Response of Turkey Poults to Soybean Lectin Levels Typically Encountered in Commercial Diets. 2. Effect on Intestinal Development and Lymphoid Organs

Y. O. Fasina,* H. L. Classen,† J. D. Garlich,‡ B. L. Black,§ P. R. Ferket,‡ Z. Uni,# and A. A. Olkowski†

*Department of Poultry Science, Auburn University, Auburn, AL 36849; †Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5A8, Canada; ‡Department of Poultry Science, North Carolina State University, Raleigh 27695-7608; §Department of Zoology, North Carolina State University, Raleigh 27695-7617; and #Faculty of Agriculture, Hebrew University, Rehovot, Israel 76-100

ABSTRACT Lectins are capable of altering intestinal morphology by binding to and disrupting the intestinal brush border membrane. They are also known to alter the weight of lymphoid organs. Therefore, we evaluated the effect of soybean lectin (SBL) on intestinal morphology and lymphoid organ weights of poults fed diets containing SBL. Dietary treatments evaluated in this study included a cornstarch and casein-based control (lectin-free) semipurified diet (PD) and semipurified diets containing 0.024 or 0.048% SBL (PDL and PDH, respectively). Experimental diets were fed from hatch to 14 d. Morphological evaluation of the intestine involved measurement of the villus height and perimeter, crypt depth, villus:crypt, and thickness of the muscle layer in the jejunum. Intestinal physical characteristics were also determined by measuring intestinal weight, length, and volume. Results indicated that 0.048% SBL in PDH increased villus:crypt and reduced total intestinal length in turkey poults. In addition, both the 0.024 and 0.048% dietary SBL levels reduced thymus weights. It was concluded that dietary SBL up to 0.048% enhanced intestinal development by increasing villus:crypt, but might alter the structural integrity of lymphoid organs.

Key words: turkey poult, soybean lectin, semipurified diet, villus:crypt, lymphoid organ

INTRODUCTION The small intestine is the major site for digestion and absorption of nutrients. The architecture of the small intestine, as it relates to digestion and absorption, has been well described (Smith, 1993; Uni, 1999; Marshman et al., 2002). The intestine is lined with a layer of epithelium that contains a continuously renewable population of columnar cells. The epithelium invaginates at different points in the intestine to form the villi and the crypts. The crypts contain stem cells that proliferate and mature into cells (enterocytes) that differentiate along the villi. Differentiation of enterocytes mainly involves the expression and formation of microvilli, expression of brush border enzymes, and the transport mechanisms at the apical membrane, also called the brush border membrane (BBM). The BBM serves to increase the surface area available for digestion and absorption of nutrients.

Dietary components (digestible and nondigestible) are known to influence the morphology of the BBM by altering villus height and crypt depth, thereby modifying the surface area available for digestion and absorption (Sharma and Schumacher, 2001). Soybean lectin (SBL), a component of soybean meal (SBM), is usually present in poultry starter diets because SBM is included in these diets as the major plant protein supplement. Lectins are carbohydrate-binding glycoproteins that, when ingested, are capable of binding to specific glycosyl receptors on the BBM. Upon binding, they may damage the BBM, affect proliferation and differentiation of epithelial cells, induce shortening or blunting of the villi, interfere with digestive and absorptive capacity of enterocytes, increase intestinal weight and size, and modulate the immune state of the digestive tract (Pusztai, 1994; Liener, 1994a,b). Previous reports have indicated that dietary incorporation of kidney bean lectin (phytohaemagglutinin) at 0.1 to 1.0% of the diet and SBL at 0.75 and 0.027% of the diet caused degenerative changes, such as villi atrophy, increased crypt depth, and increased intestinal weight in laboratory rats (Jindal et al., 1984; Rossi et al., 1984; Tajiri et al., 1988; Grant, 1989). Reports documenting the effects of SBL on gut morphology in poultry are nonexistent to the best of our knowledge. In addition, we cannot extrapolate results obtained for laboratory rats to poultry because species differences have been implicated in the response of animals to dietary lectins (Pusztai and Bar-
Experimental Design. Therefore, it was of interest to examine the effect of SBL on morphological and physical characteristics of the intestines of turkey poult fed diets containing SBL.

