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COCCI FORUM

NUMBER 7

FEATURES

IRREFUTABLE EVIDENCE

New study validates theory that vaccinating with Coccivac-B restores *Eimeria* sensitivity to diclazuril (Clinacox)

A long-held theory that vaccinating with Coccivac-B for just two cycles restores *Eimeria* sensitivity to the new-generation in-feed anticoccidial diclazuril (Clinacox) was validated recently in a large-scale field trial with a major US poultry company.

The results, according to poultry disease specialists involved in the study, could change the broiler industry's strategy for managing coccidiosis, a costly parasitic disease in poultry.

Dr. David Chapman

Dr. Harry Danforth

Previous investigations into the relationship between coccidiosis vaccination and in-feed anticoccidials — including studies by well-known researchers Dr. David Chapman of the University of Arkansas, Dr. Harry Danforth, USDA, and Dr. Greg Mathis of Southern Poultry Research in Athens, Georgia. — have demonstrated that vaccinating with Coccivac-B restores anticoccidial sensitivity in a poultry house by replacing resistant *Eimeria* organisms with ones still sensitive to in-feed anticoccidials used today.

"In addition, pen trials over the years have indicated that Coccivac-B used in a rotational program can effectively displace wild field strains of coccidia and restore sensitivity to the current in-feed anticoccidials," says Dr. Rick Phillips, director of worldwide poultry technical services, Schering-Plough Animal Health.

Those studies, however, focused on ionophore anticoccidials, not on diclazuril, now a widely-used, chemical anticoccidial.

"Despite the research and all the field reports in hand, we felt it was important to test our hypothesis in the field where the proverbial 'rubber meets the road'," Phillips says. "These latest trials with diclazuril without a doubt prove our hypothesis."

Study background and design

The study, sponsored by Schering-Plough Animal Health, was conducted at the site of a large US integrator. Independent investigators involved in the trial were the poultry company's veterinarian and Dr. Mathis of Southern Poultry Research, who conducted sensitivity testing.

The integrator, which asked not to be identified in this report, added Coccivac-B to its anticoccidial rotation to see if using the live-oocyst vaccine improved the effectiveness of diclazuril — or changed the coccidial population to one more sensitive to diclazuril — as well as conventional ionophores. The move was part of a concerted effort by the company to develop new tools and long-term strategies for managing coccidiosis.

The poultry company first collected litter samples from eight farms involved with its seven complexes to obtain baseline diclazuril sensitivity information. Prior to this, the complexes had been on a variety of in-feed anticoccidial rotation programs, all of which included diclazuril for one to two cycles the previous year, he says.

Dr. Charlie Broussard, worldwide poultry technical services manager for Schering-Plough Animal Health,

explains that after collecting initial litter samples, four of the seven complexes continued their annual rotation using ionophores or chemical-ionophore shuttle programs. The remaining three complexes incorporated two cycles of Coccivac-B vaccine into the annual rotation. Identical houses were re-sampled after two cycles of Coccivac-B or anticoccidial rotation.

To determine diclazuril sensitivity, Mathis looked at weight reduction and coccidial lesion scores in test birds and compared them to unchallenged controls. He then summarized diclazuril's efficacy as "good," "moderate" or "poor."

"Even though diclazuril was given a rest, diclazuril sensitivity did not improve or improved very little in the complexes that rotated diclazuril with other in-feed anticoccidials, rather than with the vaccine," Broussard says. "On the other hand, diclazuril sensitivity improved significantly in the complexes that used two cycles of Coccivac-B in the rotation. Not one of the samples tested scored 'poor' for sensitivity following Coccivac-B use." (See Figure 1.)

A few highlights from the trial follow, according to Broussard:

• In one complex, diclazuril sensitivity before vaccination was rated "good" in only 30% of samples. After vaccination, however, 100% of samples were rated "good."

• In another complex, only 33% of samples were rated good before vaccine use, compared to 83% after vaccination. (See Table 1.)

• In contrast, one complex where the vaccine was not used, investigators rated only 25% of samples as "good" for diclazuril sensitivity at the start of the study. After continuing on a traditional rotation program and "resting" diclazuril, 0% of the samples were rated "good" for diclazuril sensitivity.

Mathis: 'One of the most coccidiosis-significant studies'

Considering its 2-to 3-years' experience with coccidiosis vaccination and the results of this trial, the poultry company plans to continue using Coccivac and will carefully monitor results as well as assess the vaccine's role in long-term methodology. "Many other factors are involved with the selection process so we cannot say that the process is sensitivity driven, though that's a major factor," says a veterinarian for the company.

"…The take-home message is that to get the performance you got when you originally had diclazuril or other in-feed anticoccidials, you're going to have to use a vaccine."

Take-home message

Pointing to the good performance of the vaccinated flocks and the reduction in lesion scores, investigator Mathis

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KNOW THE SPORE

Identifying viable, sporulated oocysts key to a quality coccidiosis vaccine

Identifying viable, sporulated *Eimeria* oocysts and ensuring that birds get just the right amount is one of the most important steps that go into producing an effective coccidiosis vaccine.

Knight: 'It's critical to have the right balance of antigens…'

Even so, with thousands of chicks moving through the hatchery, it's difficult for busy poultry veterinarians and production managers to appreciate the technology, careful selection process, experience and rigid quality-control standards that need to go into a vaccine of this nature.

"Coccidiosis vaccination works by providing a controlled, carefully balanced dose of oocysts — coccidial eggs — to protect against the several species of *Eimeria* that cause the disease in birds," says Graham Knight, manager of coccidiosis vaccine production at Schering-Plough Animal Health Corporation's production plant in Millsboro, Delaware.

"It sounds easy, but there's more to it than gathering oocysts and putting them in a bottle. The preparation and identification of suitable oocysts is crucial," he says. "It's also critical to have the right balance of antigens, which are the individual components that lead to protection against the *Eimeria* species causing disease."

Stimulating immunity

Producers need to understand that for a coccidiosis vaccine to stimulate immunity, oocysts must be capable of releasing viable spores. "In other words, they must be sporulated. They also must be viable to be infective," Knight says.

"An infective oocyst is a sporulated oocyst, but a sporulated oocyst is not necessarily an infective one," Knight says. "We think this is an important distinction for producers to know and understand."

The reason is that sporulated oocysts age and die. In addition, some oocysts never fully develop; they are only partially sporulated, while others may be abnormal or damaged and are not infective.

"A major part of our job is the identification of fresh, fully sporulated oocysts," Knight says. "These are the oocysts that are infective and that convey immunity to birds against coccidiosis. It takes a lot of experience to build a quality coccidiosis vaccine."

