Evaluation of Growth Performance, IL-6 and Serum Biochemical Parameters of Rats Fed on Diets Containing Thyme and Ginger Powder

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Abstract: Antibiotics have been extensively used as feed additives and growth performance in animal feed industry. The use of antibiotics is hazardous due to multiple resistances of pathogens. Thyme and ginger are medicinal herbs which have anti-inflammatory, antimicrobial, and antioxidant properties and used as feed additives in animal industry. Thus, the present study carried out to estimate the effect of thyme and ginger on some biochemical and immunological parameters in addition to their effect on the growth performance. Fifteen male rats were divided into three groups, first one was fed on basal diet (the control), second group was fed on basal diet contained thyme (5%) and the third was fed on basal diet contained ginger (5%). The experiment lasts for 4 weeks and then serum sample was collected. Our results revealed the presence of significantly increase in body weight; body gain and feed efficiency values in treated groups in comparison with the control group. Total globulins and alkaline phosphatase (ALP) were significantly increased for ginger and thyme groups in comparing with control group meanwhile total proteins and albumin recorded non-significant change, GOT increased in ginger group but not in thyme one. Thyme and ginger decreased triglyceride (TG), total cholesterol (TC) and low density lipoproteins (LDL) even though high density lipoproteins (HDL) showed no significant change in either group. Thyme decreased serum urea and Creatinine meanwhile ginger increased serum Creatinine with no significant change of urea. On the other hand Thyme significantly decreases production of serum Interleukin 6 (IL-6) in mice while ginger increased its level in serum. The present study concluded that either thyme or ginger have positive effect on some biochemical and immunological parameters in addition to their effect on growth performance however they have negative effect on others, as this is the first recorded increase of ALP with the two herbs so the using of the herbs must under restriction.

Keywords: Thyme Powder, Ginger Powder, Growth Performance, Biochemical Parameter, Interleukin 6, Rats

1. Introduction

Herbs are natural alternatives to antibiotic growth promoters (AGPs) in animal nutrition due to their antimicrobial properties. Many herbs and their bio-active constituents possess a broad antimicrobial activity, and appetite and digestion stimulating effects [1]. Herbal plants have stimulatory effects on pancreatic secretions such as digestive enzymes which help to digest and absorb more amino acids from the digestive tract [2]. Herbal growth promoter (essential oil) had significant improvement of body weight, weight gain, and feed conversion in Japanese quail [3]. Thyme (Thymus vulgaris L.) is one of the popular medicinal plant mostly grown in Mediterranean region and is one of the herbal plants that have received attention as it has antioxidant and anti-bacterial [4, 5, 6], free radical scavenging properties [7], antifungal [8], antirheumatic,
carminative [9, 10], antiparastic, analgesic, hypotensive agent [11], anti-inflammatory [12], immunomodulating effect [13]. Thyme can be used traditionally for several medicinal purposes: respiratory disease, antimicrobial and antiinflammatory [14]. Supplementation with thyme oil improved the growth performance and antioxidative enzyme activities in rainbow trout (Oncorhynchus mykiss) juveniles [15]. Thyme contains volatile oil (consisting of 55% phenols) thymol and carvacral [16], thyme [10], numerous types of flavonoids and vitamin E [17, 18]. Carvacrol possesses antimicrobial, antifungal, and antioxidative activities as well as antimutagenic and anticarcinogenic effects [19, 20, 21]. Physiological and biochemical effect of the thyme was studied by many researchers, they show that thyme cause hypoglycemia and increase in appetite, also significant decrease in plasma HDL, total lipids total, cholesterol and triglycerides levels [22]. Moreover, Feeding thyme resulted in a marked increase in HDL- cholesterol concentration [23]. Dietary thyme oil increases plasma level of triglycerides, LDL-cholesterol and HDL-cholesterol in broilers [24]. Administration of ginger and garlic to broiler chickens increased their performance and boosted their immunity [25]. Ginger contains several compounds including gingerediol, gingerol, gingerdione and shogaols [26]. These compounds have been blocking the production of interleukins, and inflammatory markers [27, 28] and have antimicrobial, antioxidative and pharmacological effects [29]. Also, gingerol is the major ingredient representing a variety of bioactivities including antiinflammation and antiproliferative [30]. IL-6 has a wide variety of biological roles in numerous systems including the immune, nervous, and endocrine systems [31, 32]. IL-6 was seen to induce B-cell growth and antibody production, which is why it was originally named B-cell stimulatory factor 2, or BSF2. It is well known that a large amount of IL-6 is secreted in response to inflammatory stimuli such as Toll-like receptor ligands and proinflammatory cytokines including IL-1, IL-17, and tumor necrosis factor (TNF)-α to combat infections and, finally, to promote inflammation. Studies have shown that 6-gingerol inhibited the TNF-α, and IL-1β-induced increase in the p38-dependent NF-κB activation and expression of pro-inflammatory genes of IL-6 and IL-8 in normal prostatic epithelial cells [33]. Essential oils such thyme is effective in reducing Atopic dermatitis symptoms and for decreasing superoxide radical, degranulated mast cells, and IgE [34]. Thyme and carvacral as their principal bioactive compounds decrease levels of the proinflammatory cytokines IL-1β, IL-6, and TNF-α. However, mechanisms mediating these suppressive effects are unclear [35]. Borneol, another compound present in thyme, has been also described as an anti-inflammatory since its dietary supplementation significantly decreases the concentration of the proinflammatory cytokines IL-1β and IL-6 in mice [36]. This study was aimed to investigate the effects of using Thyme and Ginger powder on growth performance, biochemical and Immunological parameters of rats.

