



Poultry Coccidiosis

Diagnostic and Testing Procedures

THIRD EDITION

Donal P. Conway and M. Elizabeth McKenzie

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and
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The preceding editions of this handbook were designed to aid the research scientist, the diagnostician, and the veterinary practitioner to better understand the procedures used to diagnose and evaluate coccidiosis in chickens, to review the epidemiology and control of these protozoan parasites, and to outline standard procedures for testing anticoccidial drugs under laboratory conditions and in the field. These objectives have not changed in the current edition, and we have included a new chapter on anticoccidial drugs and vaccines used in the prevention and control of these parasites in chickens.

The topics developed emphasize the differential diagnosis of the major species of poultry coccidia, lesion scoring, the preparation of oocysts for laboratory tests, basic procedures for testing anticoccidial drugs, a review of the epidemiology of coccidiosis in chickens, and standard procedures for preventing this disease complex through good management practices and the use of anticoccidial drugs and vaccines.

Special acknowledgment is given to Mrs. Joyce Johnson and Dr. W. Malcolm Reid, both now deceased, and to Dr. Larry R. McDougald of the Poultry Science Department, University of Georgia, for contributing the photographs used in figures 1.2–1.7 and 2.1–2.33, for preparing appropriate descriptions used in developing the text for figures 2.1–2.33, and for their many helpful suggestions regarding the original manuscript. We are also indebted to Dr. Peter L. Long, University of Georgia, for his many constructive comments regarding the second edition.

A comprehensive review of the current edition was undertaken by Dr. Thomas K. Jeffers, and we wish to thank him very much for his contribution. His excellent comments and suggestions were of tremendous value to our final review of this edition. The advice and encouragement from Dr. Patricia C. Allen, Agricultural Research Service, U.S. Department of Agriculture, during the preparation of this edition is also greatly appreciated.

We wish to dedicate this edition to the late Joyce Johnson in honor of her participation in the development of this handbook and her many contributions in the field of poultry coccidiosis research over the years of her outstanding career. We both had the great opportunity to work with her on many occasions in the laboratory and in the field, and grew to appreciate and respect her keen eye for precision and scientific accuracy in her work.

2007

Donal P. Conway and M. Elizabeth McKenzie

The coccidia consist of a wide variety of single-celled, parasitic animals in the subkingdom Protozoa of the phylum Apicomplexa. As a group, the coccidia of the genus *Eimeria* are predominately host-specific; i.e., each species occurs in a single host species or a group of closely related hosts.

Infection by coccidia in sufficient numbers to produce clinical manifestations of disease is called coccidiosis. A light infection that does not result in demonstrable clinical effects is referred to as coccidiasis.

The species of coccidia in the chicken belong to the genus *Eimeria*. All invade the lining of the intestine or ceca. Specifically, these are *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*. The validity of two other species frequently mentioned in the literature, *E. hagani* and *E. mivati*, is under review. *E. hagani* has not been formally described since its initial description 69 years ago (Levine 1938), but Tsuji et al. (1997) were able to discriminate between the above seven species plus *E. hagani* using a two-step polymerase chain reaction (PCR) procedure. *E. mivati* was first described by Edgar and Seibold (1964), but subsequent studies led to the conclusion that this species was either a variety of *E. acervulina* (Long 1973) or a mixture of *E. acervulina* and *E. mitis* (Shirley, Jeffers, and Long 1983; Shirley 1986; Long 1987). These hypotheses were brought into question in work by Edgar and Fitz-Coy (1986) and Fitz-Coy, Edgar, and Mora (1989), who provided a more detailed

description of *E. mivati*. Recent studies by Barta et al. (1997) indicated that *E. mivati* is closely related to *E. mitis* and may be a separate species based on their analysis of the 18s ribosomal sequence. Thirteen field isolates of *Eimeria* obtained from farms in the United States were evaluated in a blind study in the laboratory using a PCR procedure (Fitz-Coy 2005). The identity of each isolate was determined by this procedure except for those subsequently identified as *E. mivati*. These results clearly indicate that further studies on the validity of this species are needed.


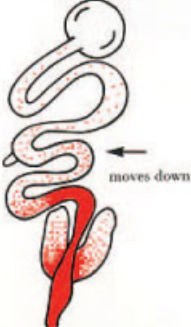



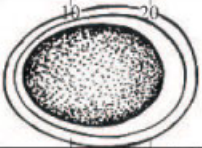
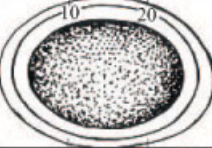

Diagnostic chart

Differential identification of each species is dependent upon the following characteristics:

1. Zone of intestine parasitized
2. Gross appearance of the lesion
3. Oocyst morphology
4. Minimum sporulation time
5. Minimum prepatent time
6. Schizont size and location of development
7. Location of parasite in the host intestinal epithelium
8. Cross-immunization tests







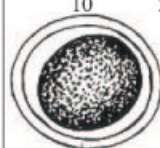
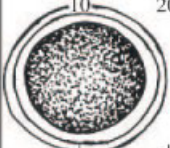
Table 1.1 provides a summary of the first six characteristics.

TABLE 1.1. Differential characteristics for eight species of chicken coccidia

	<i>E. acervulina</i>	<i>E. brunetti</i>	<i>E. maxima</i>	<i>E. mivati</i>	
MACROSCOPIC LESIONS					
	CHARACTERISTICS				
	ZONE PARASITIZED				
MACROSCOPIC LESIONS	light infection: whitish round lesions sometimes in ladder-like streaks heavy infection: plaques coalescing, thickened intestinal wall	coagulation necrosis mucoid, bloody enteritis in lower intestine	thickened walls, mucoid, blood-tinged exudate, petechiae	light infection: rounded plaques of oocysts heavy infection: thickened walls coalescing plaques	
MILLIMICRONS	10 20 30	10 20 30	10 20 30	10 20 30	
MICROSCOPIC CHARACTERISTICS					
	OOCYSTS REDRAWN FROM ORIGINALS				
	LENGTH x WIDTH µm LENGTH = WIDTH =	AV = 18.3 x 14.6 17.7 - 20.2 13.7 - 16.3	24.6 x 18.8 20.7 - 30.3 18.1 - 24.2	30.5 x 20.7 21.5 - 42.5 16.5 - 29.8	15.6 x 13.4 11.1 - 19.9 10.5 - 16.2
	OOCYST SHAPE AND INDEX - LENGTH/WIDTH	ovoid 1.25	ovoid 1.31	ovoid 1.47	ellipsoid to broadly ovoid 1.16
	SCHIZONT, MAX IN MICRONS	10.3	30.0	9.4	17.3
	PARASITE LOCATION IN TISSUE SECTIONS	epithelial	2nd generation schizonts subepithelial	gametocytes subepithelial	epithelial
	MINIMUM PREPARENT PERIOD-HR	97	120	121	93
	SPORULATION TIME MINIMUM (HR)	17	18	30	12

Compiled from various sources.

DIAGNOSTIC CHARACTERISTICS IN RED

<i>E. mitis</i>	<i>E. necatrix</i>	<i>E. praecox</i>	<i>E. tenella</i>
	large schizonts, no oocysts 		
no discrete lesions in intestine mucoid exudate	ballooning, white spots (schizonts), petechiae, mucoid blood-filled exudate	no lesions, mucoid exudate	onset: hemorrhage into lumen later: thickening, whitish mucosa, cores clotted blood
10 20 30 	10 20 30 	10 20 30 	10 20 30 
15.6 x 14.2 11.7 - 18.7 11.0 - 18.0	20.4 x 17.2 13.2 - 22.7 11.3 - 18.3	21.3 x 17.1 19.8 - 24.7 15.7 - 19.8	22.0 x 19.0 19.5 - 26.0 16.5 - 22.8
subspherical 1.09	oblong ovoid 1.19	ovoid 1.24	ovoid 1.16
15.1	65.9	20	54.0
epithelial	2nd generation schizonts subepithelial	epithelial	2nd generation schizonts subepithelial
93	138	83	115
15	18	12	18

Modified after Long and Reid (1982).

Coccidial life cycle

Development of the parasite in the host cells involves both asexual and sexual stages of multiplication. Destruction of host tissue as a result of parasite development and multiplication leads to the various clinical manifestations observed in outbreaks of disease. Development of the various species of chicken coccidia includes minor variations. A generalized life cycle is illustrated in figure 1.1.

Infection occurs when a susceptible chicken ingests a sporulated oocyst from its environment. The sporulated oocyst (figure 1.2) contains four sporocysts. Each sporocyst contains two sporozoites. The sporozoites are released by mechanical and biochemical action in the digestive tract of the chicken (Reid 1978). The liberated sporozoites (figure 1.3) invade epithelial cells in a specific zone of the intestine or ceca depending on the species involved (table 1.1). Upon entering the host cell, the sporozoite transforms in 12 to 48 hours to a feeding stage called a trophozoite. The trophozoite begins to enlarge, and the parasite nucleus divides by a process of asexual multiple division known as schizogony (merogony). At this point, the parasite stage is referred to as a schizont or meront (figure 1.4). The small parasitic stages forming within the schizont are called merozoites. The schizont ruptures when mature (day 3), releasing the merozoites. Most of these invade other epithelial cells to repeat

the process of development through the trophozoite and schizogonous stages (figure 1.5). The merozoites from the second schizogonous cycle again penetrate the epithelial cell of the host. Some or all may go through a third schizogonous cycle, depending on the species, before formation of male (microgametocytes) or female (macrogametocytes) gametocytes (figures 1.6 and 1.7).

The male gametocyte matures and ruptures, releasing a large number of minute biflagellate microgametes. The macrogametocyte grows to form a macrogamete. A thickened wall forms around the macrogamete, forming a zygote when the macrogamete is fertilized by a microgamete. This stage is the young or immature oocyst. The prepatent period varies with each species depending on the time required for each schizogonous cycle and the number of cycles.

The oocyst ruptures the host cell when mature and passes out of the bird in the droppings. Under suitable environmental conditions, four sporocysts, each containing two sporozoites, are formed within the oocyst after about 24 hours.

Diagnostic procedure

Procedures for killing birds include cervical dislocation, use of Burdizzo forceps, electrocution, and the use of euthanizing substances (Zander and Mallinson 1991).

Various techniques have been developed for post-mortem examination. Procedures basic to most techniques include (1) placing the bird on its back and severing the skin and fascia between the legs across the abdomen, (2) breaking the legs out of the acetabular articulation, (3) pulling the skin away from the musculature from the vent to the beak, (4) removing the breast, being careful not to damage internal organs or to sever large vessels, and (5) exposing the abdominal cavity and viscera for examination (Zander and Mallinson 1991).

After the bird has been opened, the intestine should be freed from the mesenteries along its entire length. The serosal surface of the unopened intestine should be examined for pinpoint-sized lesions under a strong light. These may vary in color from bright red to brown or white. Whitish plaques may be pockets of oocysts or schizonts in the upper half of the intestine. Transverse or ladderlike plaques of developing oocysts are characteristic of *E. acervulina*. In the midintestine, plaques may be pockets of *E. necatrix* schizonts. A search should be made for petechiae caused by hemorrhaging and the characteristic swelling and ballooning produced by *E. necatrix* and *E. maxima*.

When the intestine has been opened with scissors or an enterotome, the intestinal wall should be examined for thickening, petechiae, coagulation necrosis (most often seen in the lower intestine and rectum with *E. brunetti*), reddening, whitish spots, cecal cores, and bleeding. After removal of

surface ingesta, suspicious areas should be scraped and the material mounted on a slide for microscopic examination. The scrapings should be diluted with one or two drops of physiological saline. This solution is easily applied by using a polyethylene wash bottle. Intestinal debris and saline should be mixed and spread with a toothpick. A cover glass is then applied. Water is a satisfactory substitute for saline in demonstration of oocysts, but other stages of the coccidial life cycle may be destroyed.

“In a complete examination scrapings should be made from five or more locations along the gut wall. Areas with lesion should be selected if present. If no suspicious areas are located, select from (1) the duodenal area a centimeter or two below the entrance of the bile duct, (2) the middle gut region which may be located by the yolk sac diverticulum, (3) the lower intestinal area a few centimeters above the union with the cecal pouches, (4) an area from near the middle of the cecum, and (5) the rectal area” (Long and Reid 1982).

As reported by McDougald and Reid (1991), “The poultry diagnostician faces a difficult task in accurate diagnosis of coccidiosis. Four complications increase the difficulties:

1. There is overlapping of gut area parasitized by different species.
2. The gross lesions change in appearance as infection progresses during the life cycle.

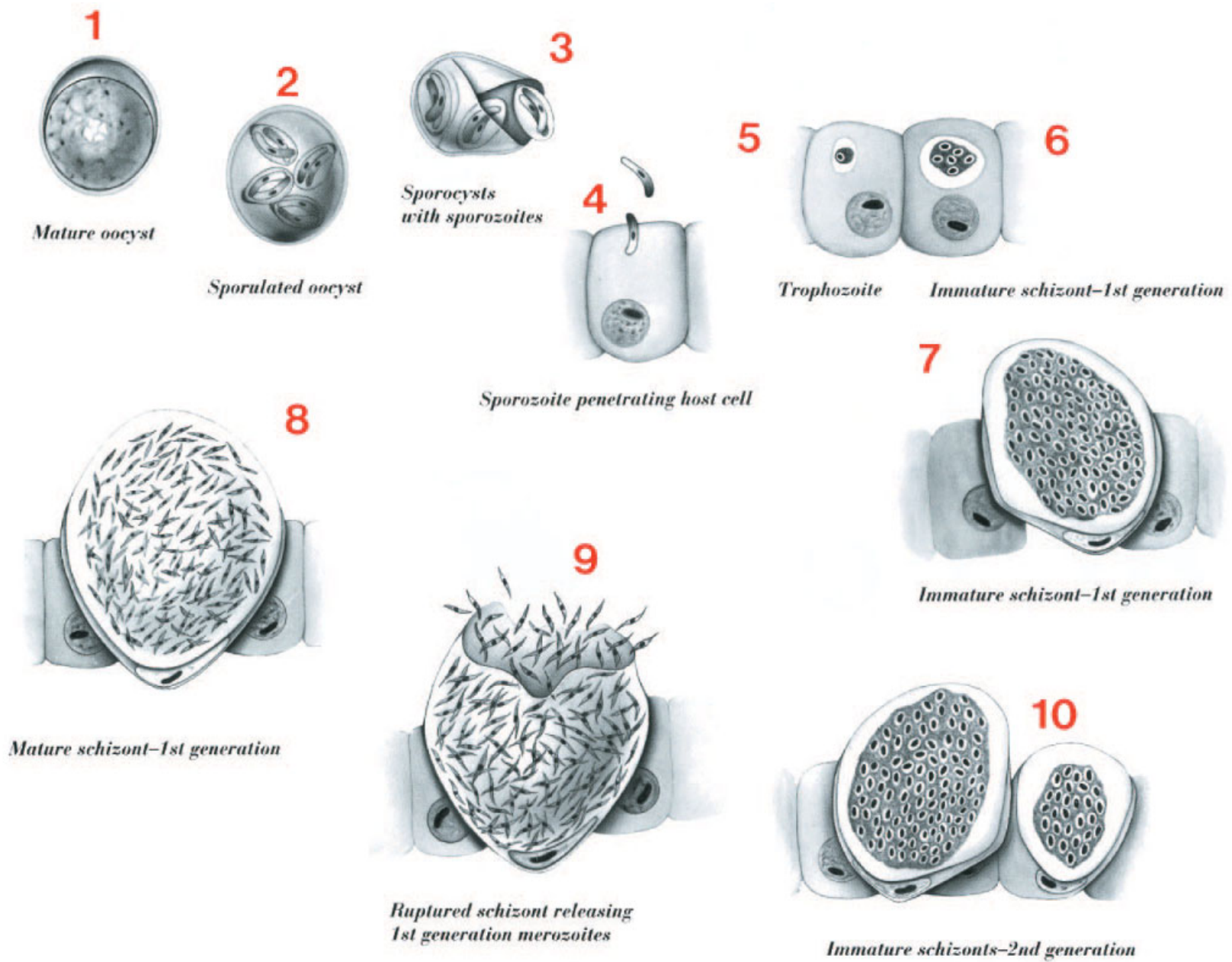


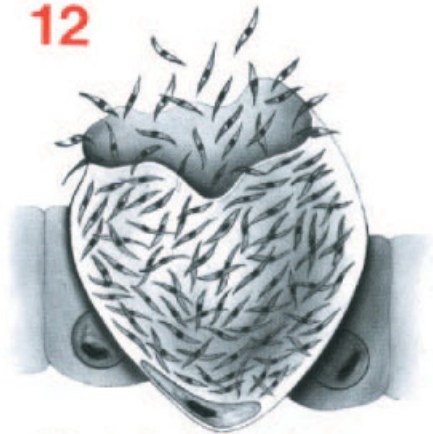
Figure 1.1. Generalized life cycle of *Eimeria* spp. in chickens (art by David J. Williams).

11



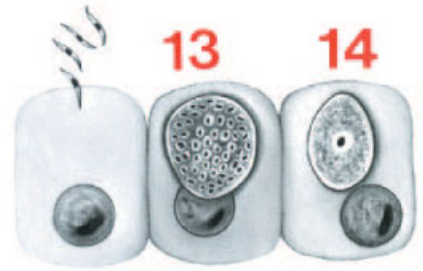
Mature schizont-2nd generation

12



Ruptured schizont releasing 2nd generation merozoites

13



Male gametocyte

14



Female gametocyte

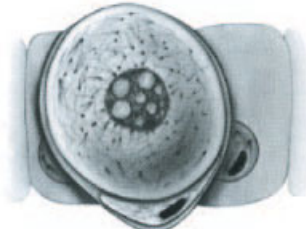
Rupture of oocyst from host epithelium

16



Male gametocyte rupturing and liberating microgametes

15

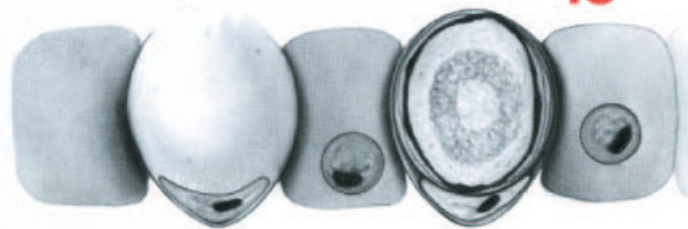


Mature male gametocyte

19

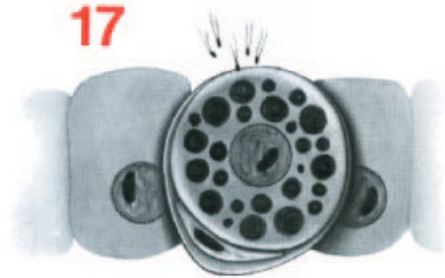


18



Developing oocyst

17



Fertilization of macrogamete

Williams

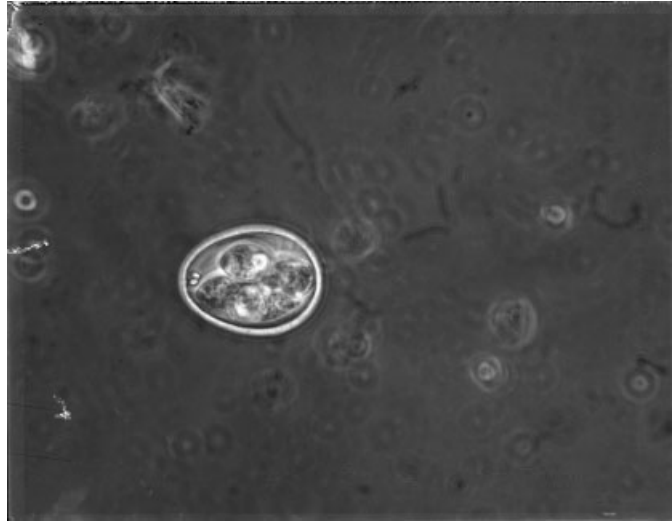


Figure 1.2. Sporulated oocyst.



Figure 1.3. Released sporocysts and sporozoites.

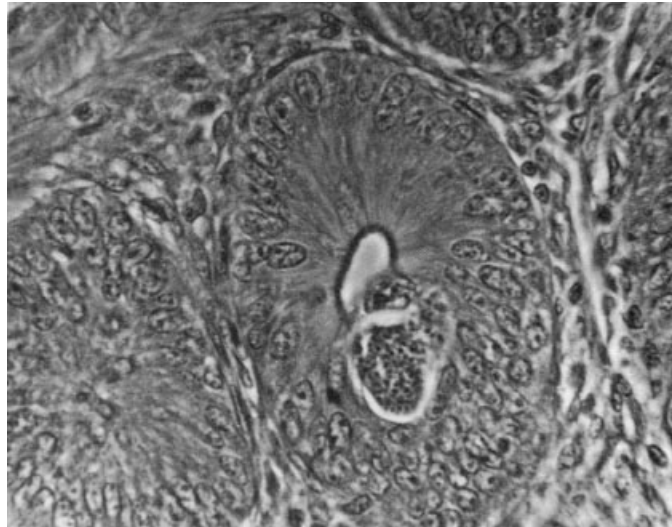


Figure 1.4. *E. tenella* schizonts, 56 h., $\times 400$.

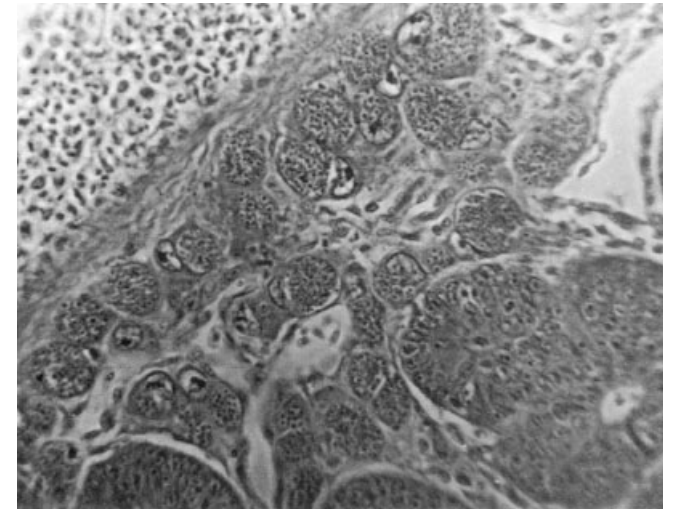


Figure 1.5. *E. tenella* schizonts, 126 h., $\times 200$.

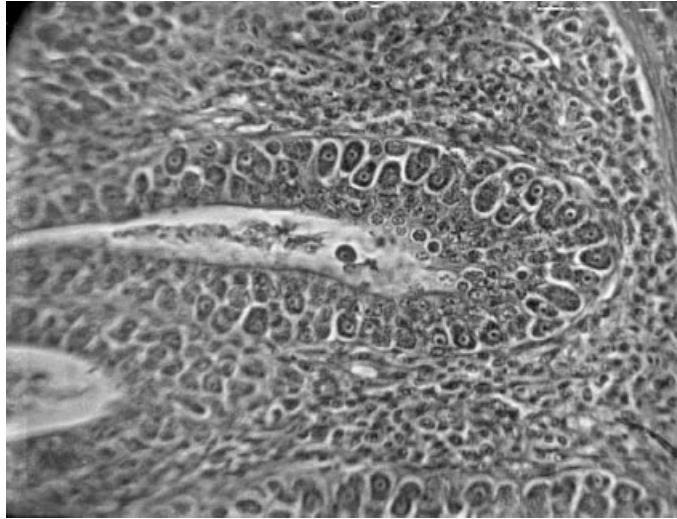


Figure 1.6. *E. tenella* gametocytes, $\times 200$.

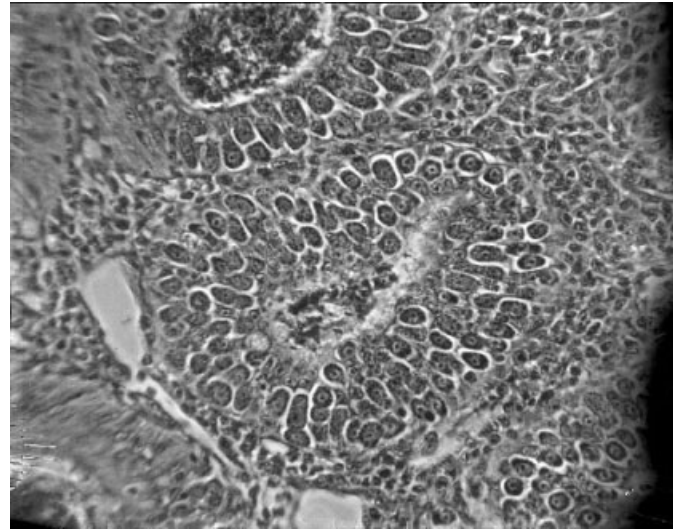


Figure 1.7. *E. tenella* gametocytes, $\times 200$.

3. The status of different birds in a flock may vary with progress of the disease—some showing acute lesions, some being in a recovery phase, and others remaining unexposed; however, diagnosis and recommendations are required on a total flock basis.
4. Distinction must be made between true clinical coccidiosis requiring treatment and mild sub-clinical coccidiasis characterized by the presence of a few lesions or a few oocysts. Coccidiasis is more common than coccidiosis. Some diagnosticians record coccidiosis for every accession if any parasitic stage is demonstrated. Thus, the seriousness of coccidiosis may be overemphasized in reports from diagnostic laboratories.”

Experienced diagnosticians may attempt a presumptive diagnosis based on gross lesions observed in the field, but a confirmatory examination conducted in the laboratory allowing a microscopic exam of scrapings obtained from the lesions is preferred. Transportation of live birds from the infected flock to the laboratory is desirable to avoid possible postmortem change that might obscure the lesions in question (McDougald and Reid 1991).

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Recognition and identification of lesions depend upon some knowledge of the species found in different parts of the intestine. Reference to table 1.1, where features of diagnostic importance for eight important species are shown in red, may prove helpful in identification.

Gross lesions for six of the more pathogenic species of *Eimeria* are given in the order in which they appear descending the intestine (figures 2.1–2.32). Lesions produced by *E. acervulina* and *E. mivati* occur primarily in the duodenal loop and the upper part of the jejunum. *E. maxima* and *E. necatrix* produce their most severe lesions in the midintestinal area, which is readily identified by the residual yolk sac diverticulum. *E. brunetti* invades the mucosa of the lower intestine and the rectum. Lesions of *E. tenella* are found mostly in the ceca, but occasionally some strains of *E. tenella* will cause lesions in the rectal area.

Lesion scoring (Johnson and Reid method)

Although some knowledge of lesions and species identification is desirable for lesion scoring, identification of species is not the primary objective of lesion scoring. Lesion scoring is a technique developed to provide a numerical ranking of gross lesions caused by coccidia (Johnson and Reid 1970). Poultry diagnosticians routinely examine the entire bird during necropsy. If coccidiosis is the only disease to be diagnosed (as in many experi-

mental situations), only the intestine needs to be examined. The entire intestine is usually pulled out unbroken from the bird. The gizzard and the rectum are left attached for orientation as to location of lesions observed in various parts of the intestine.

Beginning with the duodenum, the intestine is slit open and both the mucosal surface and the unopened serosal surface are examined for lesions. A good light source such as a surgical lamp or direct sunlight is necessary. A microscope should be available to examine smear preparations for parasites if lesions are difficult to identify.

Scoring techniques differ considerably with mixed infections, the most commonly encountered condition in the field, compared with scoring lesions involving a single species. Scoring lesions caused by one species will be discussed first. This will give a good background for later discussion of scoring lesions involving several species.

Lesions of major species

Eimeria acervulina

Eimeria acervulina commonly invades the duodenal loop of the intestine and in heavy infections may extend to infect lower levels of the jejunum and even the ileum or lower intestine.

E. acervulina +1 is shown in figure 2.1. White lesions of *E. acervulina* are clearly visible on this close-up view of the mucosal surface of the duodenal loop. The white streaks are oriented transversely across the intestine in an arrangement often described as ladderlike. This is a mild infection and would be rated as +1 using a scoring system ranging from 0 to +4. These scattered lesions do not number more than five per square centimeter. Such an infection may cause some loss of skin pigmentation but has little or no measurable effect on weight gains or feed conversion of infected birds. A scraping from one of these white lesions viewed under the microscope would reveal a mass of unsporulated oocysts and gametocytes.

E. acervulina +2 is shown in figure 2.2. The white lesions in the duodenal loop are much closer together but are still discrete. Their ladderlike orientation is less apparent than if there were fewer lesions. The lower loop of the duodenum, which has been opened, shows lesions much more clearly than the unopened upper loop. With a good light source, these distinctive transversely elongated white plaques may be readily recognized on the serosal as well as the mucosal surface. There is no thickening of the duodenal wall with a +2 lesion. Such an infection is considered mildly pathogenic and may cause some depression in weight gain in unmedicated birds.

E. acervulina +3 is shown in figure 2.3. Lesions recognizable on both opened and unopened portions of the duodenal loop are more numerous and beginning to coalesce. Some thickening of the intestinal wall is apparent, and the contents are watery because of excessive mucous secretion. This condition accounts for the consequent diarrhea. Decreases in weight gain and feed conversion efficiency occur with this level of infection in unmedicated birds.

E. acervulina +3 bordering on a +4 score is shown in figure 2.4. Lesions are coalescing in the portion of the duodenum attached to the gizzard. The lower portion shows the midintestinal area where lesions are sparse, but the lesions still exhibit a distinctive ladderlike arrangement. The more severe the infection, the further down the intestine these lesions may appear. Weight gains and feed conversion efficiency are definitely depressed with this grade of infection in unmedicated birds.



Figure 2.1. *E. acervulina* +1.

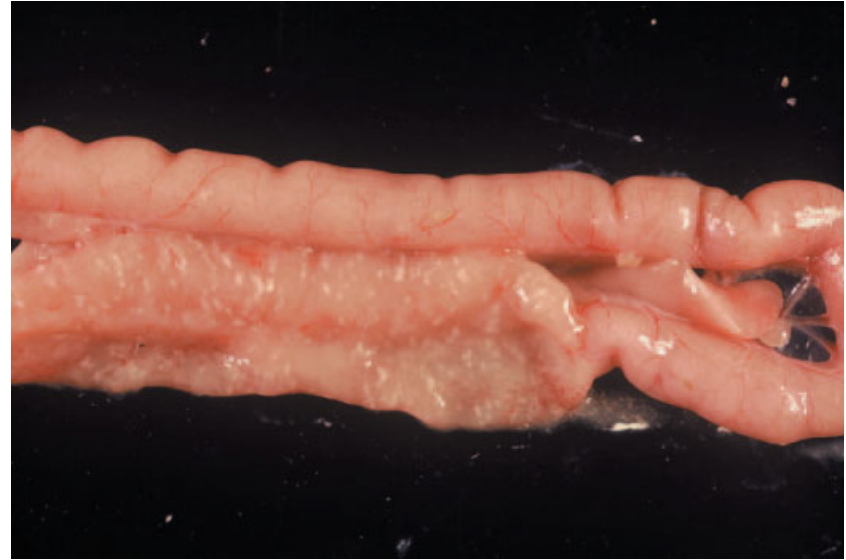


Figure 2.2. *E. acervulina* +2.

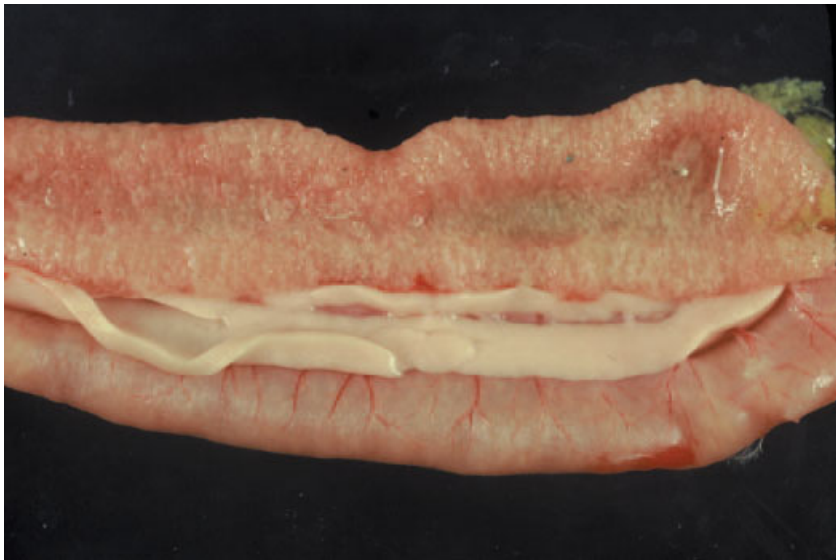


Figure 2.3. *E. acervulina* +3.



Figure 2.4. *E. acervulina* +3 bordering on a +4 score.

E. acervulina +4 is shown in figure 2.5. Coalescing of the lesions is so complete that no distinctive lesions may appear in the duodenal portion of the intestine. For this reason, such an infection may be overlooked on cursory examination. Milder infections with distinctive white plaques are more easily recognized. The intestinal wall is considerably thickened, and the roughened intestinal wall is laden with oocysts. Diarrhea, severe weight loss, poor feed conversion, and loss of skin pigmentation accompany such an infection in unmedicated birds.

Eimeria mivati

Eimeria mivati is the most recently described species of chicken coccidia, and its validity as a species has been questioned over the years (see chapter 1). It is anticipated that studies currently in progress will ultimately resolve this question. *E. mivati* is frequently confused with *E. acervulina*, and differential diagnosis of these two species is important when they differ in drug susceptibility or when using a vaccine that does not include the former species. *E. mivati* moves down the intestine more than *E. acervulina* as the infection progresses, and lesions may appear further down the intestinal tract. Although the oocysts average one or two micrometers smaller, this difference is difficult to recognize by measuring oocysts. A calibrated ocular micrometer and measurement of 10 or more oocysts will be required to recognize this difference under a microscope.

E. mivati +1 is shown in figure 2.6. Discrete rounded lesions may be readily distinguished in the opened portion of the duodenal loop and are recognizable although less distinct on the unopened serosal surface. Since the lesions are widely scattered, they have been scored as +1. Most of the lesions are more rounded than with *E. acervulina*. Light infections of this sort may induce some skin depigmentation, but effects on body weights would be difficult to measure. Scoring has been done on days 5 or 6 of infection with *E. mivati* and *E. acervulina* when lesions are most distinct.

E. mivati +2 is shown in figure 2.7. Lesions are more numerous than in the *E. mivati* +1 example (figure 2.6). Skin depigmentation and mild weight depression may accompany this degree of infection.

E. mivati +3 is shown in figure 2.8. Still more numerous lesions are present. Occasionally they are close enough to coalesce with each other. Some thickening of the intestinal wall is apparent. Moderate weight depression occurs with such an infection.

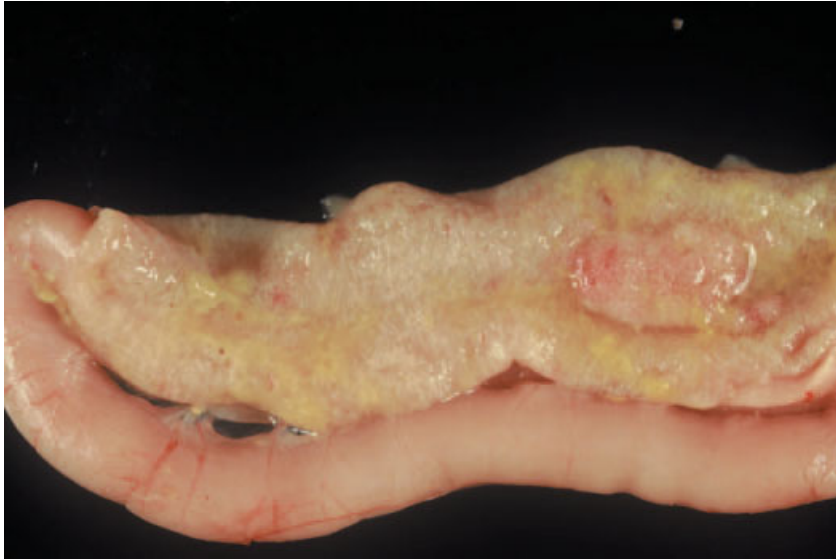


Figure 2.5. *E. acervulina* +4.