Lectins have also been implicated in altering the weights of lymphoid organs (Pusztai, 1991; Cavallede et al., 2003). Because stem cells of the avian immune system develop in the major lymphoid organs (Bursa of Fabricius and thymus), changes in weights of these organs could have irreversible and potentially detrimental consequences on the immunocompetence of animals (Ferket and Qureshi, 1999). Any impairment in the function of lymphoid organs could have detrimental consequences on the ability of the birds to exclude pathogens, resist infections, or maintain productivity during an infectious challenge.

The objective of this study was to evaluate the effect of SBL at 0.024 and 0.048% of the diet on intestinal morphology, intestinal physical characteristics, and lymphoid organ weights in turkey poult.

MATERIALS AND METHODS

Experimental Design

Intestinal tissue samples and lymphoid organ weights were collected from poult used in 2 turkey poult experiments conducted by Fasina et al. (2004). The experiments were conducted in accordance with the Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies, 1999). The University Animal Care and Use Committee of North Carolina State University also approved experimental protocols. In Experiment 1, we evaluated tissue samples, lymphoid organs, or both collected from poult fed the following dietary treatments (Table 1): 1) a casein and cornstarch-based semipurified control diet containing no SBL (PD, 2) a semipurified diet containing a low (0.024%) lectin level (PDL), and 3) a semipurified diet containing a high (0.048%) lectin level (PDH). The SBL added to the PDL and PDH diets was purified by affinity chromatography as described by Fasina et al. (2003). Each dietary treatment consisted of 4 replicate pens, and each pen contained 14-d-old poult (female Hybrid Converter strain) obtained from a commercial hatchery (Sleepy Creek Turkeys, Inc.). Duration of the experiment was 12 d, and poult were housed and raised under conditions similar to the conditions for Experiment 1.

Protocol for Experiment 1

Sampling for Histology and Lymphoid Organs.

Poult were randomly selected from the PD, PDL, and PDH treatments on d 6 (8 poult per treatment) and on d 14 (8 poult per treatment). Each poult was killed by cervical dislocation, and the small intestine was excised and placed on ice. Tissue sections (1 cm long each) were then taken from the jejunum (from the pancreo-biliary ducts to the yolk stalk), flushed with cold saline, and fixed in 10% neutral buffered formalin (Fisher Scientific, Pittsburgh, PA) for histological morphometric analysis. Lymphoid organs (spleen and Bursa of Fabricius) were also removed, freed from adhering tissue, blotted dry, and weighed individually. Relative organ weights were calculated as organ weights as percentages of BW.

Table 1. Composition of basal, semipurified diets used in Experiments 1 and 2

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>300.0</td>
<td>300</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>395</td>
<td>404</td>
</tr>
<tr>
<td>Cellulose</td>
<td>102</td>
<td>102</td>
</tr>
<tr>
<td>Lard</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ethoxyquin</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Limestone</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Salt</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Arginine-free base</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline chloride (60%)</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>ni-Methionine</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Trytophan</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Potassium-magnesium sulfate</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Potassium di H phosphate</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>SBD bicarbonate</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Selenium premix</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>5.0</td>
<td>—</td>
</tr>
</tbody>
</table>

1In both Experiments 1 and 2, soybean lectin was coated onto pellets made from the semipurified diets at 0.024, and 0.048% of the diet.
2Trace mineral premix provided the following (mg/kg diet): Zn, 120; manganese, 120; iron, 80; copper, 10; iodine, 2.5; cobalt, 1.0.
3Vitamin premix provided the following (/kg of diet): vitamin A, 13,200 IU; vitamin D, 4,000 IU; vitamin E, 66 IU; vitamin B12, 39.6 μg; riboflavin, 13.2 mg; niacin, 110 mg; pantothenic acid, 22 mg; vitamin K, 4.0 mg; folic acid, 2.2 mg; thiamine, 4.0 mg; pyridoxine, 7.8 mg; biotin, 220 μg.
4Selenium premix provided 0.2 mg of Se/kg of diet as Na2SeO3.
Table 2. Effect of dietary soybean lectin levels on jejunal morphometric indices in turkey poults (Experiment 1, d 6)

