63855	45.17443	82.86573	59.02895
Feed conversion (g feed/ g weight gain)	Mean	1.8089[a]	1.6796	1.7392[c]	1.6009 [a,c]	1.7006
	St. error	0.05197	0.04619	0.05774	0.0288	0.0231

Table 2.The effect of DL-methionine (1 or 2g/ Kg diet) and lactobacillus (1 or 2 ml/liter of water) on total albumin, alpha, beta, gamma and total protein in serum in broiler chicken during the experiment. Mean and standard error (*P<0.05*). Means with the same letter differ significantly

Protein parameters (g/dl)		Control	DL-methionine (1 g/ kg diet)	lactobacillus (1 ml / liter of water)	DL-methionine (2 g/ kg diet)	lactobacillus (2 ml / liter of water)
Albumin	Mean	1.324[a]	1.487	1.619 [a]	1.4435[d]	1.737[a.d]
	St. error	0.013	0.1	0.05	0.03	0.06
Alpha (α) globulin	Mean	1.33	0.823	1.488	1.328	1.411
	St. error	0.35	0.77	0.104	0.07	0.08
Beta (β) globulin	Mean	0.897	1.26	1.18	1.057	1.178
	St. error	0.21	0.09	0.07	0.13	0.08
Gamma (γ) globulin	Mean	0.8[a]	0.995	1.11[a]	1.044	1.07
	St. error	0.08	0.075	0.079	0.08	0.102
Total proteins	Mean	4.38[a]	5.03	5.28[a]	4.87	5.4[a]
	St. error	0.23	0.49	0.12	0.2	0.28

Table 3. The effect of DL-methionine (1 or 2g/ Kg diet) and lactobacillus (1 or 2 ml/liter of water) on superoxide dismutase (SOD) and glutathione (GSH) in serum of broiler chicken during the experiment. Mean and standard error (*P<0.05*). Means with the same letter differ significantly

Antioxidant parameter		Control	DL-methionine (1 g/ kg diet)	lactobacillus (1 ml / liter of water)	DL-methionine (2 g/ kg diet)	lactobacillus (2 ml / liter of water)
SOD (U/ml)	Mean	18.223[a]	19.13[b]	25.58	26.92[a]	30.07[a,b]
	St. error	0.22	0.4	0.76	0.47	0.41
GSH (mmol/L)	Mean	3.5[a]	3.8	3.94	4.59[a]	4.09
	St. error	0.04	0.05	0.14	0.16	0.06

DISCUSSION:

Enhancing health and production efficiency of poultry industry using natural products is now being applied as an alternative for synthetic compounds and antibiotics. Continuous search for a better and more efficient natural supplement must be performed in order to increase poultry production. The aim of the present study was to determine the better feed additive and the dose to use whether it is lactobacillus or DL-methionine to enhance weight and general health of broiler chicken. By focusing on the precise dietary needs and the cautious use of resources, producers can achieve a balance between economic efficiency and environmental stewardship.

DL- methionine is an essential amino acid crucial for protein synthesis and overall growth in broilers. Supplementing broiler diets with higher level of DL-methionine has been shown to enhance growth performance and improve feed conversion ratio by optimizing nutrient utilization and supporting metabolic processes. This finding agrees with **Agostini et al. 2016; Rehman et al. 2019 and Rubin et al. 2007.**

On the other hand, there was no significant effect in food intake, weight gain or feed conversion in the two groups supplemented with either 1 or 2 ml lactobacillus /liter of water. In line with the present results, **Fajardo et al. 2012** found no effect of probiotic *L. casei* on broiler chickens' growth, feed consumption, and feed conversion ratio. Likewise, **Rehman et al. 2020** showed no influence of multistrain probiotics (mixture of *Lactobacillus plantarum*, *Enterococcus faecium*, *Lactobacillus rhamnosus*, *Candida pintolepesii*, *Aspergillus oryzae*, and *Bifidobacterium bifidum*) on growth, feed consumption, and feed efficiency of broilers during day 0-35 of the experiment. In addition, **Sarangi et al. 2016** reported no influences of dietary inclusion of prebiotic (mannan-oligosaccharides), probiotic (*L. plantarum*, *Lactobacillus bulgaricus*, *Bifidobacterium bifidus*, *Streptococcus faecium*, and *Saccharomyces cerevisiae*), and synbiotic (mixture of *L. bulgaricus*, *S. faecium*, *L. plantarum*, *S. cerevisiae*, *B. bifidus*, and mannan-oligosaccharides) on final weight, weight gain, feed consumption, and feed conversion of broilers during the rearing for 6 weeks.