Tried and true

Although it's a tedious and important process, identifying viable sporulated oocysts isn't rocket science, Knight insists, but it does require an educated eye.

"In Millsboro, we have well-trained, seasoned technicians — many have been involved since the operation was moved to Millsboro in the 1980s. With a good microscope, they can easily differentiate a fresh, fully sporulated oocyst from an oocyst that's not," he says.

Microscopic examination is the traditional method of determining whether an oocyst is fully sporulated and has been in use since the early 1900s. "It's a straightforward and proven method, one that requires no manipulation of the sample other than dilution and needs no specialized equipment or technique," Knight explains.

Dr. Steve Fitz-Coy, now a technical

Photos by Lisa Helfert.

service representative for Schering-Plough Animal Health, agrees.

"Poultry producers have been dealing with coccidiosis for nearly 75 years, since groundbreaking work by E. E. Tyzzer in 1929. Identifying sporulated oocysts with a microscope is a tried and true process that's improved with time," he says.

Knight points out that data collected over the course of many years as well as field experience have demonstrated that coccidiosis vaccine made from fresh, fully sporulated oocysts within a fixed time frame has enough viable oocysts of each species at the end of the stated shelf-life (12 months) to be efficacious.

Complementary procedures

Several other procedures built into coccidiosis-vaccine production at Millsboro complement the identification of viable, sporulated oocysts and contribute to the vaccine's efficacy, says Knight.

One is the way in which oocysts are produced. Each coccidial species in the vaccine is grown in birds in a room dedicated to that species. The rooms are located in an antigen-production facility. The birds are not used for multiple species nor are they re-used, he says.

"Although most species of coccidial oocysts can be differentiated by trained technicians, there are exceptions. Different species have different sizes and shapes, but some overlap occurs, particularly among small oocysts. Growing each type in isolation solves this problem," Knight says.

Technicians inoculate one group of birds with known and tested seeds of one coccidial species. Excreted oocysts are then harvested and cleaned. "We don't need to differentiate oocysts — we only have to determine whether an oocyst is fully sporulated or not and how many there are.

"We need different numbers of oocysts for each species to make Coccivac. Ultimately, the oocysts are blended, but we grow them individually," he says.

Oocyst production

Nevertheless, Fitz-Coy points out, oocysts excreted by birds are not sporulated when harvested and, therefore, are not infective.

"We have to transform the harvested, non-sporulated oocysts to the sporulated and infective form under controlled conditions by providing warmth, humidity and oxygen," he says.

Adds Knight, "We mimic nature, but with the benefit of controlled conditions, we can efficiently transform the majority of oocysts over a given period of time. Because the viability of sporulated oocysts decreases with time, the harvested material is processed quickly at the Millsboro plant, which works to cGMP (current Good Manufacturing Practices)."

Knight and Fitz-Coy: 'We need different numbers of ooycsts for each species…'

After sporulation, the antigen lot is sampled and technicians identify and count the number of infective oocysts

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VACCINE CONTROLS COCCIDIOSIS, IMPROVES PERFORMANCE IN INTEGRATOR'S CONTROLLED STUDY

Editor's note: The information for this article was provided by a major US poultry company on the condition that its name and location be kept confidential. The sources mentioned in the article have nevertheless reviewed the information for technical accuracy and approved it for publication in CocciForum. It is presented here in our newest feature, Cocci Confidential, to help the poultry industry learn from these real-world experiences and improve their management of coccidiosis. If you have a story you would like to share with Cocci Confidential, please contact the editor at JFeeks@prworks.net or call 508-627-6949 (US).

The veterinarian was still skeptical. He wanted proof that vaccinating broilers to prevent coccidiosis wouldn't hurt performance.

His employer, a major poultry integrator in the US processing millions of birds annually, hadn't been vaccinating against coccidiosis for long. Initially, there were some performance irregularities, but he couldn't ignore the numbers from the third cycle — the results were stellar.

A good study was needed, but comparing a coccidiosis vaccine directly to a feed-grade anticoccidial program under true field conditions would be difficult. Non-medicated feed would have to be provided to houses with vaccinated flocks; in particular, no anticoccidials could be fed to vaccinated birds — they could destroy the vaccine oocysts that help build immunity to coccidiosis.

Anticoccidial feed would have to be delivered to other houses on the same farms with unvaccinated birds. Care would have to be taken to avoid manufacturing or delivery errors for the life of the flock. It would be a challenge for a big and busy integrator, but data was needed to determine whether changes should be made to the standard coccidiosis control program.

Study design

In the fall of 2002, a multi-farm, paired-house trial was initiated. It involved four, 4-house farms raising heavy broilers (7.5 lbs). The integrator vaccinated two houses on each farm with Coccivac-B, a live-oocyst vaccine; in the remaining houses, birds were fed an anticoccidial shuttle utilizing narasin and nicarbazin, which was the integrator's standard program and served as the study's control. Evaluations were conducted on 156,000 birds from the vaccinated group and on 156,000 birds from the control group.

The ration formulation for the two treatments was consistent except that vaccinated birds received a virginiamycin premix in the starter and finisher ration and bacitracin methylene disalicylate and roxarsone in the grower ration, while controls received narasin, nicarbazin and bacitracin methylene disalicylate in the starter ration and narasin and roxarsone in the grower and finisher rations (Table 1).

Findings

At 4 weeks (28 days) and 7.5 weeks of age (52 days), veterinarians from Schering-Plough Animal Health and Alpharma Inc. conducted posting sessions on the 16 test houses, where they looked for evidence of coccidial

species. They used microscopic evaluation for *Eimeria maxima*, since this species does not always produce distinct gross lesions and is more likely than other *Eimeria* species to impair feed conversion and weight gain. By 51 to 55 days of age, most of the lesions received mild scores of +1 and +2 (Figures 1 and 2).

In addition, the integrator processed birds from each treatment separately. The processing plant reported standard performance parameters such as the percent livability, gross pounds sold, average weight, feed conversion and average daily gain (Table 2).

The results

Vaccinated birds and the controls receiving feed-grade anticoccidials each demonstrated mild coccidial lesions at 4 weeks of age. However, the lesions had resolved by the second post-mortem exam at 7.5 weeks of age (Figures 1 and 2). Vaccination did not appear to affect either 7-day mortality or overall livability compared to controls.

