The Polyunsaturated Fatty Acid and Cholesterol Concentrations of Plasma and Aorta and Their Relationship to Avian Atherosclerosis

A. S. FEIGENBAUM, H. FISHER, G. A. LEVEILLE, H. S. WEISS, and P. GRIMINGER,
Department of Poultry Science, Rutgers, The State University, New Brunswick, New Jersey

Growing cockerels were fed diets varying in level of protein and cholesterol and in the type of fat. Their plasma and aortas were examined for cholesterol, polyunsaturated fatty acids, and atherosclerotic involvement.

1. The cholesterol levels of plasma and aorta were increased by low-protein diets and by added dietary cholesterol, singly or in combination.

2. There appeared to be an inverse relationship between cholesterol and polyunsaturated fatty acid levels in both the plasma and aorta.

3. It was again confirmed that atherosclerotic involvement is greatest in the abdominal section of the avian aorta.

4. There was no clear intergroup relationship between polyunsaturated fatty acid levels or cholesterol concentration of the blood and the aorta and atherosclerotic involvement.

5. The birds receiving the combination of coconut oil and cholesterol showed a clear trend towards greater atherosclerotic involvement than those fed corn oil.

The type of dietary fat has been prominently implicated in the regulation of blood cholesterol (1-3). Underlying this interest in the circulating cholesterol level is the possibility that atherogenesis and cholesterol deposition in the blood vessels may be controlled by regulating the blood lipids. It has also been suggested (4) that atherogenesis may be associated with a relative, essential fatty acid deficiency. In several studies we have attempted to clarify these and other hypotheses (5-9). In working with spontaneous atherosclerosis of hens in feeding trials of one to three years' duration, it was shown that atherosclerotic plaques occurred with considerable frequency in the abdominal aorta of animals that had been fed corn oil (5,6). These plaques contained high amounts of linoleic acid, much higher than are found in unaffected aortic tissues of hens not fed corn oil (8). In these same studies, in which the degree of dietary fat saturation was the major variable, little relationship could be established among differently-fed groups between either plasma cholesterol or aortic cholesterol level and aortic atherosclerosis (5,6).

The present study was undertaken to extend the previous observations, which were concerned with spontaneous atherosclerosis, to the dietary cholesterol-induced type. Degree of fat saturation (corn versus coconut oil), protein level, and cholesterol addition were the dietary variables. The major objectives were to study the relationship of blood and aorta cholesterol and polyunsaturated fatty acid levels to the extent of atherosclerotic involvement.

Methods

Day-old male chicks (Columbian x New Hampshire) were fed a practical starting ration for two weeks and then were assigned to the experimental rations for a subsequent 20-week experimental period. The composition of the various diets is given in Table I. In the experimental design different levels of protein and cholesterol and two different fats representing two levels of unsaturated fatty acids were considered. The cholesterol concentrations differed for each of the two protein levels and were chosen on the basis of the results reported by Fisher et al. (3).

The birds were separated into lots of 16 per treatment group. At the end of two weeks and again at 10 weeks blood was drawn from the heart (from 4 birds per lot) for plasma cholesterol and fatty acid analysis. At the end of 20 weeks blood samples were taken from all remaining birds. At each sampling period the birds were killed after bleeding, and their aortas were removed. The aorta was thoroughly cleaned from the heart to the iliac bifurcation and visually scored by one of us (H.S.W.), according to procedures previously discussed (7). This method of scoring gives greater weight to the height and area of lesions protruding into the lumen than it does to the appearance of gross lipid deposition. After scoring, the aortas were separated into thoracic and abdominal sections, and each of these was placed in a separate glass bottle with Teflon gasketed metal cap to be cold-extracted with chloroform-methanol (2:1) for 24 hrs. in a shaker. The extract was filtered, and made up to volume; aliquots were taken for cholesterol analysis by

---

**Table I**

<table>
<thead>
<tr>
<th>Dietary variable</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal</td>
<td>29.00</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>2.50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.50</td>
</tr>
<tr>
<td>Micro concentrate</td>
<td>1.00</td>
</tr>
<tr>
<td>Salt</td>
<td>1.00</td>
</tr>
<tr>
<td>B vitamin mix</td>
<td>0.05</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.30</td>
</tr>
<tr>
<td>A, D, and E mix</td>
<td>0.30</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose (corn)</td>
<td>to 100.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lot</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Fat-Coconut</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

---

1 Paper of the Journal Series, New Jersey Agricultural Experiment Station. Supported in part by grants-in-aid from the U. S. Public Health Service and the N. J. Heart Association.
the modified method of Zlatkis et al. (11) and for polyunsaturated fatty acid analysis, according to the procedure of Luddy et al. (12). The cholesterol analyses were made on individual aortic and plasma samples. Duplicate polyunsaturated fatty acid analyses were made on the pooled aortic fat extracts from each treatment group. The remaining plasma for each lot was also pooled, and 1-ml aliquots were pipetted into individual test tubes. These samples were dried by lyophilization and stored in the cold for later analysis of polyunsaturated fatty acids. Duplicate determinations were made by using the procedure of Morris et al. (13).