<table>
<thead>
<tr>
<th></th>
<th>PD1</th>
<th>PDH1</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villi height, μm</td>
<td>786.1</td>
<td>742.3</td>
<td>115.6</td>
<td>0.6092</td>
</tr>
<tr>
<td>Villi perimeter, μm</td>
<td>1,750.0</td>
<td>1,658.2</td>
<td>203.8</td>
<td>0.4538</td>
</tr>
<tr>
<td>Villi width, μm</td>
<td>101.4</td>
<td>106.3</td>
<td>9.1</td>
<td>0.3711</td>
</tr>
<tr>
<td>Crypt depth, μm</td>
<td>141.4</td>
<td>141.2</td>
<td>15.2</td>
<td>0.9833</td>
</tr>
<tr>
<td>Villus:crypt</td>
<td>5.56</td>
<td>5.25</td>
<td>0.54</td>
<td>0.4422</td>
</tr>
<tr>
<td>Thickness of muscle layer, μm</td>
<td>106.1</td>
<td>104.8</td>
<td>18.7</td>
<td>0.9252</td>
</tr>
<tr>
<td>Villi surface area, μm²</td>
<td>84,032</td>
<td>81,526</td>
<td>13,774</td>
<td>0.7591</td>
</tr>
</tbody>
</table>

1PD = semipurified diet (control diet); PDH = semipurified diet supplemented with 0.048% soybean lectin.
2Villus:crypt = villi height/crypt depth.

Measurement of Physical Characteristics of the Small Intestine. A second group of poults was randomly chosen on d 6 (8 poults per treatment) and on d 14 (4 poults per treatment) for measurement of physical characteristics of the small intestine. These poults were also killed by cervical dislocation, and the small intestine of each bird was divided into duodenum, jejunum, and ileum as follows: duodenum, from the gizzard to the point of entry of the pancreo-biliary ducts; jejunum, from the pancreo-biliary ducts to the yolk stalk; and ileum, from the yolk stalk to the ileo-cecal junction. Segments were then flushed with cold, deionized water, and weights of the empty segments were recorded. The length and diameter of these intestinal segments were also measured in centimeters with a clear plastic ruler. In addition, mass per unit length ratio and intestinal volume were calculated. Intestinal volume was calculated using the formula for the volume of a cylinder (πr²h), where r = half of the diameter of an intestinal segment (D/2), and h = the length of an intestinal segment.

Sample Collection for Scanning Electron Microscopy. Another set of poults (4 poults per treatment) was randomly selected on d 14 from the PD and PDH treatments. Poults were killed by cervical dislocation, and the intestine was excised. Tissue sections (1 cm long) were obtained from different parts of the duodenum (proximal, middle, and distal), jejunum (proximal, middle, and distal), ileum (middle), and cecal lobes (middle of each lobe). Sections obtained were gently flushed with cold saline and immediately fixed in 3% glutaraldehyde (Sigma Chemicals, St. Louis, MO; in 0.1 M sodium cacodylate buffer; pH 7.2) until examined.

Table 3. Effect of dietary soybean lectin levels on jejunal morphometric indices in turkey poults (Experiment 1, d 14)

<table>
<thead>
<tr>
<th></th>
<th>PD1</th>
<th>PDH1</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villi height, μm</td>
<td>1,349.1</td>
<td>1,498.7</td>
<td>189.7</td>
<td>0.3890</td>
</tr>
<tr>
<td>Villi perimeter, μm</td>
<td>2,885.1</td>
<td>3,152.5</td>
<td>192.7</td>
<td>0.0596</td>
</tr>
<tr>
<td>Villi width, μm</td>
<td>125.3</td>
<td>117.7</td>
<td>9.3</td>
<td>0.2365</td>
</tr>
<tr>
<td>Crypt depth, μm</td>
<td>255.5a</td>
<td>191.9b</td>
<td>16.3</td>
<td>0.0087</td>
</tr>
<tr>
<td>Villus:crypt</td>
<td>5.27b</td>
<td>7.78a</td>
<td>0.43</td>
<td>0.0020</td>
</tr>
<tr>
<td>Thickness of muscle layer, μm</td>
<td>167.7</td>
<td>145.4</td>
<td>20.3</td>
<td>0.2490</td>
</tr>
<tr>
<td>Villi surface area, μm²</td>
<td>171,442</td>
<td>185,627</td>
<td>12,047</td>
<td>0.0996</td>
</tr>
</tbody>
</table>

a,bMean values bearing different superscript letters within a row are significantly different (P < 0.05).
1PD = semipurified diet (control diet); PDH = semipurified diet supplemented with 0.048% soybean lectin.
2Villus:crypt = villi height/crypt depth.