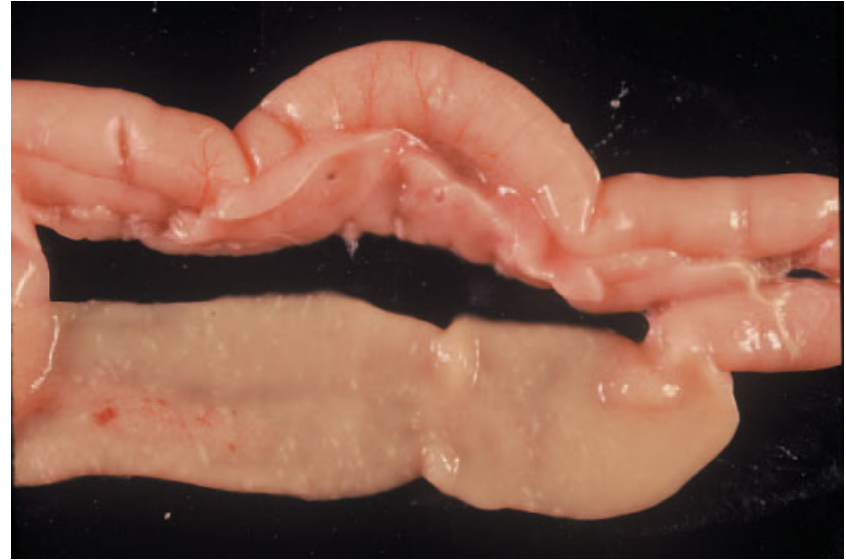


Figure 2.6. *E. mivati* +1.



Figure 2.7. *E. mivati* +2.

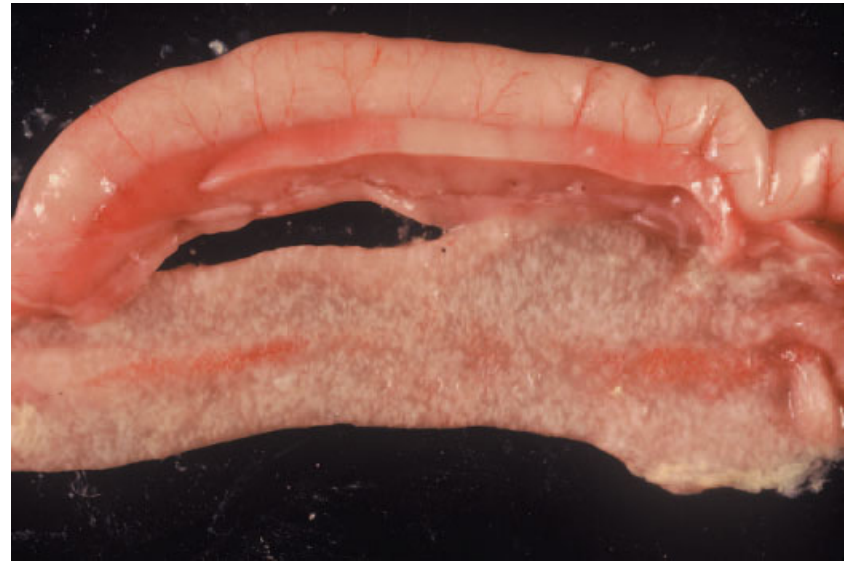


Figure 2.8. *E. mivati* +3.

E. mivati +4 is shown in figure 2.9. A severe infection produced by a high inoculum of oocysts has caused a complete fusion of lesions. Occasionally small petechiae are observed. The intestinal wall is greatly thickened, and weight loss of the bird occurs with this degree of infection.

Eimeria maxima

Eimeria maxima infections are located in the mid-intestinal area on either side of the small knob (rudimentary diverticulum) left by the yolk sac. In severe infections, lesions may extend up into the duodenum and down as far as the ileo-cecal junction.

E. maxima has been named for its large oocysts. In the center of figure 2.10 is a freshly passed *E. maxima* oocyst recognized by its large size (21–42 μm long), its distinctive brownish red color, and the irregular cellular debris on the outer surface. In contrast, the sporulated oocyst of *E. maxima* to the left and a much smaller sporulated oocyst typical of *E. mivati* or *E. mitis* to the right were recovered in the same litter sample. Sporulation of these latter two oocysts indicates they were out of the intestine in a cooler environment for at least 30 hours.

E. maxima +1 is shown in figure 2.11. Few distinctive features are obvious in mild cases of *E. maxima*. Late in the life cycle (sixth and seventh day), a few petechiae may appear on the serosal

surface of the intestine. This area is clearly indicated in figure 2.11 by the prominent yolk sac diverticulum. Intestinal contents may take on a slightly orange cast. This degree of pathogenesis may induce some weight loss and skin depigmentation.

E. maxima +2 is shown in figure 2.12. The serosal surface may show somewhat more numerous petechiae, and the intestinal contents may be more orange.



Figure 2.9. *E. mivati* +4.



Figure 2.10. *E. maxima* oocysts.

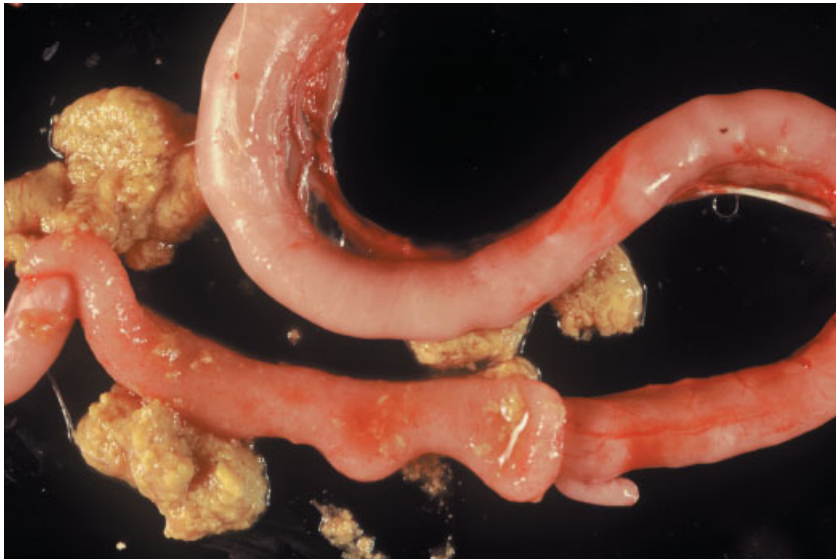


Figure 2.11. *E. maxima* +1.



Figure 2.12. *E. maxima* +2.

E. maxima +3 is shown in figure 2.13. Some thickening of the intestinal wall may be visible in heavy infections. Ballooning, a term indicating a greatly distended intestine, may occur with moderate and severe infections of *E. maxima*.

E. maxima +4 is shown in figure 2.14. Bloody intestinal contents may appear along with more numerous petechiae. Lesions from this species are more limited in the duration of their appearance (sixth and seventh day) than from some other species. Solid immunity is more rapidly established with *E. maxima* than with the other species. The adverse effect on pigmentation caused by *E. maxima* may be substantial.

Eimeria necatrix

Eimeria necatrix also becomes established in the midintestinal area. However, later development of oocysts occurs only in the ceca. This characteristic may be useful in making species diagnosis. Mild infections may be easily overlooked. This species may be observed in broiler chickens in tropical regions of the world at three to five weeks of age (McDougald and Conway 1984).

E. necatrix +1 is shown in figure 2.15. A few petechiae and white spots or plaques may be visible on the serosal surface. Little change is apparent on the mucosal surface.

E. necatrix +2 is shown in figure 2.16. Petechiae and white plaques may be more numerous on the serosal surface (“salt and pepper” appearance). Slight ballooning may appear in the area around the yolk sac diverticulum. Some increase in secretion of mucus may be apparent in the intestinal contents.

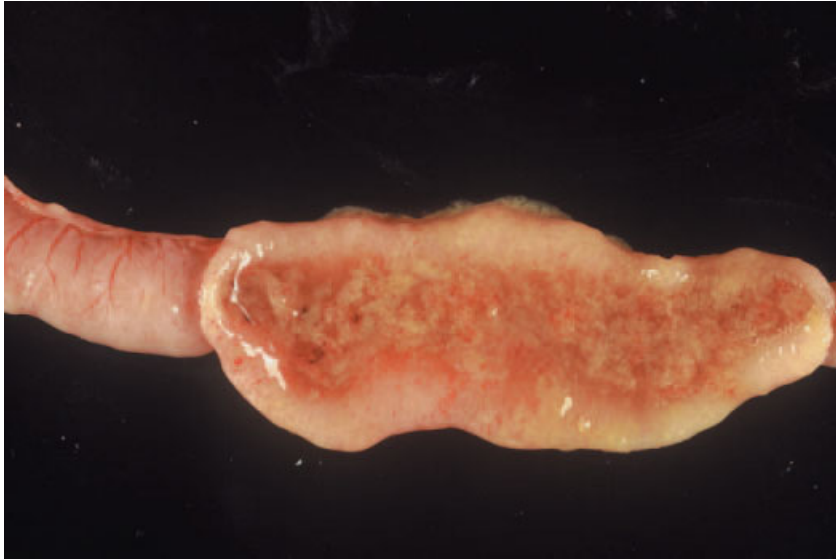


Figure 2.13. *E. maxima* +3.

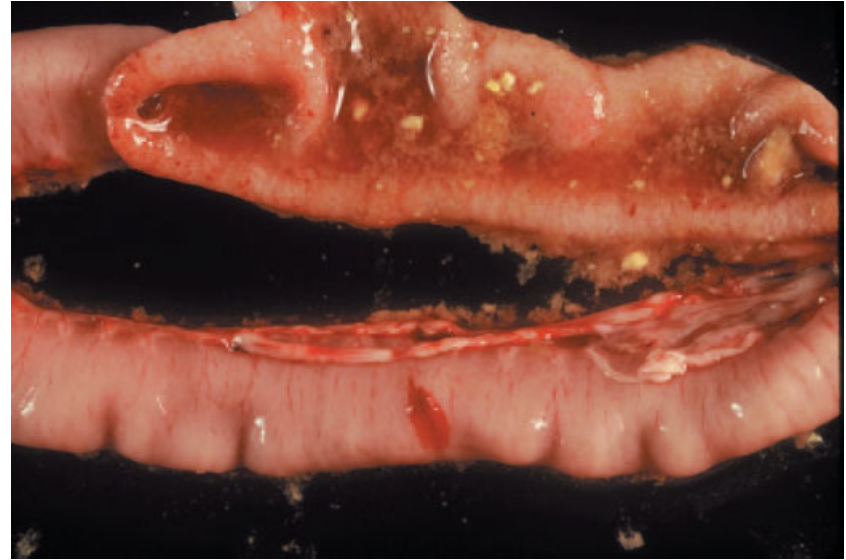


Figure 2.14. *E. necatrix* +4.



Figure 2.15. *E. necatrix* +1.

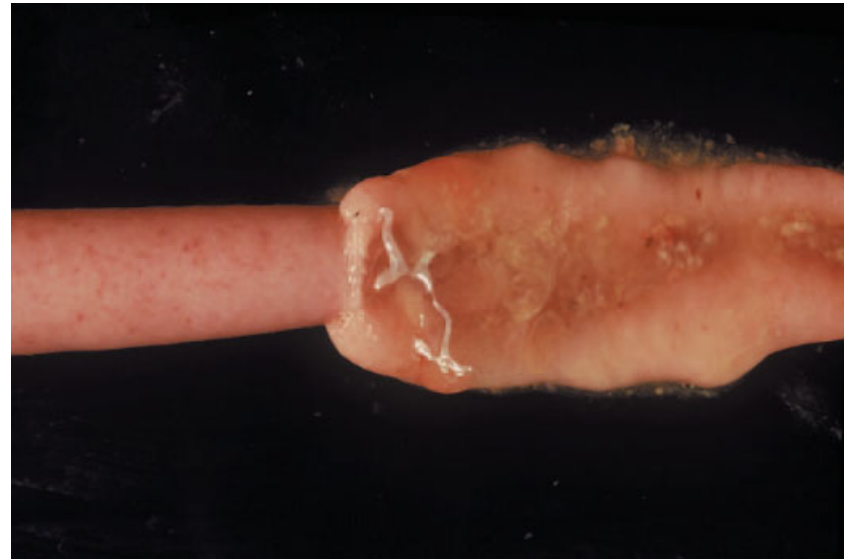


Figure 2.16. *E. necatrix* +2.

E. necatrix +3 is shown in figure 2.17. Plaques and petechiae may be much more numerous and crowded on the serosal surface. Intestinal contents may be streaked with blood and show considerable increase in mucus secretion. Ballooning may be more extensive, although it does not always appear. Weight loss and poor feed conversion occur. Birds do not eat or drink. In figure 2.18, a closer view of the same intestine shows the lesions more distinctly.

E. necatrix +4 is shown in figure 2.19. Presence of plaques and petechiae on the serosal surface may be intensified. They appear most distinctly on the fifth to seventh day of infection. Considerable blood and mucus may be present. In other cases, intestinal contents may be orange as with *E. maxima*. Ballooning may extend as far forward as the duodenum. Death may occur with *E. necatrix* +4 from the fourth to eighth day. Recovery may be slow and require two weeks or longer. For this reason, the term chronic (an unsatisfactory designation) has been applied to infections with this species.

E. necatrix schizonts under low power are shown in figure 2.20. A microscopic preparation made with a scraping of one of the white plaques shows a clumped aggregation of large schizonts easily visible under low power. Since they are deeply embedded, they may have to be located by looking on the serosal surface but scraped out from the mucosal surface. Presence of these large schizonts, which

measure about 60 μm in diameter, is diagnostic for this species.



Figure 2.17. *E. necatrix* +3.

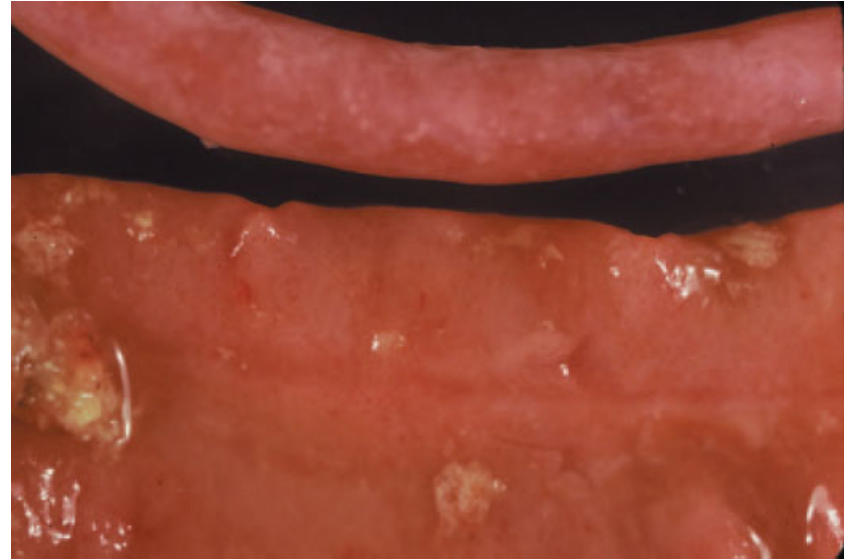


Figure 2.18. *E. necatrix* +3 (closer view).



Figure 2.19. *E. necatrix* +4.

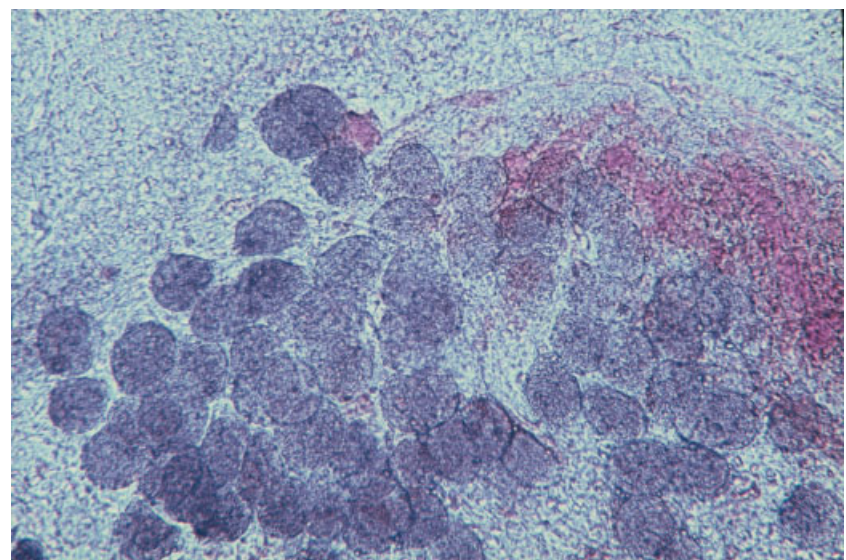


Figure 2.20. *E. necatrix* schizonts (low power).

Under higher magnification, the *E. necatrix* schizonts may show the crowded, elongated merozoites present in great numbers (see figure 2.21). A ruptured schizont shows released merozoites that swim about slowly with an undulating motion.

Eimeria brunetti

Eimeria brunetti parasitizes the lower intestine extending down into the large intestine (between the ceca) and rectum. Early stages frequently invade the midintestinal area. Weight losses are often severe, although distinctive lesions may be difficult to recognize.

E. brunetti +1 is shown in figure 2.22. The mildly infected lower intestine, shown here as the central section between the tips of the cecal pouches, may occasionally show a few petechiae although they are not always present. Petechiae are more commonly recognized on the serosal than the mucosal surface. On the mucosal surface, they may appear in small pitted areas.

E. brunetti +2 is shown in figure 2.23. Somewhat heavier infections may show more petechiae on the serosal surface, with the greatest number appearing on the fifth day after infection. A few may appear as early as three and a half days after infection and may occur anywhere from the yolk sac diverticulum downward. Mild mucosal lesions recognized by roughening of the surface of the lower

intestine may sometimes be detected more easily by feel than by sight. A dissecting instrument or the finger passed over the surface may reveal these patches of thickened material.

E. brunetti +3 is shown in figure 2.24. Small hemorrhagic streaks may appear on the mucosa, and coagulated material may be sloughed off and appear mixed with the cecal contents. Roughening of the mucosal surface is more obvious than in +2 lesions. Weight gains and feed conversion are reduced with infections of this severity.

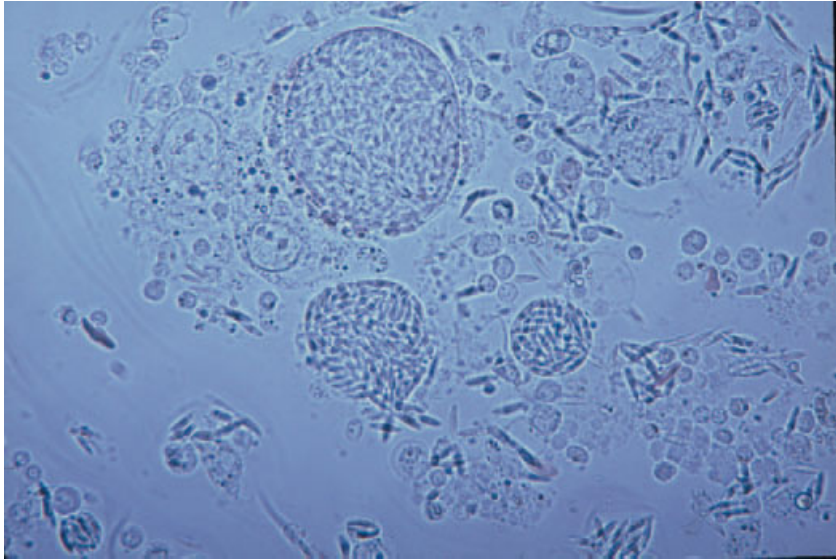


Figure 2.21. *E. necatrix* schizonts (high power).



Figure 2.22. *E. brunetti* +1.



Figure 2.23. *E. brunetti* +2.

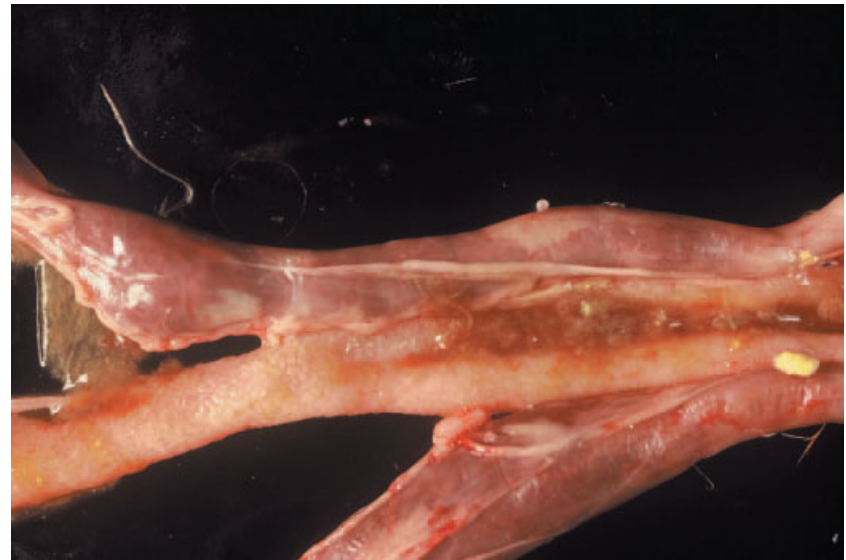


Figure 2.24. *E. brunetti* +3.

Figure 2.25 (another *E. brunetti* +3 score) shows the hemorrhagic streaks and blood-tinged contents (middle section between the two cecal pouches). The opened cecum at the bottom shows a drying up of the cecal contents, which often occurs on the sixth and seventh day of *E. brunetti* infection.

E. brunetti +4 is shown in figure 2.26. Severe coagulation necrosis may produce an erosion of the entire mucous membrane. This may appear as thickening of the intestinal wall or may cause an erosion and loosening of the entire mucosal surface (pseudomembranous necrosis). A core with the consistency of cottage cheese may form from this material. Necrosis may be severe enough in the rectal area to produce a complete blockage of the intestine and subsequent death of the bird. Although diagnostic personnel often look for severe coagulation necrosis, it is a rare flock condition. Milder (+1 to +3) lesions are more common and sometimes overlooked.

Eimeria tenella

Eimeria tenella, the well-known cause of cecal or “bloody” coccidiosis, invades the two ceca and in severe cases may also parasitize the intestine above and below the cecal junction.

E. tenella +1 is shown in figure 2.27. A few scattered petechiae, which are reddish or purple in color, are seen on the unopened cecum shown at

the bottom of the photograph. Also present, but less apparent, are petechiae on the opened cecum along the top of the photograph. Less frequently, such lesions may also extend into the lower small intestine between the ceca. There is no thickening of the cecal wall. The cecal contents (not shown in the figure) usually show a normal brownish color, although a slight amount of blood may be present. Mild clinical signs may show in infected chickens.

E. tenella +2 is shown in figure 2.28. Petechiae, which are apparent on the serosal surface, are somewhat more numerous. Bleeding, which appears on the fifth to seventh day of infection, is more marked on the mucosal surface than in a typical +1 score. In this example, bleeding is slightly more severe than in the usual +2. Except for the presence of some blood, the cecal contents are normal. Another more reliable characteristic in judging severity is the amount of thickening of the cecal wall, which is slight in this case. Clinical signs are apparent in infected chickens with this degree of infection.



Figure 2.25. *E. brunetti* +3.



Figure 2.26. *E. brunetti* +4.



Figure 2.27. *E. tenella* +1.

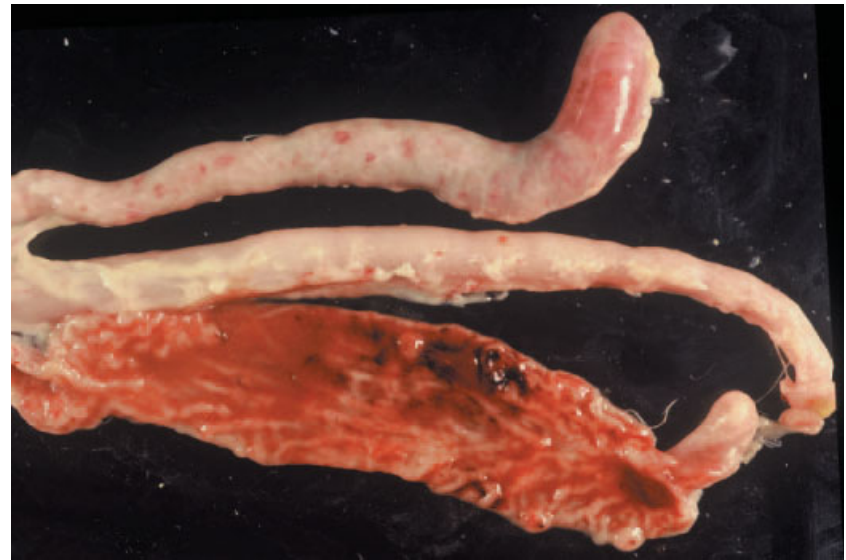


Figure 2.28. *E. tenella* +2.

E. tenella +3 is shown in figure 2.29. Bleeding is more severe, with clotting appearing in the distal end of the pouch. The clot becomes hardened as the sloughed mucosal surface joins the bloody material to form a core. There is an absence of normal cecal contents since the ceca have become practically nonfunctional. Marked thickening of the cecal wall has occurred. The serosa of the unopened cecum shows the petechiae as coalesced and eroding the entire surface. Huddling, chilling, and bloody droppings constitute clinical signs.

E. tenella +4 is shown in figure 2.30. Severe bleeding, a much thickened cecal wall, and eroding of the mucosal surface show up on the fifth day of infection. The unopened cecum is distended, with blood at the distal end, but is contracted and shortened. Chickens huddle and sometimes let out a high-pitched call. Chickens cease feeding and drinking. Death may come suddenly beginning on the fifth day, reaching the greatest number on the sixth and extending through the seventh to the tenth day of infection.

E. tenella +4 is shown in figure 2.31. By the sixth to eighth day, the cecal core is hardened and may persist for another week or more. The core may take on a more whitish cast with a huge accumulation of sloughed mucosal surface material. Microscopic examination of scrapings would show many oocysts. Purple areas denoting the presence of gangrene and rupture of the cecal wall may

occasionally occur at this stage. Dead birds are scored +4.

Eimeria mitis

Characteristics of *Eimeria mitis* are given in table 1.1. No discrete lesions are produced, but infections with this species will adversely affect bird performance and skin pigmentation (Joyner and Norton 1983; Shirley, Jeffers, and Long 1983; Novilla et al. 1987; Fitz-Coy and Edgar 1992; Gobbi and Pezzotti 2005).

Eimeria praecox

Characteristics of *Eimeria praecox* are given in table 1.1. This species does not produce gross lesions and is not considered pathogenic.

Discussion

Techniques described so far are used largely in battery experiments with the infection established by inoculation of a single species of *Eimeria*.

Figure 2.32, a photograph of the jejunum, illustrates the presence of a mixed infection commonly found under field conditions or in experimental work with field isolates. The presence of some infection is obvious due to the roughened condition and the thickening of the mucosal surface. The elongated white plaques suggest the presence of *E. acervulina*. In this region of the intestine,

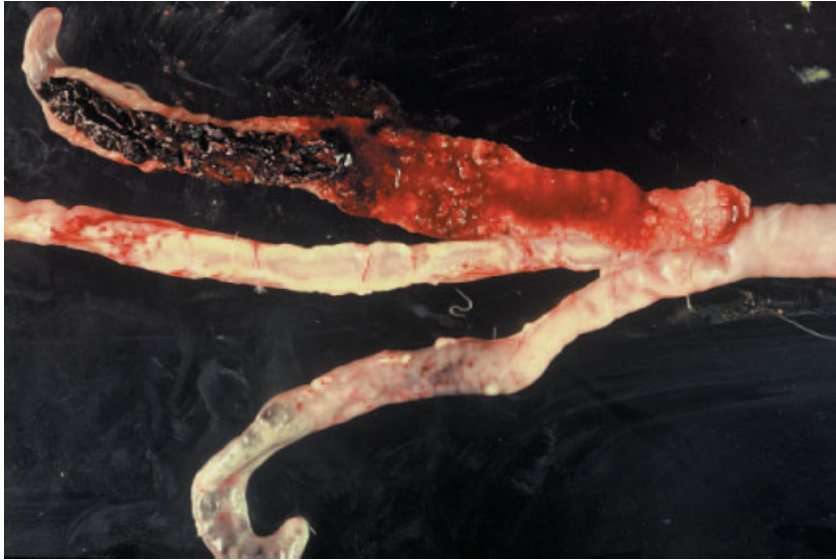


Figure 2.29. *E. tenella* +3.

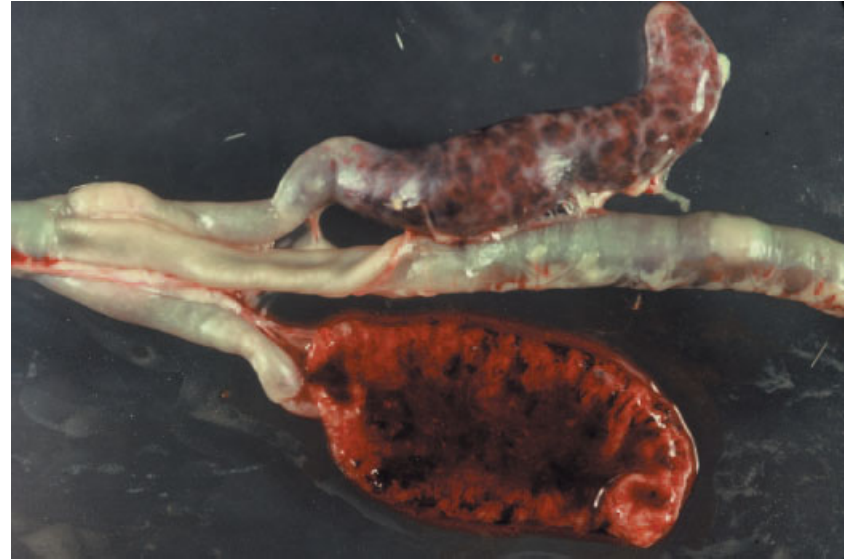


Figure 2.30. *E. tenella* +4.



Figure 2.31. *E. tenella* +4.



Figure 2.32. Mixed infection.

E. maxima and *E. necatrix* are both likely to be present. A tentative or presumptive diagnosis of the species present could be made in 10 to 20 minutes by examining scrapings under the microscope. A confirmed diagnosis might require more time, with measurement of a number of oocysts and determination of sporulation time. Such a procedure would be justified for the diagnostician in making a flock diagnosis. However, lesion scoring as a tool to make comparisons on different methods of coccidiosis control requires a comparison between a large number of birds from each treatment. To compensate for individual bird variation, replication involving several cages or pens per treatment is important. Identification of each species in each bird is often difficult, if not impossible, and is generally not recommended when lesion scoring mixed infections.

When scoring mixed infections, four areas of the mucosal surface of the intestine are individually examined (figure 2.33) in addition to the serosal surface. The serosal surface is examined first, and the intestine is then cut open to see the mucosal surface. A score of 0 to +4 is recorded for each chicken for the four regions of the mucosal surface: the duodenal and upper intestine or jejunum (U), the middle intestine (M), the lower intestine or ileum and the rectum (L), and the ceca (C).

When lesion scores are used to assess the effect of different treatments on coccidial infections, the data can be statistically analyzed by either cate-

gorical data analysis or analysis of variance models. The former model tests for differences in the distribution of individual lesion scores, i.e., the number of birds in each treatment with scores of 0, +1, +2, +3, or +4. The latter model tests for differences among the treatment means. Although both models were found comparable in work by Conway, Dayton, and Hargis (1986), categorical data analysis was found to be more sensitive in measuring differences between treatments in the shift of lesion scores up or down the scale.

A question frequently asked is, how severely affected are birds with a lesion score of +1, +2, +3, or +4? Long, Johnson, and Wyatt (1980) found that birds partially immune to *E. tenella* infection developed severe cecal lesions similar to lesions in nonimmune birds, but without experiencing a comparable reduction in either weight gain or packed erythrocyte volume. Bafundo and Donovan (1988) reported that birds inoculated with 5,000 oocysts per bird developed fairly severe lesions (mean = 2.7) without depressing weight gain, while birds inoculated with 50,000 oocysts per bird developed more severe lesions (mean = 3.2) and experienced moderate weight suppression. Conway, McKenzie, and Dayton (1990) compared the individual weight gain and lesion score in groups of unmedicated and medicated birds inoculated with single species infections of *E. acervulina*, *E. maxima*, or *E. tenella*. The results demonstrated that high lesion scores in medicated birds were associated with small changes in weight

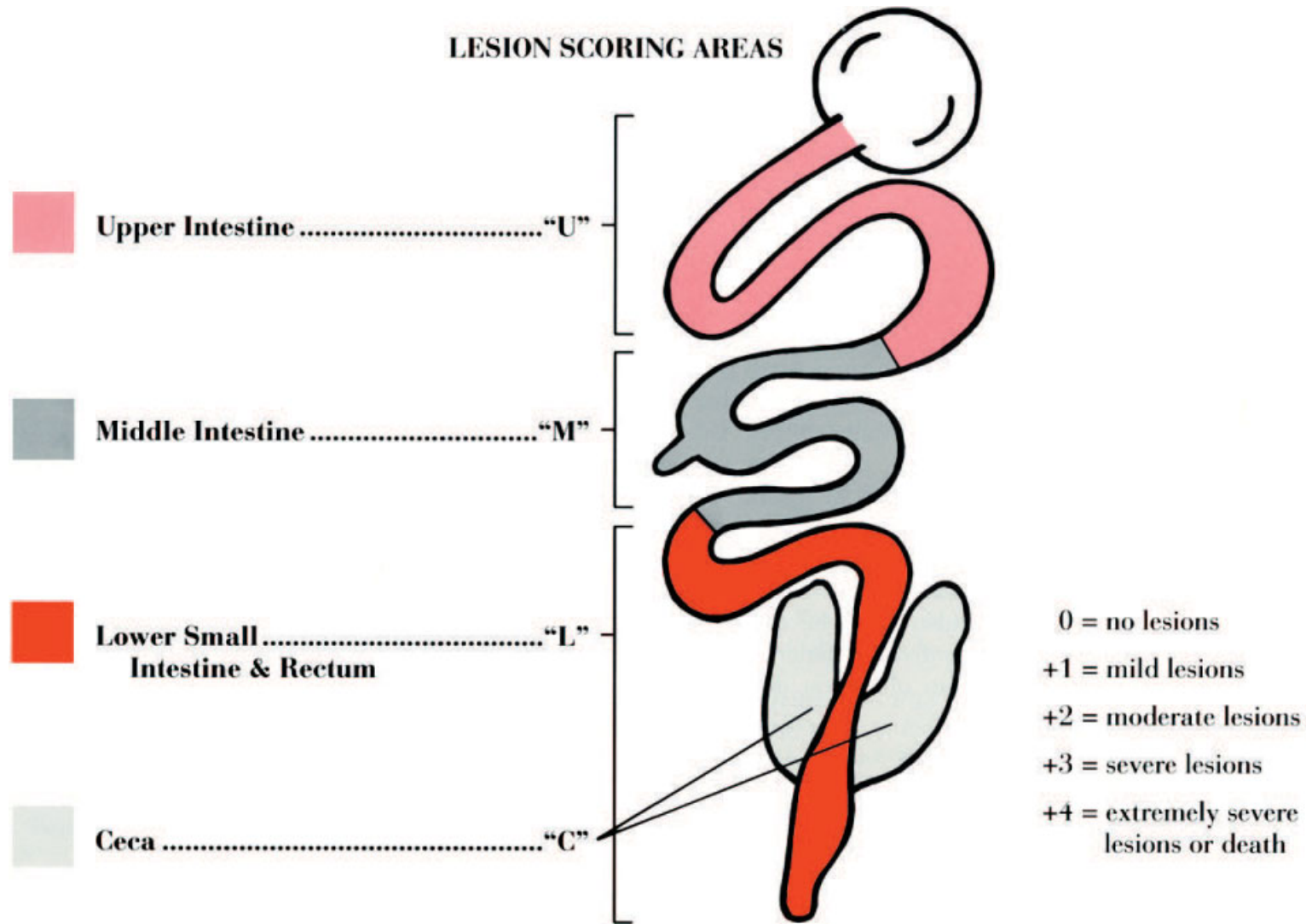


Figure 2.33. Lesion scoring areas.

gain when compared with unmedicated birds (table 2.1). More recently, Williams (2003) found that vaccinated birds had very few endogenous parasites associated with coccidial lesions found after a challenge infection, while unvaccinated, unprotected birds challenged with the same species of coccidia had great numbers of endogenous parasites associated with all lesions. Taken together, the results indicate that there are substantial limitations to the use of lesion scores alone in assessing flock performance or the efficacy of an anticoccidial program.