On a farm-by-farm basis, vaccinated flocks outperformed or equaled the performance of the control flocks for all significant parameters. Vaccinated flocks also demonstrated better performance on an averaged basis. Compared to flocks receiving standard ionophores, those that were vaccinated had:

- An average weight 9.6 points higher
- An average feed conversion 1.6 points lower
- A caloric conversion 11 calories lower
- An adjusted caloric conversion 33 calories lower
- A standard cost per pound 0.17 cents lower

Discussion & conclusion

Coccivac delivers a controlled, balanced dose of sporulated or infective oocysts of the economically significant *Eimeria* species. A new generation of oocysts develop in vaccinated birds and are then excreted, providing re-exposure to *Eimeria* oocysts. The process stimulates natural, long-lasting immunity.

Generally, it takes about two to three oocyst cycles for strong immunity to develop. Vaccinated birds may be presented with a field challenge from

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Table 1
Ration Formulation: Coccivac-B vs. Control

	Starter	Grower	Finisher
Coccivac-B	virginiamycin 20g	bacitracin methylene disalicylate 50g	virginiamycin 10g
Control	narasin 62g bacitracin methylene disalicylate 50g	nicarbazin 63g roxarsone 22g	nicarbazin 54g roxarsone 22g

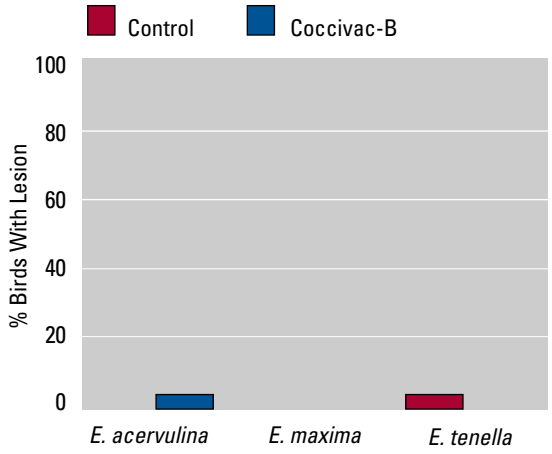

Figure 1. Birds Age 51-55 Days

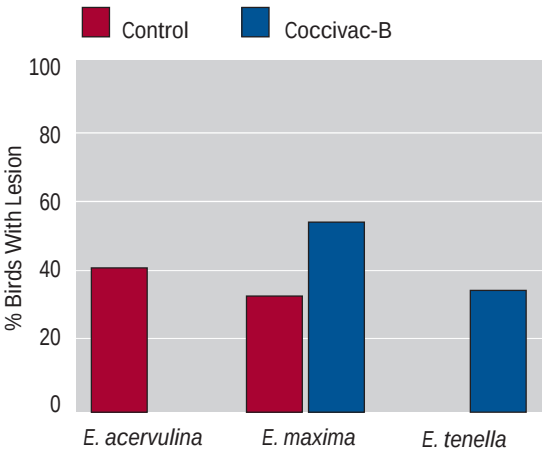

Figure 2. Birds Age 25-29 Days

SCHERING-PLOUGH'S TECH SERVICE TEAM ANSWERS QUESTIONS ABOUT MANAGING COCCIDIOSIS IN BROILERS

Charles Broussard, DVM
Steve Fitz-Coy, PhD
Lanny Howell, DVM
John McCarty, DVM
Linnea Newman, DVM
Rick Phillips, DVM
John Radu, DVM

Q. HOW PREVALENT ARE *EIMERIA MAXIMA* VARIANTS IN THE US, AND IS COCCIVAC-B CROSS-PROTECTIVE?

A. Based on a recent survey of 33 isolates of *Eimeria maxima* collected from 11 states and 18 major poultry integrators throughout the US, only three of the isolates (less than 10%) showed only partial protection by the Coccivac strain of *E. maxima*. In other words, the prevalence of *E. maxima* among US poultry integrators that is highly variant to the *E. maxima* in Coccivac is very low and is not of major significance at this time.

The results of the study also indicate that if birds are properly immunized, the vaccine strain of *E. maxima* in Coccivac-B would protect against a wide range of field isolates encountered in commercial operations in the US.

Q. IS THERE ANY MERIT TO USING AN AUTOGENOUS VACCINE FOR VARIANT SPECIES OF *EIMERIA*?

A. Autogenous vaccines are derived from uncharacterized *Eimeria* field species. These poorly defined species are not subjected to the same rigorous testing as the licensed, defined species of Coccivac. To develop a safe, consistent vaccine, it is imperative that the basic species characteristics such as pathogenicity, antigenicity and anticoccidial drug sensitivity are well defined. Using a poorly defined vaccine highly increases the risk of rapidly spreading unwanted contaminates from a few selected farms to all company farms, which could be an overnight disaster and a very expensive problem to control later; it also decreases the chances of maximizing production performance due to inconsistent product manufacturing from serial to serial.

Q. ARE YOU RE-ISOLATING THE VACCINE STRAINS FOLLOWING THE USE OF COCCIVAC?

A. Yes. All research to date supports our initial hypothesis that the vaccine strains over 2 to 3 flocks are displacing wild field strains. This is evident by the *Eimeria* population shift toward more sensitive strains as measured via anticoccidial sensitivity testing (AST).

Q. HOW STABLE IS THE POPULATION SHIFT TO *EIMERIA* STRAINS CONTAINED IN THE VACCINE?

A. It is not a permanent change — it's a shift. The vaccine strains become predominant in the absence of drug pressure. With drug pressure and time, the strains will shift back to those that are drug tolerant/resistant.

Q. HOW MANY CYCLES OF COCCIVAC USE DOES IT TAKE BEFORE NOTICING A CHANGE IN THE *EIMERIA* POPULATION?

A. It's difficult to say specifically how many, but the longer the removal of pressure from the drugs, the greater the chance of seeing a change in the *Eimeria* population. We can say, however, that there should be a minimum of two cycles and that three cycles are preferred.

Q. WHAT ABOUT COMPETITION BETWEEN THE WILD AND VACCINE STRAINS OF *EIMERIA*? WILL A SHORT LAYOUT TIME INFLUENCE THE OUTCOME?

A. Layout time will influence the desiccation rate of *Eimeria*. Coccidia will not be eliminated, but the longer the layout period, the greater the reduction in numbers.

The advantage of vaccination is that birds are exposed early in life to vaccine strains; eventually, they will develop immunity. Using a live vaccine allows us to control the dose (level of exposure) as well as the timing (day-1) of exposure. These are two major advantages in controlling any disease process.

Q. DO COCCIDIOSIS VACCINES REQUIRE SPECIAL HANDLING?

A. Yes. Make sure the vaccines are never frozen, which will kill or damage sporulated oocysts and ruin their effectiveness. If ice crystals are noticed in the liquid, the vaccine should be discarded.