Protocol for Experiment 2

From the PD, PDL, and PDH treatments, poults were randomly chosen on d 6 (4 poults per treatment) and d 12 (4 poults per treatment) of the experiment for the measurement of physical characteristics of the small intestine as done in Experiment 1. Weights of lymphoid organs (thymus, spleen, and Bursa of Fabricius) were recorded as previously described for Experiment 1. Neither histology nor scanning electron microscopy (SELM) was done in Experiment 2.

Analytical Methods

Histological Morphometric Analysis of the Jejunal Epithelium. Formalin-fixed jejunal tissue samples were dehydrated, embedded in paraffin, sectioned (5 μm), and stained with haematoxylin and eosin. Morphometric indices were determined on these sections by means of a computer-aided light microscopic image analyzer (NIH Image, National Institutes of Health, Bethesda, MD). Measurements taken included villus height (from tip of villus to the crypt opening), villus planar perimeter length, villus width at half height, crypt depth (from the base of the crypt to the level of crypt opening), villus surface area (area within the villus perimeter), and thickness of the external muscle layer. For each bird, values used for analysis were means from 10 adjacent, vertically oriented villus-crypt units per section.

SELM. Gut architecture was assessed using SELM. Tissue sections fixed in 3% glutaraldehyde were washed 5 times in deionized water. The washed samples were...
Table 4. Changes induced by soybean lectin in the physical characteristics of the poults small intestine (Experiment 1)

<table>
<thead>
<tr>
<th>Day and treatment</th>
<th>Absolute weight (g)</th>
<th>Relative weight</th>
<th>Length (cm)</th>
<th>Average M/L</th>
<th>Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Duodenum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>3.09a</td>
<td>2.01a</td>
<td>13.80</td>
<td>0.223a</td>
<td>1.543</td>
</tr>
<tr>
<td>PDH</td>
<td>2.39b</td>
<td>1.60b</td>
<td>13.00</td>
<td>0.184b</td>
<td>1.503</td>
</tr>
<tr>
<td>SEM</td>
<td>0.56</td>
<td>0.29</td>
<td>1.23</td>
<td>0.025</td>
<td>0.505</td>
</tr>
<tr>
<td>P-value</td>
<td>0.2476</td>
<td>0.1699</td>
<td>0.3850</td>
<td>0.1239</td>
<td>0.9750</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>3.34</td>
<td>2.17</td>
<td>31.45</td>
<td>0.106</td>
<td>2.531</td>
</tr>
<tr>
<td>PDH</td>
<td>2.99</td>
<td>2.01</td>
<td>27.20</td>
<td>0.110</td>
<td>1.941</td>
</tr>
<tr>
<td>SEM</td>
<td>0.48</td>
<td>0.21</td>
<td>2.18</td>
<td>0.018</td>
<td>0.447</td>
</tr>
<tr>
<td>P-value</td>
<td>0.4122</td>
<td>0.4432</td>
<td>0.0385</td>
<td>0.9150</td>
<td>0.0632</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ileum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>2.38a</td>
<td>1.55a</td>
<td>29.65a</td>
<td>0.080</td>
<td>2.176</td>
</tr>
<tr>
<td>PDH</td>
<td>1.89b</td>
<td>1.27b</td>
<td>27.35b</td>
<td>0.069</td>
<td>1.681</td>
</tr>
<tr>
<td>SEM</td>
<td>0.28</td>
<td>0.12</td>
<td>1.81</td>
<td>0.011</td>
<td>0.474</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0809</td>
<td>0.0275</td>
<td>0.0689</td>
<td>0.3536</td>
<td>0.0859</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>8.81a</td>
<td>5.73a</td>
<td>74.90a</td>
<td>0.137</td>
<td>6.251</td>
</tr>
<tr>
<td>PDH</td>
<td>7.26b</td>
<td>4.88b</td>
<td>67.55b</td>
<td>0.121</td>
<td>5.126</td>
</tr>
<tr>
<td>SEM</td>
<td>1.19</td>
<td>0.49</td>
<td>3.51</td>
<td>0.016</td>
<td>1.103</td>
</tr>
<tr>
<td>P-value</td>
<td>0.2213</td>
<td>0.1032</td>
<td>0.0390</td>
<td>0.4087</td>
<td>0.1489</td>
</tr>
<tr>
<td>d14</td>
<td></td>
<td></td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>19.95</td>
<td>5.40</td>
<td>90.83</td>
<td>0.239</td>
<td>11.373</td>
</tr>
<tr>
<td>PDH</td>
<td>18.02</td>
<td>4.83</td>
<td>84.42</td>
<td>0.228</td>
<td>9.113</td>
</tr>
<tr>
<td>SEM</td>
<td>2.77</td>
<td>0.66</td>
<td>7.27</td>
<td>0.025</td>
<td>3.02</td>
</tr>
<tr>
<td>P-value</td>
<td>0.1478</td>
<td>0.4845</td>
<td>0.2460</td>
<td>0.2731</td>
<td>0.4042</td>
</tr>
</tbody>
</table>