2. Materials and Methods

2.1. Animals

Adult male albino rats of Wistar strain weighing about 75-83 g and 8-10 weeks old were used for the present study. The animals were purchased from the animal house in King Saud University, Riyadh, Saudi Arabia. The rats were kept in ventilated, clean, sterile, plastic cages with wood shavings under conventional conditions and had free access to food and water. All animal experiments were carried for period 32 days and according to the guidelines of the Institutional Animals Ethics Committee. The animal room was well ventilated with a 12 h light/dark cycle throughout the experimental period.

2.2. Experimental Design

Rats were then divided into 3 groups of 5 animals in each group. Group I (control) rats received normal commercial basal diet contained CP 17% and Metabolizable energy 2415Kcal/kg (According to feed stuffs ingredient analysis table 2012 edition) and its composition according to table 1. Group II (thyme) rats fed with normal commercial basal diet contained 5% thyme (5gm / 100gm basal diet). Group III (Ginger) rats fed with normal commercial basal diet contained 5% ginger powder (5gm / 100gm basal diet). Weights were recorded at the beginning of experiment and at the end of the experimental period. Also at the end of experimental period, the food intake (FI), food conversion ratio (FCR) and body gain (BG) per rat in each group were calculated.

Table 1. Commercial rats Diet Composition.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa pellets</td>
<td>35%</td>
</tr>
<tr>
<td>Maize Broken</td>
<td>15%</td>
</tr>
<tr>
<td>Barley</td>
<td>15%</td>
</tr>
<tr>
<td>White Sorghum</td>
<td>10%</td>
</tr>
<tr>
<td>Sunflower white</td>
<td>5%</td>
</tr>
<tr>
<td>Sunflower black</td>
<td>5%</td>
</tr>
<tr>
<td>Wheat</td>
<td>10%</td>
</tr>
<tr>
<td>Safflower</td>
<td>5%</td>
</tr>
</tbody>
</table>

2.3. Blood Samples Collection

At the end of the experimental period, the animals were fasted for 12 hours. The blood samples were obtained from orbital venous plexus. The blood was collected and then centrifuged at 3000 g for 10 min using bench top centrifuge. Sera were separated and were collected using dry Pasteur pipette. Labeled and stored in the refrigerator at -20°C for analyses.

2.4. Biochemical Measurements

The total protein was measured by colorimetric biuret test [37], Albumin and globulins was measured by the method described by [38]. The kidney functions were indicated by measuring urea concentration according to the method of [39], and Creatinine according to method of [40]. Liver
functions were indicated by GOT and was estimated by [41], Alkaline phosphatase (ALP) was measured based on colorimetric assay with endpoint method as described by [42], Lipid profiles as total cholesterol (TC) and triacylglycerols (TG) estimated by kits according to method described by [43], high density lipoprotein (HDL) and low density lipoprotein (LDL) was measured by kits as method indicated by [44].

