INFLUENCE OF DIETARY PHYTASE AND 1,25-DIHYDROXYCHOLECALCIFEROL SUPPLEMENTATION ON THE ACTIVITY OF DIGESTIVE ENZYMES IN CHICKENS

MALGORZATA KAPICA AND IWONA PUZIO

Department of Animal Physiology, Faculty of Veterinary Medicine, Agricultural University of Lublin, 20-932 Lublin, Poland
e-mail:puzio@ursus.ar.lublin.pl

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Abstract

These studies were conducted to find out if a diet supplemented with phytase and 1,25-dihydroxycholecalciferol could change the activity of proteolytic enzymes and concentration of protein in proventriculus mucosa and pancreas in broiler chickens. The experiments were carried out on 45 broiler chickens till the age of 21 d. Animals were divided into one control group and two experimental groups. The animals from the experimental group I received diet with supplementation of phytase and the animals from the experimental group II received diet supplemented with phytase and 1,25 dihydroxycholecalciferol. A significant decrease in proteolytic activity was observed in chickens of both experimental groups as compared to controls. The supplemented diet with phytase limited high level of synthesis and secretion of proteolytic enzymes in broiler chickens fed diet rich in plant proteins.

Key words: chickens, 1,25-dihydroxycholecalciferol, phytase, proventriculus, pancreas, digestive enzyme.

Intensive growth of broiler chickens demands higher intakes of protein, calcium and other nutrients. Feeds for poultry contain mainly ingredients of plant origin. Plant seeds (cereal, legumes, oil) contain phytate, the salt of phytic acid (10). In the gastrointestinal tract of animals at low pH (about pH 2-3) phytic acid has a strong negative charge while proteins have a strong positive charge, thus phytate-protein complexes can be formed. Phytate inhibits endogenous digestive enzymes like pepsin, trypsin and amylase. It has been suggested that the reason for this inhibition is the formation of Ca phytate and therefore the reduction of Ca concentration, which is necessary for the activity of enzymes. Furthermore, phytic acid might interact with substrates for these enzymes. Phytase e.g. native phytase (occurring in some plant feed ingredients) or exogenous phytase (microbial) prevents these anti-nutritional properties of phytate.

1,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$; calcitriol], the major biologically active metabolite of vitamin D$_3$, has a well-recognized role in the regulation of mineral metabolism (8,11). Edwards et al. (4) indicated that in chickens 1,25-dihydroxycholecalciferol is two to four times as active as vitamin D$_3$. 1,25-dihydroxyvitamin D$_3$ increases gut absorption of calcium and phosphorus (14).

The aim of the present studies was to evaluate the effects of microbial phytase and 1,25(OH)$_2$D$_3$ supplementation into the diet on total protein contents and activity of gastric enzymes in broiler chickens.

Material and Methods

The experiments were carried out on 45 broiler chickens. Animals were divided into one control and two experimental groups. The animals from the experimental group I received the phytase supplemented diet (750 PTU per kg feed) and the animals from the experimental group II received diet supplemented with phytase (750 PTU per kg feed) and 1,25-dihydroxycholecalciferol (3 µg/ per kg feed).

Chickens were kept in standardised zoohygienic conditions. Feed and water were available ad libitum throughout the experimental periods. Table 1 presents the percentage composition of the feed. On the day 21 fifteen chickens from each treatment were randomly selected and euthanatized by cervical dislocation.
Table 1
Percentage composition of the diet

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<tbody>
<tr>
<td>Yellow maize</td>
<td>63.71</td>
</tr>
<tr>
<td>Soya-bean meal</td>
<td>29</td>
</tr>
<tr>
<td>Meat meal</td>
<td>4</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.22</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.7</td>
</tr>
<tr>
<td>Soya-bean oil</td>
<td>1.2</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.05</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.2</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.2</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.04</td>
</tr>
<tr>
<td>Vitamins + microelements</td>
<td>0.5</td>
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</table>

The proventriculus was separated, washed with cold water to remove traces of food, immersed in 2% NaHCO₃ solution for 10-15 min and then brought to the laboratory. The proventriculus mucous membrane was washed with cold 0.1 mol/l natrium phosphate buffer at pH 7.5 and drained on filter paper. Mucosa separated from the muscle layers was disintegrated in two passes in a glass homogenizer to a final volume of 10 ml/g (v/w) of tissue using 0.01 mol/l sodium phosphate buffer (pH 7.5) containing 0.02% of sodium azide as preservative. The homogenates were centrifuged at 5 000 x g for 15 min at 4ºC and the supernatants were stored overnight at 4ºC.

Milk clotting activity was measured according to the procedure of Berridge (1) except that the volume of milk substrate was reduced to 2 ml and 0.2 ml of the examined extract. Low-fat milk powder (S.M. Gostyn, Poland) solubilized in 0.01 mol/l Ca Cl₂ was used. The activities were expressed in coagulant units (CU). One CU was the amount of enzymes that clot 10 ml of substrate in 100 s. All the samples were analysed twice using milk powder from the same lot.