Total proteins, albumin and gamma globulins showed significant increase in groups given lactobacillus. Total protein increased levels agrees with the finding of **Astuti et al. 2022**, this can be attributed to improved protein digestibility and utilization resulting in increased concentration of blood total protein **(Mangisah et al. 2021).**

The impact of probiotic strains on the immune system is a fascinating area of study, with research indicating a variety of beneficial effects. These microorganisms can influence the host's immune responses by enhancing mucus secretion and antimicrobial peptides (AMPs), which serve as a first line of defense against pathogens **(Anderson et al. 2010; Madsen, 2012).**

They also modulate cytokine levels, which are crucial in signaling during immune responses. For instance, the strain *L. brevis* ZLB004 has been shown to reduce pro-inflammatory cytokines, potentially mitigating inflammatory responses **(Liu et al. 2015).**

The regular intake of probiotics may boost the immune system by increasing the activity of macrophages, lymphocytes, and natural killer (NK) cells, as well as the production of immunoglobulins and γ-interferon. This can lead to a more robust immune system, better equipped to fend off diseases. Additionally, different strains of *Lactobacillus* spp. have been found to have specific modulatory effects on the immune systems of poultry, which could have implications for both animal health and the poultry industry **(Koenen et al. 2004; Haghighi et al. 2006; Yang et al. 2009; Alkhalf et al. 2010).** The research by **Ahmed et al. in 2014** underscored the effects of probiotics, showing an increase in immunoglobulin M levels in broilers, while not affecting other immunoglobulins like IgG and IgA. This highlights the potential for targeted immune support through the use of specific probiotic strains. On the other hand, **Rocha et al. 2012** reported elevation in IgA and IgG levels in chicken when *Lactobacillus* spp. was administered at a concentration of 10^{10} CFU/ml.

In poultry production, stress management is a critical aspect that encompasses various factors such as management practices, nutrition, technological advancements, and environmental conditions. Oxidative stress, induced by an overproduction of free radicals, poses a significant challenge at the molecular level. To counteract this, birds have evolved a sophisticated antioxidant defense system. This system includes several layers of protection, with the primary line being antioxidant enzymes. These enzymes, such as superoxide dismutase, glutathione peroxidases, and catalase, play a vital role in neutralizing the superoxide radical - a primary biological radical - and its derivatives. Understanding and enhancing these natural defense mechanisms can lead to better stress management strategies in poultry farming, ensuring the health and productivity of the birds **(Martinez et al. 2017; Surai et al. 2019).**

The antioxidant defense system in commercial poultry production requires external assistance, such as dietary supplementation of traditional antioxidants such as vitamin E or other nutrients with regulatory functions in the antioxidant defenses. Met participation in the synthesis of glutathione have reported the high -level glutathione in heat-stressed broilers with methionine supplementation **(Santana et al. 2021; Zeitz et al. 2020; Elnesr et al. 2019).**

Studies have indicated that methionine levels can profoundly affect SOD activity in broilers, with insufficient methionine reducing SOD activity **(Wu et al. 2012),** while higher levels enhance it across various tissues **(Chen et al. 2013)**. This balance is crucial as SOD plays a vital role in protecting cells from oxidative damage. These findings underscore the importance of Met in maintaining the delicate equilibrium of the body's antioxidant defenses and immune function, highlighting its potential in improving animal health and resilience against stress-related conditions **(Martinez et al. 2017; Lugata et al. 2022).**

CONCLUSION:

The present study showed that supplementation of DL-methionine (specifically the higher dose 2 g/ Kg feed) in broiler chicken showed significant decrease in feed conversion (higher weight gain with lower feed intake) than those given normal diet or lactobacillus supplementation. On the other hand, total proteins and albumin both showed significant increase in both groups that consumed 1 and 2 ml lactobacillus /liter of water. Gamma globulin showed significant increase in the group that was supplied with 1 ml lactobacillus/liter of water. Superoxide dismutase showed significant increase in groups given DL-methionine with dose 2 g/kg diet and lactobacillus with dose 2ml /liter of water compared to control group. Glutathione showed significant elevation in the group given DL-methionine with dose 2 g/kg diet.

In other words, methionine is better in enhancing feed conversion rate while lactobacillus is better in improving health status and decreasing animal stress.

RECOMMENDATIONS:

It is recommended –based on the present study- to use methionine to get better meat yield from broiler chicken, and to use lactobacillus to improve their health status.

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