Effects of Feed Antibiotic Avoparcine on Organ Morphology in Broiler Chickens

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ABSTRACT Groups of 90 male broilers each were administered the antibiotic avoparcine mixed into feed in concentrations of 7.5, 10, and 15 ppm and achieved a higher mean body weight than the controls fed without this admixture. At the end of the 70-d fattening period, histological examination was carried out on selected individuals. The small intestine, liver, bursa of Fabricius, thymus, thyroid gland, pancreas, kidneys, heart, and skeletal muscle were observed on paraffin sections stained with hematoxylin and eosin. Cell proliferation was assessed in the liver and small intestine by means of bromodeoxyuridine labeling. The exposure to avoparcine resulted in a decreased cell proliferation in both tissues when compared to controls. In addition, hypertrophy of the hepatocytes and development of reactive lymphoid tissue in the bursa of Fabricius, which occurred in the controls, were absent in the treated animals. These observations indicate that the growth-promoting effect of avoparcine is related to a restriction in the host animals of responses to intestinal bacteria. No adverse pathological changes were observed in the examined tissues, indicating that avoparcine was well tolerated.

(Key words: avoparcine, broiler, bromodeoxyuridine, growth promotion, histology)

INTRODUCTION

Feed antibiotics have been widely used as growth promotors in poultry production. Although the growth-promoting effect of antibiotics has been commonly attributed to their efficacy against harmful intestinal bacteria (Eyssen and De Somer, 1963; Stutz and Lawton, 1984; Chaleva and Jourov, 1987; Feighner and Dashkevicz, 1987; Hornish et al., 1987), the mechanisms instrumental in the growth stimulation are poorly understood (Eyssen and De Somer, 1963). The importance of antimicrobial efficacy of feed antibiotic has been demonstrated by Eyssen and De Somer (1963), and by Muramatsu et al. (1983), who compared conventional and germ-free chickens: the germ-free chickens prospered better than the conventional chickens, unless the conventional chickens received feed antibiotics.

The potentially harmful microbial effects in chickens have not been well characterized, but several possible mechanisms have been proposed. It has been suggested that the presence of bacteria may induce a chronic inflammation, resulting in a thickening of the intestinal wall, which, in turn, impairs intestinal absorption and decreases the amount of nutrients available for the host. The findings supporting this mechanism include a thickening of the intestinal lamina propria and an increased proliferative activity of the intestinal epithelium in conventional chickens when compared to germ-free chickens (Gordon and Bruckner-Kardoss, 1961; Khoury et al., 1969; Cook and Bird, 1973; Jamroz et al. 1992 a,b).

Furthermore, bacteria may produce toxins, such as ammonia or amines, which must be detoxified in the host liver, inducing hypertrophy of the hepatocytes (McGowan et al., 1984). Bacteria may also stimulate an increased production of immunoglobulins needed to protect the biliary and upper intestinal tracts from infection (Coleman, 1987), and, by that means, reduce the body weight gain. Other possible mechanisms are the competition of bacteria with their host for nutrients, or degradation by bacteria, especially the anaerobes, of taurine, which is indispensable for conjugation of bile acids (Morris et al., 1990; Wolfram, 1991). The aim of the present study was to assess the influence of antibiotic treatment on the morphology of those organs that may be affected by the presence of microbes in broilers.

MATERIAL AND METHODS

Animals and Treatment

A large-scale feeding experiment was carried out on 360 male broilers (Hybrid Astra B 2). The birds were maintained under standard conditions on wood sawdust...
at a temperature 32°C (at the beginning), and 22°C later on, respectively. The birds were grouped in four groups of 30 birds per group in three replicates. The animals had free access to feed and drinking water. The Starter Diet, fed until 21 d of age, contained 21% protein with a 4% portion of fish flour. The Finisher Diet, fed from Days 22 to 70, contained 18% protein with a 2% portion of fish flour. The antibiotic avoparcine (Cyanamid) was added to the feed of Groups 1, 2, 3, and 4 in amounts of 0, 7.5, 10, and 15 ppm, respectively. Feed consumption was recorded and the birds were weighed on Days 49 and 70. These data were subjected to statistical analysis of variance combined with Duncan’s test. Three male chickens per dose group were randomly selected for detailed histological examination.