2.5. IL-6 Measurements

IL-6 levels were analyzed by using validated ELISA kits (ENZO® life sciences) which is a complete kit for the quantitative determination of IL-6 in biological fluids. The kit uses a monoclonal antibody of rats IL-6 immobilized on a microtiter plate to bind the IL-6 in the sample. A recombinant IL-6 standard is provided in the kit. After a short incubation the excess sample or standard is washed out and a biotinylated monoclonal antibody of IL-6 is added. This antibody binds to the IL-6 captured on the plate. After a short incubation the excess antibody is washed out and Streptavidin conjugated to Horseradish peroxidase is added, which binds to the biotinylated IL-6 antibody. Excess conjugate is washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450nm. The measured optical density is directly proportional to the concentration of IL-6 in either standards or samples [45, 46].

2.6. Statistical Analysis

All values are given as means ± S.E. Statistical analyses were performed by using SPSS VERSION 20 using one way ANOVA test for multiple groups’ comparison. Differences among means were analyzed using Duncan’s test, with $p < 0.05$ considered as significant.

3. Results

3.1. Effect of Thyme and Ginger on Growth Performance and Feed Efficiency

The obtained results showed that there were significant differences in final body weight, body gain and feed efficiency between thyme, Ginger and control groups where the highest significant value was in group supplemented with thyme followed by group supplemented by ginger in comparison with control group fed on basal diet (at $P<0.05$) as shown in table 2 and figure.1.

<table>
<thead>
<tr>
<th>Table 2. Effect of thyme and ginger on growth performance and feed efficiency.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Initial Body weight</td>
</tr>
<tr>
<td>Final body weight</td>
</tr>
<tr>
<td>Body Gain</td>
</tr>
<tr>
<td>Feed consumption</td>
</tr>
<tr>
<td>Feed efficiency</td>
</tr>
</tbody>
</table>

The groups which have different superscript letters in the same raw are significantly (P<0.05).

3.2. Effect of Thyme and Ginger on Biochemical Parameters of Rats Serum

3.2.1. Effect of Thyme and Ginger on Serum Total Proteins, Albumin, Total Globulins, ALP and GOT

Thyme and ginger recorded no significant changed of serum total proteins and albumin, meanwhile total globulins were significantly increased in comparing with control group. Moreover, there was a significant increase of serum GOT in ginger group and no significant change of it in thyme group; Meanwhile ALP was significantly increased in thyme and ginger groups as shown in Table 3.
Table 3. Effect of thyme and ginger on serum total proteins, albumin, total globulins, ALP and GOT.

<table>
<thead>
<tr>
<th></th>
<th>T. proteins (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>T. globulin (g/dl)</th>
<th>GOT (u/l)</th>
<th>ALP (u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.50 ± 0.03</td>
<td>3.74 ± 0.11</td>
<td>0.30 ± 0.30</td>
<td>30.62 ± 1.20</td>
<td>120.33 ± 10.97</td>
</tr>
<tr>
<td>Thyme</td>
<td>4.81 ± 0.20</td>
<td>3.75 ± 0.22</td>
<td>1.31 ± 0.13</td>
<td>31.51 ± 1.03</td>
<td>168.67 ± 8.99</td>
</tr>
<tr>
<td>Ginger</td>
<td>4.67 ± 0.26</td>
<td>3.46 ± 0.29</td>
<td>1.43 ± 0.14</td>
<td>42.61 ± 2.34</td>
<td>167.67 ± 10.48</td>
</tr>
<tr>
<td>F-Ratio;</td>
<td>F=0.69</td>
<td>F=0.55</td>
<td>F=9.32</td>
<td>F=16.81</td>
<td>F=7.36</td>
</tr>
<tr>
<td>P-Value</td>
<td>P=0.54</td>
<td>P=0.61</td>
<td>P=0.014</td>
<td>P=0.003</td>
<td>P=0.024</td>
</tr>
</tbody>
</table>

The groups which have different superscript letters in the same column are significantly different where the high significant group which has superscript a followed by b then c. (P<0.05).

