Physiological and Biochemical Response of Probiotics and Phytogenic Inclusion as Growth Promoters on Growing Male Rabbits

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Abstract

This experiment was conducted to study the effect of the inclusion of herbal supplementation (Thyme) and probiotic lactobacillus acidophilus on productive, physiological and immunological capabilities of growing rabbit after fattening period. It has been used in this research 30 weaning New Zealand White (NZW) rabbits at 35 days of age divided to 5 groups, 6 for each. Group 1 served as control group; Group 2 treated with 20 mg/kg b.w. of thyme aqueous extract; Group 3, 4 and 5 treated with 20, 40, 60 million bacterial count/kg b.w. of lactobacillus acidophilus, respectively. The experiment lasted for 2 month and growth performance, blood samples, liver and brain tissue were collected at the end of experiment. Revealed data demonstrated that body weight, growth performance, body weight gain, liver functions, metabolic function, and oxidative stress markers were generalized stimulated with thyme (20 mg/kg b.w.) and probiotics low dose (PBLD,20 million bacterial count/kg b.w) and confirmed via liver histological examination. In contrast probiotics medium dose (PBMD, 40 million bacterial count/kg b.w) and Probiotics high dose (PBHD, 60 million bacterial count/kg b.w) disrupt most physiological, biochemical and histopathological parameters. Thus, the study concluded that thyme (20 mg/kg b.w.) and probiotic low dose (20 million bacterial count /kg b.w) have powerful stimulatory effect in physiological and immunological performance after two months of treatments.

Key words: Thyme, Probiotic, New Zealand White (NZW) rabbits, oxidative stress markers.

Introduction

Antibiotic growth promoter is used to describe any medicine that destroys or inhibits bacteria and subsequent stimulate health performance and muscle building for domestic farm animals (Thomke and Elwinger, 1998). Worthily, antibiotic growth promoters are used to increase growing, digestion, and decrease most gastrointestinal tract disturbance. Many side effects befall antibiotics specially for destroy and kill benefit and pathogenic bacteria thus, large and most ruminants has many drawback for its effects (Allen et al. 2013).

Recently, natural probiotics act as a natural growth promoters or non-antibiotic growth promoters. They are commonly regarded as favorable alternatives to antibiotic growth promoters in livestock production. The main advantage of natural growth promoters are to low risk regarding bacterial resistance or undesired residues in animal products such as meat, milk or eggs. Generally, probiotics are live microorganisms which support the development of a beneficial gut microbiota. Probiotic bacteria (e.g. from the genera Lactobacillus, Bifidobacterium, Enterococcus) counteract undesired microorganisms such as Salmonella or E. coli by blocking receptors on the gut wall, production of antimicrobial substances or activation of the immune system (Richards et al. 2005).

Phytogenics are derived from herbs, spices or aromatic plants and have shown antimicrobial, antifungal, antiviral, antioxidant or sedative properties. They are known for their appetizing effects, since they increase the palatability of the feed and stimulate endogenous digestive enzymes. Moreover, phytogenics have a pronounced impact on the gut microflora. (Männner, 2011). The aim of the present investigation was to study the effect of the inclusion of herbal supplementation (Thyme) and probiotic lactobacilli compared with control group on productive, physiological and immunological capabilities of growing rabbit after fattening period.

Materials and methods

This experiment was done30 on New Zealand White (NZW) rabbits of one months (35 days) of age and average weight of 500g. The animals were randomly divided into five groups, each one comprise 6 animals. The 1st group preserved as normal control, 2nd group treated with 20 mg/ kg b.w. of Thyme vulgaris aqueous extract, 3rd group treated with 20 million bacterial count (MBC)/kg b.w, 4th group treated with 40 MBC/kg b.w, 5th group treated with 60 MBC/kg b.w. The experiment lasted for 2 months after weaning from mothers cages. Blood samples, liver and brain tissue were collected after two month of treatment for physiological, biochemical, and histopathological examination. The experimental rabbits were grouped and housed in a conventional clean facility according to the guidelines of the Institutional Animal Ethics. 

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Committee of NODCAR. All the experimental procedures were carried out in accordance with international guide-lines for the care and use of laboratory animals.