These results also raise the question of how many oocysts are required to generate specific scores. The relationship of oocyst dose to lesion score was examined in both monospecific and mixed infections in a number of studies (McKenzie, Conway, and Dayton 1989a, 1989b, 1989c, 1989d). This work demonstrated that the relationship is non-linear, with lesion scores increasing at a decreasing rate as the oocyst dose increases. The work by McKenzie, Conway, and Dayton (1989c, 1989d) with *E. acervulina*, for example, showed that the average lesion score for birds given 10,000 oocysts per bird was +1.38, while for birds given a 10 times higher dose the average score was +2.40.

The relationship between oocyst dose and lesion score, weight gain, feed conversion, packed cell volume, and plasma constituents in infections with *E. acervulina*, *E. maxima*, and *E. tenella* showed that plasma carotenoids, lipids, and protein were

especially sensitive to different doses of oocyst inocula (Conway et al. 1993). These data are summarized in table 2.2. Of all the response variables measured in these studies, carotenoids appeared to be the most sensitive measurement for each species as indicated by the consistently high coefficients of determination. For each species, plasma carotenoids showed some decline beginning at relatively low levels of infection. Even though the cause of decline may differ for each species (Ruff and Fuller 1975; Tyczkowski, Hamilton, and Ruff 1991; Tyczkowski, Schaefer, and Hamilton 1991), this measurement is an especially useful measurement of the pathogenic effects of coccidial infection.

Finally, as with any subjective measurement, it is important when assigning lesion scores to guard against judgments being influenced by any previous knowledge of the treatments being compared. A helper should assist by catching the birds and keeping treatment identification from the scorer. Subjective judgments from preconceived ideas about effects of a given treatment may easily color the decision between a +1 and a +2.

Table 2.1.

Pooled mean weight gain for each lesion score category within treatments

Lesion Score	UIUM		IUM		ISAL	
	No.	MWG	No.	MWG	No.	MWG
<i>E. acervulina</i> (7 isolates):						
0	271	205.8	0	–	89	179.9 ^{bc}
1	0	–	0	–	61	186.5 ^b
2	0	–	15	142.8 ^a	60	185.9 ^b
3	0	–	61	143.5 ^a	35	176.2 ^{bc}
4	0	–	203	139.4 ^a	33	170.4 ^c
<i>E. maxima</i> (5 isolates):						
0	189	240.2 ^c	0	–	19	236.0 ^c
1	0	–	15	184.9 ^a	61	217.7 ^d
2	0	–	65	173.1 ^a	62	206.2 ^{de}
3	0	–	66	154.1 ^b	32	216.6 ^d
4	0	–	43	148.5 ^b	15	194.5 ^c
<i>E. tenella</i> : (8 isolates):						
0	318	244.9 ^a	1	233.3 ^{abce}	69	230.6 ^{ef}
1	0	–	17	222.6 ^b	124	227.5 ^{bf}
2	0	–	125	189.4 ^c	73	219.1 ^{fg}
3	0	–	83	190.9 ^c	41	212.0 ^{gh}
4	0	–	45	148.3 ^d	2	164.8 ^{dh}

Source: Conway, McKenzie, and Dayton 1990.

Notes: UIUM = uninfected, unmedicated; IUM = infected, unmedicated; ISAL = infected, 60 mg of salinomycin per kilogram of ration; MWG = mean weight gain. For each species, means that do not share a common superscript element (abcdefgh) within a column or row are significantly different ($P < 0.05$).

Table 2.2.

Effects of E. acervulina, E. maxima, and E. tenella on plasma constituents, chicken performance, and lesion scores

Oocysts per Bird	Plasma Constituents Means			Mean Weight Gain (g)	Mean Feed Gain (g)	Mean Lesion Score
	Carotenoids (µg/ml)	Lipids (mg/100 ml)	Protein (mg/ml)			
<i>E. acervulina:</i>						
0	9.0 ^a	334.7 ^a	24.5 ^a	220.8 ^a	1.30 ^{ab}	0.03 ^a
10 ²	8.4 ^b	303.2 ^b	23.4 ^a	223.8 ^a	1.29 ^{ab}	0.72 ^b
10 ³	7.1 ^c	282.1 ^b	23.5 ^a	214.3 ^a	1.28 ^a	1.00 ^b
10 ⁴	5.1 ^d	210.6 ^c	20.5 ^b	212.2 ^a	1.28 ^a	2.10 ^c
10 ⁵	3.1 ^e	169.0 ^d	18.3 ^c	197.9 ^b	1.32 ^b	2.63 ^d
10 ⁶	2.6 ^e	170.0 ^d	18.3 ^c	161.9 ^c	1.47 ^c	3.50 ^e
R ²	0.977	0.943	0.901	0.889	0.893	ND
<i>E. maxima :</i>						
0	10.4 ^a	343.6 ^a	21.0 ^a	282.6 ^a	1.30 ^{ab}	0.10 ^a
6.7 × 10 ¹	10.1 ^a	331.5 ^a	20.9 ^a	289.8 ^a	1.30 ^{ab}	0.33 ^a
6.7 × 10 ²	8.2 ^b	284.2 ^b	20.0 ^a	290.7 ^a	1.28 ^a	0.89 ^a
6.7 × 10 ³	3.5 ^c	204.9 ^c	18.5 ^b	262.5 ^b	1.34 ^b	1.50 ^b
6.7 × 10 ⁴	2.4 ^d	204.0 ^c	18.6 ^b	215.0 ^c	1.52 ^c	1.62 ^b
R ²	0.986	0.927	0.647	0.895	0.900	ND
<i>E. tenella:</i>						
0	9.7 ^a	336.5 ^a	27.2 ^a	284.9 ^a	1.28 ^a	0.00 ^a
10 ²	9.6 ^{ab}	325.8 ^a	26.1 ^b	284.7 ^a	1.28 ^a	0.20 ^{ab}
10 ³	9.0 ^b	287.6 ^b	25.0 ^c	279.4 ^a	1.34 ^a	1.62 ^{bc}
10 ⁴	6.4 ^c	265.4 ^c	22.7 ^d	253.4 ^b	1.41 ^b	2.87 ^{cd}
10 ⁵	4.6 ^d	211.7 ^d	21.4 ^e	202.4 ^c	1.61 ^c	3.28 ^d
R ²	0.965	0.965	0.919	0.908	0.908	ND

Source: Conway et al. 1993.

Notes: R² = coefficient of determination for each response variable; ND = not determined. For each species, means that do not share a common superscript element (^{abcde}) within a column are significantly different ($P \leq 0.05$).

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Oocyst collection

The method used to collect oocysts depends on the purpose of the collection. In most cases, basic equipment such as a centrifuge, blender, sieve, cheesecloth, beaker, slotted spatula or spoon, tape or rubber bands, 2.5% potassium dichromate solution, and centrifuge tube or bottles are required.

Oocysts for litter oocyst counts should be collected from several representative areas of the chicken house. The litter should be collected from the top layers of material as a “handful” (approximately $3 \times 3 \times 0.5$ inches) of litter. Caked litter from areas around the drinker should be avoided. The samples are mixed together and 5 g of material are weighed. This material is soaked overnight in 2.5% potassium dichromate. The next day, the solution is poured through a small-grade sieve or tea strainer into a beaker. The solids are washed with water through the sieve into the same beaker. The remaining solid, washed materials can be discarded. The fluid sample is centrifuged at a moderate speed (e.g., 1500 rpm) to sediment the oocysts. The supernatant is discarded and the pellet is re-suspended in either potassium dichromate for storage or a known volume of saturated salt (NaCl) solution for counting.

Oocyst collection for the preparation of an inoculum is most commonly done either from samples of

fecal material collected during a patent infection or from infected intestinal tissue. In either case, the material is homogenized in a blender until the fecal material or tissues can be easily washed with water through a double layer of cheesecloth or a fine mesh sieve into a beaker. The solids in the filtrate should be allowed to settle out, either by gravity (three to four hours) or by centrifugation. The supernatant is discarded and the oocysts are re-suspended from the pellet in potassium dichromate for storage or in water for further purification procedures.

When oocysts are collected directly from the bird, scrapings are made from the lesions and rinsed into a beaker, or entire sections of the intestine are ground in a blender with potassium dichromate to release the unsporulated oocysts. The tissue suspension is filtered through cheesecloth into a beaker using a slotted spoon or spatula to agitate the suspension.

In order to separate oocysts from the washed fecal material or tissue homogenate, the oocysts can be removed from the debris in a saturated salt solution. Saturated sodium chloride is usually the most convenient material, but other saturated salt solutions may be used. The semisolid oocyst suspension should be re-suspended in the salt solution and mixed well. Plastic centrifuge bottles with tight-fitting lids are preferred for this procedure. The suspension is centrifuged at a moderate speed

(e.g., 1500 rpm) for 10–15 minutes to sediment the solids and allow the oocysts to remain suspended at the top of the supernatant. The oocysts are removed from the top layer of fluid by pipette and are re-suspended in water. The oocyst suspension should be washed, via centrifugation, three to four times to remove the salt solution. The salt-free oocyst suspension should then be stored in potassium dichromate solution.

Sporulation and storage

Oocysts must undergo sporulation before they are infective. This process occurs during a 24- to 72-hour period. Sporulation is optimized at 30°C with forced aeration. A water bath is a useful aid in the maintenance of a constant temperature during sporulation. Increasing the fluid-to-solid ratio during sporulation also enhances the rate of sporulation.

Storage of oocysts should be done in potassium dichromate solution. Refrigeration of the suspension is possible at 4°C–6°C. This temperature allows the parasites to maintain viability for two to six months, depending on the species. However, cultures stored for longer than three months will be noticeably less infective than when the same number of freshly harvested oocyst are used because of the loss in viability.

Cryopreservation of sporocysts and sporozoites of some, but not all, species is also possible in liquid nitrogen. The methods for these procedures are best followed from the description by Norton and Joyner (1968), Long (1971), and Davis (1973).

Oocyst counting

At least two methods of oocyst enumeration are commonly used. One method requires a McMaster counting chamber and is generally used in litter oocyst counting procedures since the percentage of sporulation and oocyst dimensions are not required in this measurement. A second common procedure better adapted to cleaner collections of oocysts is counting with a hemocytometer. Although the error per milliliter is higher than that of the McMaster chamber method, the hemocytometer method allows one to distinguish sporulated oocysts and make accurate measurements of the oocysts at the time of counting.

McMaster chamber method

The McMaster chamber method is documented by Hodgson (1970), Long and Rowell (1958), and Long et al. (1976).

Equipment: Centrifuge, cheesecloth (muslin), beaker, a jar with a lid, or Parafilm, McMaster counting chamber, hand tally counter, 10 or 15 ml graduated test tubes, saturated sodium chloride.

Procedure:

1. 10 g of litter are soaked in 100 ml of tap water for 24 hours at 4°C in a 200 ml beaker that is tightly covered (either with a lid or Parafilm).
2. The beaker is shaken vigorously and the litter is filtered through a single thickness of muslin (q.s. filtrate to 100 ml).
3. A 15 ml centrifuge tube is filled with filtrate to 1 cm from the top and centrifuged for five minutes at a speed that concentrates the solids.
4. The supernatant is discarded. The pellet is re-suspended in a few milliliters of saturated salt solution (NaCl) with a Vortex, or by gently tapping the tube. More salt solution is added to the original 15 ml volume, and the tube is capped and inverted several times.
5. Samples are removed with a Pasteur pipette, and a McMaster counting chamber is filled. The oocysts float to the top of the solution, and the total number is counted.

Calculation:

$$\text{Number of oocysts per gram of litter} = \frac{n}{0.15} \times \text{volume} \times 0.1$$

where n = number of oocysts counted,
 0.15 = volume of the McMaster counting chamber,
 volume = 100 ml of water that the litter is soaked in, and
 0.1 = correction for 10 g of litter originally taken.

Therefore, each oocyst counted is equivalent to 67 oocysts per gram of sample. When calculations of oocysts per bird are done, the number of oocysts per gram is divided by the number of birds in the pen to give the number of oocysts per gram per bird.

Hemocytometer method

Preparation of samples to be counted using the hemocytometer method is the same as that used for the McMaster method. The specifics of counting is the only step that differs.

The cover slip is placed on the slide, and only the silvered area under the cover slip is filled with the oocyst-containing suspension. If fluid leaks from under the silvered area into the troughs, the slide should not be counted but wiped clean and re-filled.

All sporulated oocysts located in each of the four 16-square areas and in the center 32-square area are counted. The number of oocysts counted is

multiplied by 2,000 (a factor that brings the total volume under the slide to 1 ml).

Example:

Twenty-four sporulated oocysts counted $\times 2,000 = 48,000$ oocysts per milliliter of culture. If the total number of oocysts per culture is needed, multiply the count per milliliter by the total volume of the culture (e.g., $48,000 \text{ oocysts} \times 500 \text{ ml of culture} = 2.4 \times 10^7$ total number of oocysts).

Thus, the oocyst dose per bird is calculated by dividing the number of oocysts to be inoculated per group of birds by the number of oocysts per milliliter in the culture. For example, if 10 birds should receive 250,000 oocysts each and the culture contains 40,000 oocysts per milliliter, the amount of milliliters of culture required to deliver the correct dose is calculated as follows:

$$\frac{(250,000 \text{ oocysts} \times 10 \text{ birds})}{40,000 \text{ oocysts/ml}} = 62.5 \text{ ml of suspension required for 10 birds} = 6.25 \text{ ml/bird.}$$

Assuming the oocyst dose in the preceding example is to be given orally by syringe or pipette to each bird, then the 62.5 ml of suspension in this example should be concentrated by centrifugation to provide the required number of oocysts in a total volume of 10 ml (1 ml per bird).

Propagation

It is often necessary to propagate isolates of coccidia to meet the needs of a testing program. Two to five birds raised coccidia-free in wire cages are inoculated with a small number of sporulated oocysts from the isolate. The number of oocysts to be given depends upon the *Eimeria* species involved, the pathogenicity of the isolate, and the viability of the culture. Some guidelines are given in table 3.1. Broiler chicks two to four weeks of age are ideal. A sterile droppings pan containing enough 2.5% potassium dichromate solution to cover the bottom of the pan is placed under

Table 3.1.

Recommended number of oocysts for propagation

Species	Number of Oocysts/Bird	Fecal Collections (days after infection)		Tissue Collection (days after infection)
		Begin	End	
<i>E. tenella</i>	10^4-10^5	6	9	6
<i>E. necatrix</i>	10^3-10^4	6	9	7
<i>E. acervulina</i>	10^5-10^6	5	7	—
<i>E. brunetti</i>	10^3-10^4	6	8	—
<i>E. maxima</i>	10^3-10^4	6	9	—
<i>E. mitis</i>	10^4-10^5	5	8	—
<i>E. mivati</i>	10^3-10^4	5	8	—

Source: Eckert et al. 1995.

*Days after infection. Fecal collection times may vary depending on the time of day inoculation is given and number of oocysts inoculated per bird.

the cage containing the birds during the collection period indicated. Feed is limited during this period. The droppings are collected and washed through a standard Tyler 80 mesh sieve or a double layer of cheesecloth. The filtrate containing the oocysts is concentrated and mixed with 2.5% potassium dichromate solution, as previously outlined, and prepared for sporulation.

Fecal collections as described above are best suited for *E. acervulina*, *E. maxima*, *E. mivati*, *E. praecox*, and *E. brunetti*, while propagation of *E. necatrix* and *E. tenella* is more conveniently handled by harvesting oocysts from the ceca. The entire organ including the contents is cut into small pieces and placed in 2.5% potassium dichromate. This material is subsequently blended at high speed to completely macerate all tissue. The blended material is washed through an 80 mesh sieve or a double layer of cheesecloth, and processed as already described.

Additional information on the purification of species by the isolation and propagation of a single oocyst, and the separation of oocysts from feces, is given by Davis (1973) and Long et al. (1976).

Calibration of a microscope for measuring oocysts

The following description was provided by Long and Reid (1982) for the calibration of a microscope for measuring oocysts:

Two accessories are required. These are an ocular micrometer and a stage micrometer. The former must be calibrated with all objectives used on the microscope and becomes a permanent part of the microscope equipment. The latter is used only in the calibration process. A special ocular containing a micrometer scale may be purchased or a disc with a micrometer scale may be mounted on a ridge inside the ocular after removal of the upper lens. The distance (measured in microns, abbreviated μm) which is covered by the small spaces on this scale will differ for each lens system used. The number of microns spread between the lines with each lens system thus must be determined. A stage micrometer is a microscope slide on which spaces of known length are marked with black lines. Each of the small spaces on this stage micrometer scale represents 0.01 millimeters or 10 μm .

Using low power of the compound microscope, bring the stage micrometer lines into focus and adjust the zero line of the stage micrometer to coincide with the zero line of the ocular micrometer. Find another line on the ocular micrometer which exactly coincides with a second line on the stage scale (figure 3.1). Count the number of spaces between the two lines using the ocular scale and divide this number into the number of microns represented between the two lines on the stage (number of small spaces \times 10 microns). Thus, if seven ocular spaces are equal to 10 stage spaces, the number of microns covered by each space of the ocular micrometer may be determined by dividing 7 into 100 ($10 \times 10 = 100\mu\text{m}$) which

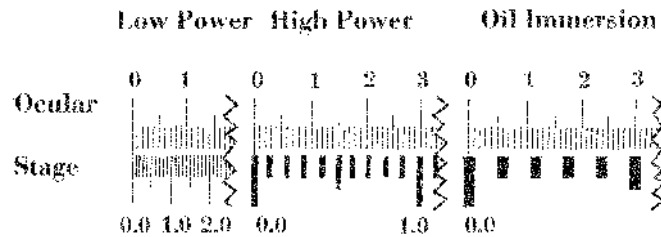


Figure 3.1. Chart for calibration of the microscope (Long and Reid 1982).

equals 14.3 μm . This unit (14.3 μm) will be used henceforth whenever low power is used for measurement. In other words, each ocular space covers 14.3 μm .

A similar calibration should be completed for each lens combination used with the microscope. A typical reading may be 3.5 microns for each space with high power and 1.6 with the oil immersion lens as illustrated (figure 3.1). Attach a permanent record of these calibration factors to the microscope. The stage micrometer will not be needed after this calibration is complete. Time may be saved by preparing a table to be kept with the microscope (table 3.2).

Table 3.2.

Example table for calculation of size in micrometers

Microscope Serial No.: _____			
Ocular Space	Size (μm)		
	Low Power (lens no.)	High Power (lens no.)	Oil Immersion (lens no.)
1	14.3	3.5	1.6
2	28.6	7.0	3.2
3	42.9	10.0	4.8
4	57.2	14.0	6.4
50	715.0	175.0	80.0

Source: Modified after Long and Reid 1982.

When you are ready to measure oocyst or other objects, place the slide with the oocysts under the rulings of the ocular micrometer. Count the number of ocular spaces covered and multiply by the factor obtained in the calibration above. Thus, use high power if the oocyst covers 9 ocular spaces, $9 \times 3.5 = 31.5$ which is the length of the oocyst in μm . (Long and Reid 1982)

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Three types of tests are generally used to study anticoccidial drugs in broiler birds. These are short-term (7–14 days) tests with birds in wire cages (battery tests), standard grow-out (6–8 weeks) tests in floor pens, and full-scale tests in commercial facilities. Each type has a different objective and value to the investigator. For example, the battery test is used most effectively to measure the efficacy of an anticoccidial drug against a variety of field isolates of coccidia. This is an efficient and relatively inexpensive testing procedure. The floor pen test is an intermediate testing procedure with a primary goal of providing statistically useful performance data under controlled conditions.

Individually, the predictive value of each test is limited. One cannot, for example, confidently extrapolate performance data in a seven-day battery test to market weight, nor can one predict from a few commercial trials the efficacy of an anticoccidial agent in preventing the lesions of major species of coccidia. As a whole, when properly conducted, the tests complement one another by providing a comprehensive picture of the efficacy, safety, and economic value of an anticoccidial agent.

The purpose of this chapter is to provide a guideline for each type of test identified in the preceding paragraphs and to reference some of the salient literature on testing procedures. A comprehensive guideline for evaluating the efficacy of anticoccidial drugs in chickens and turkeys, including

specific information relating to regulatory requirements, was recently prepared by Holdsworth et al. (2004). The following example protocols are in agreement with the requirements set forth in this reference.

Several fundamental criteria apply to all experiments (Kilgore 1970; Raines 1978):

1. Use chickens free of coccidial or other infection, and of similar age, weight, genetic background, breeder flock, and hatchery.
2. Use similar environmental conditions, e.g., lighting, floor space, housing, feeder and water space, and ventilation, for all experimental groups of chickens.
3. Use an equal number of birds in each treatment.
4. Replication of each treatment is extremely important to reduce the error caused by uncontrolled sources of variation.

Battery tests

Battery tests are used to determine the activity of new compounds in controlling coccidiosis, to precisely measure the dosage-response of an anticoccidial candidate in controlling lesions and other manifestations of coccidiosis for each of the major species, to test the efficacy of an anticoccidial drug against a wide variety of field isolates,

to determine the mode of action of an anticoccidial drug, and to measure other biological factors under carefully controlled conditions (Tyzzer 1943; Reid, Taylor, and Johnson 1969; Reid et al. 1969; Cuckler 1970; Hymas 1970; Long 1970; Shumard and Callender 1970; Waletzky 1970a; Ryley and Betts 1973; Ryley 1980; Conway et al. 1993a; Williams 1997; Chapman 1998).

The coccidial infection used in a battery test may consist of a pure isolate containing a single species or a mixture of several isolates. When using a mixture of isolates, it is best to have only one species per zone of intestine. This will facilitate scoring and permit a more precise measurement for each species studied.

The number of sporulated oocysts given per bird varies depending on the species used, the pathogenicity of the particular isolate, and the objective of the experiment. A titration of the oocyst inoculum in a small number of birds can be a great benefit in selecting an appropriate dose. A guideline for selecting the dose is given in table 4.1. The number of oocysts should be sufficient to produce some mortality (5–10%) in infections involving *E. tenella* or *E. necatrix*, and to reduce weight gains by at least 15–25% in the infected, unmedicated birds in comparison with uninfected birds.

Measurements used by various investigators to judge efficacy include mortality, weight gain, feed conversion, lesions scores, hematocrit values, dropping scores, serum or plasma carotenoids,

Table 4.1.

Suggested doses of sporulated oocysts for battery efficacy tests

Species	Number of oocysts/bird
<i>E. acervulina</i>	$1 \times 10^5 - 10^6$
<i>E. brunetti</i>	$1 \times 10^4 - 10^5$
<i>E. maxima</i>	$1 \times 10^4 - 10^5$
<i>E. mivati</i>	$1 \times 10^5 - 10^6$
<i>E. mitis</i>	$1 \times 10^5 - 10^6$
<i>E. necatrix</i>	$1 \times 10^4 - 10^5$
<i>E. tenella</i>	$1 \times 10^4 - 10^5$

and oocyst production. The most common measurements include the first four variables on the above list, with one or more of the others included at the discretion of the investigators (Natt and Herrick 1955; Kouwenhoven and van der Hort 1969; Barwick et al. 1970; Morehouse and Baron 1970; Reid 1970; Waletzky 1970a, 1970b; Joyner and Norton 1971; Yvoré and Mainguy 1972; Ruff and Fuller 1975; Sharma and Fernando 1975; Conway et al. 1993b; Conway, Dayton, and McKenzie 1999). One option in battery efficacy tests designed to assess the efficacy of an anticoccidial drug that is less than 98% efficacious against coccidia is to include two unmedicated treatments in the trial design. One unmedicated treatment would be administered a full coccidial inoculum (e.g., 1×10^5 *E. tenella* oocysts per bird), and the second would be given a dose reduced by 90% (1×10^4 *E. tenella* oocysts per bird). This allows comparison of lesion scores and

bird performance in the medicated treatments given the high oocyst dose only against a dose response curve established by the data from the two infected, unmedicated treatments. This basic design was particularly useful, for example, in tests with anticoccidial drugs of the polyether ionophore class (Conway et al. 1995).

Measurements recommended in the following example protocol include body weight, feed consumption, mortality, and lesion scores.

Title:

Battery efficacy test.

Objective:

To test the efficacy of anticoccidial drugs against selected species of *Eimeria* in broiler chickens.

Infection:

Eimeria tenella. Given *per os* directly into the crop on day 1 (24 hours after the start of test). Note that other species may be substituted as needed.

Design:

See table 4.2.

Management:

- a. Chicks/feeder space: 8 per 60 × 9.5 cm feeder.
- b. Cage space/chick: 0.057 m²/bird (67 × 68 cm cages).

Table 4.2.

Four treatments, 5 cages per treatment, 8 chicks per cage

Treatment	Route of medication	Days of medicated
Uninfected, unmedicated	—	—
Infected, unmedicated	—	—
Infected, anticoccidial drug 1 at use level	Feed	7
Infected, anticoccidial drug 2 at use level	Feed	7

- c. Feeding and watering method: *ad libitum* consumption.
- d. Housing: Petersime Finishing Batteries (manufactured by Petersime Incubator Co., Gettysburgh, Ohio 45328, U.S.A.) or similar unit.
- e. Environmental control: Air-conditioned room.
- f. Diet: Standard broiler starter ration containing 23% protein.

Experimental animals:

- a. Breed/strain: Broiler-type male chickens.
- b. Initial age: 14 days.
- c. Average initial weight/chick: 260 g.
- d. Origin: Supplied by a single commercial hatchery and breeder farm.

Procedure:

Feed chicks in each treatment with respective feed for one day (day 0) before exposure to coccidia (Note: Some workers allow 48 hours premedication before inoculating birds with oocysts). Select healthy male chicks of uniform weight from a group of chicks all within plus or minus one standard deviation of the mean of the group. Use a randomized complete block design with cage unit or deck as blocks. Birds in the uninfected treatment should be inoculated with 1 ml of pure distilled water at the same time (day 1) all other chicks are inoculated with *E. tenella*.

Rations:

Retain appropriate samples (500 g) of each ration from each mixing for drug assay. Quantities of all premixes added will be documented. Feed will be mixed to obtain a uniform distribution of the test article for each batch of feed mixed. The mixer will be cleaned between each treatment batch to prevent cross contamination of diets. Experiment diets will be prepared from a uniform basal diet. The type of mixer and mixing times will be described.

Records:

a. Body weight by cage on day 0 and at termination of the trial on day 7 (six days post infection).

- b. Record of feed added and feed remaining at end of trial for each cage for days 0–7.
- c. Daily observations of each pen. Weigh all dead chicks and retain for immediate examination. Records should include pen number, dead weight, and gross necropsy observations.
- d. Necropsy record should include sex and probable cause of death or most significant gross lesion(s). If coccidiosis lesions are observed, location and severity should be recorded.
- e. Coccidial lesion score for the ceca of surviving chicks on day 7 (six days post infection). Chicks dying of coccidiosis are given a score of +4.
- f. Records of feed mixing, feed samples obtained, and assay reports.
- g. Any mistake or change in the source data should be initialed and dated on the form and a brief statement provided as to why the change was made. Any transcribed data must be designated as such.

Statistical analysis:

Means for weight gain, feed consumption, feed conversion, lesion scores, mortality, and other variables that may be included such as plasma carotenoids will be analyzed as a randomized complete block design where the blocks are homogeneous cages in the same battery deck or tier. In this analysis, block is a random effect and the main effects for treatment (and sex if sexes are included

and separated by cage) and appropriate interactions are fixed effects. This is a mixed model and requires a mixed model analysis (SAS Institute 2001). Cage mortality percentages will be analyzed after an arcsine square root transformation. Fisher's protected least significant difference test should be used for comparisons among treatment means.

Performance will be assessed by the following equations:

Average weight gain per bird = (average final weight of live birds in a cage) – (average initial weight of all birds in that cage).

Average feed consumption per bird = [(total cage feed consumption) ÷ (surviving birds × days on test + number of days dead birds spent in cage on test)] × days on test.

Feed conversion per bird = (total feed consumption in a cage) ÷ (weight gain of surviving birds + weight gain of birds that died in the same cage).

Floor pen tests

Floor pen tests are an effective intermediate step in the progression of an anticoccidial drug or vaccine from the laboratory to commercial trials. Through

the replication that can be provided in floor pens, and the control over many sources of variation, the floor pen test can be a powerful method of measuring the effects of an anticoccidial drug or vaccine on disease control and bird performance (Reid et al. 1969; Brewer and Kowalski 1970; Cuckler 1970; Gard and Tonkinson 1970; Ott 1970; Reid 1978).

Ideally, floor pens should be identical in size, large enough to hold a minimum of 30–50 birds per pen at a density of 12–16 birds per m² (0.90–0.67 sq. ft. per bird), and located in a conventional broiler building. The central hallway should be wide enough to move and hold bagged feed conveniently. The partition between each pen should consist of boards from the floor to approximately one-half meter high, and chicken wire extending from the boards to a height of approximately two meters.

Equip each pen with a feeder (hanging or other type) and an automatic water device. Use standard equipment to ventilate or heat the building. Infrared lamps or gas brooders can be used to provide supplemental heat during the brooding period if needed. Use new litter in the pens at the beginning of each test, unless the test is specifically designed to test the efficacy of the anticoccidial drug or vaccine against a natural coccidial challenge induced by the use of naturally contaminated litter from a commercial poultry house. Place chicks on test at one day of age. The tests

should last for six to eight weeks depending on local practices.

Coccidial infections may be achieved by the use of naturally contaminated litter, as indicated above, but infections established through the use of seeder birds or inoculation of oocysts via the feed or water are more effective (Gard, Young, and Callendar 1969; Reid, Taylor, and Johnson 1969; Reid, Kowalski, and Rice 1972; Brewer and Kowalski 1970; Cuckler 1970; Gard and Tonkinson 1970; Mitchell and Scoggins 1970, Ott 1970; Ruff, Reid, and Rahn 1976; Kilgore et al. 1979; McDougald and Johnson 1979).

Measurements used by various investigators to judge performance and efficacy include weight gain, feed conversion, mortality, production costs, lesion scores, dropping scores, oocyst counts, skin pigmentation scores, plasma xanthophylls or carotenoids, plasma lipids, and immunity levels (Kouwenhoven and van der Hort 1969; Reid 1970 1978; Waletzky 1970b; Joyner and Norton 1971; Yvoré and Mainguy 1972; Ruff, Reid, and Johnson 1974; Ruff and Fuller 1975; Sharma and Fernando 1975; Yvoré 1978; Chapman and Hacker 1993; Conway et al. 1993b; McDougald, Mathis, and Conway 1996). Special measurements such as the last six variables on the preceding list are often considered optional. Of these, plasma carotenoids and lipids were demonstrated to provide a high coefficient of determination (R^2) for measuring the effects of *E. acervulina*,

E. maxima, and *E. tenella* in broiler chickens (Conway et al. 1993b). Inclusion of one or more of these measurements in a study will depend upon the experiment objectives, the anticoccidial drugs or vaccines being tested, and the preferences of the investigator.

The standard measurements of mortality, weight gains, feed consumption, and lesion scores are included in the following example protocol.

Title:

Floor pen efficacy test.

Objective:

To confirm and demonstrate the utility of an anticoccidial drug in broiler birds when fed at use level continuously for 44 days in a 49 day floor pen test (5-day drug withdrawal).

Infection:

Eimeria acervulina, *E. brunetti*, *E. maxima*, and *E. tenella*. Feed exposure method of infection on day 22 of test with oocysts of recent field isolates of each species propagated in the laboratory (Kilgore et al. 1979; Migaki and Babcock 1983). A brief description of the isolate identification, history/source, methods used to isolate, sporulate and count oocysts, and the procedure used to infect the birds in each pen will be provided.

Design: See table 4.3.

Management:

- a. Chicks/feeder space: 2 feeders, 40 cm diameter (tube type)/pen.
- b. Floor space/chick: 0.09 m²/bird (12 birds/m²) adjusted for space taken by feeders and water device.
- c. Feeding and watering method: *ad libitum* consumption.
- d. Housing: 24 floor pens with total 4.5 m²/pen. A diagram of the test facility showing the pen layout, pen size, description of equipment for feeding, and watering will be provided in the appendix of the protocol.
- e. Environmental control: Ambient humidity, 8 cm new wood-shavings litter. Clean and sanitize pens with a standard disinfectant before adding litter. Twenty-four hour lighting will be provided. Gas heaters as primary heat source with heat lamp(s) in each pen for supplemental heat as required during the brooding period. Ventilation as required during the growing period.
- f. Diet: Standard broiler starter, grower, and finisher or withdrawal rations representative of commercial practice.
- g. Other than standard vaccines administered at the hatchery (e.g., Marek's disease), no concomitant therapy is to be used during the trial. In the event of an outbreak of disease, the investigator and monitor will decide together on an appropriate course of action.

Table 4.3.

Three treatments, 8 pens per treatment, 50 chicks per pen

Treatment	Route of Medication	Days of Medicated ¹	Pens/Treatment	Chicks/Treatment ²
Unmedicated	—	—	8	400
Anticoccidial drug 1 at use level	Feed	44	8	400
Anticoccidial drug 2 at use level	Feed	44	8	400

¹ Anticoccidial drug withdrawn 5 days before trial termination.

² Equal number of each sex per treatment.

Experimental animals:

- a. Breed/strain: Broiler-type chickens.
- b. Initial age: One day.
- c. Average initial weight/chick: 40–45 g.
- d. Origin: Supplied by a single commercial hatchery and breeder farm.

Rations:

Retain appropriate samples (500 g) of each ration from each mixing for drug assay. Quantities of all premixes added will be documented. Feed will be mixed to obtain a uniform distribution of the test article for each batch of feed mixed. The mixer will be cleaned between each treatment batch to prevent cross contamination of diets. Experimental diets will be prepared from a uniform basal diet. The type of mixer, pelleting equipment, mixing times, pelleting conditions, and batching system used will be described.

Procedure:

Order at least 15% more chicks than needed for the test. Select only healthy chicks from the total order and allot them at random into 24 pens of 50 chicks each. Each treatment should consist of 4 pens of male and 4 pens of female birds or 8 pens with 25 chicks of each sex, depending on specific objectives. Equalize the total weight of the

chicks in each pen as closely as possible. Divide the floor pens into 4 blocks of 6 pens each if sexes are to be separate so that each block will contain 3 pens (subplot) of each sex. Treatments should be randomly assigned to each pen within the subplot. If pens are to contain an equal number of each sex, divide the floor pens into 8 blocks of 3 pens each, and assign treatments at random to each pen within each block (randomized complete block design). Replace all dead chicks during the first 3 days of test with healthy chicks held in clean cages for this purpose. Do not replace birds that die subsequently. These should be examined and recorded in the mortality record. Randomly select 8 birds in each pen at the start of test and identify with wing bands. Five birds from this group will be taken for lesion scoring on the designated day, 6 days after coccidial exposure.

Oocyst inoculation:

An infective dose of sporulated oocysts for each pen is prepared in accordance with the trial protocol in 100–300 ml of water. Feeders in each pen are elevated or removed for 1 hour prior to infection. Approximately 1.8 kg of dry feed is weighed out from the feeder of each pen. The feed is carefully poured into a bucket or feed tray, and 1.3 l of tap water is added while mixing the feed with a ladle to make a homogeneous wet mash. The water suspension containing the sporulated oocysts mixture for the pen is added to the wet mash while

mixing thoroughly with a ladle. The infected feed is dispensed into a chick feeder tray in the pen, and the birds are given access to only this feed until it is completely consumed. The chick feeder trays are removed, and the standard feeders are lowered or placed back in the pen after 7 hours or when all of the contaminated feed has been consumed, whichever occurs first.

Records:

- a. Bird weights by pen on days 0, 21 or 28 (optional), and 49.
- b. Feed added to each pen feeder for each feeding period, i.e., starter, grower and finisher, and feed weighed back.
- c. Daily observations of each pen. After day 3 (see procedure), weigh all dead birds and retain for immediate examination. Records should include pen number, dead weight, date, and gross necropsy observations.
- d. Necropsy record should include sex and probable cause of death or most significant gross lesions observed. If coccidial lesions are observed, location and severity should be recorded.
- e. Intestinal and cecal lesion scores of 5 birds from each pen removed on day 28 of test (6 days after exposure to oocyst-contaminated feed).
- f. Records of feed mixing, feed samples, and assay reports.

Statistical analysis:

If each sex is maintained in separate pens, the response variables for weight gain, feed consumption, feed conversion, lesion scores, and mortality will be analyzed as a split-plot design where the whole plots are sexes and the split-plots are treatments. If both sexes are initially assigned in equal numbers to each pen, the preceding response variables will be analyzed as a randomized complete block design where the blocks are homogeneous pens in the same section of the floor pen building. This is a mixed model and requires a mixed model analysis (SAS Institute 2001). Cage mortality percentages will be analyzed after an arcsine square root transformation.

Bird performance will be assessed by the following equations:

Average weight gain per bird = (average final weight of live birds in a pen) – (average initial weight of all birds in that pen).