3.2.2. Effect of Thyme and Ginger on Serum TG, TC, HDL and LDL

Thyme highly significantly decreased serum TG, while ginger significantly decreased it. Regarding to TC, thyme and ginger decreased it significantly. On the other hand, thyme and ginger groups recorded non-significant change of serum HDL, even though they revealed that a significant decrease in thyme group and a highly significant decreased of LDL in ginger group in comparing with control group as shown in Table 4.

Table 4. Effect of thyme and ginger on serum TG, TC, HDL and LDL.

<table>
<thead>
<tr>
<th></th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90.94 ± 2.03</td>
<td>58.08 ± 2.98</td>
<td>19.90 ± 0.28</td>
<td>19.29 ± 0.49</td>
</tr>
<tr>
<td>Thyme</td>
<td>36.36 ± 3.89</td>
<td>34.48 ± 5.95</td>
<td>18.23 ± 0.16</td>
<td>13.77 ± 0.66</td>
</tr>
<tr>
<td>Ginger</td>
<td>55.69 ± 5.52</td>
<td>39.89 ± 2.02</td>
<td>18.13 ± 0.96</td>
<td>9.60 ± 0.62</td>
</tr>
<tr>
<td>F-Ratio;</td>
<td>F=46.27</td>
<td>F=9.48</td>
<td>F=3.64</td>
<td>F=67.11</td>
</tr>
<tr>
<td>P-Value</td>
<td>P=0.001</td>
<td>P=0.014</td>
<td>P=0.092</td>
<td>P=0.001</td>
</tr>
</tbody>
</table>

The groups which have different superscript letters in the same column are significantly different where the high significant group which has superscript a followed by b then c. (P<0.05).

3.2.3. Effect of Thyme and Ginger on Serum Urea and Creatinine

Concerning the results of serum urea and Creatinine, which is shown in Table 5, there was a significant decreasing of serum urea in thyme group, meanwhile a non-significant change of it in ginger group in comparing with control group. On the other hand, serum Creatinine recorded a highly significantly decrease in thyme group, even though it recorded a significantly increase in ginger group.

Table 5. Effect of thyme and ginger on serum Urea and Creatinine.

<table>
<thead>
<tr>
<th></th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.64 ± 1.65</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>Thyme</td>
<td>17.25 ± 1.15</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>Ginger</td>
<td>28.87 ± 2.26</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>F-Ratio;</td>
<td>F=21.13</td>
<td>F=41.97</td>
</tr>
<tr>
<td>P-Value</td>
<td>P=0.002</td>
<td>P=0.001</td>
</tr>
</tbody>
</table>

The groups which have different superscript letters in the same column are significantly different where the high significant group which has superscript a followed by b then c. (P<0.05).

3.3. Measurement of IL6 Level in Serum Samples

In our results, Thyme had opposite effects on production of IL6; whereas there was significant decrease in serum IL-6 concentrations of the experimental group (Thyme administration) relative to the control group as shown in table 6 and Figure 2.

Meanwhile there was significant increase in serum IL-6 concentrations of the experimental group (Ginger administration) relative to the control group following at the end of week 6 of rats rearing as shown in table 6 and Figure 3.

Figure 2. Amount of serum IL-6 in control and Thyme groups.

Figure 3. Amount of serum IL-6 in control and Ginger groups.
4. Discussion

The results in table 2 and figure 1 revealed that the final body weight, body gain and feed efficiency values had significant differences between the treatments, groups supplemented with 5% thyme and 5% ginger, in comparison to Control group fed on basal diet where the best results regarding the growth performances were in group supplemented with 5% thyme then group fed on basal diet with 5% ginger in comparison with control group. These results agreed with that reported by [15, 26] but disagreed with results reported by [47].