Store Coccivac vaccines at a temperature between 36°F to 47°F (2°C and 8°C). The vaccines should be kept at these temperatures during shipping as well as during transport to farms or hatcheries. When the vaccines are refrigerated, watch for uneven temperatures that might allow partial freezing.

Q. ARE COCCIVAC VACCINES TESTED FOR POTENCY?

A. Yes. Potency testing in live birds is conducted on every serial (batch) of Coccivac manufactured. Birds are vaccinated with the serial being tested and are then challenged with every species of *Eimeria* contained in the vaccine to make sure they develop immunity. If they have developed immunity, they do not develop coccidiosis. The validity of each potency test is checked by challenging unvaccinated birds.

Q. DOES THE METHOD OF VACCINATION AFFECT THE AGE WHEN CHICKS CAN BE VACCINATED WITH COCCIVAC?

A. Yes. When the Spraycox spray cabinet is used, chicks can be vaccinated in the hatchery because the sprayer enables uniform distribution; 21 ml of coarse spray is delivered for each box of 100 chicks. Chicks “preen” to clean and dry their feathers and ingest the vaccine. Red dye mixed in with the vaccine gets their attention and stimulates preening.

An alternative to using the Spraycox applicator is feed spray application. It does not deliver the vaccine as uniformly as the Spraycox applicator, but can be used when hatchery application is not possible. Chicks must be 4 days of age, however, since younger chicks may not have developed uniform feed consumption patterns.

Q. WHAT’S THE DUAL-NOZZLE SPRAYCOX CABINET?

A. It enables simultaneous administration of Coccivac-B and Newcastle/Bronchitis (ND/IB) vaccines, which in turn provides producers with convenience and reduced labor costs. The nozzle for Coccivac delivers the coccidiosis vaccine as droplets that are ingested by preening. The nozzle for ND/IB produces a flat, even spray across the box.

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THE PATHOGENECITY OF *E. MITIS* (NOMEN OMEN?)

Dr. Luciano Gobbi
Scientific Affairs Consultant
Schering-Plough Animal Health, Italy

Eimeria mitis is one of the seven known *Eimeria* coccidial species that cause coccidiosis in chickens. It was first identified in the late 1920s. In Latin, "mitis" means mild and reflects the significance that was attributed to *E. mitis* when it was first discovered and for many subsequent years.

More attention has been given to other *Eimeria* species infecting chickens, such as *Eimeria tenella*, *E. acervulina* and *E. maxima*. These are considered common and highly pathogenic, leading to morbidity, mortality, obvious lesions, poor weight gain and serious economic losses for poultry producers. *E. mitis* remained relatively ignored since it does not cause gross lesions that can be readily identified.

In recent years, however, impressions about *E. mitis* have been changing. Several studies have demonstrated that *E. mitis* is very common in broilers and breeders. *E. mitis* does affect the lower intestines. It results in characteristically small, almost round oocysts that are smaller than those of other coccidial species — about 15 um in diameter. It is also becoming apparent that *E. mitis* is pathogenic, particularly when it occurs along with *E. acervulina*.

Table 1
Daily weight gain in birds from Phase 1 of trial

Species	Weight Gain
E.mitis	31.1 ± 4.8 gr/day/bird (p<0.01)
E. acervulina	26.7 ± 3.9 gr/day/bird (p<0.01)
Controls	38.9 ± 3.3 gr/day/bird (p<0.01)

Table 2
Feed conversion ratios from Phase 1 of trial

Species	Feed Conversion
E.mitis	1.81 ± 0.11 gr/day/bird (p<0.01)
E. acervulina	2.08 ± 0.24 gr/day/bird (p<0.01)
Controls	1.69 ± 0.06 gr/day/bird (p<0.01)

Trial

To further investigate the impact of *E. mitis*, a trial with two phases was conducted.

In the first phase, oocyst shedding and bird performance were evaluated. There were three groups of birds. At 14 days of age, 25 were infected only with *E. mitis*. To provide a positive control, another 25 were infected with *E. acervulina* since it is known to cause reduced weight gain and increased feed conversion. A third group of birds was infected with saline and served as a negative control.

On days 4 and 14 after challenge, oocysts were quantitatively counted to demonstrate that the challenge with *Eimeria* pathogens was successful and that, over time, the pattern of oocysts shedding was typical of *Eimeria* infection.

These results confirmed those of other studies demonstrating that the peak numbers of *E. mitis* oocysts are in the hundreds of thousands of oocysts per gram of feces (opg) and the peak for *E. acervulina* were in the millions of opg.

Compared to controls, infected birds in phase one had a lower daily weight gain (Table 1) despite a higher intake of feed and water and a higher feed conversion ratio (FCR) (Table 2) and lower final body weight. The final body weight of controls was statistically significantly higher than both the *E.*

mitis and *E. acervulina* groups. Controls also had higher feed intake ($p<0.01$) and water consumption ($p<0.05$) compared to the two groups of infected birds.

In the second phase of the trial, intestinal status was evaluated in three more groups of birds. At 14 days of age, 25 birds were infected with *E. mitis*, 25 with *E. acervulina* as a positive control and 25 with saline as a negative control.

The mucosal surface was evaluated for mucosal permeability. Starting at 72 hours after infection, and particularly from 96 to 144 hours post-infection, chickens infected with *E. mitis* and *E. acervulina* both exhibited differences in the colour of the mucusal surface compared to controls. This was determined by increased transfer of the colouring agent (Pontamine Sky Blue), which binds specifically with serum proteins and migrates with them outside of capillaries.

Dye leakage, evidenced by dye staining of intestinal mucosa and gut contents, was then considered as firm evidence of increased gut permeability.

Moreover, both *E. mitis* and *E. acervulina* infections caused a significant increase in the gut wall, as measured by stereoscopic microscope, due to oedema and an inflammatory reaction (see Figures 1, 2 and 3) between 72 and 144 hours post-infection.

Conclusion

The results of the trial add to growing evidence that the name attributed to *E. mitis* — the mild *Eimeria* — does not, in fact, match its real pathogenicity (NOMEN NON EST OMEN). *E. mitis* can impair performance in poultry and lead to losses for poultry producers just like other, better-known species of *Eimeria*.

To assure complete control of coccidiosis in broiler chickens, the significance of *E. mitis* must be considered and addressed in anticoccidial programs.