Average feed consumption per bird = [(total pen feed consumption) ÷ (surviving birds × days on test + test number of days dead birds spent in pen on test)] × days on test.

Feed conversion per bird = (total feed consumption in a pen) ÷ (weight gain of surviving birds + weight gain of birds that died in the same pen).

Commercial tests

Commercial tests are necessary to test an anticoccidial under typical field conditions. Many factors that may affect the usefulness of an anticoccidial cannot be introduced easily, if at all, in battery and floor pen trials. The effects of the size of bird population, automatic feeders, and controlled lighting are just a few of potentially multiple examples. In addition, a number of biological and physical factors are less clearly understood in a commercial facility, which may affect either directly or indirectly the performance of an anticoccidial drug.

Commercial trials must be carefully planned and supervised if they are to provide informative and repeatable results. The basic criteria referred to previously apply equally to commercial trials. Other factors to be considered are given by Kilgore (1970):

1. Obtain chicks from the same breeder flock source. If more than one source is used, equally divide the chicks from all sources across all treatments on the basis of breeder flock source and, if necessary, delivery date.
2. Keep the experiment design simple since there are frequently many people—e.g., the grower or caretaker, serviceman, and feed mill manager—involved in implementing the trial.

3. Give special attention to preventing errors when feed is delivered to the farm. The driver delivering bulk quantities of feed needs to be carefully instructed.
4. The feed mill manager should take special precautions to avoid errors in preparing the rations. Additions of drugs to the wrong feed, use of other drugs that may be routinely used in the normal rations, and addition of an incorrect amount of the specified drug premix are occasional problems.
5. Obtain an accurate count of the number of birds placed, the number that died or were culled on a daily basis during the test, and the number of birds produced to market.
6. A broiler house divided into two equal parts (split-house design) is generally preferred over two or more houses side by side from a standpoint of statistical validity and replication. A minimum of three to four replicates per treatment if at all possible is preferred.

Investigators generally measure bird weight, feed consumption, feed conversion, production costs and total mortality in evaluating the anticoccidial agent (Basson 1970; Cuckler 1970; Edgar 1970; Kilgore 1970; Reid 1970; Zuijdam 1970; Clarke et al. 1978). Other kinds of records include clinical observations made several times per week and necropsy of dead birds. The investigator should remove a number of birds (selected at

random) for routine necropsy in some trials. In this event, the grower should be compensated for birds removed.

Title:

Commercial test.

Objective:

To demonstrate the efficacy and utility of the anticoccidial drug in broiler chickens under commercial conditions and natural coccidial exposure in comparison with a positive control.

Design:

The number of chicks per treatment will depend on the capacity of the house. It is anticipated that straight-run (as hatched) chicks will be used in most instances, but all birds should come from the same hatchery and breeder farm. See table 4.4.

Table 4.4.

Two treatments, one house per treatment

Treatment	Total no. of birds
Anticoccidial drug 1 at use level	15,000–30,000
Anticoccidial drug 2 at use level	15,000–30,000

Management:

- a. Housing: One house per treatment or split house design if separate feeding systems (and watering systems if the drug under test is administered via the water) are available. Two identical houses side by side are the norm.
- b. Feeding and watering method: *ad libitum* consumption.
- c. Duration: Commercial broiler cycle as normally practiced.
- d. Floor space: Standard bird density for farm.
- e. Environment: New or used litter with natural contamination of coccidia oocysts as normally practiced.
- f. Rations: Standard broiler starter, grower, and finisher rations.

Procedure:

Allot birds to both treatments at 1 day of age on an equal basis with respect to breeder flock source, sex, health, number of birds, and delivery date (if necessary). Eliminate bias that might be caused by differences in location, house construction, equipment, and ventilation. Each house should be of equal size. Old litter used in each house should be from the same source. If new litter is used, the litter may be seeded by mixing in old litter from a recently vacated house. The broiler houses will be checked daily to ensure that feed, watering,

and ventilation equipment are working properly. Dead birds will be removed daily, and a record of birds removed due to death or culling will be maintained.

If coccidiosis is suspected or diagnosed, a minimum of 25 birds will be randomly selected and examined post mortem for coccidial lesions. If mortality and/or culling for reasons other than coccidiosis exceed the rate normally expected in the house, dead and culled birds will be subjected to a detailed post mortem examination to determine the cause of death or reason of poor growth.

At the end of the growing period, birds will be processed following standard procedure. The numbers of birds sent for processing should be determined as they are loaded from each house. At the processing plant, the total weight and numbers of live birds should be recorded. Records of all downgraded or condemned birds should be made (if data are available), and the total number and weights of processed birds should be recorded.

Rations:

Retain appropriate samples (500 g) of each ration from each mixing for drug assay. If feed is pelleted, samples will be taken after pelleting. Quantities of all medicated premixes added will be documented. Feed will be mixed to obtain a uniform distribution of the test article for each batch of feed mixed.

The mixer will be cleaned or flushed between each treatment batch to prevent cross contamination of diets. Experimental diets will be prepared from a uniform basal diet. The type of mixer, pelleting equipment, mixing times, pelleting conditions, procedure used for flushing the mixer, and batching system used will be described.

Records:

- a. Number of birds started and completed.
- b. Total number of birds processed including number of birds condemned and/or downgraded (if data can be obtained).
- c. Total live weight at market for each replicate or treatment.
- d. Total feed consumption for each replicate or treatment throughout the test.
- e. Dressing percentage or condemnation rate if data are available.
- f. Description of rations used.
- g. Daily record of dead birds and birds culled.
- h. Necropsy record of dead birds when possible.
- i. Examination of a representative sample ($n^{1/3}$) of birds for intestinal and cecal lesion scores at approximately 3 to 5 weeks (Gard and Tonkinson 1970). See table 4.5.

Table 4.5.

Representative sample of birds for examination of intestinal and cecal lesion scores at approximately 3 to 5 weeks

No. birds/house	Representative sample($n^{1/3}$)
1,000	10
2,500	14
5,000	17
10,000	22

Source: Gard and Tonkinson 1970.

- j. Observations made at least once weekly for possible signs of coccidiosis, wet litter, or adverse reactions to the test anticoccidial.
- k. Record of feed samples obtained and assay results.

Statistical analysis:

Assuming more than one commercial unit or house is used in the test, the response variables for mean final weight, feed consumption, feed conversion, lesion scores, and percentage mortality will be analyzed as a completely randomized design where the treatments were assigned to the commercial houses at random.

Performance will be assessed by the following equations:

Final weight per bird = (gross live weight of birds processed) ÷ (total number of birds sent to processing).

Feed consumption per bird = (total feed consumed) ÷ (total number of birds sent to processing).

Feed conversion = (total feed consumed) ÷ (gross live weight of birds processed).

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Avian coccidiosis is a common, widespread disease of growing chickens around the world. It is a disease that can seriously restrict the development of poultry production under both farm yard and modern conditions. Fortunately, much is known about the biology of the causative agent, *Eimeria* spp., and ways to control this protozoan parasite.

Factors influencing the occurrence of coccidiosis

The widespread occurrence of coccidial infections in chickens even under modern conditions of production reflects both the adaptability of the parasite and the way birds are raised. Quite typically, large numbers of day-old, fully susceptible chicks are placed together on litter in special poultry growing houses. The houses may be relatively simple in construction and only large enough to hold 1,500 to 2,500 birds, or a highly mechanized building with a capacity of 20,000 to 50,000 birds. The flock grow-out time may be a brief, five to nine weeks for broiler or meat-type birds, which make up a majority of the world poultry population, or much longer periods for other types of chickens such as replacement layers and breeder birds. Regardless of the ultimate goal, the risk of coccidiosis is indeed great to the young bird under these conditions.

Coccidial oocysts are normally introduced into new facilities through contaminated equipment or

vehicles coming from other poultry operations, or by the movement of service personnel between older and new facilities. Once a house becomes contaminated, it is virtually impossible to totally decontaminate the environment (Reid 1989). Studies with broiler birds have shown that exposure to sporulated oocysts usually begins shortly after chicks are placed on the litter (Long and Rowell 1975; Long, Tompkins, and Millard 1975; Long and Millard 1978; Braunius 1984). Litter oocyst counts are generally low during the first two to three weeks, increase rapidly to a peak between four and six weeks, and decrease to low levels again by seven to eight weeks. This pattern of infection, illustrated in figure 5.1, indicates that the peak level of infection in the growing bird is most likely to occur between three and six weeks of age. In most regions of the world, *E. acervulina* and *E. maxima* are the most commonly encountered

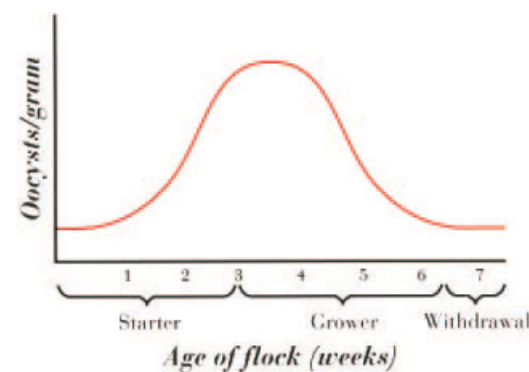


Figure 5.1. Oocysts per gram of litter by flock age (McDougald 1982 as cited by Kling, Hanssens, and Grant 1989).

species of coccidia in broiler flocks with a somewhat lower incidence of *E. tenella*, *E. mivati*, and *E. mitis*. *E. brunetti* and *E. necatrix* infections are less likely to be observed in broiler flocks, possibly because of the shorter growing times of broiler birds. As indicated in chapter 2, one exception to this is the incidence of *E. necatrix* infections that may be observed in broiler flocks in tropical regions of the world at three to five weeks of age (McDougald and Conway 1984).

Workers in the United States and Belgium have confirmed this by assessing the incidence and severity of coccidial lesions in birds selected at random from growing flocks. Dietzel (1986) found that coccidial lesions were nil or low during the first three weeks of age in studies in the southern United States, but rapidly increased to a peak by four weeks (Figure 5.2).

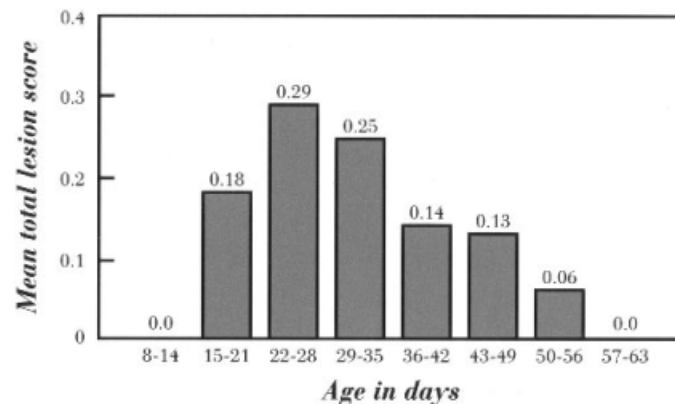


Figure 5.2. Coccidial lesion scores in broiler flocks by flock age (Dietzel 1986).

Froyman, Derijcke, and Verlinden (1987) also reported that the highest lesion scores occurred at about four weeks of age in a study extending over nine months at two broiler integrators in Belgium. These workers reported a substantial range in total average scores from flock to flock (figure 5.3).

Basic elements of coccidiosis control

Flock health care in the broadest context, including those aspects of the program that focus on the breeder flock and the hatchery, is an important influence on the vigor and immunity status of the birds during each growing cycle. The occurrence of other diseases such as complicated respiratory disease, mycotoxicosis, and infectious bursal disease can have a significant adverse impact on feed consumption and the overall efficacy of an anticoccidial program. This is especially important when the disease either precedes or occurs during the critical period of peak coccidial challenge from three to five weeks of age. The same can be said for the post-vaccination stress response associated with some viral disease vaccines given between 10 and 14 days of age, when the stress peaks during the period of peak coccidial challenge.

Fortunately, the producer does have effective ways to prevent the potentially disastrous consequences of a coccidiosis outbreak. The prevention of coccidiosis depends on a number of factors including the use of anticoccidial drugs and

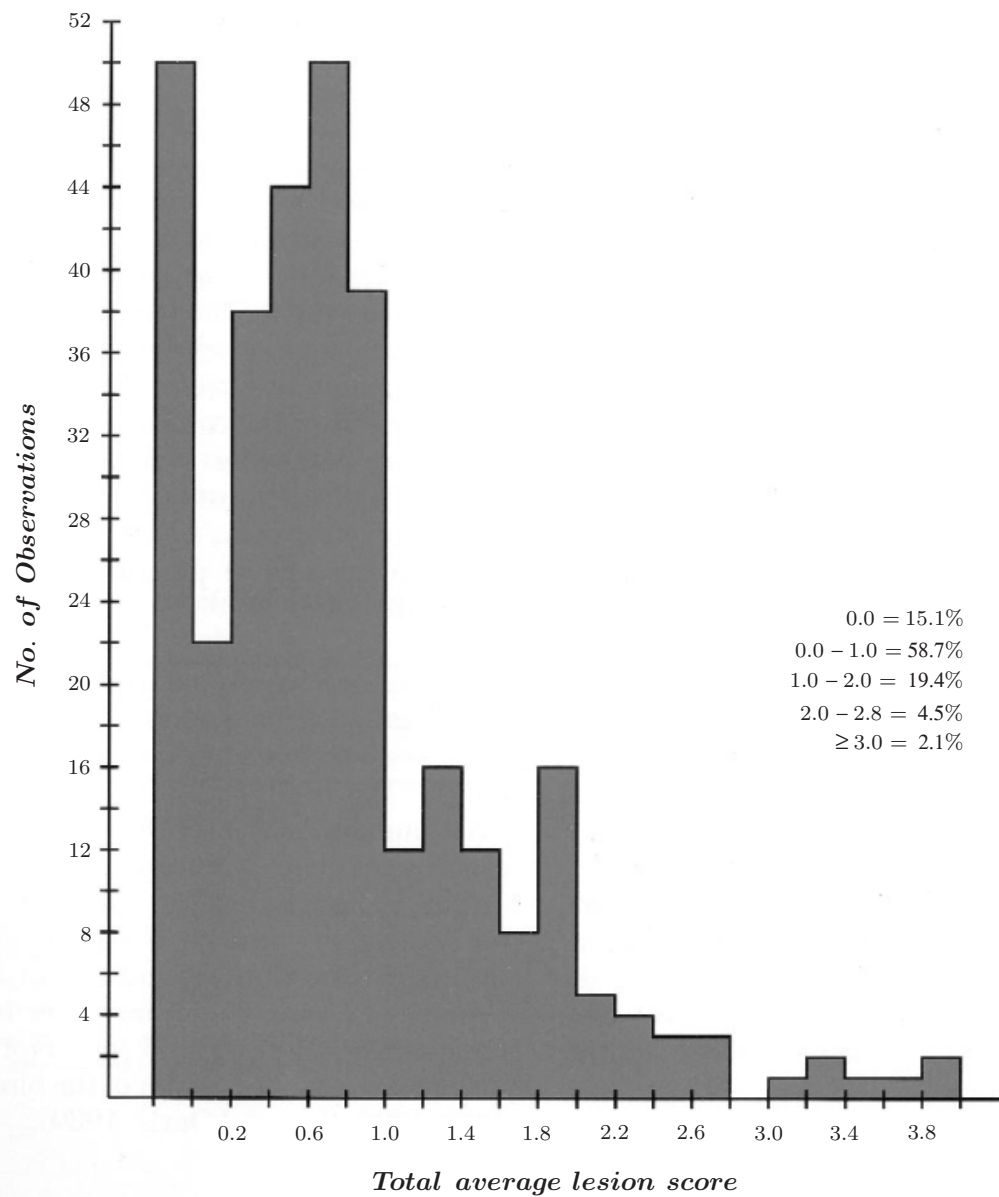


Figure 5.3. Frequency distributions of subclinical and clinical coccidiosis (Froyman, Derijcke, and Verlinden 1987). Pooled results from two integrators (329 observations from March 1 to December 31, 1986), both integrators using 110 ppm monensin continuously.

vaccines (see review in chapter 6), management of the poultry house environment, and feed quality control (Conway 1996).

Application of anticoccidial drugs and vaccines

The use of a broad-spectrum anticoccidial drug on a prophylactic basis or a vaccine is essential. Generally an anticoccidial drug is given *ad libitum* in the feed from one day of age to market. A withdrawal period of five to seven days, occasionally longer, is often observed depending on the drug being used, regulatory requirements, and practical experience. The development of a natural immunity in medicated birds depends on the anticoccidial drug being used and the level of coccidial challenge occurring during the first five to six weeks of growth, but even under the best of circumstances full immunity is not achieved until birds are seven weeks of age (Chapman 1999). Lapses in the anticoccidial drug program as a consequence of restricted feeding, skip-a-day feeding, or a withdrawal period extending beyond seven days increase the risk of coccidiosis outbreaks, and must be carefully evaluated.

Polyether ionophores should continue to play a major role in the anticoccidial programs in commercial broilers, either alone or in shuttle programs with chemical anticoccidial drugs, if consistent efficacy and performance are to be maintained (Long and Millard 1978; McDougald 1982, 1990). The use of anticoccidial vaccines in a rota-

tional program with anticoccidial drugs (see chapter 6) is also recommended to minimize the risk of anticoccidial drug tolerance or resistance problems over the long term (Williams 1998, 2002; Chapman 2002; Chapman et al. 2002; Hofacre 2003; Mathis and Broussard 2005).

The use of anticoccidial vaccines in breeder and replacement birds is probably the optimum course in most situations, and the current ability to vaccinate chicks at the hatchery either by spray cabinet or eye inoculation has made a big difference in making anticoccidial vaccines a practical option for broiler chickens as well. Vaccinated birds need to be carefully monitored to be sure that protective immunity has developed. Treatment with an anticoccidial drug via the water (e.g., amprolium, sulfaquinoxoline, and toltrazuril) may be required in vaccinated flocks if a severe coccidial challenge is diagnosed before immunity has fully developed. Reaction to *E. acervulina* and *E. maxima* in vaccines may adversely affect a pullet's ability to absorb vitamins, especially vitamins A, D, E, and K, during the vaccination period (Hofacre 2003). This may lead to the development of rickets and an increase in leg problems around four weeks of age. In view of this potential problem, the addition of vitamins to the drinking water of pullets during this period is recommended.

Anticoccidial drug shuttle programs typically use a chemical anticoccidial drug, such as nicarbazin, in the starter ration, and an ionophore in the

grower feed (Eckman 1993). More recently, improved efficacy and performance has been demonstrated with the use of an ionophore in the starter and a chemical, in this instance diclazuril, in the grower phase (Conway et al. 2001). The ultimate determination of whether to use a chemical anticoccidial drug in the starter or grower ration of a shuttle program depends on the drug itself, the season of the year, and past experience. For example, while there are no concerns over the safety of diclazuril when used in the grower phase of production, there are such concerns with nicarbazin particularly during the warmer season of the year (Buys and Rasmussen 1978; McDougald and McQuiston 1980; Keshavarz and McDougald 1981). Due to the experience over the years of drug resistance problems with chemical drugs when used continuously for three to four growing cycles, it is generally recommended that chemical anticoccidial drugs be used either in shuttle programs with polyether ionophores, or for just one or two cycles in rotation with a polyether ionophore drug or an anticoccidial vaccine.

The efficacy of any anticoccidial drug program over the long term is very dependent upon the quality of the diets fed and how well the anticoccidial drugs are mixed into each batch of feed. Feed assays should be conducted on a regular basis by validated procedures to be sure that the levels of anticoccidial drug are close to the rate of addition. If assay results are frequently below 85%

of theory, then a collaborative project should be established between the feed mill and the quality control laboratory to determine why this is occurring. Aside from the occasional misdelivery of unmedicated feed to a farm, possible reasons for low assays include the following:

1. Incorrect feed sampling and packaging of samples.
2. Inadequate grinding and extraction of the anticoccidial from the sample at the laboratory.
3. Errors in weighing the premix or bulk ingredients delivered to the mixer.
4. Premix losses during delivery from the micro-ingredient bin to the mixer.
5. Nonuniform mixing and dust losses.
6. Failure to recycle fines for inclusion in the same batch of feed.

The use of anticoccidial drug programs for replacement breeder and layer birds, in place of vaccines, presents special problems and requirements. In these instances, the anticoccidial drug program needs to be carefully monitored while natural immunity develops (Long and Jeffers 1986).

Management of the host environment

Environmental factors of importance to bird health and successful coccidiosis control in the

poultry house include temperature, ventilation, litter conditions, bird density, drinkers, lighting, and feeding equipment.

House temperature is of primary importance throughout the brooding and growing period to maintain good bird activity. If temperatures are outside of the acceptable range the adverse effect on feed consumption, and thus the consumption of the anticoccidial drug, can be significant. Ideally, temperatures in the house should be maintained at approximately 33°C–35°C (90°F–95°F) upon arrival of the chicks, and lowered 3°C (5°F) each week until the house temperature reaches 21°C (70°F) at approximately six weeks (Arbor Acres 1988). Under climatic conditions where it is difficult or impossible to maintain temperatures close to the ideal, ventilation and insulation are of paramount importance (Keshavarz 1990; Charles and Walker 2002; Donald 2003; Czarick and Lacy 2004a).

A continuous supply of fresh air is extremely important to remove moisture given off by the birds or accumulated in the litter from the droppings. Wet litter can develop quickly without proper ventilation. The need for air movement in tropical environments may even be greater because of the existing ambient humidity (Say 1987; Shane 1988). High humidity and increased litter moisture for whatever reasons contribute to increased ammonia production leading to ammonia fumes in the air above the litter. These fumes adversely affect

feed consumption and the maintenance of good health of the birds (Ross and Davis 1989; Hunton 1989–1990; Kristensen and Wathes 2000). Wet litter conditions can also lead to a chilling of the birds and increased incidence of deleterious organisms such as *Escherichia coli* and *Clostridium* spp.

Increased litter moisture in the range of 15% to 25% directly benefits oocyst sporulation in the top 5–7 cm of litter (Chapman and Johnson 1992; Waldenstedt et al. 2001). Litter moisture can be assessed by squeezing a handful of litter. Dry litter will quickly break apart when released. Wet spots caused by a leaking roof or leaking drinkers or moist litter resulting from inadequate ventilation are favorable environments for oocyst sporulation. Caked litter and wet spots should be removed whenever possible. If litter is not removed between each grow-out, an application of hydrated lime or superphosphate spread uniformly and stirred in over the old litter before applying a top dress of new litter should be considered (Harrison Poultry n.d.).

Bird density may vary from as low as 10 birds per m² (1.08 sq. ft. per bird) up to 25 birds per m² (0.43 sq. ft. per bird). Production under high-density conditions, i.e., greater than 15 birds per m², increases the risk of coccidiosis problems as a result of greater competition for feed and water, increased litter contamination and oocyst buildup, and higher litter moisture (Hamet et al. 1982).

Key factors in maximizing bird performance are the distribution of birds in the house and the need to take measures to ensure a uniform distribution (Czarick and Lacy 2004b). The farm manager can have a significant impact on the comfort level of the flock from the day of arrival to the time of processing by paying careful attention to maintaining a proper house temperature, ensuring good ventilation to provide an ample supply of fresh air during all seasons of the year, providing adequate feed and water space, and by adjusting light as required. Moisture levels in the litter are closely correlated with ventilation and the type of watering system used. As moisture levels increase in the litter, both oocyst sporulation and ammonia production are favored. This can lead to reduced consumption of feed and anticoccidial drug precisely at a time when levels of coccidial challenge are increasing. In view of this, a dryer litter (<20%) contributes to the overall efficacy of the anticoccidial drug program by reducing the level of challenge. On the other hand when an anticoccidial vaccine is being used, litter moisture levels of 20% to 25% favor the recycling of coccidial infection and the development of full immunity. Obviously, management of broiler house conditions becomes a critical issue in determining the success of the anticoccidial control program being used (Williams 2002).

The impact of any one of the factors including high-density production, limitations on feeding or drinker space per bird, a failure to maintain the

feeders and drinkers at proper heights as the birds grow, and a poor house environment can influence feed consumption and thus the consumption of the anticoccidial drug. If feed consumption is reduced because of one or more production problems, the overall efficacy of the anticoccidial program may be compromised significantly.

Feed quality control

All activities aimed toward the production and delivery of well-formulated feed with minimal batch to batch variation come under the heading of quality control (Dale 1990a, 2002; Leeson and Summers 1997, 2001). The proximate composition of new shipments of feed ingredients with respect to crude protein and moisture, for example, may be especially important. The protein level in feed should not vary by 3% or 4% from day to day on either side of the intended level. If the level is higher than intended, protein is wasted, while a suboptimal level adversely affects performance. Either way, production costs increase. Maintaining feed quality with respect to proper levels of protein, energy, minerals, essential nutrients, and feed additives such as the anticoccidial drug will have a significant benefit on flock performance and production costs. By maintaining nutrient quality at an optimum level, the chances for the anticoccidial drug or vaccine to provide its full benefit are substantially enhanced. A review by Dale (2002) provides an excellent summary of the costs and benefits of using currently

available procedures to establish an effective and economical quality control program. Maintaining good feed quality also extends to the conditions of bulk ingredient storage, and the potential presence of toxic factors such as mycotoxins and ergot (Huff et al. 1986; Wilcox and Unruh 1986; Bondi and Alumot 1987; Doerr 1987; Hoerr 1991; Leeson, Diaz, and Summers 1995; Dale 2002; Collett 2004). Bulk ingredients should be routinely assayed for mycotoxins, especially for those produced by *Aspergillus* and *Fusarium* spp. since the presence of either of these mycotoxins can adversely affect feed consumption and the overall performance of an anticoccidial program. A similar concern applies to the potential adverse effect of biogenic amines, particularly histamine, in sit-

uations where fish meal is used as a protein source. Bulk ingredients with high levels of these toxic contaminants should be avoided or diluted with safer ingredients.

Some example causes of variation in feed and feed ingredient quality are listed in table 5.1. The grower, integrator, and independent feed mill manager should take these types of factors into consideration when establishing and monitoring a coccidiosis control program. The success or failure of an anticoccidial program often depends not just on the efficacy of the anticoccidial drug or vaccine, but on how successful the producer is in achieving consistent quality control in the feeding program.

Table 5.1.

Examples of special causes and common causes of variation in feed and feed ingredient quality

Feed/Ingredient	Special Causes of Variation	Common Causes of Variation
Corn	High level of aflatoxin	Mild insect damage
Soybean meal	Severely overprocessed	Low level protein
Fat	High peroxide value	—
Sorghum	Bird resistant variety	—
Meat and bone meal	—	Low bone content resulting in high protein and lower calcium and phosphorus
Phosphate	—	Varying availability
Vitamin premix	—	Time in storage, storage conditions
Mixed feed	Wrong feed delivered to farm; forgot to add vitamins, anticoccidial drug, etc.; double recommended amount of limestone	Suboptimal mixing time, overloaded mixing equipment, small weighing errors, mold contamination

Source: Dale 1990b.

Conclusion

In view of these many and varied relationships between broiler management and the overall efficacy of the anticoccidial program, the production manager needs to take a comprehensive approach in managing anticoccidial drug and vaccine programs to achieve success over the long term.

In conclusion, the prevention and control of coccidiosis will be dependent upon the use of anticoccidial drugs and vaccines integrated with a comprehensive program focusing on high standards of bird health, nutrition, and management of the production environment.

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Anticoccidial drugs

Prior to the 1930s, the poultry producer had little hope of controlling outbreaks of coccidiosis in growing chickens other than a reliance on flowers of sulfur, dry or liquid skim milk, and buttermilk remedies (Beach and Freeborn 1927; Herrick and Holmes 1936; Ryley and Betts 1973), and the development of a natural immunity through the course of infection. In the third decade of the last century, studies by various workers clearly demonstrated that sulfonamide drugs had significant antibacterial and anticoccidial potential (Levine 1939, 1940; Hawking 1963). This pioneering work ultimately led to the discovery, development, and introduction of a wide range of anticoccidial drugs. The chemistry, safety, and efficacy of many of these products are summarized below (Chemical Abstracts Service registry number provided in brackets). A number of the anticoccidial drugs reviewed have been withdrawn over the years because of safety or efficacy issues, but our goal is to provide a more comprehensive picture of the many interesting discoveries that were developed and marketed for coccidiosis control and prevention. Sources of information include reviews by Joyner, Davies, and Kendall (1963), Reid (1972, 1975), Ryley and Betts (1973), MacPherson (1978), McDougald (1982), Long (1988), McDougald and Roberson (1988), and McDougald and Reid (1991) and key papers on specific anticoccidials or groups of an-

ticoccidials as cited. *The Merck Index* (Merck & Co. 2001), *The Merck Veterinary Manual* (Merck & Co. 1991), and the *Feed Additive Compendium* (Miller Publishing Co. 1990, 2004) are the primary sources of product and chemical names, dosage, and chemical structures for each of the anticoccidial products reviewed. Tables 6.1 and 6.2 summarize the recommended dose rates for most of the anticoccidial drugs currently available in different regions of the world.

Amprolium

Chemical name: [121-25-5] 1-[(4-amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride, hydrochloride; 1-[(4-amino-2-propyl-5-pyrimidinyl)methyl]-2-picolinium chloride, hydrochloride.

Product name: Amprol.

Chemical structure: See figure 6.1.

Safety: Amprolium appears to be well tolerated when used continuously in chickens in the range of 125–250 ppm (Rogers et al. 1960; Rogers 1962). Studies testing amprolium at 250 and 500 ppm indicated no adverse effects on broiler chicken mortality or performance (Ott, Dickinson, and Van Iderstine 1960; Morrison et al. 1961; Ryley and Betts 1973). Feeding of amprolium up to and including 1,000 ppm to layer chickens did not adversely affect feed intake, egg production, hatch

Table 6.1.

Anticoccidial drugs recommended for the therapeutic treatment of coccidiosis in chickens

Chemical Name	Route of Application	Use Level	Treatment Schedule
Amprolium	Feed	250 ppm	2 weeks
	Water	0.006%	1–2 weeks
	Water	0.012%–0.024%	3–5 days
Sulfadimethoxine	Water	0.05%	6 days
Sulfaguanidine	Feed	10,000–15,000 ppm	5–7 days
Sulfamethazine	Feed	4,000 ppm	3–5 days
	Water	0.1%	2 days
	Water	0.05%	4 days
Sulfaquinoxaline	Feed	1,000 ppm	2–3 days on, 3 days off; then 500 ppm for 2 days on, 3 days off, and 2 days on
	Feed	500 ppm	3 days on, 3 days off, 3 days on
	Water	0.04%	2–3 days; then 3 days on plain water; then 0.025% for 2 days on, 3 days off, and 2 days on
Sulfaquinoxaline + pyrimethamine	Water	0.005% + 0.0015%	2–3 days on, 3 off, and 2 days on
Furazolidone	Feed	110 ppm	5–7 days; then 55 ppm for 2 weeks
Nitrofurazone	Feed	110 ppm	5 days
	Water	0.0082%	5 days
Toltrazuril	Water	0.0025%	2 days continuous medication
		0.0075%	6–8 h./day for 2 days

Note: Follow local regulatory requirements for required withdrawal periods prior to slaughter. Some of these drugs may not be available in specific countries because of regulatory limitations.

of fertile eggs, embryo mortality, weight or quality of egg shell, yolk, and albumen, but a large percentage of chicks hatched from hens receiving 1,000 ppm were dead or weak at the time of hatch (Polin et al. 1962). Studies by Polin, Wynosky, and Porter (1963) also demonstrated that amprolium interferes with the absorption of thiamine in

laying hens when fed at relatively low levels (e.g., greater than 25 ppm) as measured by thiamine levels in the egg yolk. Feeding 125 ppm amprolium for 60 weeks beginning at 16 weeks of age to laying hens had no adverse effect on egg production, but the average Haugh unit score of eggs from the medicated hens was significantly ($P < 0.01$)

Table 6.2.

Contemporary anticoccidial drugs used for the prevention of coccidiosis in chickens

Chemical Name	Approved Poultry Type		Use Level in Feed (ppm)
	Broilers	Layers	
Chemical drugs:			
Amprolium	Yes	Replacements only	125–250
Amprolium + ethopabate	Yes	Replacements only	125–250 + 4
Arprinocid ¹	Yes	No	60
Clopidol	Yes	Replacements only	125
Decoquinat	Yes	No	30
Diclazuril	Yes	No	1
Dinitolmide (zoalene)	Yes	Replacements only	125
Halofuginone hydrobromide	Yes	No	3
Nequinat	Yes	Replacements only	20
Nicarbazin	Yes	No	125
Robenidine hydrochloride	Yes	No	33
Polyether ionophores:			
Lasalocid	Yes	No	75–125
Maduramicin	Yes	No	5–6
Monensin	Yes	Replacements only	100–120
Narasin	Yes	No	60–80
Narasin + nicarbazin	Yes	No	54–90 of the 2 drugs
Salinomycin	Yes	No	44–66
Semduramicin	Yes	No	25
Natural extract:			
Steroidal sapogenins ²	Yes	No	3.4–13.6

Notes: Products listed are those known to be used with some frequency in Europe, Latin America, Asia/Pacific region, and/or North America. The primary source of information was FDA 21 CFR part 558 subpart B, Specific New Animal Drugs for Use in Animal Feeds. In all instances, *local labeling should be consulted*.

¹Information obtained from European Medicines Evaluation Agency/Poultry Health Services Ltd. (<http://www.poultry-health.com/library/antimicrobials/eufeed99.htm>).

²Information obtained from Mathis (2001), Walker (2002), and the manufacturer (Distributors Processing Inc., 17656 Avenue 168, Porterville, CA 93257 U.S.A.).

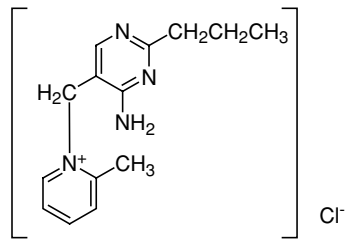


Figure 6.1. **Amprolium.**

lower than that of eggs from unmedicated hens (Stephens and Barnett 1970). The Haugh score means for both treatments, however, were higher than the minimum value needed for classification as Grade AA eggs.

Efficacy: Amprolium blocks the absorption of thiamine in coccidia at relatively low levels (Rogers 1962) and acts early in the asexual generation against the first through the second stage schizonts of coccidia (Warren and Ball 1963; Reid 1972). Morrison et al. (1961) reported that 125 ppm amprolium provided good efficacy based on percentage survival, weight gain, lesion score, and oocyst score against a mixed inoculum of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* in two studies with broiler chicks in battery cages. Amprolium at 125 ppm was highly efficacious in preventing *E. tenella* infection in birds inoculated with different levels of oocysts (McLoughlin and Gardiner 1962a). Some level of immunity development was also reported by these workers in the amprolium-medicated birds sufficient to prevent

coccidiosis mortality after a challenge inoculation. Peterson and LaBorde (1962) reported that amprolium at 25, 50, 75, 100, 125, and 150 ppm in the feed of White Rock chicks was highly efficacious against separate infections of *E. tenella* and *E. necatrix*. Amprolium given in the feed at 125 ppm was only moderately efficacious against *E. necatrix*, *E. brunetti*, *E. mivati*, and *E. maxima* in studies reported by Ryley (1967a), but efficacy against *E. tenella* and *E. acervulina* was excellent. The addition of liquid amprolium to the drinking water of replacement pullets on alternate days when amprolium-medicated feed was fed in a restricted (skip-a-day) feeding program provided good efficacy against *E. acervulina*, *E. maxima*, and *E. tenella* (Ruff, Chute, and Garcia 1991). The combination of 240 ppm amprolium and 180 ppm sulphaquinoxaline in the drinking water was found to be highly efficacious in treating infections of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* (Horton-Smith and Long 1965).

Resistance and partial resistance to amprolium by *Eimeria* isolates collected in the field have been reported in a number of instances in chickens (Warren, Ball, and MacKenzie 1966; Hodgson et al. 1969; Chapman 1976c, 1989c; Mathis, McDougald, and McMurray 1984; McDougald et al. 1987b; Rotibi, McDougald, and Solis 1989). In studies with field isolates of *E. acervulina* with reduced sensitivity to amprolium, the percentage sporulation of oocysts from amprolium-medicated

birds was comparable to the sporulation percentage of oocysts from unmedicated birds (Mathis and McDougald 1981). Laboratory studies have indicated that the development of resistance to amprolium occurred slowly and only partially even when a coccidial isolate was serially passed in chickens fed a diet containing suboptimal levels of the drug (McLoughlin and Gardiner 1968; Chapman 1978a, 1978b, 1982a, 1989b). Using four anticoccidial drugs in sequence in broiler chickens, McLoughlin and Chute (1975) produced a strain of *E. tenella* resistant to 125 ppm amprolium following exposure to the drug 10 times on an intermittent basis. Similar results were also reported by Tamas, Schleim, and Wilks (1991).