a,bMean values bearing different superscript letters in a column are significantly different (P < 0.05).

1Data are presented as means per treatment. Number of observations per mean (n = 4).

2PD = semipurified diet (control diet); PDH = semipurified diet supplemented with 0.048% soybean lectin.

3Grams per 100 g of BW.

4M/L = mass per unit length.

dehydrated with increasing concentrations of acetone and finally washed 3 times in 100% acetone. Following this, the samples were critically point dried, mounted on aluminum stubs (with longitudinally fractured surfaces of the tissue exposed), sputter-coated with gold, and examined on a Philips 505 scanning electron microscope (Philips, Holland).

**Statistical Analysis**

Data were subjected to ANOVA for completely randomized designs (GLM procedure; SAS Institute, 1994). Significant differences among means were determined with the Duncan option of the GLM procedure (Waller and Duncan, 1969; SAS Institute, 1994). Statements of statistical significance were based on P < 0.05.

**RESULTS**

Histological parameters assessed are presented in Tables 2 and 3. There were no differences in the morphometrical measurements during the first week. However, on d 14, pouls fed PDH had lower crypt depth and a higher villus: crypt than did pouls fed the control PD.

Physical characteristics of the small intestine are presented in Tables 4 and 5. Results will be interpreted in terms of “total values” obtained (the sum of duodenum, jejunum, and ileum) for each parameter assessed. In Experiment 1 (Table 4), total length was smaller (P < 0.05) for the PDH treatment compared with the PD treatment on d 6. However, on d 14 of Experiment 1 (Table 4) and throughout Experiment 2 (Table 5), no differences were observed among treatments for all parameters assessed. Regardless, it was noted that pouls fed the SBL-supplemented diets (PDH or PDL) had numerically lower values for all physical parameters measured when compared with the control PD treatment (Tables 4 and 5). Scanning electron microscopy revealed no major changes in the architecture of the gut, except in the ceca. At the end of Experiment 1 (d 14), villi size in the ceca of pouls fed PDH was larger compared with that of pouls fed PD (Figure 1).

Absolute and relative weights of lymphoid organs are presented in Table 6. In both experiments, there were no differences observed in the absolute and relative weights of the Bursa of Fabricius and spleen of pouls. However, in Experiment 2, pouls fed the SBL-supplemented diets (PDL and PDH) had lower thymus weights (P < 0.05) compared with pouls fed PD.