Figure 1. Measurement of gut wall 72 hours post-infection

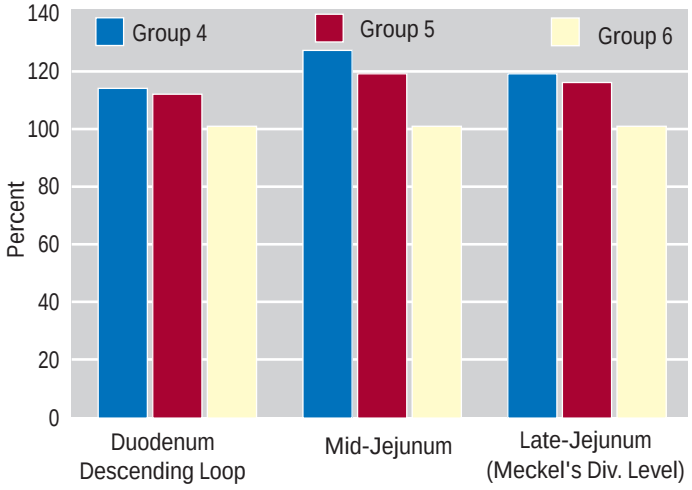


Figure 2. Measurement of gut wall 96 hours post-infection

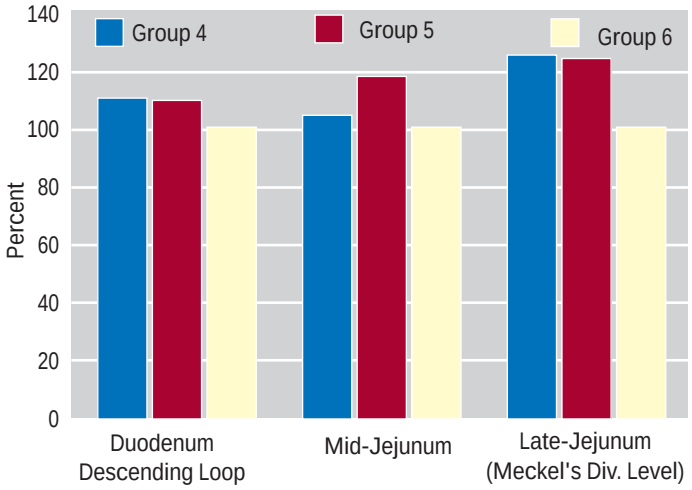
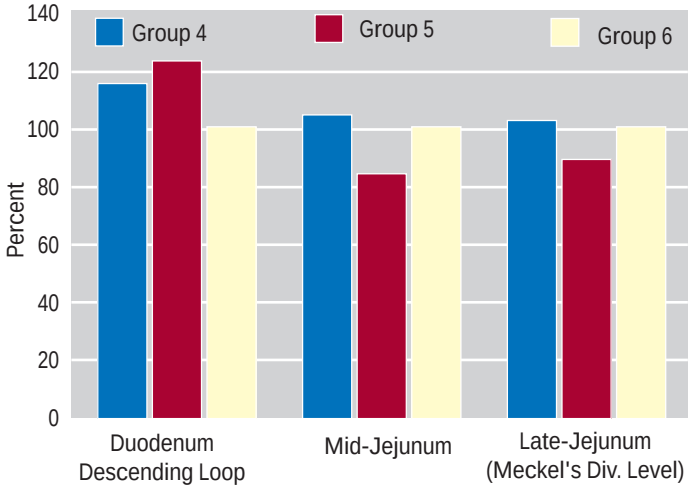


Figure 3. Measurement of gut wall 144 hours post-infection



THWARTING NECROTIC ENTERITIS

Controlling coccidiosis, good flock management keys to prevention

Necrotic enteritis (NE) has long been a concern for poultry producers, but evidence is now emerging that the disease can be thwarted by providing early and uniform protection against another disease that's hard on the gut — coccidiosis.

"Producers know the signs of NE well," says Dr. Luciano Gobbi, a veterinarian and scientific affairs consultant in Italy for Schering-Plough Animal Health.

Infected birds have feces that are looser, with more fluids. "They are less consistent and display a lighter color," he explains. They are viscous and sticky and the water-to-feed ratio is increased."

Birds with NE also show reduced feed intake, which leads to a host of problems. "Subclinical NE leads to impaired performance and loss of group uniformity, while the full-blown clinical form of NE causes mortality or condemnation at slaughter," Gobbi explains (Figures 1, 3 and 4).

Figure 1. Effect of clostridial enteritis infection on the daily gain of broilers

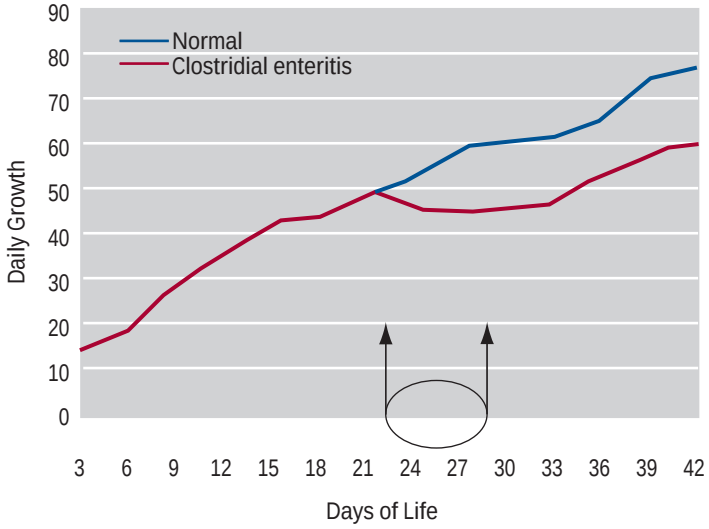
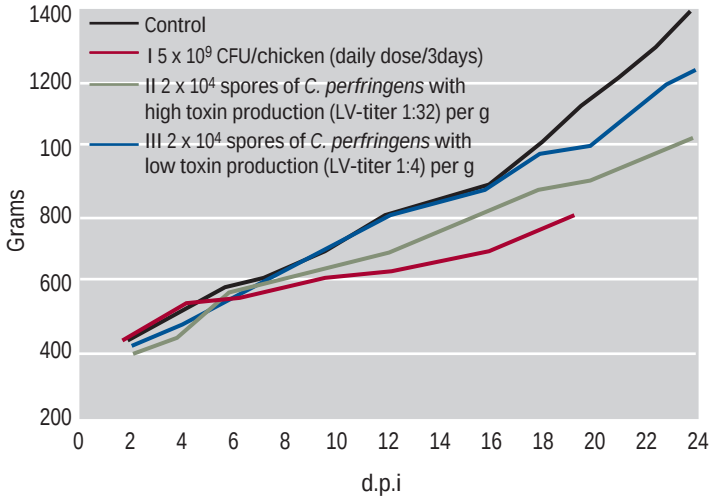


Figure 2. Development of body weight of chickens in relation to concentration of *C. perfringens* in feedstuffs



Strategy for control

Not all the factors involved in the development of NE are fully understood, but it's well known that *Clostridial perfringens* is the organism responsible for the disease and that other bacteria may play a role. In addition, there is an association between clostridial toxins and gut irritation or disruption, he says.