**Herbals extraction:**
Approximately 100 g of *Thyme vulgaris* leaves was placed in a clean, flat-bottomed glass container and soaked in ten volume of distilled water. The container with its contents was sealed and kept for 3 days. Then extraction was carried out using ultrasonic sound bath accompanied by sonication (40 minutes). The content filtrated by a piece of clean, white cotton material. The extract then was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and dried by electric oven at 45°C temperature and continued up to obtain aqueous (14.95 g) extract. The gummy extract was stored in an air tight container (Algohary et al. 2016).

**Body weight and daily weight gain:**
Body weight and daily weight gain were determined at the beginning of experiment and after two month of treatment at the end of experiment. Daily weight gain was calculated by difference between final and initial weight divided by 60 days.

**Measurement of blood Biochemical parameters:**
**Measurement of serum liver function enzymes:**
Hepatic dysfunction was assessed by measuring the elevation in serum levels of Aspartate transaminase (AST) and Alanine transaminase (ALT) using commercially available kits. The results were expressed in U/L. (Reitman and Frankel, 1957).

Serum total protein and albumin determined using commercially available kits according to the method of Doumas et al. (1971). The globulin value was obtained by subtracting the value of albumin from the corresponding value of total protein. The albumin to globulin ratio (A/G) was calculated by dividing A/G values. The results were expressed in g/L according to Bradford (1976).

**Determination of Metabolic hormones:**
**Triiodothyronine (T3), Thyroxin (T4), and Thyroid stimulating hormone (TSH):**
Determination of serum T3 and T4 (Saxema et al., 1968) TSH (Olayemi, 2007) by ELISA (Enzyme Linked Immunosororbant Assay), the kit was obtained from Fortrees Diagnostic Limited, United Kingdom and North Ireland.

**Determination of oxidative stress markers:**
**Determination of Malondialdehyde (MDA) in liver tissue by HPLC:**
Malondialdehyde (MDA) standard was prepared by dissolving 25 μL 1,1,3,3 tetraethoxypropane (TEP) in 100 ml of distilled water to give 1 mM stock solution. Working standard was prepared by hydrolysis of 1 ml TEP stock solution in 50 ml 1% sulfuric acid and incubation for 2 h at room temperature. The resulting MDA standard of 20 nmol/ml was further diluted with 1% sulfuric acid to yield the final concentration of 1.25 nmol/ml to get the standard for the estimation of total MDA (Karatep, 2004). The samples were analyzed on an Agilent HP 1100 series HPLC apparatus (USA). The analytical column Supelcosil C18 (5 μm particle and 80 Å pore size) (250 x 4.6 ID). Mobile phase consists of 30 mmol KH2PO4 and methanol (65%-35%, H2PO4 by pH 4), and the mobile phase at a 1.5 ml/ min flow rate, wavelength 250 nm. According to the method of Karalas et al. (2002).

**Determination of oxidized glutathione (GSSG) and reduced glutathione (GSH) in liver tissue by HPLC:**
The thiols compounds of oxidized and reduced glutathione were detected by HPLC using the method of Jayatilleke and Shaw (1993). Glutathione (oxidized and reduced) reference standard purchased from Sigma Chemical Co. Dissolved in 75% methanol in stock 1mg/ml and diluted before application to HPLC. The HPLC system of Agilent consisted of quaternary pump, a column oven, Rheodine injector and 20μl loop, UV variable wavelength detector. The report and chromatogram taken from Chemstation program purchased from Agilent. Synergi RP Max column 3.9 at wavelength 210 nm with flow rate 2ml/min was used. Pot. Phosphate buffer - acetonitrile at PH 2.7 was used as an isocratic mobile phase.

**Determination of nitrite / nitrate (NO) of liver tissue by HPLC:**
Nitrites and nitrate was determined according to the method of Papadoyannis et al. (1999) by HPLC. Sodium nitrite and sodium nitrate used for the reference standard preparation with stock concentration 1mg/ml. A standard mixture of nitrite and nitrate was used to determine the retention times and separation of the peaks. Nitrite and nitrate concentrations were equal in the mixture solution. HPLC analysis: The samples were analyzed on an Agilent HP 1200 series HPLC apparatus (USA). The analytical column was anion exchange PRP-X100 Hamilton, 150 x 4.1 mm, 10 μm. The mobile phase was a mixture of 0.1 M NaCl - methanol, at a volume ratio 45:55. The flow rate of 2 mL/min, wavelength adjusted to 230 nm.