**Results**

*Plasma.* The results of the plasma analyses are shown in Table II. Only the cholesterol values are given in complete detail for each of the three sampling periods. For greater clarity the presentation of the polyunsaturated fatty acid data has been restricted to the di- and tetraenoic acids with the tri-, penta-, and hexaenoic acids pooled together. For further clarification purposes, since there were no discernible differences between sampling periods, an average value for all three periods was computed.

Comparison of the effect of dietary fat on the polyunsaturated fatty acids of the plasma showed, as expected on the basis of the dietary fat composition, a lower dienoic and tetraenoic acid content for those birds receiving the coconut oil than for those receiving the corn oil. Within the coconut oil-fed groups those on the low-protein level generally had less of the various polyunsaturated fatty acids in the plasma than the groups receiving the higher protein diets. Adding cholesterol to the low-protein diets resulted in a further decrease of polyunsaturated fatty acid levels. In the corn oil-fed groups the feedings of low-protein diets generally resulted in lower polyunsaturated fatty acid levels as compared to the high-protein diets. The addition of cholesterol to the diet also accounted for a general decrease in fatty acid levels in comparison to the respective groups not getting cholesterol.

Irrespective of the type of dietary fat, the plasma cholesterol level was elevated on the low-protein diets as compared to the high-protein diets, and this elevation was further accentuated when cholesterol was included in the diet. At the first sampling period (5 weeks) the plasma cholesterol levels of the coconut oil-fed birds were higher than the levels for the respective corn-oil fed groups. At the final sampling period (20 weeks) the levels for the coconut oil-fed groups had decreased and those for the corn oil-fed groups had increased so that at this time the levels for the corn oil-fed groups were actually higher than the levels for the respective coconut oil-fed groups.

**Thoracic Aorta.** The results of the analysis on the aortic segments are presented in Tables III and IV. As for the plasma values, the polyunsaturated fatty acids have been averaged for all three periods. The di- and tetraenoic acids are presented separately while the tri-, penta-, and hexaenoic acids have been combined into one pooled value. The visual scores were also averaged for the three sampling periods since there was excellent agreement between periods.

In the thoracic section of the aorta the coconut oil-fed groups had lower di- and tetraenoic acid levels than the corn oil-fed groups while there were no appreciable differences in the tri-, penta-, and hexaenoic acid fractions. With but one exception and irrespective of other dietary variations, the low protein-fed groups showed lower levels of the polyunsaturated fatty acids. While either low-protein or cholesterol-supplemented diets produced variable effects on the dienoic acid level, the combination of dietary cholesterol and low protein invariably resulted in lower dienoic acid concentrations.

---

### Table II

**Effect of Dietary Fat, Cholesterol, and Protein Level on Plasma Lipid Constituents**

<table>
<thead>
<tr>
<th>Dietary Variables</th>
<th>Protein</th>
<th>Cholesterol</th>
<th>Plasma Values</th>
<th>Fatty Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>5 wk.</td>
<td>10 wk.</td>
</tr>
<tr>
<td>Coconut, 5</td>
<td>10</td>
<td>0</td>
<td>28.2 ± 2.3</td>
<td>25.1 ± 2.1</td>
</tr>
<tr>
<td>Coconut, 5</td>
<td>25</td>
<td>0</td>
<td>17.8 ± 2.1</td>
<td>12.7 ± 1.7</td>
</tr>
<tr>
<td>Coconut, 5</td>
<td>10</td>
<td>2</td>
<td>114.1 ± 12.3</td>
<td>125.4 ± 11</td>
</tr>
<tr>
<td>Coconut, 5</td>
<td>25</td>
<td>2</td>
<td>59.2 ± 3.9</td>
<td>139.5 ± 23</td>
</tr>
<tr>
<td>Corn oil, 5</td>
<td>10</td>
<td>0</td>
<td>287 ± 25</td>
<td>226 ± 25</td>
</tr>
<tr>
<td>Corn oil, 5</td>
<td>25</td>
<td>0</td>
<td>178 ± 11</td>
<td>153 ± 7</td>
</tr>
<tr>
<td>Corn oil, 5</td>
<td>10</td>
<td>2</td>
<td>109 ± 138</td>
<td>1399 ± 38</td>
</tr>
<tr>
<td>Corn oil, 5</td>
<td>25</td>
<td>2</td>
<td>314 ± 3</td>
<td>727 ± 7</td>
</tr>
</tbody>
</table>

---

### Table III

**Effect of Dietary Fat, Cholesterol, and Protein Level on Atherogenesis and Lipid Components of the Thoracic Aorta**

<table>
<thead>
<tr>
<th>Dietary Variables</th>
<th>Thoracic Aorta Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>Protein</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Coconut, 5</td>
<td>10</td>
</tr>
<tr>
<td>Coconut, 5</td>
<td>25</td>
</tr>
<tr>
<td>Coconut, 5</td>
<td>10</td>
</tr>
<tr>
<td>Coconut, 5</td>
<td>25</td>
</tr>
<tr>
<td>Corn oil, 5</td>
<td>10</td>
</tr>
<tr>
<td>Corn oil, 5</td>
<td>25</td>
</tr>
<tr>
<td>Corn oil, 5</td>
<td>10</td>
</tr>
<tr>
<td>Corn oil, 5</td>
<td>25</td>
</tr>
</tbody>
</table>

---

* Pooled values for tri-, penta-, and hexaenoic acids.