Amprolium + Ethopabate

Chemical name: [121-25-5] 1-[(4-amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride, hydrochloride (amprolium); and [59-06-3] 4-acetamido-2-ethoxybenzoic acid methyl ester (ethopabate).

Product name: Amprol Plus, Amprol Hi-E.

Chemical structure: See figure 6.1 for amprolium and figure 6.2 for ethopabate.

Safety: Means for final weights and feed conversion were not affected in broiler chickens fed the combination of 125 ppm amprolium + 4 ppm ethopabate for 9 weeks in a floor pen trial not

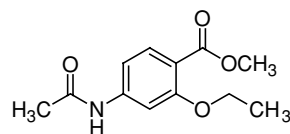


Figure 6.2. **Ethopabate.**

involving a coccidial infection (Morrison et al. 1967). In two experiments in which ethopabate was fed at varying levels from 10 to 300 ppm in combination with 100 ppm amprolium for 8 weeks, levels up to 100 ppm ethopabate were well tolerated (Kobow, Akkilič, and Lüders 1969). Higher ethopabate levels in combination with 100 ppm amprolium depressed weight gains.

Efficacy: Ethopabate is a folate antagonist that blocks a step in the synthesis of *p*-aminobenzoic acid (Rogers et al. 1964) and is combined with amprolium to improve the spectrum of efficacy. Morrison et al. (1967) demonstrated that the combination of 125 ppm amprolium + 4 ppm ethopabate was highly efficacious against single and mixed infections of *E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella* based on mortality, weight gain, lesion score, and oocyst score data. This combination was not efficacious against *E. brunetti* in two battery trials based on bird performance, mortality, and lesion score data (Kowalski and Reid 1972). The application of 240 ppm amprolium + 16 ppm ethopabate given in the drinking water (7.75% + 0.5% concentration) for 5 days beginning 2 days after infection was more

effective than toltrazuril or sulfaquinoxaline + pyrimethamine in reducing lesions caused by recent field isolates of *E. tenella* (Chapman 1989a). The combination of 125 ppm amprolium + 40 ppm ethopabate is recommended when the combination of *E. acervulina*, *E. maxima*, and *E. brunetti* is expected to occur (Miller Publishing Co. 2004).

Isolates of *E. tenella* and *E. acervulina* obtained from the field beginning in the 1970s were frequently resistant to 125 ppm amprolium + 4 ppm ethopabate in surveys conducted by Jeffers (1974b, 1974c) and Mathis and McDougald (1982). Less than a decade later, 46% of the field isolates collected from 99 broiler farms in the United States (12 states) were found to be resistant to this combination, and another 15% were partially resistant or manifested a reduced sensitivity (McDougald, Fuller, and Solis 1986). A large number of field isolates of *E. maxima* were also found to be resistant to this combination in a survey by Chapman (1980).

Amprolium + Ethopabate + Sulfaquinoxaline

Chemical name: [121-25-5] 1-[(4-amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride, hydrochloride (amprolium); [59-06-3] 4-acetamido-2-ethoxybenzoic acid methyl ester (ethopabate); and [59-40-5] 4-amino-N-2-quinoxalinybenzenesulfonamide (sulfaquinoxaline).

Product name: Pancoxin.

Chemical structure: See figure 6.1 for amprolium, figure 6.2 for ethopabate, and figure 6.12 for sulfaquinoxaline.

Safety: This combination was developed to take advantage of the synergistic benefits of the three chemicals at relatively safe levels of each drug by itself. Reviews of the safety of amprolium and ethopabate are provided above, and that of sulfaquinoxaline is given below.

Efficacy: The combination of 80 ppm amprolium + 60 ppm ethopabate + 5 ppm sulphaquinoxaline given in the feed was highly efficacious against single-species infections of *E. acervulina*, *E. maxima*, *E. mivati*, *E. necatrix*, *E. brunetti*, and *E. tenella* in studies by Ryley (1967a). Morrison et al. (1967) reported superior efficacy with this combination drug against a mixed infection of *E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella* in comparison with buquinolate, amprolium + ethopabate, and zoalene.

Field isolates of *E. acervulina*, *E. brunetti*, *E. maxima*, and *E. tenella* recovered from the United Kingdom were found to be resistant to this combination in separate studies by Warren, Ball, and MacKenzie (1966), Hodgson et al. (1969), and Chapman (1980). Many of these isolates were also cross resistant with other commonly used anticoccidial drugs.

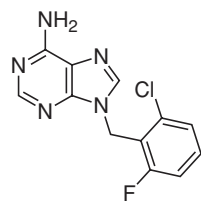


Figure 6.3. **Arprinocid.**

Arprinocid

Chemical name: [55779-18-5] 9-[(2-chloro-6-fluorophenyl)methyl]-9H-purin-6-amine; 9-(2-chloro-6-fluorobenzyl)adenine.

Product name: Arpocox.

Chemical structure: See figure 6.3.

Safety: Arprinocid had no adverse effect on weight gain or feed conversion (feed:gain) when fed continuously to broiler chickens for 8 weeks at 50 ppm, and only a small adverse effect on these measurements when fed at a level of 100 ppm (Miller et al. 1977).

Efficacy: Studies testing a range of doses in the feed demonstrated that 60 ppm or greater of arprinocid was highly efficacious in preventing infections of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* (Miller et al. 1977). Efficacy against *E. tenella* was improved in these studies, based on the reduction in mean lesion scores and oocyst counts, at dose levels of

70 and 80 ppm arprinocid. Further battery efficacy and floor pen studies with broiler chickens by a number of workers confirmed these findings and demonstrated that 60 to 70 ppm of arprinocid was the optimum dose range (Muniz et al. 1977; Ortega and Muniz 1977; Kilgore et al. 1978; Olson et al. 1978; Ruff, Anderson, and Reid 1978; Schindler et al. 1979; Schroder, Smith, and Harvey 1980). It was also reported that oocysts of *E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella* harvested from birds fed different levels of arprinocid, even at levels substantially below the proposed use level of 60 ppm, had a decreased capacity to sporulate (Solis and Muniz 1977; Tamas et al. 1978). Related work by Ruff, Anderson, and Reid (1978) demonstrated that oocysts of *E. acervulina*, *E. brunetti*, and *E. tenella* recovered from medicated birds and subsequently sporulated were less infective when inoculated into susceptible chicks than oocysts obtained from unmedicated chicks. Studies evaluating the efficacy of arprinocid against different developmental stages of *Eimeria* species *in vivo* found the highest activity against the first and second asexual generations (McManus, Olson, and Pulliam 1980; McQuiston and McDougald 1981). These results indicated that arprinocid was predominantly coccidiocidal in action. Mode-of-action studies indicated that arprinocid inhibited nucleic acid formation by blocking the hypoxanthine transport across parasite cell membranes during parasite development in the host (Wang, Simashkevich, and Stotish 1979; Wang et al.

1979; Wang and Simashkevich 1980). Studies by Wang, Simashkevich, and Fan (1981) have also indicated that cytochrome P-450 mediated microsomal metabolism involving arprinocid-1-N-oxide, a metabolite of arprinocid, may be part of the mechanism of action of arprinocid, causing a destruction of the parasite endoplasmic reticulum leading to cell death.

Drug resistance to arprinocid was reported for the first time in England in an *E. tenella* isolate from a broiler flock where the anticoccidial drug had been used for seven successive growing cycles (Chapman 1982a). Laboratory tests with this isolate demonstrated that arprinocid was not effective at 60 and 75 ppm, but at 90 ppm arprinocid, mortality was reduced in comparison to the infected, unmedicated treatment. Field isolates of *E. acervulina*, *E. maxima*, and *E. tenella* collected from farms in Holland and Germany where arprinocid had been used during three of four growing cycles were found to be resistant to the 60 ppm level of medication (Braunius, Greuel, and Sézen 1984). A similar picture emerged in France where resistant isolates of *E. acervulina* and *E. tenella* were recovered from poultry farms within the first three years of the official registration of arprinocid (Hamet 1986).

Clopidol (meticlorpindol, clopindol)

Chemical name: [2971-90-6] 3, 5-dichloro-2, 6-dimethyl-4-pyridinol.

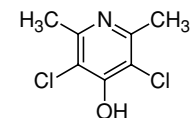


Figure 6.4. **Clopidol (meticlorpindol, clopindol).**

Product name: Coyden 25.

Chemical structure: See figure 6.4.

Safety: The effects of 125, 250, and 500 ppm clopidol on egg production, egg size, fertility, and hatchability were assessed in layer birds in a three-generation life-cycle study and a two-year dietary study (Bucek 1969). Egg production, fertility, and hatchability were not influenced by clopidol, but egg size was smaller in the 125 and 250 ppm clopidol treatments in comparison with the control treatment.

Efficacy: Stage-of-action studies testing the efficacy of clopidol against *E. tenella* demonstrated a coccidiostatic action against the parasite rather than a cidal effect (Ryley 1967b). Only the sporozoite was affected after it invaded the host cell, and development resumed just as soon as clopidol was withdrawn from the feed. Long and Millard (1968) reported a similar outcome in work with *E. acervulina*, *E. mivati*, *E. maxima*, *E. brunetti*, *E. praecox*, and *E. tenella*. When medication was not initiated until 48 hours or later after oocyst inoculation, parasite development was relatively unaffected. Clopidol needs to be in the feed on the

day of infection or inoculation to obtain full anticoccidial activity (Reid 1972). Evidence that oocyst sporulation of *E. tenella* was depressed by continuous medication with 62.5 ppm clopidol was reported by Arakawa et al. (1991).

Studies involving infections of *E. acervulina*, *E. mivati*, *E. praecox*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* demonstrated that 125 ppm clopidol was highly efficacious in maintaining weight gain, preventing mortality, and reducing lesions caused by these species of coccidia (Long and Millard 1967; Stock, Stevenson, and Hymas 1967). Long and Millard (1967) reported that the efficacy of clopidol against *E. acervulina* was improved substantially at 250 ppm in comparison with 125 ppm. Ryley (1967a) also reported improved efficacy against severe infections of *E. acervulina*, *E. maxima*, and *E. mivati* at 250 ppm clopidol in comparison with 125 ppm. The efficacy of 125 ppm clopidol against severe mixed infections of *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* was greater than 125 amprolium + 4 ppm ethopabate, 125 ppm nicarbazin, and 125 ppm zoalene as measured by bird performance and clinical signs of disease (Reid and Brewer 1967). A series of studies by Norton and Joyner (1968) confirmed that 125 ppm clopidol was coccidiostatic in action against laboratory strains of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella*, and demonstrated that the level of efficacy varied among species. Based upon oocyst shedding and

weight gain data, *E. brunetti* was the most susceptible species to control by clopidol, while efficacy against *E. acervulina* and *E. necatrix* was reduced in that some oocyst shedding was observed. *E. maxima* infections in medicated birds resulted in oocyst shedding and some weight loss, and infection of medicated birds with *E. tenella* resulted in fairly high oocyst counts and substantially reduced weight gains. Large scale tests in the United States and United Kingdom demonstrated that 125 ppm clopidol provided superior performance in comparison with other anticoccidial drugs under a range of field conditions (Stock, Stevenson, and Hymas 1967).

Field isolates of *Eimeria* spp. from chickens showing resistance to 125 ppm clopidol have been described in several reports (Jeffers 1974a, 1974b, 1974c; Oikawa et al. 1975; Chapman 1976c, 1980; Mathis and McDougald 1982; McDougald, Fuller, and Solis 1986; McDougald et al. 1987; Chapman and Hacker 1994). Jeffers and Challey (1973) demonstrated that selection for clopidol resistance in two laboratory strains of *E. acervulina* resulted in increased sensitivity to 4-hydroxyquinoline drugs with resistance to clopidol. On the contrary, subsequent selection for resistance to decoquinate in those strains did not produce resistance to clopidol. In laboratory studies with *E. tenella*, the number of generations needed to develop resistance to clopidol was found to range from 7 to 13 passages (Chapman 1978b).

Clopidol + nequinat (methyl benzoquat)

Chemical name: [2971-90-6] 3, 5-dichloro-2,6-dimethyl-4-pyridinol (clopidol); and [13997-19-8] 6-butyl-1, 4-dihydro-4-oxo-7-(phenylmethoxy)-3-quinolinecarboxylic acid methyl ester (nequinat).

Product name: Lerbek.

Chemical structure: See figure 6.4 for clopidol and figure 6.17 for nequinat.

Safety: Use of these two drugs in combination at levels slightly below the recommended level of each drug when used alone provided improved safety and efficacy (Küther 1987).

Efficacy: Clopidol and nequinat are structurally related in that both chemicals possess a pyridone ring. Nequinat is a member of the 4-hydroxyquinoline class (see below) and must be in the feed at the time of initial infection to obtain optimum efficacy (Ryley 1967b; Reid 1972). Evidence indicates that this combination (100 ppm clopidol + 8 ppm nequinat) acts synergistically, giving a greater level of protection against coccidial infection than either drug by itself (Ryley 1975).

Field isolates of *E. acervulina* and *E. tenella* resistant to 100 ppm clopidol + 8 ppm nequinat were collected from broiler farms in Holland and

Germany (Braunius, Greuel, and Sézen 1984) and France (Hamet 1986) within a few years of product introduction. *E. maxima* field isolates resistant to this combination were reported by Chapman (1980), but isolates sensitive to one of the two drugs in the combination were sensitive to Lerbek.

Folate antagonists and inhibitors

This class of anticoccidial compounds is composed of ethopabate (see amprolium + ethopabate above), a select group of 2,4-diaminopyrimidines (DAPs), and a number of sulfonamides. As described in a review by Bevill (1988), the enzyme dihydrofolate reductase (DHFR) catalyzes the reduction of dihydrofolic acid to tetrahydrofolic acid. The latter is the biologically active form of folic acid and has an important role in the synthesis of purines and pyrimidines in coccidial parasites. Some DAP derivatives inhibit the action of DHFR, and consequently have a lethal effect on bacteria or protozoa as a result of thymine deficiency. Sulfonamides act by inhibiting the enzyme-catalyzed condensation of *p*-aminobenzoic acid (PABA) and 2-amino-4-hydroxytetrahydropteridine, an essential step in the synthesis of folic acid. Lux (1954) demonstrated that the combination of an active DAP with an active sulfonamide drug was clearly synergistic in efficacy against *E. tenella*. The levels of both drugs could be reduced substantially when given in combination without affecting the overall

efficacy of either drug alone at much greater levels. Studies on the time of peak activity of sulfonamides and the combination of ormetoprin + sulfadimethoxine indicated that second-generation schizonts are most affected by the action of these drugs (Reid 1973). An important concern associated with the use of sulfonamide drugs has been their potential for toxic effects in chickens at levels that are close to or within the range of their recommended use levels, especially at dose rates recommended for therapeutic treatment (Peckham 1978; Julian 1991). Care in estimating feed and/or water consumption in preparing an accurate therapeutic dosage is critically important. Signs of toxicity due to sulfonamide drugs are generally associated with hemorrhagic syndrome, bone marrow depression, thrombocytopenia, and depression of the immune system.

Since folate antagonists and inhibitors were among the first anticoccidial drugs to be used in commercial poultry production, it is no surprise that the first reports of drug-resistant *Eimeria* isolates from chickens involved members of this class (Waletzky, Neal, and Hable 1954; Tsunoda 1963; Warren, Ball, and MacKenzie 1966; Hodgson et al. 1969; Chapman 1976c; Mathis and McDougald 1982; Mathis, McDougald, and McMurray 1984). Resistant or partially resistant species identified most frequently in these studies were *E. tenella* and *E. acervulina*, but resistant isolates of *E. maxima* and *E. brunetti* were also recovered in the

studies by Warren et al. (1966) and Hodgson et al. (1969).

Although a substantial number of compounds in this group were tested over the years, those included in the following review did attain some level of commercial use.

Diaminopyrimidine

Chemical name: [5355-16-8] 5-[(3, 4-dimethoxyphenyl)methyl]-2, 4-pyrimidinediamine (diaveridine); [58-14-0] 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine); and [6981-18-6] 2, 4-diamino-5-(6-methylveratryl) pyrimidine (ormetoprim) used in combination with sulfonamides for improved efficacy.

Product name: Darvisul (sulfaquinoxaline + diaveridine); Whitsyn 10, 5, and S (sulfaquinoxaline + pyrimethamine); and Rofenaïd 40 (sulfadimethoxine + ormetoprim).

Chemical structure: See figure 6.5 for diaveridine, figure 6.6 for pyrimethamine, and figure 6.7 for ormetoprim.

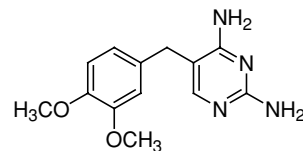


Figure 6.5. **Diaveridine.**

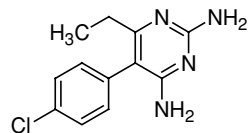


Figure 6.6. **Pyrimethamine.**

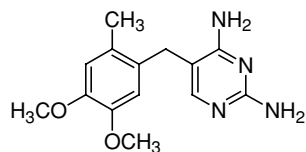


Figure 6.7. **Ormetoprim.**

Safety: Pyrimethamine has been reported to adversely affect growth, feathering, and development of the hock joint (perosis) when given to chickens in the ration at levels of 25, 50, and 100 ppm (Arundel 1959). Red cell counts and hemoglobin were also adversely affected in chickens at the 50 and 100 ppm levels of pyrimethamine in this work. The level of pyrimethamine in finished feed in the product Whitsyn 5 falls in the lower end of this range (30 ppm) and as a consequence may adversely impact bird performance (Gardiner and McLoughlin 1963a). The combination of sulphaquinoxaline + diaveridine (Darvisul) fed to growing chicks at levels up to 8 times the recommended levels of 80 ppm sulphaquinoxaline + 10 ppm diaveridine did not adversely affect weight gain in a 7-week study with layer chicks (Clarke 1962). The feeding of Rofenaïd at its use level (125 ppm sulfadimethoxine + 75 ppm ormetoprim), and at 2 and 4 times

the use level, showed no adverse effects on bird performance and health after 8 weeks, nor on egg production, hatchability, or egg quality when fed to layer birds from day of hatch to 20 weeks (Marusich et al. 1969). The feeding of ormetoprim at very high levels (500 ppm) to laying hens resulted in drug residues in the eggs within 4 days of onset of medication (Furusawa 2001).

Efficacy: Studies by Lux (1954) demonstrated that a number of diaminopyrimidines had significant activity in preventing *E. tenella* infection. The most active antifolics in the various analogs examined by Lux were the *p*-chloro derivatives of the 5-phenyl series. Studies by Arundel (1959) demonstrated that the efficacy of 1,000 ppm sulfaguandine, 200 ppm sulfapyrazine, or 500 ppm sulfadimidine was substantially improved when given in combination with 50 ppm pyrimethamine based on chick mortality and weight gain data. In these studies, the feeding of the medicated diets did not begin until 48 hours after coccidial inoculation because of concerns about the toxic effects of 50 ppm pyrimethamine when fed for more than 3 to 4 days.

Studies by Clarke (1962, 1964) indicated that a mixture of 10 ppm diaveridine + 80 ppm sulphaquinoxaline (Darvisul) administered in the feed beginning 1 or 2 days before infection was highly efficacious against single-species infections of *E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix*, and *E. tenella* in studies with layer-type

chickens. Ball and Warren (1965) reported that the combination of 0.0043% weight/volume (w/v) diaveridine + 0.005% w/v sulfaquinoxaline given in the drinking water was just as effective as 0.043% w/v sulfaquinoxaline in the prevention of infection by *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* in chickens based on various measurements including bird performance, oocyst output, and mortality data. Short-term continuous treatment, particularly beginning two or three days after infection, provided the best overall efficacy. The combination of 30 ppm pyrimethamine + 100 ppm sulfaquinoxaline (Whitsyn 5) provided excellent efficacy against *E. tenella* based on mortality data and pathologic index, but weight gain means in both infected and uninfected treatments were depressed (Gardiner and McLoughlin 1963a). It was concluded by these workers that the weight gain depression was due to the toxic effect of pyrimethamine. In the same studies, the combination of these two drugs at reduced levels (Whitsyn 10) was much less efficacious. Whitsyn S given in the drinking water (0.0065% concentration) of broiler chickens for six consecutive days beginning two days after infection with isolates of *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mivati*, *E. necatrix*, and *E. tenella* (two isolates) provided moderate to good efficacy in comparison with a number of chemical anticoccidial drugs (Mitrovic and Schildknecht 1973). One of the *E. tenella* isolates used in this series of trials was a sulfonamide-resistant strain that caused 15% to 45% mortality in the

treatments medicated with various sulfonamide drugs, but mortality was totally prevented by the combination product. In a more recent study, the combination of sulfaquinoxaline + pyrimethamine in the drinking water (45 + 14 ppm) for 3 days beginning 48 hours after inoculation was less efficacious than amprolium + ethopabate in reducing lesions and improving weight gain in chickens infected by recent field isolates of *E. tenella* (Chapman 1989a).

Battery and floor pen trials with 125 ppm sulfadimethoxine + 75 ppm ormetoprim (Rofenaid) given continuously in the feed beginning 48 hours before infection was reported by Mitrovic, Schildknecht, and Fusiek (1969) to be highly efficacious against single- and mixed-species infections of *E. acervulina*, *E. mivati*, *E. brunetti*, *E. maxima*, *E. necatrix*, and *E. tenella* based on performance data, mortality prevention, and average degree of infection. These results were subsequently confirmed in a series of well-replicated battery efficacy trials conducted at three different locations in the United States by Orton and Hambly (1971).

Sulfadimethoxine

Chemical name: [122-11-2] 4-amino-N-(2, 6-dimethoxy-4-pyrimidinyl) benzenesulfonamide; N¹-(2, 6-dimethoxy-4-pyrimidinyl) sulfanilamide.

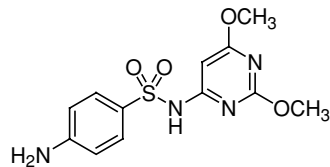


Figure 6.8. **Sulfadimethoxine.**

Product name: Agribon, Albon, Rofenaid 40 (a 5:3 mixture with ormetoprim), and many other names.

Chemical structure: See figure 6.8.

Safety: Sulfadimethoxine given in the drinking water had no adverse effect on performance when given at levels up to 0.05% to broiler chickens (Mitrovic and Bauernfeind 1967). Higher levels appeared to be unpalatable, reducing water intake and weight gains as a consequence. Medication of laying hens with 500 ppm sulfadimethoxine in the feed resulted in drug residues in the eggs within 4 days after the start of feeding (Furusawa, 2001).

Efficacy: Administration of sulfadimethoxine in the water at a level of 0.05% beginning 3 days after infection and continuing for 6 days was highly efficacious against *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* in chickens (Mitrovic and Bauernfeind 1967). Some coccidiosis mortality (5%) did occur in the study with *E. necatrix*, indicating it may be the dose determining species. Sulfadimethoxine given in the drink-

ing water (0.05%) of broiler chickens for 6 consecutive days beginning two days after single-species infections with isolates of *E. acervulina*, *E. mivati*, *E. brunetti*, *E. maxima*, *E. necatrix*, and *E. tenella* (two isolates) provided excellent efficacy, with one exception, in comparison with a number of chemical anticoccidial drugs (Mitrovic and Schildknecht 1973). The exception occurred with one of the *E. tenella* isolates that was sulfonamide resistant and caused 15% mortality in the sulfadimethoxine treatment.

Sulfaguanidine

Chemical name: [57-67-0] 4-amino-N-(aminomethyl)benzenesulfonamide.

Product name: Diacta, Ganidan.

Chemical structure: See figure 6.9.

Safety: In a review by Peckham (1978), the toxic effects of 10,000 ppm sulfaguanidine feeding to growing chickens for 8 days was reported to include hemorrhagic infarcts, necrosis, and swelling of the spleen. Sulfaguanidine was the least toxic in

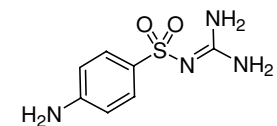


Figure 6.9. **Sulfaguanidine.**

this respect compared with 5,000 ppm sulfamethazine and 5,000 ppm sulfamerazine.

Efficacy: The continuous feeding of 5,000 ppm sulfaguanidine beginning 3 days before to 14 days after infection of chicks with *E. tenella* markedly reduced mortality (Allan and Farr 1943). Medication via the feed with 10,000 ppm sulfaguanidine for 3 days following infection with *E. tenella* reduced coccidiosis mortality in comparison with the unmedicated treatment by 76% in one study and 97% in a second study reported by Seeger (1946). Sulfaguanidine was less efficacious than 4,000 ppm sulfamethazine for 3 days in these studies. Sulfaguanidine medication in the feed at 10,000 ppm was less efficacious in preventing mortality due to *E. tenella* infection than 2,500 ppm sulfamethazine, and medication had to be initiated within 72 hours after oocyst inoculation to obtain optimum results (Barber 1948).

Sulfamethazine (Sulphamezathine, Sulfadimerazine, Sulfadimidine)

Chemical name: [57-68-1] 4-amino-N-(4, 6-dimethyl - 2 - pyrimidinyl) benzenesulfonamide; N¹-(4,6-dimethyl-2-pyrimidinyl)sulfanilamide.

Product name: Diazil, Sulfadine, Sulmet, S-Dimidine.

Chemical structure: See figure 6.10.

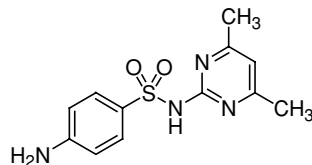


Figure 6.10. **Sulfamethazine (sulphamezathine, sulfadimerazine, sulfadimidine).**

Safety: One percent (10,000 ppm) sulfamethazine given in the diet to uninfected birds for 3 days reduced average weight gain by 40% in comparison with unmedicated, uninfected birds (Hawkins and Kline 1945). In a review by Peckham (1978), the toxic effects of 5,000 ppm sulfamethazine fed to growing chickens for 8 days included hemorrhagic infarcts, necrosis, and swelling of the spleen. Sulfamethazine was also found to reduce egg production in laying birds (Peckham 1978). Bird performance was normal in a 6-week toleration study involving the feeding of 2,500 ppm sulfamethazine from 1 day of age (Tugwell 1955).

Efficacy: Sulfamethazine given in the ration at levels of 1% to 2%, or in a saturated solution in place of the drinking water, was found to be effective in reducing mortality due to severe infections of *E. tenella* (Horton-Smith and Taylor 1942, 1943, 1945). Medication was initiated in these studies as soon as the first deaths from acute coccidiosis were observed. Additional studies in the latter report by these authors demonstrated

that surviving medicated birds had acquired a strong immunity to *E. tenella*. Subsequent studies by Horton-Smith (1948) demonstrated that the action of sulfamethazine against *E. tenella* was directed against the second generation schizonts by inhibiting nuclear division and the formation of merozoites. Medication of chickens in the feed with 2,000 to 4,000 ppm sulfamethazine for a minimum of 2 days following infection with *E. tenella* was found to be highly efficacious in preventing mortality due to coccidial infection (Seeger, 1946). Continuous feeding of sulfamethazine at a level of 2,500 ppm provided good efficacy in preventing mortality caused by *E. tenella* in growing chickens, but medication had to begin no later than 72 hours after inoculation to achieve satisfactory efficacy (Barber 1948). In contrast, a study by Tugwell (1955) demonstrated that 2,500 ppm sulfamethazine given in the ration beginning 7 days before infection failed to prevent high mortality (25.3%) in chickens due to *E. tenella*. Sulfamethazine given in the drinking water (0.1%) of broiler chickens for six consecutive days beginning two days after single-species infections with isolates of *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mivati*, *E. necatrix*, and *E. tenella* (two isolates) provided excellent efficacy, with one exception, in comparison with a number of chemical anticoccidial drugs (Mitrovic and Schildknecht 1973). The exception occurred with one of the *E. tenella* isolates that was sulfonamide resistant, and caused 20% mortality in the sulfamethazine treatment.

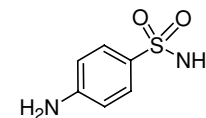


Figure 6.11. **Sulfanilamide.**

Sulfanilamide

Chemical name: [63-74-1] 4-aminobenzenesulfonamide; *p*-anilinesulfonamide; *p*-sulfamidoaniline.

Product name: Prontosil, Prontylin.

Chemical structure: See figure 6.11.

Safety: The weight gain of growing chickens was depressed by the inclusion of sulfanilamide in the daily ration at levels ranging from 1,000 to 5,000 ppm (Levine 1939). All levels of medication depressed bird performance, and the degree of weight depression was directly proportional to the level of drug in the feed. The adverse effects of sulfanilamide reported by a number of workers included reduced egg production and poor egg shell development in laying birds (Peckham 1978).

Efficacy: The first description of the efficacy of sulfonamide drugs against coccidiosis in chickens was reported by Levine (1939). In this seminal work, Levine demonstrated that sulfanilamide given daily at the rate of 0.3 g/bird was highly efficacious against *E. acervulina*, *E. praecox*,

E. mitis, *E. hagani*, and *E. maxima* based upon the suppression of oocyst counts during the course of infection. Once medication was discontinued, oocysts appeared in the feces but the peak oocyst counts were considerably lower than counts recorded in control birds. Sulfanilamide was not effective when given at the rate of 0.3 g daily against infections of *E. tenella* and *E. necatrix* based upon the severity of lesions in the medicated birds and mortality. In separate studies with *E. mitis*, *E. praecox*, and *E. hagani*, sulfanilamide was fed in the mash at a level of 3,000 ppm, and the “coccidiostatic effect was evidenced as long as the drug was fed” (Levine 1939). Levine concluded from this work that some derivative of sulfanilamide may become available that will provide a comparable level of efficacy without the toxic effects on performance.

Ball (1966b) reported that sulfanilamide was highly efficacious when given in the feed at levels of 1,000 and 2,000 ppm in preventing oocyst production in separate groups of chickens inoculated with two different strains of *E. acervulina*. Efficacy against a third strain was variable, with good efficacy at the higher use level.

Sulfaquinoxaline

Chemical name: [59-40-5] 4-amino-N-2-quinoxalinylnbenzenesulfonamide; N¹-(2-quinoxalinyln) sulfanilamide.

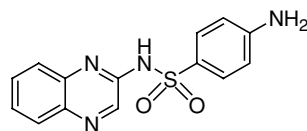


Figure 6.12. **Sulfaquinoxaline.**

Product name: Aviochina (sodium salt), Quina-trol, SQ, Sulquin.

Chemical structure: See figure 6.12.

Safety: The feeding of 2,500 ppm sulfaquinoxaline (SFQ) causes a severe anemia in chickens with hemorrhages on the legs, breast muscle, and in abdominal organs (Merck & Co. 1991). Toxicity is more likely to be observed when medication is given in the water during hot weather. Feeding 300 ppm SFQ to growing chickens for 8 weeks reduced the weight gain of female birds (Singsen, Scott, and Matterson 1948), but adverse effects were not observed when SFQ was administered to growing chickens at 500 ppm in various feeding schedules (Grumbles, Delaplane, and Higgins 1948b; Cuckler and Ott 1955). Continuous feeding of 500 ppm SFQ indicated that this level of medication may be slightly toxic for male chicks (Bletner et al. 1953). Subsequent studies showed that medication of growing chicks with 125 ppm SFQ in the feed for 6 weeks caused a slight decrease in feed consumption and weight, and lesions in the liver, bile duct, kidney, bone marrow, and thyroid gland (Newberne and Buck

1956). A decrease in the level of glucocorticoids in the adrenal glands of broiler chickens was observed when 125 ppm SFQ was fed for 8 weeks (Brown 1958). Administration of 0.025% SFQ in the drinking water continuously for up to 12 weeks had no adverse effects on growing chickens in studies by Cuckler and Ott (1955), but a concentration of 0.0645% SFQ in the drinking water for 5 days was toxic for chickens (Peckham 1978). Studies by Grumbles, Delaplane, and Higgins (1948b), Dickinson (1949), and Smith, Delaplane, and Wiley (1952) have demonstrated that feeding at levels up to 500 ppm SFQ will not affect egg production or hatchability when given to laying birds. The blood clotting time was significantly increased in New Hampshire chicks fed 300 ppm SFQ continuously from 1 day to 4 weeks of age (Sweet, Romoser, and Combs 1954), and similar results were reported by Cuckler and Ott (1955) after feeding 4,000 ppm SFQ continuously to chickens for 3 weeks. The feeding of 2,500 ppm SFQ to layer birds for 8 to 10 days was found to adversely affect egg production and bird performance (Peckham 1978).

Efficacy: Delaplane, Batchelder, and Higgins (1947) first reported that SFQ given in the diet at 500 and 1,000 ppm was highly efficacious in preventing mortality due to *E. tenella*. According to these workers, the greatest protection was achieved when medication was initiated from 1 day before or up to 2 days after inoculation. Cuckler and Ott (1947) found that 1,000 ppm SFQ

in the diet had a lethal effect on sporozoites and first generation merozoites of *E. tenella*, but efficacy was not complete. They observed that second generation schizonts produced merozoites, but the second generation merozoites appeared to be degenerated. Third generation schizonts, gametocytes, and oocysts did not develop in medicated chicks in their studies. Use of SFQ on a prophylactic basis at the level of 500 ppm in the feed 2 days of each 5 gave good protection against *E. tenella* and *E. necatrix* in broiler birds (Grumbles, Delaplane, and Higgins 1948b). In subsequent studies, these same workers (Grumbles, Delaplane, and Higgins 1948a) reported that continuous feeding of 125 ppm SFQ was highly efficacious in preventing naturally acquired cecal and intestinal coccidiosis (predominantly *E. tenella* and *E. necatrix*) in broiler chickens on a commercial farm. In contrast, a study by Tugwell (1955) demonstrated that 125 ppm SFQ given in the ration beginning 7 days before infection failed to prevent high mortality (17%) in chickens due to *E. tenella*. SFQ use levels of 150, 300, and 500 ppm provided good efficacy against *E. acervulina* in studies with layer pullets (Dickinson, 1949). Horton-Smith and Long (1959b) found that 125 and 500 ppm SFQ in the diet beginning 2 days before infection was only moderately efficacious in preventing mortality caused by a severe *E. necatrix* infection. Excellent efficacy was observed at these levels in the diet by the same workers against a severe infection of *E. acervulina*. Studies by Norton and Joyner (1968) demonstrated that 125 ppm SFQ,

also given in the ration beginning 2 days before infection, was highly efficacious against single-species infections of *E. acervulina*, *E. maxima*, and *E. brunetti* based on weight gains and oocyst counts. Ball (1966b) reported that SFQ was highly efficacious when given in the feed at levels of 30 and 60 ppm in preventing oocyst production in separate groups of chickens inoculated with two different strains of *E. acervulina*. A third strain of *E. acervulina* (Andover) required a much higher level of SFQ (250 ppm) to obtain comparable efficacy. In the same series of studies by Ball, high efficacy against different strains of *E. tenella* required use levels of 250 ppm or greater. The overall efficacy advantages of sulfaquinoxaline in comparison with other sulfonamides were attributed to the fact that it is more readily absorbed than other sulfonamides when given in the feed (White and Williams 1983).

Medication with 0.04% SFQ via the water beginning no later than 24 hours after infection with *E. necatrix* and continuing for a minimum of 5 days after infection was highly efficacious in preventing mortality and sustaining good weight gain (Peterson and Hymas 1950). Peterson and Hymas (1950) found that a dose rate of 0.03% SFQ via the water was needed to achieve maximum therapeutic efficacy. SFQ given in the drinking water (0.04%) of broiler chickens for 6 consecutive days beginning 2 days after single-species infections with isolates of *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mivati*, *E. necatrix*, and *E. tenella*

(two isolates) was only moderately efficacious, with one exception, in comparison with a number of chemical anticoccidial drugs (Mitrovic and Schildknecht 1973). In one trial involving a sulfonamide-resistant isolate of *E. tenella*, SFQ was not efficacious. White and Williams (1983) reported that a combination of 33 mg/l of trimethoprim + 99 or 100 mg/l of sulfaquinoxaline given for 5 days in the water beginning on the day of infection, or within 24 to 48 hours following infection, provided good efficacy against infections of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella*.

Glycarbylamide

Chemical name: [83-39-6] 1H-imidazole-4,5-dicarboxamide.

Product name: Glycamide.

Chemical structure: See figure 6.13.

Safety: The feeding of glycarbylamide to White Leghorn hens at levels ranging from 200 to 1,600 ppm for 21 days caused a decrease in fertility and

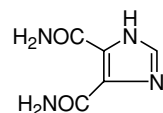


Figure 6.13. Glycarbylamide.

embryo mortality at levels in excess of 400 ppm (Polin, Porter, and Cobb 1961).