**Determination of serotonin [5-hydroxytryptamine (5HT)] in brain tissue by HPLC:**
The HPLC system consisted of quaternary pump; a column oven, Rheodine injector and 20μl loop, UV variable wavelength detector. The report and chromatogram taken from data acquisition program purchased from chemstation. The sample was immediately extracted from the trace elements and lipids by the use of solid phase extraction Chromabond column NH2 phase cat. No.730031. the
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Histopathological examination

Samples were taken from the liver of rabbits in different groups and fixed in 10% formal saline for twenty four hours. After that all tissue washed via tap water then serial dilutions of alcohol to dehydrate samples. Cleared specimens embedded in xylene then paraffin twenty four hours at 56°C in hot air oven. Paraffin blocks were prepared for sectioning at 4 μm by slidge microtome. The obtained tissue sections were deparaffinised and stained on glass slide by hematoxylin and eosin stains for histopathological examination via electric light microscope according to Banchroft et al. (1996).

Statistical analysis:

Statistical analysis of the obtained data was performed using the general linear model (GLM) produced by Statistical Analysis Systems Institute (SAS, 2004). Significant differences among means were evaluated using Duncan’s Multiple Range Test. The following linear model was applied:

\[ Y_{ij} = \mu + \alpha_i + \xi_{ij} \]

where: \( Y_{ij} \) = Observation measured; \( \mu \) = Overall mean; \( \alpha_i \) = Effect of treatment; \( \xi_{ij} \) = Experimental error assumed to be randomly distributed \((\sigma^2 = 0)\).

Results:

1- Body weight and daily gain:

As shown in Table 1 generalized increase \((P<0.05)\) in body weight and body weight gain in groups treated with Thyme and PBLD in comparing with control group and did not show any significant in other groups after two month of treatments. In addition, thyme and PBLD showed significant differences increase of body weight gain in comparing with control group. In contrast, PBMD and PBHD did not show any significant effect compared with control group.

2- Liver function:

The obtained biochemical data in Table 2 did not show any significant effect of thyme and PBLD treatment in liver function such as ALT and AST. On the other hand, PBHD showed significant hepatocytes infiltration resembling in AST concentration. Level of protein production resembling in TP, Glob and A/G showed significant increase \((P<0.05)\) in thyme group in comparing with control group. Indeed, PBLD showed stimulation in protein production in comparing with control group but PBMD and PBHD showed mild inhibition \((P>0.05)\) in protein production in comparing with control group.

Table 1. Effect of thyme, low, medium, and high dose of probiotics on growing rabbit's growth after two months of treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g) after 2 month of treatment</th>
<th>Body weight gain(g/day)at 4-12 Wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1923 ± 52.3b</td>
<td>16.7 ± 0.448b</td>
</tr>
<tr>
<td>Thyme</td>
<td>1984 ± 52.68ab</td>
<td>19.5 ± 0.549a</td>
</tr>
<tr>
<td>PBLD</td>
<td>2099 ± 59.33a</td>
<td>20.2 ± 0.576a</td>
</tr>
<tr>
<td>PBMD</td>
<td>1949 ± 52.2ab</td>
<td>16.6 ± 0 .44b</td>
</tr>
<tr>
<td>PBHD</td>
<td>1920 ± 52.36b</td>
<td>15.5 ± 0.417b</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± S.E.M for 6 rabbits/group.

Thyme = Thyme extract 20 mg/kg b.w, PBLD=Probiotics low dose 20 MBC/kg b.w , PBMD=Probiotics medium dose,40 MBC/kg b.w) and PBLD Probiotics high dose (PBHD, 60 MBC/kg b.w).

a, b, c, d means having different superscript letters in the same column differ significantly \((P<0.05)\).