One key NE-control measure, therefore, is to minimize gut lesions by protecting birds from other sources of gut irritation, particularly coccidiosis.

"This can be accomplished easily and effectively with a coccidiosis vaccine such as Paracox-5, which provides early protection uniformly among flocks. The vaccine also eliminates concerns about anticoccidial resistance and drug residues in the birds or feeders," Dr. Gobbi says.

Other steps producers can take to control NE include using a good brooding system and paying careful attention to the house environment, including the temperature, ventilation and stocking density. Litter type should be well monitored as well, he says.

Consider clostridial sources

Considering the sources of clostridial infection may also help control NE.

In broilers, the hatchery can be a source of *C. perfringens*, he says. Dr. Gobbi cites a study reported in *Avian Diseases* in which the percentage of *C. perfringens*-positive samples from three hatcheries ranged from 13% to 23%, with an overall incidence of 20%; positive samples were consistently found on each of 9 sampling days.

The organism was found in eggshell fragments and chick fluff and in paper pads that were stored in the hatchery before use and after placement beneath chicks for 1 hour, Dr. Gobbi says.

Another vehicle for the spread of *C. perfringens* and NE is dust in closed environments such as hatcheries and poultry houses, he notes.

"Disinfectants have limited efficacy in destroying bacterial spores, and hatchery sanitation programs that seek to eliminate sources of the organism such as eggshell fragments may be helpful only if they incorporate dust control," Dr. Gobbi cautions.

Tests conducted in Germany indicate that feed can be a source of *C. perfringens* (Figure 2). Feed ingredients, especially raw diet material, should be screened properly for *C. perfringens*. Feedstuffs containing 1,000 to 100,000 spores of *C. perfringens* cause NE, he says.

"NE has been linked to diets high in wheat, so avoid feeding formulas with too much wheat," Dr. Gobbi says, "and use an appropriate enzyme."

Recent information indicates that certain chicken lines may be susceptible to *C. perfringens*, so producers may want to consider the lines of chickens they raise. NE has been linked to rapid bird growth and, perhaps, to an insufficient intestinal blood supply, Dr. Gobbi notes.

There is still a lot to learn about NE, he says, but the best strategy is to view NE as a multi-factorial disease and to implement different control measures simultaneously.

"If we pay careful attention to good flock management, prevent insults to the gut through measures such as vaccination, reduce factors that suppress the immune system such as stress and viral diseases and employ tactical antibiotic use when appropriate, we can make significant strides in NE control," Dr. Gobbi says.

Figure 3. Small intestine with no detectable gross lesions (score lesion = 0)

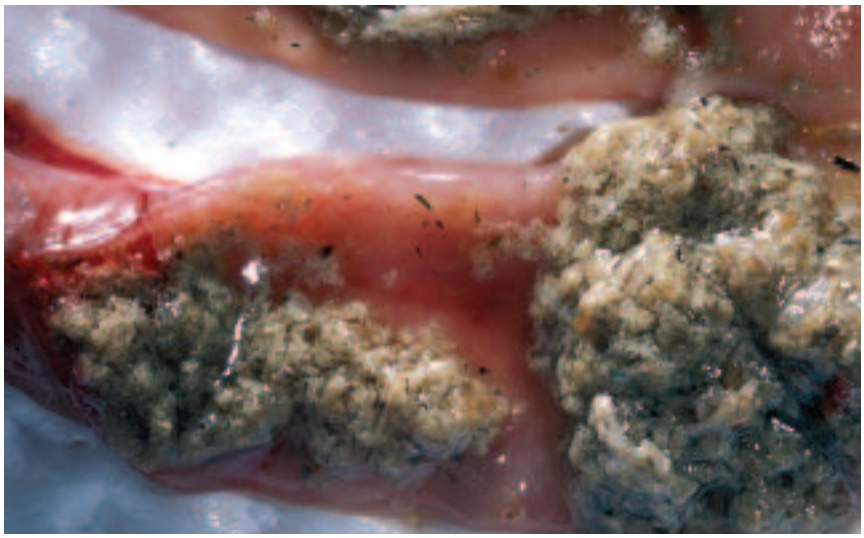
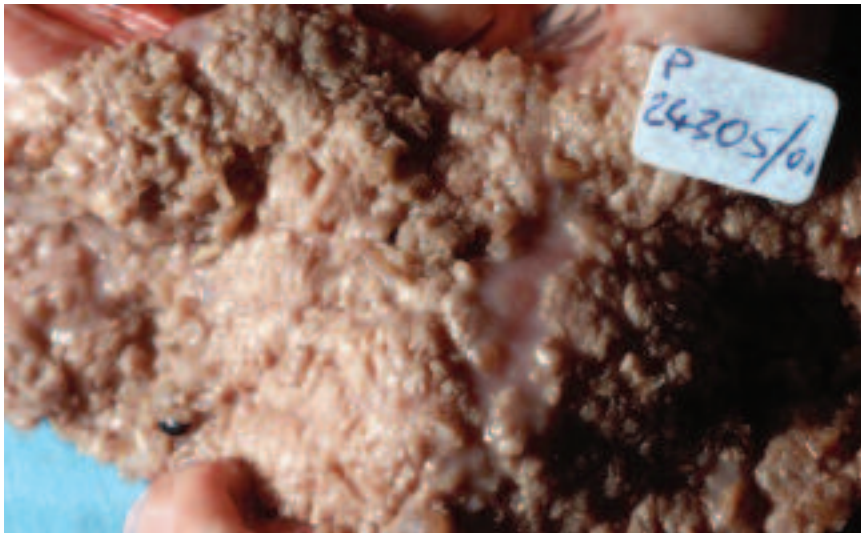


Figure 4. Severe and extensive necrosis on intestinal mucosa (severe fibronecrotic enteritis) (score lesion = 4)



YEAR OF THE VACCINE

China's producers are shifting to biological controls to manage coccidiosis, avoid drug concerns

Coccidiosis is one of the most severe parasitical diseases of broilers and broiler breeders in China, often presenting with severe clinical signs, including red blood in the feces, poor flock uniformity and mortality.

The costly parasitic infection can cause subclinical disease, too, characterized by signs such as poor weight gain. Subclincal disease is more likely to occur when other conditions, particularly necrotic enteritis, are present.

"In the past, we mainly adopted three methods for control of coccidiosis," says Tai Youhua, DVM, director of the Animal Health Center for Zhucheng Foreign Trade Broiler Co. Ltd, the second-largest broiler integrator in China's Shandong Province.