**DISCUSSION**

The intestinal samples evaluated in this study were obtained from pouls used in a nutrition study conducted.
Table 5. Changes induced by soybean lectin in the physical characteristics of the poult small intestine (Experiment 2)1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total absolute weight (g)</th>
<th>Relative weight3</th>
<th>Length (cm)</th>
<th>Average M/L4 (g/cm)</th>
<th>Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>8.92</td>
<td>5.78</td>
<td>73.25</td>
<td>0.137</td>
<td>4.57</td>
</tr>
<tr>
<td>PDL</td>
<td>8.50</td>
<td>5.93</td>
<td>68.50</td>
<td>0.137</td>
<td>2.80</td>
</tr>
<tr>
<td>SEM</td>
<td>1.11</td>
<td>0.63</td>
<td>4.85</td>
<td>0.016</td>
<td>1.15</td>
</tr>
<tr>
<td>P-value</td>
<td>0.6111</td>
<td>0.7907</td>
<td>0.2155</td>
<td>0.9681</td>
<td>0.0720</td>
</tr>
<tr>
<td></td>
<td>d 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>18.87</td>
<td>6.61</td>
<td>94.13</td>
<td>0.229</td>
<td>9.86</td>
</tr>
<tr>
<td>PDL</td>
<td>16.40</td>
<td>6.40</td>
<td>92.73</td>
<td>0.201</td>
<td>7.28</td>
</tr>
<tr>
<td>SEM</td>
<td>2.76</td>
<td>1.05</td>
<td>6.14</td>
<td>0.022</td>
<td>2.17</td>
</tr>
<tr>
<td>P-value</td>
<td>0.2526</td>
<td>0.7832</td>
<td>0.7502</td>
<td>0.1417</td>
<td>0.1424</td>
</tr>
</tbody>
</table>

1Data are presented as means per treatment. Number of observations (n = 4).
2PD = semipurified diet (control diet); PDL = semipurified diet supplemented with 0.024% soybean lectin.
3Grams per 100 g of BW.
4M/L = mass per unit length.

by Fasina et al. (2004). These poults were fed either a cornstarch and casein-based semipurified diet [control (PD) treatment] or the semipurified diet supplemented with 0.024 or 0.048% SBL (PDL or PDH treatments, respectively). It was established that the PD diet is nutritionally satisfactory when compared with a conventional corn and SBM poult starter diet (Fasina et al., 2004).

The SBL levels incorporated into PDL (0.024%) and PDH (0.048%) simulated the range of SBL levels (0.22 to 0.67 mg of lectin/g of meal) found in a variety of desolventized toasted SBM samples across the United States (Maenz et al., 1999). Soybean meal is usually incorporated at about 40% of poult starter diets. Inclusion of SBM that contains 0.22 or 0.67 mg of lectin/g into separate poult starter diets at 40% of the diet will result in diets containing approximately 0.100 and 0.270 mg of SBL/g of diet. In turn, these dietary SBL levels correspond to starter diets that contain 0.010 and 0.027% SBL, respectively. In addition, SBM processed by alternative methods (other than conventional desolventizing-toasting) may contain higher SBL levels. For instance, Maenz et al. (1999) found that flash-desolvenitized meals may contain up to 3 mg of SBL/g of meal.

The intestine is able to respond to changes in the diet by altering its weight, length, absorptive area, and rate of enterocyte turnover (Bedford, 1996). Thus, in this study, we examined intestinal morphological and physical attributes to determine the effect of SBL on intestinal development. The higher villus:crypt in poults fed PDH vs. PD on d 14 of Experiment 1 is probably due to the larger villi surface area in poults fed PDH (Table 3). Crypt depth and villus height are useful indicators of the size of these poults were fed either a cornstarch and casein-based semipurified diet [control (PD) treatment] or the semipurified diet supplemented with 0.024 or 0.048% SBL (PDL or PDH treatments, respectively). It was established that the PD diet is nutritionally satisfactory when compared with a conventional corn and SBM poult starter diet (Fasina et al., 2004).

The SBL levels incorporated into PDL (0.024%) and PDH (0.048%) simulated the range of SBL levels (0.22 to 0.67 mg of lectin/g of meal) found in a variety of desolventized toasted SBM samples across the United States (Maenz et al., 1999). Soybean meal is usually incorporated at about 40% of poult starter diets. Inclusion of SBM that contains 0.22 or 0.67 mg of lectin/g into separate poult starter diets at 40% of the diet will result in diets containing approximately 0.100 and 0.270 mg of SBL/g of diet. In turn, these dietary SBL levels correspond to starter diets that contain 0.010 and 0.027% SBL, respectively. In addition, SBM processed by alternative methods (other than conventional desolventizing-toasting) may contain higher SBL levels. For instance, Maenz et al. (1999) found that flash-desolvenitized meals may contain up to 3 mg of SBL/g of meal.