Efficacy: Horton-Smith and Long (1959a) reported that 30 ppm glycarbylamide given in the feed beginning 48 hours before inoculation with a severe challenge of either *E. tenella* or *E. necatrix* was highly efficacious in preventing mortality in 23-day-old chickens. These results were consistent with the outcome of studies with *E. tenella* reported by McLoughlin and Chester (1959). Efficacy of glycarbylamide was substantially reduced or ineffective if medication was delayed to 3-day-old infections. These workers also found that the efficacy of 30 ppm glycarbylamide against *E. acervulina* was substantially less than either 125 ppm nicarbazin or 125 ppm sulphaquinoxaline based on oocyst counts up to the 15th day of infection. Medication of chicks with 30 ppm glycarbylamide beginning 24 hours prior to infection was highly efficacious against *E. tenella* based on bird performance, mortality percentage, and mean cecal lesion scores (McLoughlin, Gardiner, and Chester 1960). Peterson (1960) reported that 30 ppm glycarbylamide in the feed provided complete or nearly complete protection against infections by either *E. tenella* or *E. necatrix* in 3-week-old chicks.

Resistance to 30 ppm glycarbylamide developed in strains of *E. tenella* in less than 10 passages in laboratory tests conducted by McLoughlin and Gardiner (1961), and was evident by the 12th pas-

sage in work by Ball (1966a). Subsequent studies by Gardiner and McLoughlin (1963b) with the glycarbylamide-resistant strain of *E. tenella* demonstrated that sensitivity to glycarbylamide did not increase even following serial propagation of the isolate nine times in unmedicated birds.

Halofuginone hydrobromide

Chemical name: [64924-67-0] *rel*-7-bromo-6-chloro-3-[3-[(2R, 3S)-3-hydroxy-2-piperidinyl]-2-oxopropyl]-4(3H)-quinazolinone hydrobromide.

Product name: Stenorol.

Chemical structure: See figure 6.14.

Safety: Use of 3 ppm halofuginone in the diet of growing chickens resulted in a greater incidence ($P \leq 0.05$) of skin rips in female birds at the processing plant in comparison with birds given 100 ppm monensin (Angel et al. 1985). A subsequent study by Casey, Crosley, and Smith (1992) testing the effect of 0, 1.5, 3, and 6 ppm halofuginone in the diet on skin tensile strength in two strains of broiler chickens demonstrated that the

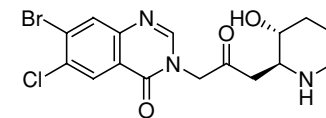


Figure 6.14. **Halofuginone hydrobromide.**

use level was inversely correlated with skin tensile strength in both sexes of the two strains. The adverse effect of halofuginone on broiler skin tensile strength could be reversed by observing a 7-day withdrawal period at the end of the growing cycle or by using halofuginone in the starter feed and changing to a different anticoccidial drug in the grower/finisher period in work by Crosley et al. (1992), but subsequent studies by Pinion, Bilgili, and Hess (1995) and Pinion et al. (1995) demonstrated that the use of 3 ppm halofuginone continuously or only in the starter feed decreased skin strength and increased the incidence of skin tears during processing. Higher dietary levels of proline or supplementation with ascorbic acid and zinc did not prevent the adverse effects of halofuginone on skin quality in the study by Pinion, Bilgili, and Hess (1995). This negative effect of halofuginone on skin strength appears to be due to interference with collagen synthesis (Christensen et al. 1994). Feeding of 3 ppm halofuginone to pullet and layer chickens had no adverse effect on pullet growth, nor on egg production percentages, average egg weight, shell coloring, fertility, hatchability, and hatching weight of chicks (Hamet 1979a, 1979b). Halofuginone medication via the feed at levels of 3, 6 and 9 ppm reduced body weight in a 56-day floor pen trial, with a significant ($P < 0.01$) linear regression for this effect (Morrison, Ferguson, and Leeson 1979).

Efficacy: Early studies with halofuginone demonstrated that a use level of 3 ppm in the diet pro-

vided complete protection against infections of *E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella* in chickens (Yvoré et al. 1974). Comparable results were reported by Morrison, Ferguson, and Leeson (1979) using single and mixed infections of the same species, and by Ryley and Wilson (1975) in laboratory studies involving severe infections of *E. necatrix*, *E. brunetti*, and *E. tenella*. Halofuginone was highly efficacious against recent field isolates of *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* when given at 3 ppm in the ration of broiler chickens in battery cages based on bird performance, lesion score, dropping score, mortality, and oocyst production (Edgar and Flanagan 1979). These authors also found that halofuginone was not as effective against *E. acervulina* as against the other species, and that the drug effect for all species was cidal rather than static.

A small percentage of coccidia isolates collected from broiler houses in the southeastern United States was found to be resistant to 3 ppm halofuginone in a survey reported by Mathis and McDougald (1982), but a high percentage of field strains of *E. acervulina* and *E. tenella* collected from broiler farms in France from 1975 to 1984 was resistant to 3 ppm halofuginone (Hamet 1986). Many field isolates of *E. acervulina*, *E. maxima*, *E. brunetti*, *E. mitis*, and *E. tenella* collected from broiler farms in northern Germany were also found to be resistant to 3 ppm halofuginone (Stephan et al. 1997).

4-Hydroxyquinolines

Investigations by Ryley (1967b) demonstrated that buquinolate and methyl benzoquate act primarily to prevent development of the coccidial parasite rather than exerting a coccidiocidal effect. This work was confirmed in further studies by a number of workers (Brewer and Reid 1967; Challey and Johnson 1968; Leathem and Engle 1970), including work with decoquinolate. Subsequent studies by Wang (1975, 1976) indicated that anticoccidial drugs in this class act by inhibiting the respiration of coccidial sporocysts and sporozoites. Studies by Williams (1997) added further insight on the mode of action of decoquinolate by demonstrating that in addition to the inhibitory effect on sporozoites and trophozoites of *E. tenella* and *E. acervulina*, 40 ppm decoquinolate was cidal in action against first stage schizonts. While the inhibitory effect was found to be reversible upon withdrawal of the anticoccidial, confirming that the effect of drugs in this class is static rather than cidal upon the coccidial population in the host, the cidal effect of decoquinolate against some of the first stage schizonts partially reduces the residual level of infection.

Resistance development to members of this class of anticoccidial drugs became a major concern within a short time following their introduction for use in chickens (McManus, Campbell, and Cuckler 1968; Jeffers and Challey 1973; Jeffers, 1974a, 1974b, 1974c; Oikawa et al. 1975;

Chapman 1976c, 1980; Mathis and McDougald 1982). Strains found to be resistant in these studies included *E. acervulina*, *E. tenella*, *E. maxima*, *E. brunetti*, *E. necatrix*, and *E. praecox*. Laboratory studies subsequently demonstrated that resistance development to different strains of *E. tenella* generally occurred within eight or fewer passages (McLoughlin and Chute 1971, 1973a; Chapman 1975, 1978b, 1989b).

Buquinolate

Chemical name: [5486-03-3] 4-hydroxy-6,7-bis(2-methylpropoxy)-3-quinolinecarboxylic acid ethyl ester; 4-hydroxy-6,7-diisobutoxy-3-quinolinecarboxylic acid ethyl ester; ethyl 6,7-diisobutoxy-4-hydroxyquinoline-3-carboxylate.

Product name: Bonaid.

Chemical structure: See figure 6.15.

Safety: Feeding of buquinolate at 82.5 or 110 ppm in the ration for 2 months had no adverse effects on bird performance or mortality percentages of broiler chickens in floor pen trials (Lott 1966;

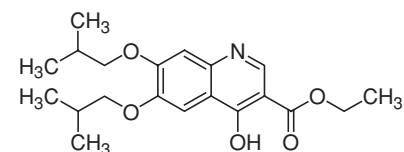


Figure 6.15. **Buquinolate.**

Raines 1968). The feeding of 70 ppm buquinolate to broiler chickens in floor pens had no adverse effect on means for final body weight or feed conversion after 9 weeks (Morrison et al. 1967). Buquinolate feeding to growing chickens from 1 day to 10 weeks of age at levels up to 440 ppm was not toxic according to Sadler et al. (1968). Pullets fed 110 ppm buquinolate during the growing period prior to production, or during both the growing and laying periods produced fewer ($P < 0.01$) eggs than pullets in the unmedicated treatment (Stephens, Barnett, and Butters 1967). Pullets in the unmedicated treatment required less feed per dozen eggs, but there were no differences between treatments in egg quality (Haugh units). Eggs were heavier from hens medicated during the laying period, but there were no differences between treatments in egg fertility or hatchability.

Efficacy: Administration of buquinolate in the feed at levels ranging from 55 to 110 ppm was highly efficacious against single and mixed infections of *E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella* (Morrison et al. 1967). In a study involving infections by a single species, means for coccidial lesion and oocyst scores were much lower for the first three species when compared with the data for *E. tenella*. Engle, Humphrey, and Johnson (1967) reported buquinolate provided excellent efficacy against these same species when used at levels of 82.5 and 110 ppm, and that levels as low as 27.5 ppm provided good efficacy against these species based on weight gain, mortality, and oocyst

count data. The efficacy of 82.5 ppm buquinolate against severe, mixed infections of *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* was superior to 125 ppm amprolium + 4 ppm ethopabate, 125 ppm nicarbazin, and 125 ppm zoalene based on performance and morbidity data (Brewer and Reid 1967). Studies by Ryley (1967a) demonstrated that buquinolate at dose levels of 62.5 and 125 ppm provided good to excellent protection against a severe infection of *E. tenella*, and that the efficacy of 82.5 and 125 ppm buquinolate was excellent against infections of *E. acervulina*, *E. maxima*, *E. brunetti*, and *E. mivati*. In a series of studies involving the above five species plus *E. praecox*, *E. mitis*, and *E. hagani*, 82.5 and 110 ppm buquinolate was highly efficacious against all eight species based upon bird performance and coccidiosis mortality (Edgar and Flanagan 1968). Sadler et al. (1968) reported that 82.5 ppm buquinolate provided good to excellent efficacy against *E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella* in comparison with 125 ppm amprolium + 4 ppm ethopabate, 30 ppm sulfanitran + 25 ppm nitro-mide + 5 ppm roxarsone, 125 ppm nicarbazin, and 125 ppm zoalene in separate studies with each species. Overall, buquinolate was more efficacious than the other anticoccidial drugs in these studies.

Decoquinolate

Chemical name: [18507-89-6] 6-decyloxy-7-ethoxy - 4 - hydroxy - 3 - quinolinecarboxylic acid

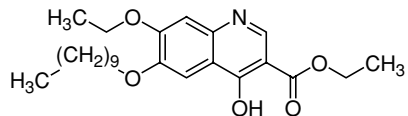


Figure 6.16. **Decoquate.**

ethyl ester; ethyl 6-(n-decyloxy)-7-ethoxy-4-hydroxyquinoline-3-carboxylate.

Product name: Deccox.

Chemical structure: See figure 6.16.

Safety: The metabolism of decoquate was evaluated in chickens and found to be poorly absorbed from medicated feed (Semán et al. 1989). The small amount of decoquate that was absorbed was rapidly cleared from blood and tissues.

Efficacy: The efficacy of 10, 20, and 40 ppm decoquate against *E. acervulina* "type," *E. maxima*, *E. brunetti*, and *E. tenella* was first described by Ball et al. (1968). Decoquate at 20 and 40 ppm was consistently superior in these tests to 125 ppm amprolium, 125 ppm clopidol, the combination of 80 ppm amprolium + 60 ppm sulfaquinoxaline + 5 ppm ethopabate, and 125 ppm zoalene. McLoughlin and Chute (1971) found that 30 ppm decoquate was highly efficacious against 10 different isolates of *E. tenella*, nine of which were resistant to other anticoccidial drugs. The efficacy of decoquate was reduced slightly against one isolate that was resistant to buquinolate. These

workers also found that resistance to decoquate could be demonstrated after six serial passes of an *E. tenella* isolate in birds given a suboptimal level (15 ppm) of decoquate during the first five passages. This isolate was also cross resistant to buquinolate.

Nequinate (Methyl Benzoquate)

Chemical name: [13997-19-8] 6-butyl-1,4-dihydro-4-oxo-7-(phenylmethoxy)-3-quinolinecarboxylic acid methyl ester; 7-(benzyloxy)-6-n-butyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid methyl ester.

Product name: Statyl.

Chemical structure: See figure 6.17.

Safety: Ryley (1967a) reported that the performance of birds fed nequinate at levels from 10 to 100 ppm for 10 weeks was not affected, and that egg production, egg weight, and shell color was not affected when laying hens were given nequinate at levels of 10 to 1,000 ppm for a period of 14 days. These results indicated that nequinate is a

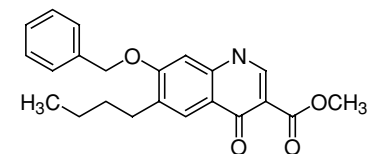


Figure 6.17. **Nequinate (methyl benzoquate).**

well-tolerated and safe compound for use in broiler chickens at a use level in the range of 10 to 20 ppm.

Efficacy: Ryley (1967a) reported that 10 ppm nequinatate was highly efficacious against severe infections of *E. tenella* based on mortality control and weight gain data, and at 10 to 20 ppm nequinatate was highly efficacious against *E. necatrix*, *E. acervulina*, *E. maxima*, *E. mivati*, and *E. brunetti* based on weight gain and oocyst count data. Studies by Long and Millard (1968) demonstrated that 2 ppm nequinatate inhibited the development of sporozoites of *E. acervulina*, *E. mivati*, *E. maxima*, *E. brunetti*, *E. praecox*, and *E. tenella*. Nequinatate use at 20 ppm provided excellent protection against susceptible strains of *E. tenella* in a number of studies in comparison with other anticoccidial drugs (McLoughlin and Chute 1973a).

Nicarbazin

Chemical name: [330-95-0] N, N'-bis(4-nitrophenyl)-urea, compounded with 4,6-dimethyl-2(1H)-pyrimidinone (1:1); 4, 4'-dinitrocarbanilide compounded with 4,6 dimethyl-2-pyrimidinol (1:1).

Product name: Nicarb, Nicoxin, and Nicrazin.

Chemical structure: See figure 6.18.

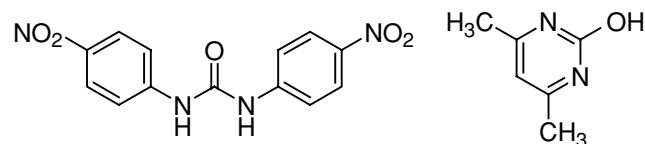


Figure 6.18. **Nicarbazin.**

Safety: Continuous medication with nicarbazine via the feed at 75, 150, and 300 ppm up to 12 weeks of age was well tolerated by growing chicks (Cuckler, Malanga, and Ott 1956). Medication at 600 ppm in this study resulted in depressed weight gain but did not cause any mortality or signs of toxicity. Early studies by Ott et al. (1956) and Sherwood, Milby, and Higgins (1956) demonstrated that nicarbazine in the feed of breeder or layer birds may adversely affect egg-shell pigmentation, egg production, and egg hatchability depending on the level of nicarbazine fed. In addition, levels of nicarbazine as low as 50 ppm in the feed of layer birds caused extensive mottling of egg yolks (Polin, Ott, and Siegmund 1957). More recently, Jones et al. (1990) concluded from a study in broiler breeders fed nicarbazine at levels of 20, 50, and 100 ppm that as the level of nicarbazine increased there was a linear decrease in hatchability, and that egg-shell depigmentation was directly related to the level of nicarbazine fed starting at 50 ppm. In a second study, these workers reported that feeding 125 ppm nicarbazine to broiler breeders reduced egg production and caused a severe decrease in hatchability.

Bird mortality increased substantially during periods of hot weather in broiler chickens fed 125 ppm nicarbazin (Buys and Rasmussen 1978; McDougald and McQuiston 1980b; Keshavarz and McDougald 1981). Studies by Beers et al. (1989) subsequently demonstrated that broiler birds fed 125 ppm nicarbazin experienced increased body temperature during heat stress, with significant adverse effects on blood acid-base balance, blood lactate, and heart rate than in birds fed an unmedicated ration.

Efficacy: Cuckler et al. (1955) first described the anticoccidial activity of this complex involving 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). They reported the efficacy of DNC against *E. tenella*, *E. acervulina*, and *E. necatrix* in chickens was improved "at least tenfold when it was complexed with HDP" (Cuckler et al. 1955). In subsequent work by Cuckler, Malanga, and Ott (1956), continuous feeding of nicarbazin at levels of 100 to 200 ppm was highly efficacious in preventing infections by *E. tenella* and *E. necatrix* in chickens. Further studies by several workers (Ott et al. 1956; Rubin et al. 1956; McLoughlin and Chester 1959; McLoughlin, Gardiner, and Chester 1960; Gardiner and McLoughlin 1963a) confirmed that 125 ppm nicarbazin was highly efficacious against *E. tenella* in chickens. The efficacy of 125 ppm nicarbazin against this species was found to be superior to the efficacy of 125 ppm sulfaquinoxaline and comparable to the efficacy of 110 ppm

nitrofuazone (Ball 1959). In this work, the efficacy of nicarbazin against *E. tenella* was reduced substantially if medication was delayed beyond the first 24 hours after inoculation. Timed medication studies testing the efficacy of 125 ppm nicarbazin against *E. tenella* indicated the greatest effect occurred against the second generation schizont, but earlier stages were also affected (McLoughlin and Wehr 1960). The suppressive effect persisted as long as medication was fed, but development of the parasite resumed upon drug withdrawal. Horton-Smith and Long (1959b) found that 125 ppm nicarbazin was highly efficacious in preventing mortality against a severe infection of *E. necatrix*, but slightly less efficacious against a severe infection of *E. acervulina*. Medication was initiated prior to coccidial infection in both studies. Morrison et al. (1961) reported that 125 ppm nicarbazin provided good efficacy based upon percentage survival, weight gain, lesion score, and oocyst score against a mixed inoculum of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* in two studies with broiler chickens in battery cages. McManus (1970) found that 125 ppm nicarbazin was highly efficacious in preventing infections of *E. mivati*, *E. hagani*, and *E. praecox* based on chick growth, mortality, the severity of pathological lesions, and numbers of oocysts produced.

The addition of liquid amprolium to the drinking water of replacement pullets on the days when medicated feed was restricted provided good

protection against infections of *E. acervulina*, *E. maxima*, and *E. tenella* (Ruff, Chute, and Garcia 1991). Subsequent challenge studies with birds given amprolium in the drinking water demonstrated that a protective immunity had developed in these birds.

Extensive surveys of field isolates from all major broiler-producing areas in the United States found a fairly high percentage (57.6%) of the *E. acervulina* isolates were resistant to 125 ppm nicarbazin (Jeffers 1974b, 1974c). The incidence of *E. tenella* among these field isolates from farms using nicarbazin either alone or in shuttle programs was quite low, indicating that nicarbazin maintained a high efficacy against this species. Laboratory studies with *E. tenella* have demonstrated that development of resistance to this species is at best slow and quite variable (McLoughlin and Gardiner 1967; Tamas, Schleim, and Wilks 1991). Surveys conducted in Europe, Latin America and in North America showed a slow increase over time in the incidence of nicarbazin resistance among field isolates of the above species and *E. maxima* (Mathis and McDougald 1982; McDougald, Fuller, and Solis 1986; McDougald et al. 1987b; Chapman 1989c; Rotibi, McDougald, and Solis 1989; Stephan et al. 1997). The overall evidence indicates that the slow development of resistance to this drug coupled with its more frequent use in shuttle programs in rotation with other anticoccidial drugs has preserved its high efficacy over the years.

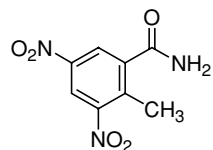


Figure 6.19. **Dinitolmide (zoalene).**

Nitrobenzamides

Dinitolmide (zoalene)

Chemical name: [148-01-6] 2-methyl-3,5-dinitrobenzamide; 3,5-dinitro-*o*-toluamide.

Product name: Zoamix; DOT, DNOT.

Chemical structure: See figure 6.19.

Safety: Accidental feeding of dinitolmide at levels of 400 to 860 ppm in the feed of broiler chickens and replacement pullets caused a range of toxic signs including extended necks, vertigo, incoordination, tumbling, stunted growth, and reduced weight gains (Bigland, Howell, and DaMassa 1963; Cameron and Spackman 1982). The former workers induced similar signs in chickens within 14 days by giving dinitolmide in the feed at levels of 500, 725, 1,000, and 1,250 ppm. Gross and microscopic pathological changes were not found in affected birds, and the neurological symptoms and depressed weight gain disappeared within 24 hours after withdrawal. Dinitolmide feeding to utility pigeons on farms in Australia resulted in severe neurological problems and a substantial

reduction in squab production (Reece and Hooper 1984).

Efficacy: Dinitolmide acts against the asexual merozoite stages of the coccidial parasite and must be present during this time of infection to have its maximum effect (Hymas and Stevenson 1960; Joyner 1960). The time of greatest activity of dinitolmide was designated as early as day 3 of infection by Reid (1973). Studies by several workers demonstrated that 125 ppm dinitolmide provided good efficacy against *E. tenella* when medication via the feed commenced at the time of infection and was maintained for at least 7 days (Joyner 1960; Gardiner and McLoughlin 1963a). Peterson (1960) reported 125 ppm dinitolmide provided excellent efficacy against both *E. tenella* and *E. necatrix*. Morrison et al. (1961) found that 125 ppm dinitolmide provided good efficacy against a mixed inoculum of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* in two studies with broiler chickens in battery cages based upon percentage survival, weight gain, lesion score, and oocyst score data. A subsequent study by these workers (Morrison et al. 1967) found that dinitolmide was less effective than buquinolate, amprolium + ethopabate, and amprolium + ethopabate + sulfaquinoxaline against a mixed infection of *E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella*. Studies by Ryley (1967a) demonstrated that 125 ppm dinitolmide was highly efficacious against *E. tenella*, but efficacy against *E. acervulina*, *E. maxima*, *E. mivati*,

E. necatrix, and *E. brunetti* was poor based on mortality, weight gain, and oocyst count data.

In a laboratory study, the development of resistance by an isolate of *E. tenella* following serial passage in the presence of dinitolmide was relatively slow based on mortality, performance, and pathologic index data (McLoughlin and Gardiner 1962b). Mean weight gain was slightly reduced in birds fed 125 ppm dinitolmide and challenged with the isolate following 11 passages, but weight gain reduction was greater in medicated birds challenged with the isolate following 19 passages. On the contrary, McLoughlin and Chute (1975) using four anticoccidial drugs in rotation in chickens produced a strain of *E. tenella* resistant to dinitolmide after exposure to the drug just 10 times on an intermittent basis. A survey conducted in 1964–1965 in Great Britain found that fairly high percentages of the *E. tenella* (68%), *E. acervulina* (86%), *E. brunetti* (69%), and *E. maxima* (73%) isolates from dinitolmide-medicated flocks were resistant to dinitolmide (Warren et al. 1966). In the years that followed, there appeared to be an increasing frequency of field isolates of *E. acervulina*, *E. maxima*, and *E. tenella* manifesting resistance to dinitolmide (Chapman 1976c, 1989c; Mathis and McDougald 1981, 1982).

Aklomide + Sulfanitran

Chemical names: [3011-89-0] 2-chloro-4-nitrobenzamide (aklomite); and [122-16-7] N-[4-

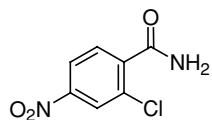


Figure 6.20. **Aklomide.**

[[[(4-nitrophenyl)-amino]sulfonyl]phenyl]acetamide; 4'-[(*p*-nitrophenyl)sulfamoyl]acetanilide; N⁴-acetyl-N¹-(*p*-nitrophenyl)sulfanilamide; 4-acetaminobenzenesulfon-4'-nitroanilide (sulfanitran).

Product name: Alklomix, Novastat.

Chemical structure: See figure 6.20 for aklomide and figure 6.21 for sulfanitran.

Safety: Weight gain means for chickens given 250 ppm aklomide + 200 ppm sulfanitran and those in the unmedicated treatment were not different in battery studies reported by McLoughlin and Chute (1966). The same combination of 250 ppm aklomide + 200 ppm sulfanitran was found to have a substantial weight-depressing effect when fed to broiler chickens in combination with furazoli-

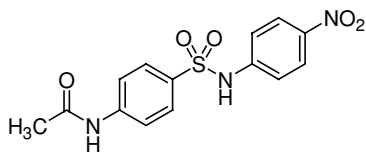


Figure 6.21. **Sulfanitran.**

done at levels greater than 110 ppm (Morrison, Tremere, and Standish 1974).

Efficacy: Aklomide is closely related to zoalene and nitromide. The anticoccidial activity of nitrobenzamides is chiefly directed against the first generation merozoites, and as work with zoalene has demonstrated medication must be provided in the feed prior to coccidial exposure to obtain maximum efficacy (Hymas and Stevenson, 1960; Joyner 1960). The mixture of 250 ppm aklomide + 200 ppm sulfanitran was highly efficacious against single-species infections of *E. acervulina*, *E. necatrix*, and *E. tenella* based on weight gain, mortality, and fecal score data in trials reported by Baron and Morehouse (1963). McLoughlin and Chute (1966) found that 250 ppm aklomide + 200 ppm sulfanitran was highly efficacious against *E. tenella* in four battery trials with 3-week-old chicks.

Twelve field isolates of *E. tenella* that manifested resistance to different anticoccidial drugs were tested in a series of studies with Novastat at its recommended use level (McLoughlin and Chute 1973b). Novastat was efficacious against seven of the twelve drug-resistant strains. The five strains of *E. tenella* that were resistant to Novastat were also resistant to nicarbazin, nitrofurazone, Trithiadol, Unistat, and zoalene based on previous testing. In a separate study reported in the same reference, resistance to Novastat was clearly evident

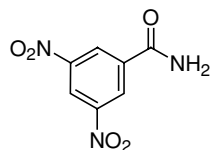


Figure 6.22. **Nitromide.**

within five passages of an *E. tenella* isolate not previously exposed to drugs.

Nitromide + Sulfanitran + Roxarsone

Chemical name: [121-81-3] 3,5-dinitrobenzamide (nitromide); [122-16-7] N-[4-[(4-nitrophenyl)-amino]sulfonyl]phenyl]acetamide (sulfanitran); [121-19-7] 4-hydroxy-3-nitrophenylarsonic acid (roxarsone).

Product name: Unistat-3, Tristat, and Unistat.

Chemical structure: See figure 6.22 for nitromide, figure 6.21 for sulfanitran, and figure 6.28 for roxarsone.

Safety: See safety sections for roxarsone and ak-lomide + sulfanitran for specific information on the safety of these drugs. Very little information has been published on the safety of nitromide. It is classified as a carcinogen (Shappell, Larsen, and Bakke 1999), and a 5-day withdrawal period is required with its use in chickens (Miller Publishing Co. 1990). Earlier studies in chickens demonstrated that nitromide residues decreased

to less than 0.010 ppm in 5 days (Zietlow et al. 1969), and more recent work in chickens with ^{14}C -labelled nitromide indicated that the main route of elimination was in the urine, with 58% of the dose recovered after 48 hours (Shappell, Larsen, and Bakke 1999). These results indicated a significant portion of the dose was absorbed from the gastrointestinal tract. All birds were sacrificed 72 hours after dosing and tested for nitromide residues. At 72 hours, the gastrointestinal tracts contained 1%–4% of the dose, livers had less than 0.1%, and radioactivity was not detected in the carcasses. The major metabolite in both urine and feces was found to be 3-acetamido-5-nitrobenzamide.

Efficacy: The combination of 250 ppm nitromide + 300 ppm sulfanitran + 50 ppm roxarsone was highly efficacious against *E. tenella* based upon bird performance, coccidiosis mortality percentages, and pathologic index in studies by Gardiner and McLoughlin (1963a). This drug combination acts early in the life cycle of coccidia. In two battery cage trials, each involving a mixed inoculum of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella*, 1,000 ppm nitromide in the feed by itself was only moderately efficacious in preventing infection based upon mortality, weight gain, lesion score, and oocyst score data (Morrison et al. 1961). These results were consistent with earlier studies by Morehouse and McGuire (1959). McLoughlin and Chute (1975) using four anticoccidial drugs in

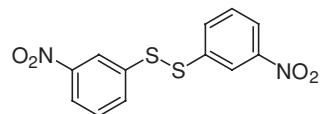


Figure 6.23. **Nitrophenide.**

sequence in broiler chickens produced a strain of *E. tenella* resistant to Unistat following exposure to the drug 10 times on an intermittent basis.

Nitrophenide

Chemical name: [537-91-7] bis(3-nitrophenyl)disulfide; m,m'-dinitrodiphenyl disulfide.

Product name: Megasul

Chemical structure: See figure 6.23.

Safety: Nitrophenide at high levels (700–1600 ppm) in the feed beginning at 10 days of age produced severe signs of toxicity during 3 weeks of treatment (Waletzky, Hughes, and Brandt 1949). All clinical signs of toxicity were reversed after termination of drug administration. In the same studies, 400 ppm nitrophenide fed to male and female birds from 4 to 12 weeks of age had no adverse effects on viability, weight gain, or subsequent reproductive capability. In contrast, 14-week-old birds fed 400 ppm nitrophenide did manifest signs of toxicity, including ataxia and reduced feed consumption, beginning 4 days after onset of feeding (Peckham 1978). Excellent

weight gains were obtained in comparison with unmedicated birds when 125 ppm nitrophenide was fed for 6 weeks beginning at 1 day of age in a toleration study not involving coccidial infection (Tugwell 1955). The feeding of 200 ppm nitrophenide to growing cockerels from 2 days to 10 weeks of age significantly ($P < 0.001$) depressed the growth of chicks, and the mean weights of the spleens and livers were below that of the unmedicated control birds (Schoettle et al. 1956).

Efficacy: Waletzky, Hughes, and Brandt (1949) found that nitrophenide at levels of 500 to 750 ppm in the feed beginning at the time of inoculation or 24 to 48 hours before inoculation with *E. tenella* was highly efficacious in preventing mortality, reducing lesion severity, and maintaining weight gain. Lower levels of nitrophenide in the range of 100 to 150 ppm were substantially less efficacious in preventing mortality in very severe infections. These workers also found that 750 ppm nitrophenide was equally efficacious against mixed infections of *E. necatrix* and *E. tenella*. Use of 300 ppm nitrophenide in the feed provided good efficacy in preventing mortality due to infection by *E. necatrix*, but weight gain was adversely affected by this level of medication in work by Peterson and Hymas (1950). The efficacy of 125 ppm nitrophenide was poor in preventing mortality caused by an *E. tenella* (Tugwell 1955), and only moderately efficacious at this level in preventing mortality against a severe infection of *E. necatrix* (Horton-Smith and Long 1959b). In

a series of studies by Gardiner, Farr, and Wehr (1952) testing 750 ppm nitrophenide in the feed, it was concluded that medication during the period from the 49th through the 96th hour after inoculation provided the greatest level of protection against *E. tenella*. These authors observed that the longer periods of medication provided greater benefit, and that their results indicated that nitrophenide was coccidiostatic in action. Oocyst sporulation was markedly reduced in birds medicated only during the last two days of the sexual cycle (97–144 hours). They also noted that the second generation schizonts were affected by nitrophenide medication, but it wasn't clear if nitrophenide partially destroyed the second generation schizonts or simply retarded their development. A recent mode-of-action study indicated that nitrophenide targeted the development of *E. tenella* at the sexual stages by inhibiting mannitol-1-phosphate dehydrogenase (Allocco et al. 2001). It was demonstrated in these studies that nitrophenide was specific in targeting the mannitol cycle in the sexual stages with a substantial reduction ($\geq 90\%$) in oocyst shedding in chickens given 500 ppm and 1,000 ppm nitrophenide in the feed. Remaining oocysts were abnormal in development and essentially incapable of further development.

Nitrofurans

Members of this class of chemicals were used in a wide variety of products for the treatment and

prevention of coccidiosis in poultry. The spectrum of efficacy was somewhat limited, and safety was a concern in some instances. Resistance development to members of this class was also a concern. Gardiner and McLoughlin (1963c) successfully induced resistance to nitrofurazone in a strain of *E. tenella* after seven passages of the isolate. Field studies also demonstrated the early development of resistance to nitrofurazone by many isolates of *Eimeria* spp. from poultry (Cuckler and Malanga 1955; Warren, Ball, and MacKenzie 1966; Krylov and Zaients 1981).

Furazolidone

Chemical name: [67-45-8] 3-[[[(5-nitro-2-furyl)-methylene]amino]-2-oxazolidinone; 3-(5-nitrofurfurylideneamino)-2-oxazolidinone.

Product name: NF-180, Furovag, Furoxane, Furoxone, Giarlam, and many others.

Chemical structure: See figure 6.24.

Safety: Chicks receiving 110 to 200 ppm furazolidone in the feed demonstrated an increased growth rate, and at 330 ppm a slight reduction in growth after 4 weeks (Harwood and Stunz 1954).

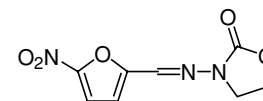


Figure 6.24. **Furazolidone.**

Extensive toxicological studies in various animal species indicated that the toxicity of furazolidone when given at fairly high dosages on a daily basis was evident primarily in the central nervous system (Rogers et al. 1956; Ali and Bartlet 1982), with some pathological changes also occurring in the heart, liver, and testes (Khan et al. 1995). Ali and Bartlet (1982) proposed “that furazolidone antagonized the utilization of thiamin, perhaps by inhibiting its phosphorylation.” Generally, the toxic effects of furazolidone in various animal species were reversible upon discontinuance of medication. Male broiler breeder birds fed furazolidone at levels of 150, 250, or 350 ppm showed decreased testicular weights at the two highest levels, and mean plasma testosterone levels were reduced at all levels of medication (Andrabi, Ahmad, and Shahab 1998). The data indicated that furazolidone adversely affected sexual maturation in male birds. Medication of egg and meat type breeder birds with 220 ppm furazolidone did not affect egg production, egg weight, egg fertility, or egg hatchability percentages after 4 weeks of medication in a study by Kondra and Guenter (1968). However, in studies by Sauer, Jensen, and Shutze (1969), egg weight gain was reduced by the continuous feeding of furazolidone to laying hens at levels ranging from 3.1 to 110 ppm. This effect was substantial at levels of 12.3 ppm furazolidone and above. Furazolidone feeding to laying hens resulted in drug residues in the eggs within 6 days of the start of feeding (Furusawa 2001).

Efficacy: Furazolidone given at 110 ppm in the diet controls mortality due to *E. tenella* if given no later than 48 hours after infection (Harwood and Stunz 1954). It is recommended for the prevention of coccidiosis caused by *E. acervulina*, *E. necatrix*, and *E. tenella* when fed continuously at 55 ppm, or for the control of coccidiosis outbreaks caused by these species when used at 110 ppm for a minimum of 5–7 days followed by use at 55 ppm for 2 weeks to prevent recurrence.

Nihydrazone

Chemical name: [67-28-7] acetic acid [(5-nitro-2-furyl)methylene]hydrazide; 5-nitro-2-furaldehyde acetylhydrazone; acetic acid 5-(nitrofurfurylidene)-hydrazide.

Product name: Furiton, Nidrafur.

Chemical structure: See figure 6.25.

Safety: Feeding White Leghorn pullets 110 ppm nihydrazone for 14 months commencing at 21 weeks of age had no adverse effects on egg production, egg quality, feed efficiency, livability, fertility, hatchability, and Haugh units (Boone and Barnett 1965).

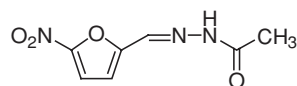


Figure 6.25. Nihydrazone.

Efficacy: Timed medication studies using a diet containing 330 ppm nihydrazone indicated this compound was coccidiostatic against first and second generation schizogenous stages of *E. tenella* in chickens (Johnson and Van Ryzin 1962). Gross and microscopic studies by these workers indicated that nihydrazone reached the parasitized host cells via the blood stream. Dietary levels of 82.5 to 220 ppm nihydrazone demonstrated activity against *E. necatrix* and *E. tenella*, but activity against *E. acervulina* was poor (Johnson and O'Connor 1965). In two battery cage studies each involving a mixed inoculum of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella*, 110 ppm nihydrazone in the feed was only moderately efficacious in preventing infection based upon mortality, weight gain, lesion score, and oocyst score data (Morrison et al. 1961). Nihydrazone at 110 ppm in the feed was only partially efficacious in preventing infection by *E. tenella* based on the incidence of cecal lesions and poor weight gain in medicated birds (McLoughlin and Gardiner 1965).