After 10 wk (70 d) of fattening the animals were injected intraperitoneally with BrdU (bromodeoxyuridine) diluted in 0.9% NaCl, at a concentration of 15 mg BrdU/10 mL solution, using the dose of 1 mL/100 g body weight. To avoid a possible effect the circadian rhythm on cellular proliferation, the injection and necropsy were done between 0900 and 1100 h, following the approach of Tanaka et al. (1990). The chickens were killed by exsanguination 1 h following the administration of BrdU and subjected to necropsy. After recording the weight of the carcass, tissue samples from the small intestine (duodenum), liver, bursa of Fabricius, thymus, thyroid gland, pancreas, kidneys, heart, and skeletal muscle (Pectoralis) were preserved in neutral buffered 4% formalin. One chicken of control Group 1 had to be excluded from the experiment because of an accident during transportation.

Processing of Tissues

Duration of fixation was 24 h for the BrdU reaction and 7 d for the histological examination. The tissues were dehydrated, embedded in paraplast, and sectioned at 4 to 5 μm. For histological examination, the sections were stained with hematoxylin and eosin. The BrdU reaction was carried out on sections deparaffinized with a graded alcohol series, incubated at 37°C for 60 min with anti-BrdU antibody diluted 1:1000, and detected with rabbit anti-mouse immunoglobulin using diaminobenzidine and hematoxylin counterstain.

Evaluation

The assessment of organ morphology was carried out under a light microscope. The thickness of intestinal wall (measured from muscularis mucosae to the tip of villus) was determined with an eyepiece scale under the microscopic enlargement × 53 on the sections stained with hematoxylin and eosin. Five randomly selected areas were measured of each specimen. Because the mitotic activity of the hepatocytes was very low, large areas of liver tissue had to be examined. In each animal, the BrdU-positive hepatocytes found in a field of 50 mm² were counted under a light microscope. To quantify the BrdU-labeled cells in the small intestine, an automatic morphometric system of Olympus with program “CUE 3” was used. On five randomly selected areas of 0.172 mm² per specimen all labeled cells were automatically counted. Because the number of available individuals was limited, the results were not analyzed statistically, but the observed differences are considered to be biologically meaningful.

RESULTS

Analysis of mean body weight of all 360 chickens showed significantly increased gain in all groups fed the antibiotic avoparcine compared to the control. The beneficial effect of avoparcine was more pronounced at Day 49 than at the end of the fattening prolonged to Day 70 (Table 1). The feed:gain index was 2.56, 2.56, 2.62, and 2.51 for Groups 1 to 4, respectively, for the period from 0 to 49 d, and 2.98, 3.01, 2.95, and 2.89 kg for the period of 0 to 70 d. The state of health was good in all groups throughout the experiment and mortality did not exceed 4%. When compared to the controls, the mean numbers of BrdU-labeled epithelial cells in the small intestine were decreased in the treated groups. The mean value for the control group was 113 labeled cells per 0.86 mm². In the treated groups (2, 3, and 4) this value decreased to 24, 18, and 56% of the control value, respectively. A characteristic example of the difference in proliferative activity in the intestine is shown in Figure 1. Likewise, examination of BrdU-stained liver specimens revealed decreased proliferative activity in all treated groups. The mean value in the control group was 72 labeled cells per 50 mm². In the treated groups (2, 3, and 4) this value decreased to 24, 18, and 56% of the control value, respectively. Analysis of the thickness of the intestinal wall of small intestine showed slightly higher mean values in treated groups. The value for the control group was 165 μm and the values for the treated groups (2, 3, and 4) were increased to 103, 115, and 104%, respectively. Examination of hematoxylin and eosin stained samples from lobus sinister lateralis of the liver showed a slight hypertrophy (manifested by a

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight at 49 d (g)</th>
<th>Weight at 70 d (g)</th>
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<tbody>
<tr>
<td>1</td>
<td>1,866</td>
<td>3,047</td>
</tr>
<tr>
<td>2</td>
<td>1,991 ± 6.7</td>
<td>3,085 ± 12</td>
</tr>
<tr>
<td>3</td>
<td>2,000 ± 7.2</td>
<td>3,170 ± 4.3</td>
</tr>
<tr>
<td>4</td>
<td>1,992 ± 6.7</td>
<td>3,054 ± 0.2</td>
</tr>
</tbody>
</table>

I.Cyanamid (UK) Holdings PLC, Gosport, Hampshire, PO13 OAS, UK.
2BrdU and anti-BrdU were obtained from BrdU and anti-BrdU were obtained from Bio-Science Products AG, Biochemica and Diagnostica, 7303 GC Apeldoorn, The Netherlands.
FIGURE 1. Small intestine from a control chicken (left), and from a chicken fed with 15 ppm avoparcine (right). The number of BrdU-positive cells (dark nuclei) in the control animal is considerably higher than in the animal fed with avoparcine. BrdU = bromodeoxyuridine, 380x. (Bar = 100 μm).

larger cell-body size when compared to treated groups) of the hepatocytes in the chickens from control Group 1 (Figure 2).