Total protein content was analysed using the Bradford method (2). Proteolytic activity of pancreatic tissue was determined by the spectrophotometric technique using casein as substrate. One unit of the activity was defined as the amount of enzyme that hydrolysed 1 mg of casein/20 min incubation/mg protein. The trypsin activity was measured using a micromodification of the original method of Erlanger (5), based on longer time of incubation. The trypsin activity was expressed as units (U) and defined as the amount of enzyme that hydrolysed 1mmol of substrate/15 min/mg of protein. The amylolytic activity was determined by the method described by O’Sullivan (9). The activity of amylase was expressed as units (U) and calculated in mmol of maltose released from the starch solution.

The activity of proteolytic enzymes and concentration of protein were expressed as averages (mean, SE) and statistical analysis was performed using ANOVA (Tukey-Kramer multiple comparison) test.

Results

It was demonstrated that both experimental diets decreased significantly the concentration of protein (P<0.05) and proteolytic activity (P<0.001) in homogenates of the proventriculus mucous membrane in comparison with controls (Table 2). As seen from the Table the protein content was 153.6 mg/g of wet mucosa in control group, 135.2 mg/g of wet mucosa in experimental group I and 132.7 mg/g of wet mucosa in experimental group II. The proteolytic activity of gastric enzymes was the highest in control group (0.73 CU/mg) and the lowest in chickens receiving the diet supplemented with phytase and 1,25-dihydroxycholecalciferol (0.48 CU/mg).

Diet with supplementation of phytase induced the increase of chicken body weight and was on the average at the level of 746.4g ± 10.6 in comparison with the control group 665.8 g ± 14.85.

Table 2
Mean total protein content and enzyme activity in homogenates of proventriculus mucosa

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Experimental group I</th>
<th>Experimental group II</th>
</tr>
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<tbody>
<tr>
<td>Concentration of protein (mg/g wet mucosa)</td>
<td>153.6 ± 6.6</td>
<td>135.2 ± 8.17</td>
<td>132.7 ± 9.11*</td>
</tr>
<tr>
<td>Proteolytic activity (CU/mg of protein)</td>
<td>0.73 ± 0.13</td>
<td>0.55 ± 0.14***</td>
<td>0.48 ± 0.11***</td>
</tr>
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</table>

* P < 0.05; *** P < 0.001; ± SE

Table 3
Relative mean weight of the pancreas and liver (g/kg b.w.)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Experimental group I</th>
<th>Experimental group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>3.42 ± 0.19</td>
<td>3.33 ± 0.13</td>
<td>3.43 ± 0.15</td>
</tr>
<tr>
<td>Liver</td>
<td>30.41 ± 0.85</td>
<td>27.73 ± 0.92</td>
<td>28.13 ± 0.36</td>
</tr>
</tbody>
</table>

± SE
No significant differences were noted in the relative pancreas weight. Diet with supplementation of phytase and 1,25-dihydroxycholecalciferol induced the decrease in relative liver weight and was on the average at the level of 28.13 per kg live b.w. In the experimental group I the lowest value of relative liver weight was observed at the level of 26.13 g per kg b.w. (P<0.05). Control diet induced an enlargement up to the value of 30.41 g per kg b.w.

Total protein contents amounted to 51.91 mg/g of pancreatic tissue of control group, 65.36 mg/g in the experimental group I and 69.48 mg/g in the experimental group II. The phytase supplemented diet significantly increased the protein contents of pancreatic tissue.

The amylolytic activity in homogenates of the pancreas was higher in the chicken of the control group. It was lower in the first experimental group and the lowest in the animals of the second experimental group. The proteolytic and the trypsin activity showed the same tendency.

**Discussion**

The concentration of protein in homogenates of stomach mucosa suggests that in the short period (21 d) of the assay and with the level consumed (both phytase and 1,25-dihydroxycholecalciferol) there were no statistically significant effects on the concentration of the protein.

The high level of proteolytic activity in stomach mucosa of control group may be the result of the formation of a phytate-protein complex in which sites on the protein are less susceptible to enzymatic attack. Our data support the reports in the literature indicating that phytate-protein complexes are more resistant to proteolytic digestion (6). This inhibitory effect was reduced by the supplementation of the diet with phytase.
The pH of stomach digesta is favourable for high phytase activity, whereas the pH in the small intestine, especially the higher pH in the lower part of the small intestine, is not favorable for high phytase activity (12).

During the processing and preparation of phytate-containing foods (i.e. cereals and legumes), phytate may be chemically or enzymatically dephosphorylated to varying degrees, resulting in myo-inositol esters having 1 to 5 attached phosphate groups. In vitro studies with phytate hydrolases indicated that myo-inositol phosphate esters significantly inhibit the peptic and tryptic digestion of some proteins (6, 13).

Knuckles et al. (7) showed that phytate is capable to produce a small but significant decrease in in vitro digestion of casein and bovine serum albumin by pepsin.

Calcitriol exerts rapid effects (seconds to minutes) on the plasma membrane, activating several transmembrane signalling systems that results in stimulation of calcium influx and release of calcium from intracellular stores (3).

Phytase supplementation diet in doses of 750 PT U per kg feed limited the high level of the secretion of stomach and pancreas enzymes in broiler chickens, which were fed diet rich in plant proteins containing phytate. Diet supplemented with phytase and 1,25-(OH)₂D₃ can intensify these effects. Both experimental diets decreased total protein content in the proventriculus mucosa and relative liver weights, but increased total protein content in pancreatic tissue. Phytase supplemented diet increased also the body weight of the examined chickens.

References