Nitrofurazone

Chemical name: [59-87-0] 2-[(5-nitro-2-furyl)-methylene]hydrazinecarboxamide; 5-nitro-2-furaldehyde semicarbazone.

Product name: nfz, Amifur, Furacin, Furazol, Nitrofur, Coxistat, and many others.

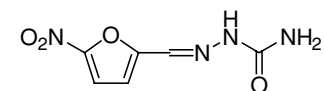


Figure 6.26. **Nitrofurazone.**

Chemical structure: See figure 6.26.

Safety: Administration of 400 and 500 ppm nitrofurazone in the feed depressed bird performance (Peterson and Hymas 1950). Feeding of 220 ppm nitrofurazone continuously for 14 days reduced growth in chickens (Peckham 1978). Reduced growth, depression, and ruffled feathers are typical signs of nitrofurazone toxicity in chicks (Julian 1991). Good weight gain was recorded for chickens given 56 ppm nitrofurazone for 6 weeks beginning at 1 day of age in a toleration test not involving a coccidial infection (Tugwell 1955).

Efficacy: The prophylactic efficacy of 67 ppm nitrofurazone against natural outbreaks of cecal and intestinal coccidiosis in chickens was found to be very good in a series of experiments based on improved weight gains and substantially reduced mortality in medicated birds in comparison with unmedicated birds (Harwood and Stunz 1950). Mortality due to *E. necatrix* infection was prevented in chicks receiving 200 ppm nitrofurazone (Peterson and Hymas 1950). Feeding of 56 ppm nitrofurazone was only moderately efficacious in

preventing infections of *E. tenella* based on weight gain and mortality data (Tugwell 1955). Horton-Smith and Long (1959b) reported that 100 ppm nitrofurazone was only moderately efficacious against severe infections of *E. necatrix* and *E. acervulina* based on mortality data for the former species, and performance, mortality, and oocyst count data for the latter species. Nitrofurazone demonstrated good control of oocyst production in birds infected with *E. maxima*. The efficacy of nitrofurazone against *E. necatrix* was improved slightly with the combination of 110 ppm nitrofurazone + 16 ppm furazolidone (Bifuran) according to these workers. Trials with a water formulation (Furacin) demonstrated that a concentration of 0.008% was effective in reducing mortality due to *E. tenella* infection in chickens (Johnson 1960). Higher levels (0.01% and 0.02%) were more efficacious in these studies, but toxicity was evident by a depressing effect on weight gain. Nitrofurazone given in the water at 0.008% concentration for a period of 6 days beginning 2 days after infection was highly efficacious against single-species infections of *E. tenella* (two strains) and *E. necatrix*, but efficacy against single-species infections of *E. brunetti*, *E. maxima*, and *E. mivati* was at best moderate (Mitrovic and Schildknecht 1973). Stage-of-action studies indicated nitrofurazone was coccidiostatic against first and second generation schizogonous stages of *E. tenella* in chickens (Johnson and Van Ryzin 1960; Reid 1973).

Organic arsenicals

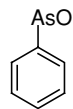


Figure 6.27. Oxophenylarsine (arsenosobenzene).

Oxophenylarsine (arsenosobenzene)

Chemical name: [637-03-6] arsenosobenzene; phenyl arsenoxide; phenylarsine oxide.

Product name: Arzene.

Chemical structure: See figure 6.27.

Safety: Basic studies on integrin-cytoskeleton linkages indicated that phenylarsine oxide (arsenosobenzene) inhibited the strengthening of cytoskeletal linkages (Choquet, Felsenfeld, and Sheetz 1997). This effect may help to explain the occurrence of leg weakness problems observed under some conditions with the use of arsenical drugs.

Efficacy: Studies by Peterson (1960) testing arsenosobenzene at 10 to 30 ppm found it to be highly efficacious in preventing infections of *E. tenella*, but efficacy against *E. necatrix* was weak. Gardiner and McLoughlin (1963a) also reported good efficacy against *E. tenella* in trials testing arsenosobenzene at its use level of 20 ppm.

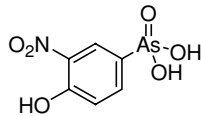


Figure 6.28. **Roxarsone.**

Roxarsone

Chemical name: [121-19-7] 4-hydroxy-3-nitrophenylarsonic acid; 4-hydroxy-3-nitrobenzenearsonic acid; 3-nitro-4-hydroxyphenylarsonic acid.

Product name: Roxarsone, 3-Nitro-10 (also 20 and 50), Sodium Arsanilate.

Chemical structure: See figure 6.28.

Safety: The growth-promoting capabilities of roxarsone in chickens were described by Morehouse (1949) and Schoettle et al. (1956). Chickens fed levels of roxarsone from 6.61 to 92.59 ppm performed better than unmedicated birds, and optimum performance was observed at the 90 ppm level. The feeding of roxarsone at levels greater than 50 ppm depressed performance and increased the arsenic concentration in the kidneys of crossbred chicks in a study by Czarnecki and Baker (1982). Broiler chickens given a diet containing 50 ppm roxarsone showed an increased incidence ($P < 0.10$) of tendon lesions, with a higher frequency in female birds (Laster et al. 1999). Studies by other workers (Waldroup et al. 1995; Rath et al. 1998) concluded that 50 ppm roxar-

sone had no negative effect on leg development, but in both sets of studies only male birds were used. Further research is needed on this topic. The accumulation and ultimate environmental impact of roxarsone residues in poultry litter in the form of the more toxic residue arsenate is a growing concern wherever this chemical is used in broiler production (Bednar et al. 2003; Garbarino et al. 2003; Rutherford et al. 2003).

Efficacy: The anticoccidial activity of roxarsone appears to occur during the first 48 to 72 hours post inoculation (Reid 1972). Morehouse and McKay (1951) demonstrated that the administration of 0.01% roxarsone in the drinking water was efficacious in the control of *E. tenella* when medication was started prior to or no later than the time of infection. Roxarsone at 50 ppm was moderately efficacious against *E. brunetti* based on means for weight gain, feed conversion, and coccidial lesions in two battery cage studies (Kowalski and Reid 1972). Inclusion of 50 ppm roxarsone in the diet significantly improved pigment levels in blood plasma of broiler chickens inoculated with *E. maxima* in a battery cage experiment, and in both plasma and skin pigment levels of broiler chickens when exposed to litter seeded with a mixture of *E. acervulina*, *E. brunetti*, *E. hagani*, *E. maxima*, *E. necatrix*, *E. praecox*, and *E. tenella* in two floor pen trials (Kowalski and Reid 1975). Roxarsone is one of three chemicals included in a feed additive in combination with sulfanitran and butynorate (Polystat

3) with efficacy claims for *E. acervulina*, *E. necatrix*, and *E. tenella* in chickens. Roxarsone is frequently used in combination with other anticoccidial drugs, and in particular the ionophorous anticoccidial drugs, to improve efficacy and skin pigmentation (McDougald, Keshavarz, and Rosenstein 1981; Mitrovic, Schildknecht, and Trainor 1977).

Polyether ionophores

The discovery and development of polyether ionophorous antibiotics, starting with monensin in the latter half of the 1960s, brought new life to the field of poultry coccidiosis prevention and control. It was demonstrated that these antibiotics are capable of forming lipophilic complexes with cations such as Na^+ , K^+ , Ca^{++} , and Mg^{++} and transporting them into and through biological membranes (Pressman 1976; Smith and Strout 1979). In the studies by Smith and Strout (1979) with *E. tenella*, it was found that both narasin and lasalocid caused a swelling and ultimate destruction of the sporozoite with no apparent effect on the host cell. They concluded that the destructive mechanism of ionophores against the sporozoite was directed toward the pellicle membrane. Their work also demonstrated that *E. tenella* sporozoites accumulated and retained lasalocid and narasin prior to invasion of the host cells. Studies with monensin, salinomycin, and lasalocid demonstrated that these three ionophores caused an increase in the levels of sodium and a decrease in potassium

levels across animal cells by altering the sodium-potassium pump in the cytoplasmic membrane (Smith and Rozengurt 1978; Austic and Smith 1980; Smith and Galloway 1983). The influx of sodium into the parasite exceeded the capacity of the parasite cell to remove it, thus leading to the death of the parasite. Studies involving lasalocid, monensin, narasin, and salinomycin showed that the pretreatment of *E. tenella* sporozoites with these ionophores significantly inhibited the ability of the sporozoites to invade the host cell or subsequently develop in the host cell even in the absence of any drug in the host cell environment (Smith, Galloway, and White 1981). These results indicated that the cidal action of ionophorous antibiotics may be more dependent upon extracellular exposure of the parasitic stages rather than drug exposure within the host cell. Studies by Long and Jeffers (1982) with monensin, lasalocid, and salinomycin provided further support to this conclusion, and in separate studies by these workers it was demonstrated that the lethal effects of monensin also included second generation merozoites. The efficacy of monensin, lasalocid, salinomycin, and narasin were compared when the drugs were given in the feed beginning at 96 hours (*E. acervulina*) or 120 hours (*E. brunetti*, *E. maxima*, or *E. tenella*) after inoculation by Guyonnet, Johnson, and Long (1991). The results demonstrated that all four ionophores improved bird performance and reduced oocyst counts, indicating some activity against the late stages of each species.

Because of the relatively narrow therapeutic index of ionophorous anticoccidials in chickens, the diagnosis of toxicity has been an ongoing concern (Dowling 1992; Novilla 1992). Toxic effects in chickens include reduced appetite, weight depression, leg weakness, diarrhea, recumbency, and death according to these authors. Key findings at necropsy include “focal degenerative cardiomyopathy, skeletal muscle necrosis, and congestive heart failure,” according to Novilla (1992). A review of the toxicity of polyether ionophores, especially in species other than the chicken, found that maduramicin was the most toxic and salinomycin the least toxic of the ionophores (Oehme and Pickrell 1999). In this review, lasalocid was more toxic than salinomycin, but less toxic or equal to narasin, which in turn was less toxic or equal to monensin.

Polyether ionophores have two distinct advantages that contribute significantly to their continued efficacy and usefulness in the field. First, their mode of action does not facilitate the rapid selection of resistant populations as is often seen with chemical anticoccidials (Chapman 1982b, 1984a, 1984b, 1986a; Jeffers 1978a, 1978b, 1981, 1989; Augustine et al. 1987; Bafundo and Jeffers 1990). Long-term exposure of a single coccidial isolate to a specific ionophore through multiple passages may result in reduced efficacy of the ionophore when medicated birds are given high levels of oocyst inocula derived from that isolate population, but complete resistance generally has not

been the case. This is substantially different from many chemical anticoccidial drugs, among which complete resistance has been demonstrated often after exposing the coccidial population to the drug for just 5 to 10 passages. Over the years, there have been a number of reports of field isolates showing reduced sensitivity or resistance to different ionophores based upon a variety of measurements (e.g., Jeffers 1974b, 1984; Chapman 1976b, 1976c, 1979, 1986b; Weppelman et al. 1977; McDougald 1981; Bedrnik 1983; Braunius, Greuel, and Sézen 1984; Fuller, McDougald, and Solis 1986; Hamet 1986; McDougald, Fuller, and Solis 1986; McDougald et al. 1987b; Stallbaumer and Daisy 1988; Bedrnik et al. 1980; Chapman and Shirley 1989; Munoz, Rodriguez, and Wang 1989; Rotibi et al. 1989; Tamas et al. 1991; Chapman and Hacker 1994; Peeters et al. 1994; Stephan et al. 1997; Dauschies, Gasslein, and Rommel 1998; Danforth et al. 1999; Yadav and Gupta 2001). Much of the variation in results with field isolate tests is related to the fact that the ionophores generally allow some level of infection to occur even under conditions of relatively low challenge. As a consequence, in laboratory tests involving severe levels of challenge, the level of infection that ultimately occurs in medicated birds may be sufficient to cause a 15% to 30% reduction in weight gain and only a small reduction of lesion scores in comparison with infected, unmedicated birds. This type of problem with the ionophores has been illustrated best over time by monensin, particularly in laboratory tests

involving *E. tenella*. The design of laboratory tests, especially with respect to the number of birds per cage and cages per treatment, and the number of oocysts given per bird in the challenge dose are important factors to consider (Holdsworth et al. 2004). One recommendation in laboratory tests involving polyether ionophores has been to include at least two infected, unmedicated treatments in the trial design, with each unmedicated treatment given a different number of oocysts (e.g., 1×10^4 and 1×10^5 of *E. tenella*), to provide a standard curve for comparison with the medicated treatments inoculated at the highest level of challenge only (Conway et al. 1995).

A second advantage of polyether ionophores as indicated above is that ionophores do not completely control infection in the field, but usually allow a low level of infection of the indigenous coccidial population to occur. It has been postulated that the low-level infections observed under field conditions when using ionophores decrease the selective pressure on the coccidial population cycling through the flock, and allow for a gradual development of immunity to coccidial infection in the broiler bird (Jeffers 1989; Magee 1992; Chapman and Hacker 1993; Eckman 1993; Chapman 1999). As immunity develops, the immune response to the coccidial challenge coupled with the efficacy of the ionophore anticoccidial work together to establish an ongoing control of the infection. Since the immune response acts with equal effect against drug-susceptible and drug-resistant

strains of a coccidial population, drug resistance is also less likely to occur under these conditions.

Lasalocid

Chemical name: [25999-31-9] [2R-[2 α [2S*(3R*, -4S*, 5S*, 7R*), 3S*, 5S*], 5 α , 6 β]]-6-[7-[5-ethyl-5-(5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyran-2-yl)tetrahydro-3-methyl-2-furanyl]-4-hydroxy-3,5-dimethyl-6-oxononyl]-2-hydroxy-3-methylbenzoic acid; 3-methyl-6-[7-ethyl-4-hydroxy-3,5-dimethyl-6-oxo-7-[5-ethyl-3-methyl-5-(5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl)-2-tetrahydrofuryl]hepty]salicylic acid, sodium salt.

Product name: Avatec.

Chemical structure: See figure 6.29.

Safety: Lasalocid sodium is a monocarboxylic ionophore produced by a strain of *Streptomyces lasaliensis* isolated from a soil sample collected in Hyde Park, Massachusetts, U.S.A. Use of lasalocid at levels up to 225 ppm in floor pen trials with

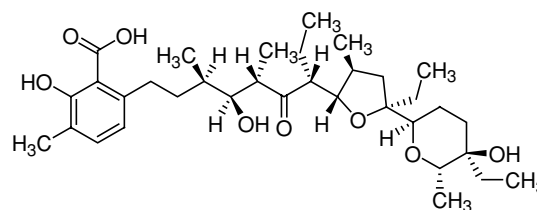


Figure 6.29. Lasalocid.

broiler chickens was found to be relatively safe with only slight growth depression occurring at the highest level tested (Taylor et al. 1974). The accidental feeding of poultry feed containing 125 ppm lasalocid to horses was not toxic, and the amount of lasalocid consumed by horses given medicated poultry feed was well below the LD₅₀ of approximately 21.5 mg/kg of body weight when orally dosed (Hanson, Eisenbeis, and Givens 1981). The accidental feeding of lasalocid-medicated feed at 125 ppm to breeder hens caused a substantial decrease in fertility and hatchability of incubated eggs (Aebi 1989). The feeding of 110 and 125 ppm lasalocid to broiler chickens in floor pen trials was reported to cause an increase in litter moisture (Ward and Brewer 1981; Ouart, Damron, and Christmas 1995). Lasalocid given in the feed at 125 ppm was incompatible with sulfadimethoxine given in the water at 500 mg/l (Frigg, Broz, and Weber 1983), and with chloramphenicol (Downing 1992). Lasalocid was found to be the only ionophore compatible with 125 and 250 mg/l tiamulin in studies reported by Laczay et al. (1989).

Efficacy: The anticoccidial activity of lasalocid was first described by Mitrovic and Schildknecht (1974). Based on weight gain, feed conversion, and mortality data, medication via the feed with lasalocid at 75 and 100 ppm was highly efficacious against infections caused by *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix*, *E. brunetti*, *E. praecox*, and *E. tenella*. Several different isolates of each species were used in these stud-

ies involving single species and mixed infections. Lower levels of lasalocid (25 and 50 ppm) were included in each trial, but the efficacy was reduced at these levels particularly against infections of *E. necatrix* and *E. tenella*. The efficacy of 50 and 75 ppm lasalocid against severe and moderate infections of a mixture of species (*E. acervulina*, *E. mivati*, *E. praecox*, *E. maxima*, *E. necatrix*, *E. brunetti*, *E. hagani*, and *E. tenella*) was excellent and comparable to 121 ppm monensin in floor pen studies reported by Reid, Johnson, and Dick (1975). The efficacy of 75, 100, and 125 ppm lasalocid was compared with 99 and 121 ppm monensin against 11 field isolates of *E. tenella* from different geographic areas of the United States (Mitrovic, Schildknecht, and Trainor 1977). Based on weight gain, feed conversion, coccidiosis mortality, average degree of infection, and oocyst counts, both ionophores were highly efficacious at all levels tested and comparable to each other. These workers also included treatments with each ionophore with and without 50 ppm roxarsone, and concluded that the combination with roxarsone provided further improvements in reducing lesions and oocyst counts in comparison with the ionophores alone. The efficacy of lasalocid at levels of 75, 90, 100, and 125 ppm against mixed infections of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* in broiler chickens in floor pens was excellent at all levels given, although the performance results at 75 to 100 ppm lasalocid were superior to 125 ppm (Bains 1980). In tests comparing the efficacy

of 75 ppm lasalocid, 60 ppm salinomycin, and 100 ppm monensin against various mixtures of *Eimeria* spp. in broiler chickens, the efficacy of lasalocid was superior to both salinomycin and monensin in tests involving *E. brunetti* and *E. maxima*, but it was less efficacious than either of the other ionophores in preventing lesions of *E. acervulina*, *E. necatrix*, and *E. tenella* (Migaki, Chappel, and Babcock 1979).

Peak activity of lasalocid was found to occur when medication at 100 ppm was initiated within 24 hours after inoculating broiler chickens with *E. tenella* and 48 hours after inoculation with *E. acervulina* (Guyonnet, Johnson, and Long 1990). There were no significant differences in bird performance or lesion scores when medication was withdrawn after 48 hours with *E. tenella* and 72 hours with *E. acervulina*. Initiation of medication at various intervals after 48 hours was found to reduce oocyst output for both species. Overall, these results indicated that lasalocid acted primarily early in the life cycle against sporozoites and merozoites, but activity against later generations of merozoites and possibly gamonts was evident.

Maduramicin ammonium

Chemical name: [84878-61-5] (2R,3S,4S,5R,6S)-6-[(1R)-1-[(2S,5R,7S,8R,9S)-2-[(2S,2'R,3'S,5R,5'R)-3'-[(2,6-dideoxy-3,4-di-o-methyl- β -l-arabino-hexopyranosyl)oxy]-octahydro-2-methyl-5'-[(2S,3S,5R,6S)-tetrahydro-6-hydroxy-3,5,6-tri-

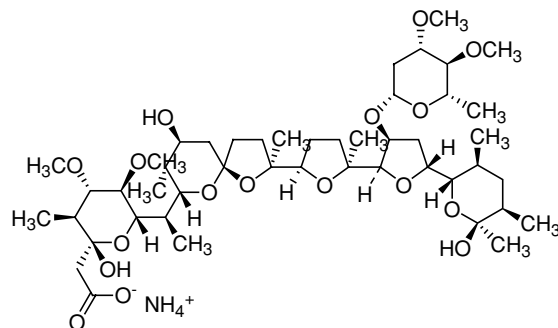


Figure 6.30. Maduramicin.

methyl-2H-pyran-2-yl][2,2'-bifuran]-5-yl]-9-hydroxy-2,8-dimethyl-1,6-dioxaspiro [4.5]dec-7-yl]ethyl]tetrahydro-2-hydroxy-4,5-dimethoxy-3-methyl-2H-pyran-2-acetic acid, monoammonium salt.

Product name: Cygro.

Chemical structure: See figure 6.30.

Safety: Maduramicin is a monoglycoside polyether ionophore produced by *Actinomadura yumaensis* isolated from a soil sample collected in Arizona in the United States. The performance of male broiler chickens was reduced significantly ($P \leq 0.05$) when given a ration medicated with 15 ppm maduramicin over a 49-day period (Kantor and Schenkel 1984). The performance of cockerels in the 10 ppm maduramicin treatment in this study was slightly reduced in comparison with the 5 ppm maduramicin treatment, but the differences

between these two treatments in means for weight gain and feed conversion were not significant. Studies by Singh and Gupta (2003) indicated that the feeding of 5 and 10 ppm maduramicin to chickens resulted in growth retardation at 21 days, and adversely affected various hematological values, including hemoglobin concentration (both levels), and packed cell volume (10 ppm level only). In a 52-day floor pen test with broiler chickens, birds fed 5 ppm maduramicin for 49 days followed by a 3-day withdrawal period consumed significantly ($P < 0.05$) more water than birds in the unmedicated and 60 ppm salinomycin treatments (Radu et al. 1987). Birds in the salinomycin treatment consumed less ($P < 0.05$) water than birds in the unmedicated treatment in this study, but unfortunately measurements of litter moisture were not obtained. Medication with 250 ppm tiamulin via the water on days 14 to 19 of a floor pen study depressed weight gains in broiler chickens fed 5 ppm maduramicin (Kantor 1986). Subsequent studies demonstrated that tiamulin given in the drinking water at 125 and 250 mg/l is incompatible with the feeding of 5 ppm maduramicin in broiler chickens (Laczay et al. 1988, 1989). A toxic interaction between maduramicin and the antioxidant duokvin was reported by Varga et al. (1994). Serious outbreaks of toxicity have been described in cattle and sheep given poultry litter in the feed that was sourced from poultry farms using maduramicin (Fourie et al. 1991; Bastianello et al. 1995) and from the misadministration of maduramicin premix in cattle

feed (Shlosberg et al. 1997). Key signs of toxicity in cattle and sheep included sudden mortality, congestive heart failure, and a stiff gait (in sheep only).

Efficacy: Maduramicin (prinicin) was reported to be highly efficacious against mixed infections of *Eimeria* spp. in broiler chickens when fed at levels in the range from 4 to 7 ppm (Kantor and Schenkel 1984; Kantor, Schenkel, and Kennett 1984; McDougald et al. 1984; Schenkel et al. 1984). Efficacy at 2.5 ppm maduramicin was reduced in comparison with the higher levels tested in these studies. Overall, the data indicated that the efficacy of 5 ppm maduramicin was comparable to 100 and 120 ppm monensin, 70 ppm narasin, and 60 ppm salinomycin against infections of *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix*, *E. mitis*, *E. brunetti*, and *E. tenella*. Maduramicin at 5 ppm was superior to both levels of monensin in battery efficacy studies involving separate infections of *E. acervulina*, *E. maxima*, and *E. tenella* based on the reduction of coccidial lesions. These results were further corroborated in a series of floor pen studies comparing the efficacy of 4, 5, 6, and 7 ppm maduramicin with 100 ppm monensin and 60 ppm salinomycin against mixed infections of six *Eimeria* spp. in broiler chickens (Wang, Kobland, and Ingle 1986). Based on performance data, coccidial lesion control, and coccidiosis-related mortality in these studies, 5 ppm maduramicin was more efficacious than monensin and comparable to salinomycin. In a study

comparing the efficacy of 3 ppm halofuginone, 125 ppm lasalocid, 5 ppm maduramicin, 120 ppm monensin, and 66 ppm salinomycin, maduramicin was the least effective against *E. maxima* (Folz et al. 1988). Maduramicin at 5 to 7 ppm was reported to be more efficacious than monensin and narasin, and about equal to salinomycin in reducing lesions and mortality and maintaining good performance in studies with *Eimeria* isolates that were partly resistant to ionophores (McDougald et al. 1987a). Stage-of-action studies testing 5 ppm maduramicin indicated that medication must be present at the time of infection to achieve optimum efficacy against *E. tenella* (Kantor and Schenkel 1984). When medication was delayed to day 2 post infection, bird mortality and lesion scores increased and mean weight gain reduced. Some activity against later stages of development beyond day 3 of infection was also evident in these studies.

Monensin sodium

Chemical name: [17090-79-8] 2-[5-ethyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl]-2-furyl]-2-furyl]-9-hydroxy- β -methoxy- α , γ ,2,8-tetramethyl-1,6-dioxaspiro-[4.5]decane -7-butyric acid, sodium salt.

Product name: Coban.

Chemical structure: See figure 6.31.

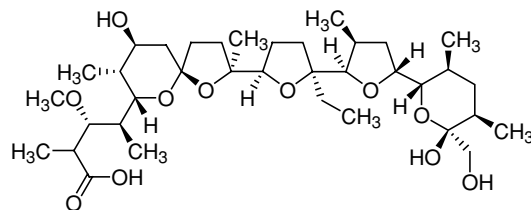


Figure 6.31. **Monensin.**

Safety: Produced by fermentation by a strain of *Streptomyces cinnamonensis* originally isolated from a soil sample collected in Arizona in the United States, monensin is characterized as a monocarboxylic acid (Haney and Hoehn 1967). Broiler chickens given feed medicated with 120 ppm monensin weighed less ($P < 0.01$) than chickens in the 0 or 100 ppm monensin treatments in a 48-day floor pen test (Gard et al. 1975). Damron et al. (1977) also reported a significant ($P < 0.05$) reduction in body weight of broiler chickens fed 99 and 121 ppm monensin for 56 days in comparison with unmedicated and lasalocid-medicated treatments. In their studies, weight reduction was greater in the 121 ppm monensin treatment than in the 99 ppm monensin treatment. Parsons and Baker (1982) concluded from their studies that the growth depression observed in broiler chickens fed 121 ppm monensin was due largely to reduced feed consumption. They also found that much of this effect on weight gain reduction could be offset by avoiding deficient levels of dietary protein. Subsequent studies by McDougald and Mathis (1984) showed that monensin intake

actually increased (mg intake/kg of body weight) when broiler chickens were fed diets low in energy and protein (low density) due to increased feed intake. As a consequence, the efficacy of reduced levels of monensin against *E. tenella* was superior in chickens on a low-density diet than on a high-density diet. In countries where poultry rations typically were lower in protein and energy than in the United States, it was found that the use of monensin at slightly reduced levels (90 to 100 ppm) worked very well without compromising efficacy. Studies by several workers demonstrated that compensatory growth would occur over a 5- to 7-day period following the withdrawal of monensin when given at 100 to 121 ppm to broiler chickens in floor pens because of increased feed consumption during the withdrawal period (McDougald 1980; McDougald and McQuiston 1980a; Metzler, Britton, and McDougald 1987). Studies by Cervantes, Jensen, and Brenes (1982) using diets supplemented with high levels of fish meal, animal protein, or poultry byproduct meal with and without monensin demonstrated that the growth depression sometimes observed under conditions of commercial production in broiler chickens could be eliminated by supplementing the diet with potassium carbonate. Monensin was found to be incompatible when given at levels of 100 to 120 ppm with a number of antibiotics given via the water including tiamulin (125 and 250 mg/l), sulfaquinoxaline (200 mg/l), sulfamethazine (750 mg/l), sulfadimethoxine (500 mg/l), and erythromycin (200 mg/l) according to

studies by Frigg et al. (1983) and Laczay et al. (1989). In addition, monensin has been reported to be incompatible with sulfachlorpyrazine, chloramphenicol, and oleandomycin (Dowling, 1992). Finally, a toxic interaction between monensin and a dihydroquinoline-type antioxidant known as duokvin was reported by Prohászka et al. (1987) and Varga et al. (1994).

There was a concern for a number of years following the introduction of monensin that feather pecking on the back (bareback condition) and the consumption of floor feathers may be increased by monensin feeding in broiler chickens. These observations led to the suspicion that the use of monensin caused an increased requirement for sulfur amino acids in the diet. The results of a number of studies failed to support these concerns (Gard et al. 1981; Wellenreiter 1981; Baker 1983; McDougald and Keshavarz 1984).

Feeding 100 ppm monensin to Arbor Acres broiler breeders for 10 days resulted in a reduced fertility for days 3 through 14 of the withdrawal period in a study by Jones et al. (1990). The accidental feeding of monensin-medicated feed to horses can result in toxicity and death (Matsuoka 1976; Hanson, Eisenbeis, and Givens 1981).

Efficacy: Shumard and Callender (1968) demonstrated that 121 ppm monensin in the diet provided excellent efficacy against a number of

isolates each of *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* based on bird mortality, performance, lesion score (*E. necatrix* and *E. tenella*) and oocyst count (all other species) data. The levels of infection in nine trials with *E. tenella* by these workers resulted in high mortality (35%–100%) in the infected, unmedicated treatments, while the mortality percentage in the infected, monensin treatments was 0% in all except two trials when 5% mortality was recorded. Coccidiosis-related mortality in the five trials with *E. necatrix* ranged from 45% to 100% in the infected, unmedicated treatments, while in the infected, monensin treatments mortality was 20% in one trial and 0% in the remaining trials. Monensin medication at either 100 or 120 ppm in a series of floor pen trials provided good efficacy against mixed infections of *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix*, *E. brunetti*, *E. hagani*, *E. praecox*, and *E. tenella* (Reid, Kowalski, and Rice 1972). Lesion control in these studies was best in the upper (*E. acervulina* and *E. mivati*), middle (*E. maxima* and *E. necatrix*), and lower (*E. brunetti*) intestine according to the authors, but monensin “did permit lesions due to *E. tenella* under conditions favorable to coccidiosis.” The coccidial cultures used in these studies were derived from a live vaccine (Coccivac-D, Sterwin Laboratories, Inc.). The efficacy of 121 ppm monensin was excellent against severe, single-species infections of *E. acervulina*, *E. necatrix*, and *E. brunetti*, but reduced against a severe inoculum (4×10^5 oocysts/bird) of *E. tenella* in trials reported

by Ryley and Wilson (1975). The level of *E. tenella* challenge used in their studies caused 94% mortality in the infected, unmedicated treatment, and 19% mortality in the 121 ppm monensin treatment. The use of overwhelming levels of coccidial challenge in this series of studies made interpretation of the results more difficult. Monensin given in the diet at 100 ppm was highly efficacious against *E. mitis* based on chick performance and intestinal scores (abnormalities) in studies by Watkins, Bafundo, and Donovan (1990). The efficacy of monensin at 100 ppm against *E. tenella* and *E. necatrix* appears to be greatest against the early stages of the parasite during the first 48 hours after inoculation for *E. tenella*, and 72 hours after inoculation for *E. necatrix* based on lesion scores data, but some efficacy was observed in terms of total oocyst production even when medication was delayed by as much as 96 hours after inoculation for *E. tenella* and 120 hours after inoculation for *E. necatrix* (Long and Jeffers 1982). In a study comparing the efficacy of 3 ppm halofuginone, 125 ppm lasalocid, 5 ppm maduramicin, 120 ppm monensin, and 66 ppm salinomycin, monensin was the least effective against *E. tenella* and ranked low against *E. necatrix* (Folz et al. 1988). The efficacy of monensin against resistant field isolates of *Eimeria* from commercial broiler chickens was significantly improved based on means for weight gain and oocyst counts following the use of a live vaccine (Coccivac-B) by the broiler farms where the resistant isolates were originally obtained (Chapman 1994).

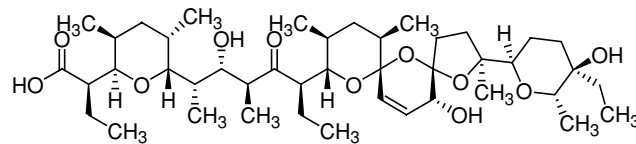


Figure 6.32. **Narasin.**

Narasin

Chemical name: [55134-13-9] (4S)-4-methylsalinomycin.

Product name: Monteban.

Chemical structure: See figure 6.32.

Safety: This polyether monocarboxylic acid product is produced by *Streptomyces aureofaciens* described by Boeck et al (1977) and Berg and Hamill (1978). Jones et al. (1990) using Arbor Acres males and females reported that feeding 70 ppm narasin for 10 consecutive days had only a slight adverse effect on egg production, but hatchability was reduced significantly ($P < 0.05$) beginning on days 9 through 10 of the feeding period. Narasin also reduced egg weight in this study. Narasin at its recommended use level in the feed was incompatible when given in combination with the antibiotic tiamulin in the water at levels of 125 and 250 mg/l (Frigg et al. 1983; Laczay et al. 1989). Narasin has also been found to be incompatible with erythromycin, sulfachlorpyrazine, sulfaquinoxaline, sulphadimethoxine, and chlo-

ramphenicol (Dowling, 1992). A toxic interaction between narasin and the dihydroquinoline-type antioxidant duokvin used in feed formulations has been described by Varga et al. (1994).

Efficacy: The efficacy of 40, 60, 80, and 100 ppm narasin against single- and mixed-species infections of *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* demonstrated that use levels in the range of 60 to 100 ppm were more efficacious than 40 ppm narasin (Ruff et al. 1979). The study with a mixture of these species also indicated that 60 to 100 ppm narasin was more efficacious than 99 ppm monensin particularly in the overall control of coccidial lesions. These results were confirmed in subsequent trials in floor pens using various mixed infections including *E. acervulina*, *E. mivati*, *E. maxima*, *E. brunetti*, *E. necatrix*, *E. mitis*, *E. praxox*, and *E. tenella* (Ruff et al. 1980; Jeffers et al. 1988a). The pooled results of 17 floor pen trials conducted in five countries in Europe demonstrated that the optimum dose was in the range of 60 to 80 ppm narasin based on means for final weight and feed conversion (Walters, Bentley, and Jones 1981). The control of coccidial lesions, due primarily to *E. acervulina*, *E. maxima*, and *E. tenella*, in the 60 to 80 ppm range was comparable to 100 ppm monensin. In a summary of 104 battery efficacy trials involving both laboratory strains and recent field isolates of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella*, 80 ppm narasin was more efficacious than 60 ppm narasin based

on improved weight gains in studies involving *E. tenella* plus a mixture of intestinal species (Jeffers, Tonkinson, and Callender 1988). Experience in the field, however, definitely indicated that the optimum range for narasin was 60 to 80 ppm (Jeffers et al. 1988b). The efficacy of 60 or 80 ppm narasin against severe infections of *E. tenella* was improved when combined with 50 ppm roxarsone in floor pen trials conducted by Bafundo et al. (1989).

Narasin + Nicarbazine

Chemical name: [55134-13-9] (4S)-4-methylsalinomycin (narasin); and [330-95-0] N, N'-bis(4-nitrophenyl)-urea, compounded with 4,6-dimethyl-2(1H)-pyrimidinone (1:1) (nicarbazine).

Product name: Maxiban⁷².

Chemical structure: See figure 6.32 for narasin and figure 6.18 for nicarbazine.

Safety: The introduction of this combination product in the late 1980s was designed to improve efficacy, particularly against strains of *Eimeria* spp. resistant to nicarbazine or ionophores alone, and to improve bird performance. These goals were achieved (see below), but concerns regarding the effect of nicarbazine under heat-stress conditions on broiler chickens were not eliminated. Long, Johnson, and McKenzie (1988) reported that heat-stress mortality generally asso-

ciated with the feeding of 125 ppm nicarbazine (McDougald and McQuiston 1980b) was substantially reduced with the combination of narasin + nicarbazine (50 + 50 ppm). A subsequent study with male broiler chickens fed 50 ppm narasin + 50 ppm nicarbazine, and maintained under conditions in which the ambient temperature was cycled from 27°C (80.6°F) to a high of 37°C (98.6°F) each day for 21 days with a relative humidity at 50%, indicated that the combination product does not eliminate the nicarbazine toxicity in the male bird due to heat distress (Wiernusz and Teeter 1991).

Efficacy: The combination of 50 ppm narasin plus 50 ppm nicarbazine was found to be highly efficacious against a mixed infection of *E. acervulina*, *E. maxima*, *E. mitis*, *E. brunetti*, *E. necatrix*, and *E. tenella* (Long, Johnson, and McKenzie 1988). Narasin at 70 ppm alone or in combination with nicarbazine (50 + 50 ppm) improved performance in comparison with birds in the 125 ppm nicarbazine and unmedicated treatments. A report summarizing the results of nine trials in seven countries demonstrated that the combination of 40 ppm narasin + 40 ppm nicarbazine in either continuous or shuttle programs resulted in greater body weights in broiler chickens than birds in a 125 ppm nicarbazine to 60 or 70 ppm narasin shuttle program treatments (Guneratne and Gard 1991). The beneficial effects of nicarbazine on improving skin pigmentation of broiler chickens in the absence and presence of coccidial infection

were also reported to occur in broiler birds given a 50/50 ppm combination of narasin + nicarbazin (Bafundo 1989).