Table 2. The effect of thyme extract, low, medium, and high dose of probiotics on growing rabbit's liver function after 2 months of treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (u/L)</th>
<th>ALT (u/L)</th>
<th>TP (g/dL)</th>
<th>Alb (g/dL)</th>
<th>Glob (g/dL)</th>
<th>A/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.54 ± 0.782b</td>
<td>36.1 ± 1.006b</td>
<td>6.75 ± 0.18bc</td>
<td>3.98 ± 0.11b</td>
<td>2.76 ± 0.07b</td>
<td>1.52 ± 0.04a</td>
</tr>
<tr>
<td>Thyme</td>
<td>24.95 ± 0.696c</td>
<td>37.15 ± 0.987b</td>
<td>7.13 ± 0.19a</td>
<td>3.81 ± 0.10b</td>
<td>3.31 ± 0.09a</td>
<td>1.08 ± 0.02b</td>
</tr>
<tr>
<td>PBLD</td>
<td>31.56 ± 0.886b</td>
<td>41.36 ± 1.094a</td>
<td>7.51 ± 0.21a</td>
<td>4.45 ± 0.12a</td>
<td>3.06 ± 0.08ab</td>
<td>1.48 ± 0.04a</td>
</tr>
<tr>
<td>PBMD</td>
<td>32.1 ± 0.903ab</td>
<td>48.3 ± 1.303a</td>
<td>6.45 ± 0.17cd</td>
<td>3.82 ± 0.10b</td>
<td>2.63 ± 0.07b</td>
<td>1.58 ± 0.04a</td>
</tr>
<tr>
<td>PBHD</td>
<td>34.8 ± 0.927a</td>
<td>36.51 ± 1.017b</td>
<td>6.26 ± 0.17d</td>
<td>3.99 ± 0.11b</td>
<td>2.27 ± 0.06b</td>
<td>1.81 ± 0.05a</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± S.E.M for 6 rabbits/group.

Thyme = Thyme extract 20 mg/kg b.w, PBLD= Probiotics low dose 20 MBC/kg b.w , PBMD=Probiotics medium dose,40 MBC/kg b.w) and PBLD Probiotics high dose (PBHD, 60 MBC/kg b.w).

a, b, c, d means having different superscript letters in the same column differ significantly \((P<0.05)\).

AST= Aspartate transaminase, ALT = alanine transaminase, TP= Serum total protein, ALB = albumin, glob=globulin and A/G = albumin / globulin ratio.
3- Thyrotrophic hormones (TSH) and Thyroid hormones (T3 and T4):

Results depicted in Table 3 showed generalized stimulation in T3 (P<0.05) for all treatments in compared with control group; at the same time PBHD showed (P<0.05) markedly increase in T4 in comparing with control group. On the whole, TSH did not affected by any treatments after the end of experiment.

4- Antioxidant and oxidative stress markers in liver tissue:

Malondialdehyde, reduced glutathione, oxidized glutathione and nitric oxide:

It is clear from Table 4 that PBMD and PBHD showed significant increase (P<0.05) in MDA, GSSG and NO in comparing with control group with actual mean of 57.25 ± 1.611 (μmol/g) and 3.26 ± 0.086a (μmol/g), respectively and NO with actual mean of 0.498 ± 0.014 (μmol/g) and 0.533± 0.014 (μmol/g) respectively. That Thyme and PBLD increase endogenous antioxidant substance and decrease oxidative and nitrositive radicals after two months of treatments in comparing with control group. As shown, an increase (P<0.05) in GSH treated with Thyme and PBLD in comparing with control group with actual mean of 19.84 ± 0.527 (μmol/g) and 16.87 ± 0.446 (μmol/g), respectively also PBMD and PBHD markedly decrease GSH in comparing with control group with actual mean of 13.59 ± 0.367 (μmol/g) and 11.02 ± 0.307 (μmol/g), respectively.

5- Brain serotonin (5-hydroxytryptamine-5HT):

As shown in Figure 1, PBMD and PBHD showed significant (P<0.05) decrease of 5HT in comparing with control group with actual mean of 0.413 µg/g, 0.398 µg/g, respectively. Thyme and PBLD treated group increase (P<0.05) appetite marker (5HT) in comparing with control group with actual mean 0.632, 0.712 µg/g, respectively.