Veterinarians, nutritionists and producers in China fill room at recent meeting to learn more about coccidiosis vaccination.

"First we managed the rearing style, such as rearing birds on net. Second, we rotated and shuttled the anticoccidials in feed. Third, we administered various anticoccidial medicines, including diclazuril, or sulfa drugs," he says.

"But none of these methods generated the same efficacy as they had before. Due to resistance, in-feed anticoccidials such as ionophores and synthetic chemicals are no longer as efficacious as they once were, and it was time to adopt a new way of thinking in coccidiosis control by developing immunity through vaccination," Tai adds.

Medication problems

Besides resistance, he says, controlling coccidiosis with medications raises another problem: Residues. Sulfa drugs such as sulfaquinoxaline and sulfadimidine are highly efficacious in reducing mortality and controlling symptoms. As a result, sulfa drugs are frequently the product of choice in China when coccidiosis breaks out due to their efficacy and cheaper price.

"But there are restrictions on sulfa drug residues in poultry to be exported to Japan," Tai says. "It is difficult for us to monitor the choice of anticoccidial by contract growers, so residues are a big concern for poultry meat exporters."

In addition, there are restrictions on residues in exported broiler meat for medicines including clopidol and nicarbazine, which along with sulfaquinoxaline have been banned by the Ministry of Agriculture of China. It is likely that more in-feed anticoccidials will be banned in the future, he predicts.

The solution: vaccination

Because resistance and residue were serious concerns for export-oriented and "green" bird integrators, Zhucheng started vaccinating day-old chicks with Coccivac-B, which provides lifelong protection against four leading species

of *Eimeria* that affect broilers. The birds are vaccinated in the hatchery with a specially designed spray cabinet that provides uniform dosing.

The result, according to Tai, has been good control of coccidiosis. There has been a significant reduction in the need for treatment and subsequent concern about residues. The vaccine also has replaced field-resistant oocysts with highly sensitive vaccine oocysts, he says.

Vaccinating for coccidiosis, Tai continues, also provides an excellent method of avoiding problems with resistance and residues. The oocyst species used to produce the vaccine were isolated before in-feed anticoccidials on the market were launched and are therefore highly sensitive to all approved ionophores and chemical treatments. That's why replacing resistant field strains with oocysts in the vaccine is beneficial, he says.

"Since the end of 2001, we have vaccinated about 25 million birds with Coccivac-B," reports Wang Chunming, chief veterinarian of the Animal Health Center. "In fact, we mandated that all birds reared on the floor must be vaccinated at day of age by Coccivac-B through spraying in the hatchery.

"Our growers have accepted the concept of vaccination, especially since they saw the results obtained with the first 1.3 million birds either vaccinated or medicated respectively," he adds.

Those results showed that vaccinated birds had an equal or better performance index than non-vaccinated birds. "As a result, growers no longer use anticoccidials in feed for prevention or in drinking water for treatment, except for the occasional use of diclazuril to control mild post-vaccination reactions. We are free of coccidiosis and residues in broilers," he says.

Technical service crucial

Because biological prevention against coccidiosis is a new concept for the broiler industry in China, successful implementation of coccidiosis vaccination required good technical service.

According to Tai, Schering-Plough Animal Health's technical personnel introduced the advantages of coccidiosis vaccination and created interest in Coccivac-B. They defined a trial protocol based on local conditions with clients and taking into account the location, number of birds, groupings and measurement index. The team of specialists also provided crucial support

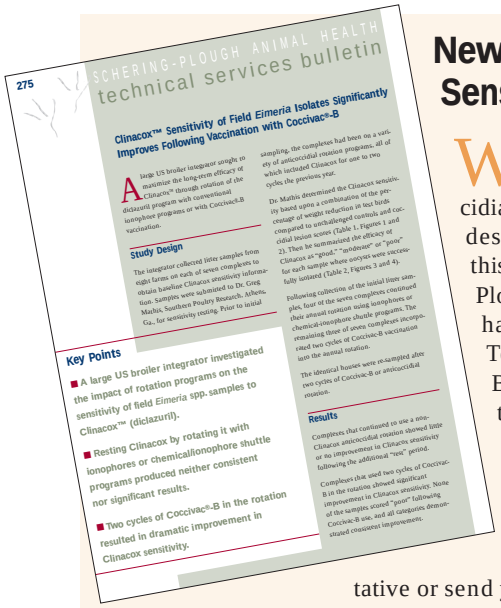
continued on page 22

Zhucheng hatchery workers use a specially designed spray cabinet to administer coccidiosis vaccine to day-old chicks.

Broilers vaccinated for coccidiosis have performed as well as or better than medicated birds.

Broadway Blizzard

This was the scene at Times Square, New York City, as 41 representatives from top European poultry companies emerged from a Schering-Plough Animal Health symposium on managing nutrition in birds vaccinated for coccidiosis. The surprise snowstorm eventually dumped more that a foot of snow on the Big Apple while attendees — many of them from more temperate climates — braved the elements for a blustery night on the town. Watch for the next issue of *CocciForum* magazine for complete coverage of the meeting.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SCHERING-PLOUGH ANIMAL HEALTH

technical services bulletin

Clinacox™ Sensitivity of Field *Eimeria* Isolates Significantly Improves Following Vaccination with Coccivac®-B

A large US broiler integrator sought to maximize the long-term efficacy of Clinacox™ through rotation of the diclazuril program with conventional ionophore programs or with Coccivac®-B vaccination.

Study Design

The integrator collected litter samples from eight farms on each of seven complexes to obtain baseline Clinacox sensitivity information. Samples were submitted to Dr. Greg Mathis, Southern Poultry Research, Athens, Ga., for sensitivity testing. Prior to initial sampling, the complexes had been on a variety of anticoccidial rotation programs, all of which included Clinacox for one to two cycles the previous year.

Dr. Mathis determined the Clinacox sensitivity based upon a combination of the percentage of weight reduction in test birds compared to unchallenged controls and coccidial lesion scores (Table 1, Figures 1 and 2). Then he summarized the efficacy of Clinacox as "good," "moderate" or "poor" for each sample where oocysts were successfully isolated (Table 2, Figures 3 and 4).

Following collection of the initial litter samples, four of the seven complexes continued their normal rotation using ionophores or chemical-ionophore shuttle programs. The remaining three of seven complexes incorporated two cycles of Coccivac®-B vaccination into the normal rotation.

The identical houses were re-sampled after two cycles of Coccivac®-B or anticoccidial rotation.