Salinomycin sodium

Chemical name: Sodium salt $C_{42}H_{69}NaO_{11}$ [55721-31-8].

Product name: Bio-Cox, Coxistac, Sacox, Salgain, Salocin.

Chemical structure: See figure 6.33.

Safety: Salinomycin is produced by a strain of *Streptomyces albus* isolated from a soil sample collected in Shizuoka Prefecture, Japan (Kinashi et al. 1973, 1975; Miyazaki et al. 1974). It is a monocarboxylic acid polyether antibiotic that preferentially mediates the transport of monovalent alkali cations, e.g., Cs^+ , K^+ , Na^+ , and Rb^+ (Mitani, Yamanishi, and Miyazaki 1975; Mitani et al. 1976). The feeding of 80 ppm salinomycin to broiler chickens in floor pens caused a reduction ($P \leq 0.05$) in weight gain as a direct result of reduced feed intake (Migaki and Babcock 1979; Keppens and De Groote 1980; Yvoré et al.

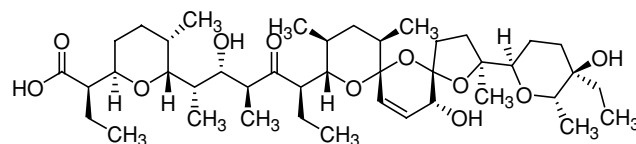


Figure 6.33. Salinomycin.

1980). Body weight and feed consumption were depressed ($P < 0.05$) in broiler chicks fed salinomycin at 77 or 88 ppm for 21 days, but no adverse effects on performance were observed at lower concentrations (44, 55, and 66 ppm) of salinomycin (Harms and Buresh 1987). Broiler chickens fed 60 ppm salinomycin for 5 or 6 weeks followed by a withdrawal period of 1 or 2 weeks showed increased feed consumption during the withdrawal period and improved feed conversion ($P < 0.05$) in comparison with unmedicated birds (McDougald and McQuiston 1980a). More recent tests with broiler chickens in a 46-day floor pen test showed that the withdrawal of salinomycin (60 ppm) on day 39 of test resulted in an increase ($P < 0.05$) in feed intake per bird in comparison with birds still on the drug, but no benefit on weight gain or feed conversion (Chapman et al. 1993). Floor pen studies with male and female broiler chickens demonstrated that salinomycin had no effect on the dietary requirements for methionine over a 49-day grow-out (Leeson and Summers 1983). Jones et al. (1990) using Arbor Acres broiler breeders reported that feeding 60 ppm salinomycin for 10 consecutive days significantly ($P < 0.05$) reduced egg hatchability during days 1 through 6 of the withdrawal period. Sixty ppm salinomycin was incompatible with tiamulin medication in the water (125 and 250 mg/l) in studies by Frigg et al. (1983) and Laczay et al. (1989), and the dihydroquinoline-type antioxidant (6,6'-ethylidene-bis/2,2, 4-trimethyl-1,2-dihydroquinoline) when used to stabilize

poultry diets (Proháska et al., 1987; Proháska and Rozsnyai, 1990; Varga et al., 1994). Salinomycin has also been found to be incompatible with erythromycin, sulfachlorpyrazine, sulfaquinoxaline, and sulphadimethoxine (Dowling 1992).

Efficacy: A comparison of the efficacy of 60 ppm salinomycin, 100 ppm monensin, and 75 ppm lasalocid against different combinations of *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix*, *E. brunetti*, and *E. tenella* demonstrated that salinomycin was equal to or more efficacious than either of the other two ionophores (Migaki, Chappel, and Babcock 1979). Comparable results were also reported by a number of workers comparing 60 ppm salinomycin with varying levels of monensin ranging from 90 to 120 ppm (Bedrnik et al. 1980; Greuel and Raether 1980; Migaki et al. 1980). Means for 56-day weight gain and feed conversion, coccidial lesion scores, hematocrit, and serum optical density were comparable for 60 ppm salinomycin, 3 ppm halofuginone, and 100 ppm monensin against a mixed infection of *E. acervulina*, *E. necatrix*, and *E. tenella* in a floor pen test with broiler chickens (Yvoré et al. 1980). The latter three measurements in this test were obtained at 7 and 14 days after the start of infection on day 13. A comparison of 66 ppm salinomycin, 100 and 121 ppm monensin, and 75 and 125 ppm lasalocid indicated the three ionophores were comparable in efficacy at the levels tested against mixed infections of the six *Eimeria* spp. indicated above (McDougald, Keshavarz, and Rosenstein

1981). A pooled analysis of 54 field trials comparing 60 ppm salinomycin with 100 ppm monensin in many different countries demonstrated that means for final weight and feed conversion (feed:gain) for broiler chickens in the salinomycin treatment were superior ($P < 0.05$) in comparison with birds in the monensin treatment (Hamant et al. 1984). Comparable findings were also reported by these workers in 13 trials comparing 60 ppm salinomycin with 3 ppm halofuginone. In nine field trials comparing 60 ppm salinomycin with 90 ppm lasalocid, final weights between the two treatments were not different, but feed conversion was improved ($P < 0.05$) in the salinomycin treatment. Studies with *E. acervulina*, *E. maxima*, and *E. tenella* demonstrated that salinomycin had a cidal effect against sporozoites and schizonts, including late schizogonous stages (Chappel 1979; Conway et al. 1993). In studies with *E. acervulina*, there was no improvement in bird performance when medicated feed was given longer than 72 hours.

Semduramicin

Chemical name: [113378-31-7] (2R,3S,4S,5R,6S)-tetrahydro-2,4-dihydroxy-6-[(1R)-1-[(2S,5R,7S,8R,9S)-9-hydroxy-2,8-dimethyl-2-[(2S,2'R,3'S,5R,5'R)-octahydro-2-methyl-5'-[(2S,3S,5R,6S)-tetrahydro-6-hydroxy-3,5,6-trimethyl-2H-pyran-2-yl]-3'-[[2S,5S,6R)-tetrahydro-5-methoxy-6-methyl-2H-pyran-2-yl]oxy][2,2'-bifuran]-5-yl]-1,6-dioxaspiro[4.5]dec-7-yl] ethyl]

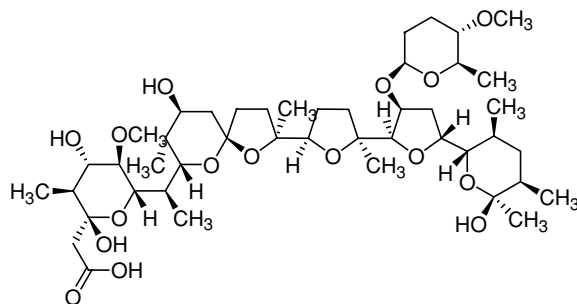


Figure 6.34. **Semduramicin.**

-5-methoxy-3-methyl-2H-pyran-2-acetic acid;
sodium salt [119068-77-8].

Product name: Aviax.

Chemical structure: See figure 6.34.

Safety: The discovery of semduramicin began with the fermentation of *Actinomadura roseorufa* isolated from a soil sample in Japan. The polyether ionophore isolated from this culture proved to have excellent anticoccidial efficacy, but the safety profile was not acceptable. As a consequence, a semisynthetic program was initiated to determine if the safety profile could be improved by chemical modification of the original ionophore. This effort proved successful with the removal of a glycone ring to form semduramicin (Glazer et al. 1992). Once it was demonstrated that the safety profile of semduramicin was significantly improved and did indeed meet requirements, a major effort was initiated to obtain a mutant of *A. roseorufa*

that would yield semduramicin directly. This involved subjecting a population of the original culture to selective mutagens over hundreds of generations, ultimately leading to a strain of *A. roseorufa* that produced semduramicin exclusively in large scale fermentation (Glazer et al. 1990; Tynan et al. 1990). Broiler chickens fed 0, 25, 50, or 75 ppm semduramicin in a 49-day floor pen trial demonstrated a dose-related decrease in feed consumption with a concomitant decrease in weight gain at the two highest levels of semduramicin (Conway 1996). Hematological and pathological results were normal in all treatments, and litter quality was not affected nor was bird mortality. Juvenile feathering was observed in some birds fed 50 and 75 ppm semduramicin in this study because of the reduced feed intake. A series of floor pen trials was conducted to determine if the feeding of 25 ppm semduramicin would affect the performance of broiler chickens at different dietary levels and sources of protein, methionine levels, and electrolyte balance (Pesti et al. 1999a, 1999b, 1999c). The overall conclusion from these studies was that semduramicin had no adverse effects or interactions on these factors. More recent floor pen performance studies by Pesti et al. (2002) with male broiler chickens indicated that the feeding of 25 ppm semduramicin in the starter and grower feeds for either 34 or 39 days followed by a 15- or 10-day withdrawal period, respectively, resulted in improved feed conversion at 49 days of testing. The improvements in feed conversion observed in these studies were a result

of reduced feed consumption during the starter and grower phases of production. The feeding of semduramicin to broiler breeders at several levels up to 25 ppm for periods greater than one week caused a decrease in cumulative egg production, percentage shell, and cumulative fertile hatchability, and an increase in early embryonic mortality (Brake et al. 2001).

Efficacy: Dose titration trials demonstrated that the optimum use level of semduramicin for the prevention of coccidiosis caused by *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, *E. mitis*, and *E. tenella* was 25 ppm (McKenzie et al. 1993). Studies comparing the efficacy of 25 ppm semduramicin with 60 ppm salinomycin, 5 ppm maduramicin, and 100 ppm monensin against recent field isolates of different *Eimeria* spp. demonstrated that semduramicin was, with one exception, equal to or superior in efficacy to the other ionophores against the different isolates tested (Logan et al. 1993). The one exception occurred in the comparisons between semduramicin and maduramicin. In these instances, semduramicin was more efficacious than maduramicin in controlling *E. acervulina* and *E. maxima*, but less efficacious than maduramicin in controlling *E. tenella*. The high efficacy of 25 ppm semduramicin against *E. maxima* was further demonstrated in separate studies using two levels of oocyst inocula (Conway et al. 1995). In these studies, the efficacy of semduramicin was demonstrated to be greater than 90% in controlling *E. maxima* based

on improvements in bird performance, plasma carotenoid concentrations, and coccidial lesion control. The efficacy of 25 ppm semduramicin, 66 ppm salinomycin, and 110 ppm monensin against a mixed infection of *E. acervulina*, *E. maxima*, and *E. tenella* was compared in three floor pen trials (Quarles et al. 1992). In a pooled analysis of the final results (at day 43), weight gain was improved in all three anticoccidial treatments in comparison with the infected, unmedicated treatment, and feed conversion was improved in the semduramicin and salinomycin treatments in comparison with the monensin treatment. Semduramicin was more efficacious than either salinomycin or monensin in the prevention of *E. maxima* lesions, and more efficacious than monensin in the prevention of *E. acervulina* and *E. tenella* lesions. Floor pen studies comparing the efficacy of 25 ppm semduramicin, 66 ppm salinomycin, and 110 ppm monensin against mixed infections of *E. acervulina*, *E. maxima*, and *E. tenella* demonstrated that semduramicin was more efficacious than monensin and salinomycin in improving bird performance and shank pigmentation scores (McDougald, Mathis, and Conway 1996). In these studies, semduramicin was more efficacious than salinomycin in controlling upper intestinal coccidial lesions, more efficacious than monensin in controlling midintestinal lesions, and comparable to the other two ionophores in controlling lesions in the ceca. Stage of action studies with *E. acervulina* and *E. tenella* demonstrated that semduramicin had a cidal effect early in the coccidial

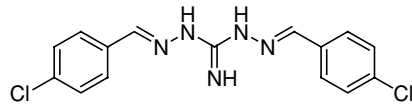


Figure 6.35. **Robenidine.**

life cycle against sporozoites and schizonts, including late schizogonous stages (Conway et al. 1993). Semduramicin was more efficacious than salinomycin in controlling lesions caused by both species tested in these studies.

Robenidine hydrochloride

Chemical name: [25875-51-8] bis[(4-chlorophenyl)-methylene]carbonimidic dihydrazide; 1,3-bis [(*p*-chlorobenzylidene)amino]guanidine; hydrochloride [25875-50-7].

Product name: Robenz, Cycostat.

Chemical structure: See figure 6.35.

Safety: In a series of three floor pen trials, the performance of broiler chickens fed different levels of robenidine from 33 to 330 ppm for 56 to 63 days was not adversely affected at levels up to 198 ppm (Berger et al. 1974). Means for weight gain and feed conversion were adversely ($P < 0.01$) reduced in these studies at the highest level (330 ppm). A 5-day withdrawal period is required in broiler chickens to prevent residues, and to avoid

an adverse taste of the meat. It is also contraindicated in laying hens to prevent residues in the egg.

Efficacy: Robenidine acts early in the life cycle against the trophozoites, primarily toward the end of the first asexual stage, and if medication is extended to 7 days or longer it is cidal in action by its inhibition of respiratory activity in cell mitochondria (Ryley and Wilson 1971; Reid 1972). First generation schizonts were initially arrested in development by robenidine, and for a short time these schizonts can resume development if medication is withdrawn from the feed. Several studies testing the efficacy of 33 ppm robenidine in the diet of broiler chickens demonstrated it was highly efficacious against all major *Eimeria* spp. in chickens (Kantor et al. 1972; Kennett, Kantor, and Gallo 1974). Robenidine at 33 ppm was highly efficacious against severe infections of *E. necatrix*, *E. brunetti*, and *E. tenella* in laboratory studies reported by Ryley and Wilson (1975).

Resistance to robenidine was induced in the Weybridge strain of *E. maxima* within seven passages by Joyner and Norton (1975). These workers also found that the resistant strain was robenidine dependent, and could only be satisfactorily established in birds receiving robenidine in the diet. Resistance did not develop as quickly in a second strain tested in their studies, nor did it show drug dependence. Strains of *E. tenella* and *E. acervulina* showing varying degrees of resistance to robenidine were described subsequently by several

workers (Chapman 1976a, 1976c; Lee and Fernando 1977; McLoughlin and Chute 1978; Hamet 1986; Chapman and Hacker 1994). These data indicate that robenidine sensitivity to the above species is best maintained when the drug is used sporadically in rotation with other anticoccidial drugs. A good illustration of this was demonstrated in a survey conducted by Chapman (1989c) in which all of 30 isolates of *E. tenella* obtained from broiler and breeder farms were sensitive to robenidine.

Steroidal sapogenin

Chemical name: U.S. Patent 6,569,843.

Product name: Cocci-Guard.

Chemical structure: Information is not available.

Safety: Steroidal sapogenins are extracted from plants in the Liliaceae, Amaryllidaceae, and Dioscoraceae families. Many different chemical variations of the steroidal sapogenin structure have been identified. Feeding steroidal sapogenin to chickens for 42 days at levels of 3.32, 9.96, and 33.21 ppm had no adverse effect on performance or mortality. A study in broiler chickens given a diet containing 3.4 ppm steroidal sapogenin with a chromic oxide marker indicated that steroidal sapogenin was not absorbed nor metabolized. Tissue residue studies in chickens have also confirmed these findings. Extensive field studies in

Brazil, Mexico, and Peru have demonstrated that steroidal sapogenin is safe and effective in chickens in comparison with other anticoccidial programs (Walker 2002).

Efficacy: Steroidal sapogenin at 3.4 ppm is slightly less efficacious than 66 ppm salinomycin and 99 ppm monensin against *E. acervulina*, comparable to salinomycin and monensin against *E. maxima*, and slightly more efficacious than salinomycin and monensin against *E. tenella* (unpublished data). A study involving a mixture of these three species demonstrated that the efficacy of 3.4 ppm steroidal sapogenin was comparable to 66 ppm salinomycin, 99 ppm monensin, and 5 ppm maduramicin based on bird performance, coccidial lesion control, and mortality (Mathis 2001). This author also reported that 3.4 ppm steroidal sapogenin was moderately efficacious against a mixed infection of *E. necatrix*, *E. brunetti*, and *E. mitis*. The efficacy of 6.8 ppm steroidal sapogenin was substantially improved, particularly against *E. tenella*, in comparison with the 3.4 ppm use level in a study involving a mixture of *E. acervulina*, *E. maxima*, and *E. tenella* (unpublished data).

Triazines

Diclazuril and toltrazuril belong to the triazine class of compounds that have demonstrated a high degree of anticoccidial activity at relatively low levels of medication when given either in the feed

or drinking water (Chappel, Howes, and Lynch 1974; Ryley, Wilson, and Betts 1974; Haberkorn and Stoltefuss 1987). Compounds in this class are coccidiocidal rather than coccidiostatic.

Diclazuril

Chemical name: [101831-37-2] 2, 6-dichloro- α -(4-chlorophenyl)-4-(4, 5-dihydro-3, 5-dioxo-1, 2,4-triazin-2(3H)-yl)benzeneacetonitrile; (*p*-chlorophenyl)[2,6-dichloro-4-(4,5-dihydro-3,5-dioxo-*as*-triazin-2(3H)-yl)phenyl]acetonitrile.

Product name: Clinacox.

Chemical structure: See figure 6.36.

Safety: Body weight and feed conversion were not significantly affected ($P \leq 0.05$) in birds fed diclazuril at 1, 5 and 10 ppm for 42 days in comparison with uninfected, unmedicated birds in floor pen studies by Vanparijs, Marsboom, and Desplenter (1989). These studies demonstrated that diclazuril was well tolerated up to 10 times the recommended dose of 1 ppm.

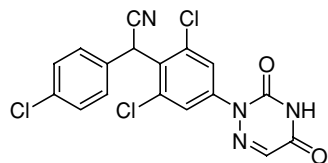


Figure 6.36. Diclazuril.

Efficacy: Initial studies testing the efficacy of diclazuril in the range of 0.5 to 10 ppm clearly demonstrated that high efficacy was achieved in preventing infections of *E. acervulina*, *E. maxima*, *E. necatrix*, *E. mivati*, *E. mitis*, *E. brunetti*, and *E. tenella* when used at a level of 1 ppm (Mathis and McDougald 1988; Vanparijs, Marsboom, and Desplenter 1989; Vanparijs et al. 1989; McDougald, Mathis, and Seibert 1990). Variables measured in these studies to determine the efficacy of diclazuril included bird performance, mortality, coccidial lesion scores, dropping scores, and oocyst counts in medicated and unmedicated birds. The results of these tests indicated that 1 ppm diclazuril was not as efficacious against *E. maxima* as it was against the other species in controlling coccidial lesions, and best lesion control was against infections of *E. acervulina* and *E. tenella*. Studies in floor pens confirmed these findings and further demonstrated the high efficacy of 1 ppm diclazuril against a wide selection of recent field isolates of *Eimeria* species (Kutzer, Lowenstein, and Mitterlehner 1988; Braem 1989; McDougald et al. 1990; Montemayor, Casas, and Moreno 1990; Vanparijs et al. 1990; Conway et al. 2001).

Studies by Maes et al. (1988, 1989) and Verheyen et al. (1988, 1989) demonstrated that diclazuril has a cidal effect against both asexual and sexual stages of *E. tenella*, the late schizont stages of *E. necatrix* and *E. acervulina*, the gametocytes of *E. brunetti*, and the zygote of *E. maxima*. The latter finding helps to explain why diclazuril was less

efficacious in controlling lesions caused by *E. maxima* in comparison with the other species since the primary lesions due to this species develop at an earlier stage in the life cycle. The ultimate effect of diclazuril regardless of the coccidial species is that the life cycle is interrupted and the parasite is destroyed.

Diclazuril has been found to have some residual efficacy lasting for several days following drug withdrawal against *E. acervulina*, *E. maxima*, *E. necatrix*, *E. mitis*, *E. brunetti*, and *E. tenella* (McDougald and Seibert 1998). This effect appears to be unique among a broad range of chemical and polyether ionophore anticoccidial drugs.

Three field isolates of *E. acervulina*, two isolates of *E. acervulina* and *E. maxima* mixture, and one isolate of *E. tenella* were found to be resistant to 1 ppm diclazuril, and four additional isolates of these species were partially resistant, in a survey conducted in Brazil approximately two years after its introduction for commercial use (Kawazoe and Di Fabio 1994). Two field isolates of *E. acervulina* and one isolate of *E. maxima* were found to be resistant to 1 ppm diclazuril in a survey conducted in northern Germany (Stephan et al. 1997). Studies by Chapman (1989b) demonstrated that an incomplete resistance to diclazuril was induced within 10 serial passages of *E. acervulina* and *E. tenella* under laboratory conditions using increasing concentrations of drug in the diet.

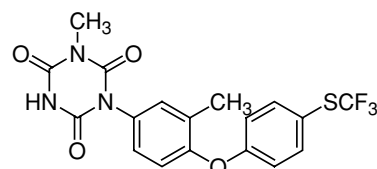


Figure 6.37. Toltrazuril.

Toltrazuril

Chemical name: [69004-03-1] 1-methyl-3-[3-methyl-4-[4-[(trifluoromethyl)thio]phenoxy]phenyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione; 1-methyl-3-[4-[p-[(trifluoromethyl)thio]phenoxy]-m-tolyl]-s-triazine-2,4,6(1H,3H,5H)-trione.

Product name: Baycox.

Chemical structure: See figure 6.37.

Safety: The LD₅₀ of toltrazuril in chicks was reported to be 1,000 mg/kg (Vázquez and Vázquez 1990). This is approximately 143 times the recommended therapeutic dose rate.

Efficacy: Medication with 20 ppm toltrazuril in the drinking water for days 2 and 3 post inoculation provided good efficacy against mixed infections of *E. acervulina*, *E. maxima*, and *E. tenella* in chickens (Haberkorn and Stoltefuss 1987). Intermittent medication with 50 ppm toltrazuril in the drinking water was very effective when

given on two to three occasions at weekly intervals against repeated inoculation with a line of *E. tenella* resistant to monensin (Chapman 1987). Birds inoculated with high doses of oocysts during the period of medication were found to be immune to subsequent challenge. Toltrazuril at 25 ppm in the drinking water (2.5% concentration) beginning 48 hours after infection for 2 days was efficacious in preventing mortality and oocyst production, and in reducing lesions in chickens infected by recent field isolates of *E. tenella* in a study by Chapman (1989a). Medication with toltrazuril in the drinking water at 25 ppm for 2 days or at 75 ppm for 8 hours each day for 2 days was found to be highly efficacious against *E. acervulina*, *E. maxima*, *E. brunetti*, *E. necatrix*, *E. mitis*, and *E. tenella* in chickens in a series of laboratory and field studies summarized by Mundt (1989). Toltrazuril was more effective than sulphachlorpyrazine in the control of cecal lesions caused by *E. tenella* when treatment was initiated 24 hours after infection, but when treatment was delayed to 72 hours after infection the sulphonamide was more efficacious (Laczay, Voros, and Semjen 1995). The efficacy of 37.5, 75, and 150 ppm toltrazuril in the drinking water was more efficacious than 1.5, 3, and 6 ppm halofuginone given via the feed against *E. tenella* based on performance data, lesion control, and oocyst counts (Ramadan, Abo el-Sooud, and el-Bahy 1997). A well-replicated floor pen study comparing various in-feed anticoccidial programs with and without toltrazuril

medication in the drinking water at 7 mg/kg of body weight on days 18 and 19 demonstrated that toltrazuril was highly efficacious against a mixed infection of *E. acervulina*, *E. maxima*, and *E. tenella* (Mathis et al. 2003). The results of this study indicated that toltrazuril provided significant benefits in performance, lesion control, and reduction of oocyst counts when used either as a supplement to in-feed anticoccidial programs or as the sole anticoccidial program with unmedicated feed.

The intermittent use of toltrazuril as the only anticoccidial during five consecutive growing cycles with broiler birds resulted in the development of resistance in some *Eimeria* isolates (Vertommen, Peek, and van der Laan 1990). In a survey of *Eimeria* field isolates from northern Germany, several isolates were found to be partially resistant to toltrazuril and diclazuril, and one isolate was found to be resistant to both drugs (Stephan et al. 1997).

It has been reported that toltrazuril acts against all intracellular stages of coccidia, and that electron microscopic exams have shown that the drug interferes with nuclear division and with mitochondrial activity (Vázquez and Vázquez 1990). These workers reported that toltrazuril causes a severe vacuolization of intracellular stages of coccidia due to a swelling of the endoplasmic reticulum.

Anticoccidial vaccines

More than 50 years have passed since the introduction of the first live anticoccidial vaccine Coccivac for use in poultry in 1952, and another 33 years before a second live vaccine, Immucox, came into the marketplace (Shirley and Long 1990; Williams 2002b). These are nonattenuated strains of field isolates of *Eimeria* spp. as described below. Since then, five more live vaccines have been launched commercially in different regions of the world. The first two of the newer vaccines, Paracox and Livacox, are composed of attenuated strains of *Eimeria* spp. that are characterized for their shortened life cycle and reduced pathogenicity (Long 1972, 1974; Jeffers 1975, 1986; McDougald and Jeffers 1976; Shirley 1993). A third vaccine, Nobilis COX ATM, is a nonattenuated mixture of wild-type *Eimeria* spp. that is “relatively” tolerant to ionophores (Vermeulen, Schaap, and Schetters 2001). The fourth and fifth vaccines, Advent and Inovocox, are nonattenuated mixtures of *E. acervulina*, *E. maxima*, and *E. tenella*. Inovocox was designed for administration *in ovo*. Several other vaccines have been reported to be under development (Williams 2002a).

The effectiveness of any live vaccine program is dependent on the initial controlled vaccination either at the hatchery or farm followed by controlled cycling of each *Eimeria* species in the vaccinated birds for up to four weeks to allow full immu-

nity development (Williams 2002a). Environmental conditions in the poultry house need to be carefully monitored during this period to ensure both a good immunity development and that the birds achieve industry-standard weight gains and feed conversion.

Initially, the greatest application of live vaccines was in layer and broiler breeder birds at time of placement as chicks. This began to change in the last decade of the 20th century, and there is currently a growing use of live anticoccidial vaccines in broiler birds as well (Chapman 2000; Shirley 2000; Williams 2002a). An important factor contributing to this growing use in broiler flocks is the ability to apply vaccines in an economically effective way at the hatchery by spray cabinet or *in ovo* inoculation.

Vaccines provide a very practical and important alternative to the exclusive use of anticoccidial drugs for two excellent reasons. First, it has been demonstrated in a number of studies that vaccines give a comparable level of coccidiosis protection to the growing broiler chicken as current anticoccidial programs (Danforth et al. 1997; Danforth 1998; Williams et al. 1999; Williams 2002a; Mathis and Lang 2002; Williams and Gobbi 2002; Fitz-Coy 2005). An exception to this may be in grow-out times of less than 42 days (Waldenstedt et al. 1999). Second, it has been demonstrated that the use of live vaccines, with the exception of Nobilis COX ATM, leads to a replacement of the

indigenous populations of coccidia in the broiler growing facility with susceptible coccidial populations carried over from the vaccine (Jeffers 1976; Chapman 1994, 2000; Williams 1998; Mathis and Broussard 2005; Peek and Landman 2005). Under these conditions, the efficacy of anticoccidial drugs is extended substantially and can be used very effectively in rotation programs with vaccines (Williams 2002a).

Research and development of recombinant vaccines has not been very successful to date for a number of reasons (Allen and Fetterer 2002). CoxAbic, just recently introduced, is the only example of a subunit vaccine for coccidiosis that is commercially available at this time (Belli et al. 2004).

Nonattenuated vaccines

Coccivac

Coccivac contains a mixture of live, wild-type strains of *Eimeria* spp. that are sensitive to anticoccidial drugs. The *Eimeria* isolates used in the vaccine were isolated prior to the use of most if not all of the anticoccidial drugs used today. The numbers of oocysts of each species are administered in a controlled dose carefully calculated to induce sufficient infection to produce an appropriate immune response without causing a pathogenic effect following two to three life cycles of coccidia.

Day-old chicks are vaccinated via a high-volume (21 ml) very coarse spray through the manufacturer's spray cabinet (SprayCox) or handheld spray applicator (SprayCox Junior). The birds ingest the oocysts in the vaccine while preening themselves immediately after vaccination. A red dye, mixed with the vaccine at the hatchery, serves to further attract their attention as they preen themselves. Vaccination via the SprayCox cabinet has enhanced the uniformity of initial application, vastly improving the vaccination response, and making other methods of vaccination such as feed spray, drinking water application, and eye spray methods much less efficient.

Coccivac vaccines may be used in rotation with in-feed anticoccidial drug medication to repopulate poultry houses with anticoccidial drug-sensitive strains of *Eimeria* or as a stand-alone coccidiosis control program.

Coccivac-D contains a mixture of *E. acervulina*, *E. brunetti*, *E. hagani*, *E. maxima*, *E. mivati*, *E. necatrix*, *E. praecox*, and *E. tenella* for use in birds grown beyond 8 weeks such as layer and breeder replacement birds.

Coccivac-B is a mixture of *E. acervulina*, *E. maxima*, *E. mivati*, and *E. tenella* specified for use in broiler chickens.

Coccivac-T contains a mixture of *E. dispersa*, *E. meleagrimitis*, *E. adenoides*, and *E. gallopavonis*

for vaccination of commercial and breeder turkeys.

Immucox

Immucox is a live vaccine composed of wild-type *Eimeria* spp. as described below. The vaccine is administered by water or by an edible gum based gel (Immunogel). Vaccination by gel is accomplished by placing the gel in the chick boxes at the hatchery on the day of hatch. The gel should be completely consumed before delivery. Administration of the vaccine by gel was reported to be superior to immunization by gavage, spray cabinet, or by conventional delivery in the water in studies by Danforth et al. (1997). In a floor pen trial reported by these workers, the performance of immunized birds was comparable to nonimmunized birds given an anticoccidial drug in the feed. Field studies in Texas comparing the use of Immucox with an anticoccidial drug program (halofuginone-salinomycin shuttle) demonstrated that means for final weights and feed conversion were slightly improved in the Immucox-vaccinated flocks in comparison with the flocks medicated with anticoccidial drugs (Lee 1989).

Immucox for Chickens 1 (broilers and roasters) contains a mixture of *E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella*.

Immucox for Chickens 2 (breeders and egg layers) contains a mixture of the above species plus *E. brunetti*.

Nobilis COX ATM

This vaccine contains a mixture of ionophore-tolerant strains of *E. acervulina*, *E. tenella*, and *E. maxima* (two antigenically different strains). The reason for using ionophore-tolerant strains of coccidia in the vaccine is that it allows the use of an ionophore anticoccidial drug in the feed during the first 3 to 4 weeks of production while immunity is developing to prevent any significant outbreak of coccidiosis (Vermeulen et al. 2001).

Advent

Advent coccidiosis control vaccine contains a mixture of *E. acervulina*, *E. maxima*, and *E. tenella*. The selected isolates are sensitive to commonly used anticoccidial drugs, and the vaccine is intended for use in broiler flocks either by itself or in rotation with anticoccidial drugs. The vaccine is administered either by spray cabinet at the hatchery or by application directly on the feed at the time chicks arrive in the production unit. Advent has been demonstrated to be safe and efficacious in broiler chickens in studies involving both natural exposure and controlled coccidial challenge (Cherry et al. 2003; Dibner et al. 2003c). Broiler chickens immunized with Advent have demonstrated a high level of resistance to challenge with $3 \times 10^5 E. acervulina$ and $4 \times 10^4 E. maxima$ or *E. tenella* based upon the reduction of lesion scores and bird performance in immunized compared with nonimmunized birds in studies reported by Dibner, Quiroz, and Knight (2005a). Studies on commercial broiler farms have shown that the use

of Advent through five successive grow-outs resulted in improved performance in two subsequent grow-outs when an anticoccidial drug was used in comparison with flocks on a farm where the vaccine was not used (Cherry, Quiroz, and Bray 2005).

The quality of coccidial vaccines is only as good as the viability of the oocysts in the vaccine. Experience has shown that the viability of the oocysts in the vaccine can be affected adversely during shipment and storage. An *in vitro* assay procedure (Viacyst) was recently described by Dibner et al. (2003a, 2003b; Dibner, Quiroz, and Knight 2005b) to measure the viability of *E. acervulina*, *E. maxima*, and *E. tenella* sporocysts in Advent. The Viacyst assay procedure accurately quantifies the number of live sporulated oocysts given at the time of vaccination, thus ensuring proper protection levels for birds vaccinated with Advent.

Inovocox

Inovocox is a live oocyst vaccine containing a mixture of *E. acervulina*, *E. tenella*, and two strains of *E. maxima* intended for administration *in ovo* on day 18 of incubation using an automated *in ovo* injection device (Inovoject System). Studies with *E. acervulina* oocysts, sporocysts, and sporozoites given *in ovo* on day 18 of embryo development demonstrated that each stage of coccidia resulted in infection in the chick upon hatching as demon-

strated by oocyst output and evidence of protective immunity (Doelling et al. 2001). Field studies with Inovocox administered to commercial broiler embryos on day 18 of incubation demonstrated the vaccine to be safe and equal in efficacy to a standard polyether ionophore anticoccidial drug program given in the feed to unvaccinated birds (Avakian et al. 2001). Comparable results were also reported by Poston et al. (2001) in a separate set of field trials conducted through three successive grow-outs. Two 49-day floor pen studies were conducted comparing Inovocox vaccinated birds with nonvaccinated birds given 66 ppm salinomycin in the feed (Martin et al. 2005a). In one study, the birds were placed on clean litter while in the second study built up litter was used. At the end of each study, the means for feed conversion and body weight for birds in each treatment were equivalent. Studies by Doelling et al. (2005) have shown that full immunity had developed by days 21–22 in birds vaccinated with Inovocox based on improvements in weight gain and reduced lesion scores in comparison with nonvaccinated birds.

The strains of coccidia used in the Inovocox vaccine have been demonstrated to be sensitive to the most commonly used anticoccidial drugs such as salinomycin, monensin, diclazuril, and nicarbazin in studies by Martin et al. (2005b). *In ovo* administration of Inovocox on day 18 of incubation at the same time with either a commercial infectious bursal disease (IBD) vaccine or a commercial

HVT/SB1 Marek's disease vaccine (MDV) demonstrated that interference did not occur in the development of immunity to these respective pathogens (Doelling et al. 2004; Poston et al. 2004).

Attenuated vaccines

Livacox

Livacox vaccines are composed of attenuated strains of *Eimeria* spp. as indicated for a very select use in the field. These vaccines are administered in the drinking water at 1 to 10 days of age or in the hatchery by intra-ocular spraying (Chapman 2000; Williams 2002a).

Livacox T for broilers and breeders contains a mixture of *E. acervulina*, *E. maxima*, and *E. tenella*.

Livacox Q for broilers contains a mixture of *E. acervulina*, *E. maxima*, and *E. tenella*.

Livacox D for caged chickens contains a mixture of just *E. acervulina* and *E. tenella*.

Paracox

Upon its introduction in 1989, Paracox became the first commercially available vaccine containing attenuated strains of *Eimeria* spp. (Francis 2000). The strains selected for this vaccine have a shortened life cycle and are relatively non-

pathogenic (Shirley, 1993). Paracox vaccine can be administered in the hatchery (SprayCox), by spraying on the feed, or by being mixed in the drinking water. Vaccination in the feed should be done within the first 24 to 48 hours after the chicks are placed. Water vaccination is recommended at 3 days of age. Vaccination by spraying on the feed at 1 day of age, as opposed to a later time, appears to provide a substantial benefit in stimulating an earlier development of immunity in broiler birds grown out to just 6 or 7 weeks of age (Williams 2000).

Paracox-5 contains strains of *E. acervulina*, *E. maxima* (2 strains), *E. mitis*, and *E. tenella* for use primarily in broiler chickens.

Paracox-8 contains the above species plus *E. brunetti* and *E. necatrix* for use in free range chickens, broiler birds produced without drug treatment for the organic market, capons, layers, and breeder replacement birds (Frémont 2000).

Subunit vaccines

CoxAbic

This vaccine is prepared from affinity purified gametocyte antigens isolated from *E. maxima* (Wallach et al. 1995). According to Belli et al. (2004), production of this vaccine is expensive, and a recombinant subunit vaccine substitute for

CoxAbic is desirable. Their work indicated the development of a recombinant subunit vaccine meeting “the antigenic and immunogenic properties of the native protein vaccine, CoxAbic, is feasible” (Belli et al. 2004). Breeding hens vaccinated intramuscularly twice with CoxAbic during rearing produced chicks that demonstrated immunity to infections of *E. acervulina*, *E. maxima*, *E. mitis*, and *E. tenella* (Finger and Michael 2005).

Testing anticoccidial vaccines

Guidelines for evaluating the efficacy and safety of live anticoccidial vaccines were recently reviewed by Chapman et al. (2005). These guidelines provide recommendations on the design of laboratory and field trials with vaccines and information on regulatory requirements in the European Union and the United States.

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Notes