Table 3. Effect of thyme extract, low, medium, and high dose of probiotics on growing rabbit’s metabolic and appetite function after 2 months of treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>T3 (pg/ml)</th>
<th>T4 (ng/dl)</th>
<th>TSH (mlU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>137 ± 3.77b</td>
<td>1.94 ± 0.05b</td>
<td>0.86 ± 0.02a</td>
</tr>
<tr>
<td>Thyme</td>
<td>145 ± 4.11a</td>
<td>1.86 ± 0.05b</td>
<td>0.90 ± 0.02a</td>
</tr>
<tr>
<td>PBLD</td>
<td>147 ± 4.91a</td>
<td>1.81 ± 0.05b</td>
<td>0.95 ± 0.02a</td>
</tr>
<tr>
<td>PBMD</td>
<td>145 ± 3.98a</td>
<td>1.97 ± 0.05b</td>
<td>1.10 ± 0.03a</td>
</tr>
<tr>
<td>PBHD</td>
<td>130 ± 3.52c</td>
<td>2.64 ± 0.07a</td>
<td>0.92 ± 0.03a</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± S.E.M for 6 rabbits /group.

Thyme = Thyme extract 20 mg/kg b.w, PBLD = Probiotics low dose 20 MBC /kg b.w , PBMD =Probiotics medium dose,40 MBC/kg b.w) and PBHD Probiotics high dose (PBHD, 60 MBC /kg b.w).

a, b, c, d means having different superscript letters in the same column differ significantly (P<0.05).

T3= Triiodothyronine, T4= Thyroxin and TSH= Thyroid stimulating hormone

Table 4. Effect of thyme extract, low, medium, and high dose of probiotics on growing rabbit’s oxidative stress markers after 2 months of treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA(nmol/g)</td>
</tr>
<tr>
<td>Control</td>
<td>49.08 ± 1.299b</td>
</tr>
<tr>
<td>Thyme</td>
<td>52.5 ± 1.464b</td>
</tr>
<tr>
<td>PBLD</td>
<td>48.53 ± 1.362b</td>
</tr>
<tr>
<td>PBMD</td>
<td>57.25 ± 1.611a</td>
</tr>
<tr>
<td>PBHD</td>
<td>63.24 ± 1.685a</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± S.E.M for 6 rabbits /group.

Thyme = Thyme extract 20 mg/kg b.w, PBLD= Probiotics low dose 20 MBC /kg b.w , PBMD =Probiotics medium dose,40 MBC/kg b.w) and PBHD Probiotics high dose (PBHD, 60 MBC /kg b.w).

a, b, c, d means having different superscript letters in the same column differ significantly (P<0.05).

MDA=Malondialdehyde, GSH= reduced glutathione and GSSG= oxidized glutathione and NO= Nitrite / Nitrate level.
Fig1: Effect of thyme extract, low, medium, and high dose of probiotics on growing rabbit's after 2 months of treatment of Serotonin.

Histopathological examination
Histological examination of liver tissue showed normal hepatocysts for control, thyme, and probiotic low dose. In contrary, probiotics medium and high dose showed alteration in hepatocysts and initial liver manifestation for portal vein and cell leakages.
Physiological and biochemical response of probiotics and phytogenic inclusion as

Discussion

Probiotics are used to replace the antibiotics in the diet, the zootechnical traits are scarcely improved but with promoting the development and maintenance of the caecal flora. Obtained data demonstrated that the rabbit's body weight and daily weight gain are accelerated for PBLD (20 MBC/kg b.w) and Thyme (20 mg/kg b.w) group in comparing with control group, which reflected to potentially utilization of feed intake and best accumulation for nutrients in the body storage pathway. Presented data are in agreement with Pogany et al. (2009), who reported that probiotic stimulated body weight after one month of treatment under heat stress condition for NZW weanling rabbit. Similarly results were obtained by Kritas and Morrison, (2005) who reported the beneficial effect of probiotic supplementation in broiler diet which increase body weight and feed conversion. The improving in body weight and other weight parameters may due to a natural physiological way and improving digestion by balancing the resident gut microflora as they can improve the integrity of the intestinal mucosal barrier, digestive and immune functions of intestine. Improvement of absorption through intestine of nutrient transportation and immune functions of intestine. Improvement of integrity of the intestinal mucosal barrier, digestive and immune functions of intestine. Improvement of absorption through intestine of nutrient transportation and immune functions of intestine.

The activities of ALT and AST were measured as indicators of hepatocellular damage. The results of present study revealed that there were insignificant changes in ALT and AST activities in treated rabbits with PBLD in compare with control group. Osman and Morgan (2007) reported that the supplementation of Bio-Mos, Bio-Plus or their mixture in rabbit diets reduced liver enzymes (ALT and AST) which associated with a greater improvement in liver enzymes in comparing with un supplemented group. In the enzymatic level of liver tissues the probiotics improved liver function through decreasing serum ALT and AST. On the other hand, high dose of probiotic (PB) markedly inhibited liver function through significantly elevated the liver biomarker enzymes activities, which was reflected by a significant increase in serum levels of AST, ALT, total protein and albumin. Obtained data indicated that PBHD (60 MBC/kg b.w) have stressful effects on the hepatic cells, which are in consistence with Osman and Morgan (2007).