Observation of the bursa revealed minimal to moderate hypocellularity, which indicated a suppression of development of reactive lymphoid tissue in four of seven examined chickens from Groups 2, 3, and 4 (Table 2). Owing to low density of the lymphocytes in comparison to the control group, the size of bursal lobules in these individuals was considerably decreased as well. The presence of reactive lymphoid tissue was seen in the control group (Figure 3).

In the thymic cortex of some treated birds, there was a minimal decrease in the number of lymphocytes. Because this finding was seen in one control chicken as well, no relevance was attributed to this difference. In the thyroid gland, neither in the control Group 1, nor in the Groups 2, 3, and 4 were there signs of increased activity of secretory epithelium. Hypertrophy would be manifested as cells with an increased amount of cytoplasm and cuboidal to prismatic form. Examination of the pancreas and kidneys did not show differences between the control group and Groups 2, 3, and 4. In the heart and skeletal muscle, there were no signs of an increased burden on the myofibers due to the rapid growth of the young chickens. The histological features in treated animals were comparable to those seen in the control animals.

DISCUSSION

The results indicated that the addition of avoparcine to chicken feed caused a decrease in mitotic activity of

<table>
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<tr>
<th>Group</th>
<th>Dose of avoparcine (ppm)</th>
<th>Minimal</th>
<th>Moderate</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1/3</td>
<td>0/3</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>1/2</td>
<td>0/2</td>
</tr>
</tbody>
</table>
the epithelial cells both in the epithelial cells of small intestine (duodenum) and in the liver cells (hepatocytes). This finding is in accordance with the previous observation that in germ-free animals or in animals fed antibiotics, the mitotic activity and the cell migration rate of intestinal epithelial cells were decreased (Khoury et al., 1969). Morphological changes in the intestinal wall, such as inflammation, and an increase in thickness of the lamina propria, length of the villi, weight of the intestine, and mitotic activity of the epithelial cells are reportedly associated with a higher number of bacteria in the intestine (Gordon and Bruckner-Kardoss, 1961; Khoury et al., 1969; Cook and Bird, 1973; Jamroz et al., 1992 a,b). In contrary, we observed a higher thickness in the intestinal wall in treated animals, possibly reflecting decreased cellular turnover, as indicated by decreased mitotic activity. Moreover, in the control group, there was no evidence of inflammatory changes that could produce intestinal thickening.

The mostly quiescent hepatocytes in adult animals can be induced to proliferate by hormonal or metabolic imbalance. Certain intermediary metabolites can stimulate protein and DNA synthesis in the hepatocytes (McGowan et al., 1984). The role of ammonia, the level of which in portal blood depends on kind and amount of bacteria in the gut (putrefaction), is yet undetermined. Our results show a slight hypertrophy of hepatocytes in control chickens, indicating their activity to be higher than that of treated animals. Their proliferative activity was higher as well. This result suggests that in the control chickens, there were more bacteria present in the gut and that there were more nitrogenous end-products to be detoxified in the liver cells. The hepatocytes are also known to produce immunoglobulins that protect the biliary ducts and digestive tube against infections (Coleman, 1987). The hypertrophy of these cells in our study correlates with the results of Klasing and Austic (1984), who showed increased protein synthesis in the liver, bursa, spleen, and thymus in chickens infected with Escherichia coli.

In the present study, most chickens fed avoparcine had minimal to moderate suppression of the develop-
ment of reactive lymphoid tissue in the bursa. This result indicates that there may be lack of stimulation of the immune system mediated by gut microorganisms in the antibiotic-treated birds (Thorbeke et al., 1957; Gordon and Bruckner-Kardoss, 1961; North, 1975; Furuta et al., 1980).

In conclusion, our observations indicate that the growth-promoting effect of avoparcine could be related to a restriction in the host animals of responses to intestinal bacteria, such as increased renewal of epithelial intestinal cells, increased liver activity, and increased immune response. Moreover, addition of avoparcine to the chicken feed does not evoke adverse pathological changes in the small intestine, liver, bursa, thymus, thyroid gland, pancreas, kidneys, heart, and skeletal muscle.

REFERENCES