The results in table 3 revealed that thyme and ginger insignificantly changed serum total protein and albumin which agree with [23] who recorded that thyme powder didn’t affect serum protein and albumin of broiler chicks but this result disagree with [48]. Moreover Ginger significantly increased serum total globulins of rats in comparison with control one which agrees with the result of [48] who showed that increased serum globulins of rats received ginger. Using of thyme and ginger increase serum level of GOT and ALP in comparison with control which disagree with [78] who stated that ginger (1%) decreased serum AST and ALP of rats and [49] who reported that the increased serum AST of injected rat with CCl4 restored to normal by ginger, the difference may be due to the used dose. also, [50] revealed that thyme 2gm/Kg recorded decrease of serum AST of broiler chicken our result may differ as difference in dose. ALP is commonly found in biliary tree and bile ducts, a blockage in this system will cause an elevated ALP [51]. In concern to the results in table 4 which revealed deceased serum level of TC, TG and LDL which agree with [22, 52, 53] meanwhile these results disagree with [24] who recorded increase plasma level of triglycerides and LDL-cholesterol in broilers with thyme oil and [23] who stated no significant influence of thyme on LDL-cholesterol in broilers but [54] indicated that Ginger may improve hypercholesterolemia by modifying lipoprotein metabolism enhanced uptake of LDL by increasing LDL receptors. Reduction of triglycerides and cholesterol noticed with thyme was attributed to the lowering effect of thymol on HMG-Co A reductase the rate-limiting enzyme of cholesterol synthesis [55-56]. [57] Suggested that ginger stimulates the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body. Cholesterol is eliminated from the body either unchanged or after conversion to bile acids. Small changes in the composition of bile can result in crystallization of cholesterol as gall stone also cholesterol is a major constituent of gall stones [58]. This is the first record for increasing of ALP with ginger use which may indicate that overuse of ginger may be predisposing factor of gall stone as it may change the bile composition by increasing formation of bile acids to eliminate the cholesterol, moreover [59] reported that immune activation of some herbal agents is one of suspected mechanism of liver injury. Regarding to the results in table 5 recorded that thyme reduced serum urea and Creatinine which is in agreement with [60, 61] as thyme maintains normal kidney functions by maintaining normal level of oxidative stress parameters, reduces MDA, and prevents histopathological changes in the kidney [61]. On the other hand, ginger insignificantly reduced serum urea and significantly increased serum Creatinine, these results were in disagreement with the results of [62] who recorded that a beneficial effect of ginger for urea and Creatinine taking away from plasma of normal rats and [63] who stated that ginger extracts (twice a week for six consecutive weeks) reduced urea and Creatinine levels in normal rat when compared to control one. The disagreement may be due to the difference in the administration regime. As the increased of serum Creatinine considered one of the indicator for kidney injury [64] and misusing of ginger must be avoided.

Interleukin 6 act as a pro-inflammatory cytokine, it is encoded by the IL-6 gene. IL-6 secretion during infection leads to inflammation and stimulation of the immune response [65]. The key finding of this study is that ginger increase level of serum IL-6 concentrations in rats, these results was agreed with [66] who mentioned that the administration of ginger resulted in over increased in the production of IL-6. Also [67] illustrated that the oral administration of squeezed ginger increased the production of IL-6 in rat leukemic monocytes. While [26] mentioned that ginger contains several compounds and enzymes including gingerdiol, gingerol, gingerdione and shogaols. These compounds have been blocking the production of interleukins, and inflammatory markers [27, 28] and have antimicrobial, antioxidative and pharmacological effects [29]. Thyme significantly decrease production of serum IL-6 in mice and these results agreed with [68] who mentioned that IL-6 gene expression in mice fed with any of thyme extracts was reduced until level of nonactivated control cells which expression was decreased to half compared to activated cells. Thyme may act as effective inhibitors of LDL-induced proinflammatory cytokines (TNF-α, IL-1β, and IL-6) secretion.

5. Conclusion

Nowadays medicinal herbs are used extensively and many believed that they are safe. On our best of knowledge these the first study which recorded the increasing of ALP with GOT with ginger and increase of ALP with thyme even their positive effect on the other concerned parameters in our study. Ginger increase level of serum IL-6 concentrations, while thyme decrease its level in serum. Although Thyme supplementation group improved the growth performance.

| Table 6. Effect of thyme and ginger on amount of serum IL6. |
|----------------|----------------|
|                | Control     | Thyme       | Ginger      |
| IL6 pg/ml      | 945.98±2.15b| 923.46±8.9^a| 1153.70±18.95^a|
| F-Ratio; P-Value | =124.71; P=0.001 |

The groups which have different superscript letters in the same row are significantly different where the high significant group which has superscript a followed by b (P<0.05).
Acknowledgment

I am deeply grateful to Dr. Ola Talkhan, associate professor of biochemistry, faculty of Science, Hail University, KSA for making it possible to carry out the biochemistry part of this work in their department.

References