The intestine is able to respond to changes in the diet by altering its weight, length, absorptive area, and rate of enterocyte turnover (Bedford, 1996). Thus, in this study, we examined intestinal morphological and physical attributes to determine the effect of SBL on intestinal development. The higher villus:crypt in poults fed PDH vs. PD on d 14 of Experiment 1 is probably due to the larger villi surface area in poults fed PDH (Table 3). Crypt depth and villus height are useful indicators of the size

Table 6. Effect of dietary soybean lectin levels on the absolute and relative weights of lymphoid organs1

<table>
<thead>
<tr>
<th></th>
<th>PD2</th>
<th>PDL2</th>
<th>PDH2</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bursa of Fabricius</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute weight, g</td>
<td>0.411</td>
<td>0.368</td>
<td>0.444</td>
<td>0.118</td>
<td>0.2611</td>
</tr>
<tr>
<td>Relative weight3</td>
<td>0.119</td>
<td>0.113</td>
<td>0.121</td>
<td>0.031</td>
<td>0.8199</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute weight, g</td>
<td>0.305</td>
<td>0.279</td>
<td>0.347</td>
<td>0.084</td>
<td>0.1449</td>
</tr>
<tr>
<td>Relative weight3</td>
<td>0.088</td>
<td>0.084</td>
<td>0.093</td>
<td>0.020</td>
<td>0.5070</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute weight, g</td>
<td>0.406a</td>
<td>0.272b</td>
<td>0.315b</td>
<td>0.031</td>
<td>0.0005</td>
</tr>
<tr>
<td>Relative weight3</td>
<td>0.142a</td>
<td>0.099b</td>
<td>0.108b</td>
<td>0.013</td>
<td>0.0025</td>
</tr>
<tr>
<td>Bursa of Fabricius</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute weight, g</td>
<td>0.403</td>
<td>0.321</td>
<td>0.409</td>
<td>0.062</td>
<td>0.1365</td>
</tr>
<tr>
<td>Relative weight3</td>
<td>0.141</td>
<td>0.116</td>
<td>0.140</td>
<td>0.022</td>
<td>0.2432</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute weight, g</td>
<td>0.420</td>
<td>0.366</td>
<td>0.403</td>
<td>0.066</td>
<td>0.5281</td>
</tr>
<tr>
<td>Relative weight3</td>
<td>0.146</td>
<td>0.134</td>
<td>0.139</td>
<td>0.022</td>
<td>0.7491</td>
</tr>
</tbody>
</table>

1Mean values bearing different superscript letters within a row are significantly different (P < 0.05).
2PD = semipurified diet (control diet); PDL = semipurified diet supplemented with 0.024% soybean lectin; PDH = semipurified diet supplemented with 0.048% soybean lectin.
3Data were recorded on d 14 in Experiment 1 and on d 12 in Experiment 2. Values are presented as means per treatment (n = 8 in Experiment 1; n = 4 in Experiment 2).
4Weight expressed in grams per 100 g of BW.
of the proliferative and absorptive compartments in the intestinal mucosa (Sharma and Schumacher, 2001). A high villus:crypt is associated with a well-differentiated intestinal mucosa with high digestive and absorptive capabilities (Jeurissen et al., 2002). Thus, the superior feed efficiency reported by Fasina et al. (2004) for poults fed PDH (feed efficiency = 0.975; P < 0.05) compared with poults fed the control PD (feed efficiency = 1.030) may be attrib-
uted to the presence of a better differentiated intestinal mucosa with higher digestive and absorptive capabilities in pouls fed PDH.

Physical characteristics of the intestine in this study indicated that SBL reduced intestinal length in Experiment 1. A smaller intestinal tract is an indication of higher absorptive efficiency per unit of intestinal weight, thus allowing for greater feed efficiency (Mitchell and Smith, 1991). Furthermore, Bedford (1996) reported that maintaining the rate of digestion with a smaller gastrointestinal tract would enable a greater proportion of absorbed energy to be utilized for carcass accretion, as the nutrient requirement for maintenance of the intestine would be reduced. The smaller intestinal tract observed in pouls fed PDH in this study again corroborates the findings reported by Fasina et al. (2004) that pouls fed PDH had superior feed efficiency when compared with pouls fed PD.