Thyme extract (20 mg/kg b.w) may play a protective role against mediated liver injury through its antioxidant and free radical scavenging properties (Loiene et al. 2007) due to the presence of phenolic compounds thymol and carvacrol (Lee and Shibamoto, 2002; Miura et al. 2002).

Obtained data showed the effect of probiotics on thyroid function by generalized stimulation in T3 for treated groups compared with control group, at the same time PBHD (60 MBC/kg b.w.) showed markedly increase (P<0.05) in T4 in comparing with control group. On the whole, TSH did not show any effect by different treatments at the end of experiment.

The relationship between the thyroid hormone and body weight is due to responsibility of thyroid metabolic function, as the increase in level of thyroid hormones leads to over stimulation of metabolism which accordingly leads to decrease in body weight, on the other hand the decrease in the level of thyroid hormones leads to inhibition of metabolism which accordingly increases the body weight. (Khan et al. 2013)

In the present study, Thyme (20 mg /kg b.w) and PBLD (20 MBC/kg b.w) increased endogenous
antioxidant substance and decrease oxidative radicals after two months of treatments in comparison with control group due to presence of phenolic phytochemicals in Thyme that posses antioxidant and free radical scavenging effects thereby protecting cellular components against free radical induced damage (El-Nekety et al., 2011). Moreover, they are likely to possess different antioxidant capacities due to their diverse chemical structures. On the other hand, PBMD (40 MBC/kg b.w) and PBHD (60 MBC/kg b.w) showed significant increase (P<0.05) in Malondialdehyde (MDA), oxidized glutathione (GSN) and nitric oxide (NO) which may be attributed to the imbalance between oxidant and antioxidant leading to many pathological changes including cellular damage in comparing with control group; also PBMD (40 MBC/kg b.w) and PBHD (60 MBC/kg b.w) markedly decrease (P<0.05) reduced glutathione (GSH) in comparing with control group.

Increased malondialdehyde (MDA) level in livers similar biochemical alterations were observed by Cemal et al. (2016) who reported that the failure of the antioxidant defense mechanism is due to the overproduction of free radicals decreased activities of the scavenging enzymes or both. Thyme has antimicrobial and antioxidant properties which effect may due to its active components, enhances appetite and has been reported to promote growth performance (Hippenstiel et al. 2011. Abdel-Wareth et al. 2012 Raskovic et al. 2015). Moreover, thyme oil can have a beneficial impact on animal performance, health status, and welfare under hot environmental conditions (Attia et al. 2017).

Serotonin is primarily found in the enteric nervous system located in the gastrointestinal tract (GI tract). However, it is also produced in the central nervous system (CNS). Serotonin is the hormone of happiness and appetite improvement, which lead to good food intake and accordingly the improvement in the weight gain (Demir et al. 2008). Our study proved that thyme (20 mg /kg b.w) and PBLD (20 MBC/kg b.w) have a positive effect on the level of serotonin, so good appetite occur and finally good advance in the body weight. In the present study, there were significant differences (P<0.05) in appetite level resembling in the enhancer of feed intake, there is a difference in favor of the PBLD (20 MBC/kg b.w) and thyme (20 mg /kg b.w) and in comparing with other groups. This may be attributed to improved digestion and absorption of nutrient in the digestive tract due to the presence of thyme riches with flavonoids, PB (probiotic) riches with Lactobacillus. These findings are consistent with a series of experimental studies (Sims and Sefton, 1999) which revealed that dietary probiotics (Midilli et al., 2008) increase feed intake of broiler chickens. However, it is in contrast with the findings by (Yeo and Kyuul, 1997) who reported that dietary additions of probiotics did not affect feed intake of broiler chickens.

Conclusion
This study concluded that thymus vulgaris (20 mg /kg b.w) and probiotic low dose (20 MBC/kg b.w) have powerful stimulation in physiological and immunological performance after two months of treatments.

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