Scanning electron microscopy revealed that cecal villi were larger in pouls fed PDH compared with pouls fed PD (Figure 1). A similar effect was observed in rats when endocytosis of kidney bean lectin resulted in a rapid increase in mucosal cell protein synthesis (Oliveira et al., 1988). The larger villi size in ceca of pouls fed PDH is probably an indication that active SBL reached the large intestine, bound to cecal epithelium, and was endocytosed. Thirty-three and 13% increases were observed in the weight and crypt depth of the ceca, respectively, when rats were fed a lactalbumin-based diet containing 0.75% phytohaemagglutinin compared with a lactalbumin-based control diet (Bardocz et al., 1995). The lectin-induced increase in villi size was probably due to an increase in mucosal cell protein synthesis and hyperplasia of crypt cells, both caused by accumulation of polyamines (mostly spermidine) that stimulate cellular proliferation (Grant et al., 1987; Oliveira et al., 1988; Bardocz et al., 1990).

Measurement of lymphoid organ weights is an accepted method of assessing the integrity of lymphoid organs and immunocompetence (i.e., ability to respond to invading pathogens) of birds (Dietert et al., 1994; Ferket and Qureshi, 1999). The relative lymphoid organ weights (reported in g/100 g of BW) obtained in this study were comparable with those reported in the literature. For instance, the relative weights of Bursa of Fabricius (0.12 ± 0.028 to 0.14 ± 0.024) and spleen (0.09 ± 0.019 to 0.14 ± 0.031) of 14-d-old pouls fed PD in Experiments 1 and 2 (Table 6) were comparable with the bursa (0.16 ± 0.019 to 0.17 ± 0.010) and spleen (0.09 ± 0.010 to 0.09 ± 0.010) weights previously reported for 10-d-old female turkey pouls (Phelps et al., 1987). The lower thymus weights induced by SBL in PDL and PDH are indications that dietary SBL can alter or affect structural integrity of the thymus in turkey pouls. A similar finding has been reported by Oliveira et al. (1988). Those researchers found that thymus weights were smaller (P < 0.05; 0.15 ± 0.03) in rats fed 0.3% kidney bean lectin compared with rats fed the control, lectin-free diet (0.20 ± 0.02). In addition, they observed that the lectin-fed rats had lower blood insulin levels and, thus, speculated that the lectin-mediated reduction in thymus weight may be indirectly linked to the reduced insulin levels.

Dietary kidney bean lectin and, to a lesser extent SBL, can reduce blood insulin concentration in rats soon after reaching the small intestine (Pusztai et al., 1986; Grant et al., 1987). They bind to insulin receptor of cells and induce muscle catabolism such that there is reduction in the fractional rate of protein synthesis without a similar decrease in protein degradation rate with a resulting net loss in muscle protein in some tissues (Palmer et al., 1987; Pusztai and Bardocz, 1996). This lead Pusztai and Bardocz (1996) to conclude that lectin-induced muscle atrophy is a result of the abrogation of the stimulatory effect of insulin on muscle protein synthesis because the receptor sites for insulin were blocked by the lectin. However, because we did not measure insulin levels in this study, we hesitate to assert that the SBL-induced thymic atrophy observed was linked to insulin levels. Regardless, our thymic weight results indicate that SBL may alter cell-mediated immune responses because the thymus is the primary site for T-lymphocyte maturation, and T-cells are one of the major cells involved in cell-mediated immune responses.

We have described the effects of SBL on intestinal development and lymphoid organs. Inclusion of SBL at 0.048% of the turkey poult diet enhanced intestinal development (i.e., digestive and absorptive capacity) by increasing intestinal villus:crypt. Conversely, presence of SBL (both 0.024 and 0.048%) in the diet reduced thymus weight. Therefore, although SBL can increase the digestive and absorptive capacity of the intestine in pouls, it may alter the structural integrity of lymphoid organs. It is recommended that studies should be conducted to evaluate the effect of dietary SBL levels on thymic function or cell-mediated immunity.

ACKNOWLEDGMENTS

The authors thank Carole Morris, Annette Israel, and Riswana Ali from the Department of Poultry Science, North Carolina State University and Dawn Abbott from the Department of Animal and Poultry Science, University of Saskatchewan, Canada for technical assistance.

REFERENCES