Results

Complexes that continued to use a non-Clinacox anticoccidial rotation showed little or no improvement in Clinacox sensitivity following the additional "test" period.

Complexes that used two cycles of Coccivac®-B in the rotation showed significant improvement in Clinacox sensitivity. None of the samples scored "poor" following Coccivac®-B use, and all categories demonstrated consistent improvement.

Key Points

- A large US broiler integrator investigated the impact of rotation programs on the sensitivity of field *Eimeria* spp. samples to Clinacox™ (diclazuril).
- Resting Clinacox by rotating it with ionophores or chemicalionophore shuttle programs produced neither consistent nor significant results.
- Two cycles of Coccivac®-B in the rotation resulted in dramatic improvement in Clinacox sensitivity.

New TSB Details Sensitivity Trial

Want more information on the anticoccidial sensitivity trial described on page 4 of this issue? Schering-Plough Animal Health has published a new Technical Service Bulletin summarizing the key data from the study, complete with tables and color charts. For a free copy, contact your company representative or send your request to Fabio Paganini. Email: fabio.paganini@spcorp.com. Fax: 908-629-3206. Ask for publication SPAH-PBU-275 and remember to include your full name and address.

Got a Story Idea for *CocciForum*?

The editors of *CocciForum* welcome news tips and story ideas from its readers around the world. If there's a particular subject you'd like to see covered in a future issue — perhaps a specific area of coccidiosis management — please let us know. Write to JFeeks@prworks.net or call 508-627-6949 (US). We want to hear from you.

Producers Learn About Managing Intestinal Health and Coccidiosis Vaccination at Ultrecht Seminar

Representatives from over 60 European poultry companies attended a Schering-Plough Animal Health seminar in Ultrecht, The Netherlands, to learn more about poultry intestinal health and coccidiosis vaccination.

The seminar, held in conjunction with VIV Europe 2003, was entitled "Management of the Intestinal Health in the Modern European Poultry Industry."

The meeting started off with a talk by Dr. Joaquim Bruffau, head of animal nutrition at IRTA Research Centre in Spain, on the challenges and opportunities of European poultry production. Dr. W. J. M. Landman, of the Animal Health Service, Poultry Health Centre, The Netherlands, followed with a presentation about anticoccidial sensitivity in European *Eimeria spp*. isolates.

Dr. Luciano Gobbi of Italy, a veterinary consultant for Schering-Plough Animal Health, discussed managing intestinal health and broiler performance without feed additives based on the Italian experience, and Dr. César Carnicer, of Schering-Plough Animal Health, Spain, spoke on the feasibility of using Paracox-5 in standard production.

Excellent Attendee Response

The response to the seminar among attendees was excellent, says Fabio Paganini, marketing manager for Europe, the Middle East, Africa and Asia/Pacific.

"The seminar presented new ways of managing intestinal health and coccidiosis. The use of Paracox-5 requires structural changes, but brings several opportunities in terms of improving performance on a long-term basis. We demonstrated how Paracox-5 is technically feasible and safe," Paganini says.

Attendees also heard about Schering-Plough Animal Health's plans to provide producers with enhanced technical services. "We have assembled an impressive team and this year will expand our efforts to offer their expertise to producers," he says.

Irrefutable Evidence continued from page 5

Phillips: 'These are powerful findings that could revolutionize the way the industry controls coccidiosis for years to come.'

notes that two cycles of Coccivac-B prompted a dramatic shift toward increased diclazuril sensitivity.

"We followed exactly the same houses before and after diclazuril was used, then used Coccivac so we could pinpoint for sure whether we were replacing resistance," he says. "The results demonstrate that we can replace or at the very least dilute the amount of *Eimeria* resistance that's out there by using Coccivac for several cycles and making it part of a long-term control program."

Mathis, who calls the landmark trial "one of the most coccidiosis-significant studies" he's worked on in more than 20 years, thinks the results will go a long way toward directing coccidiosis management in the future.

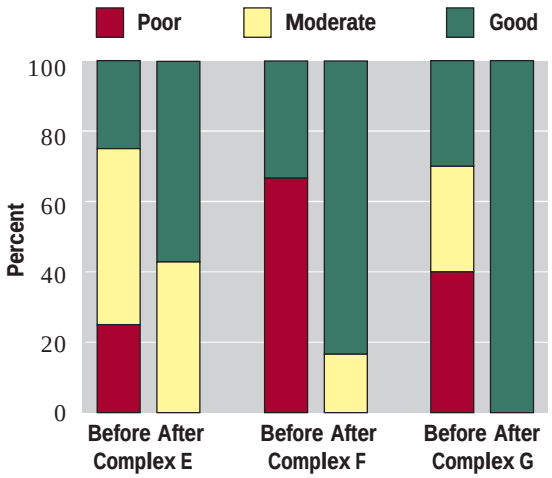
"The take-home message is that to get the performance you got when you originally had diclazuril or other in-feed anticoccidials, you're going to have to use a vaccine. Coccivac is the only coccidiosis vaccine that has clearly demonstrated that it can replace resistance."

Phillips agrees and says the results are almost an exact duplication of past

Table 1
The Results of Sensitivity Testing at the Seven Complexes in the Study

		Clinacox Sensitivity (%)		
Complex	**Rotation Program**	**Good**	**Moderate**	**Poor**
A - Before	Anticoccidial	25	12.5	62.5
After	Anticoccidial	0	12.5	87.5
B - Before	Anticoccidial	25	12.5	62.5
After	Anticoccidial	16.7	33.3	50
C - Before	Anticoccidial	16.6	0	83.3
After	Anticoccidial	0	50	50
D - Before	Anticoccidial	0	80	20
After	Anticoccidial	20	40	40
E - Before	Anticoccidial	25	50	25
After	Coccivac-B	57	42.8	0
F - Before	Anticoccidial	33.3	0	66.6
After	Coccivac-B	83.2	16.6	0
G - Before	Anticoccidial	30	30	40
After	Coccivac-B	100	0	0

Figure 1. Rotation with Coccivac-B for two cycles dramatically improved sensitivity of field isolates to Clincox.



pen study trial results. "These are powerful findings that could revolutionize the way the industry controls coccidiosis for years to come," he adds.

"For the first time in decades, vaccination is being viewed as a foundation to successful coccidiosis management — one that can be used either year-round or in a carefully planned, long-term rotation with in-feed anticoccidials to maximize their impact."

Know the Spore continued from page 7

per ml. "We get at least two independent counts on freshly produced material using light microscopy," Knight says.

The final product includes non-sporulated and partially sporulated oocysts, but only oocysts that are fully sporulated are counted as infective, he says.

Fitz-Coy says, "We must keep in mind that oocyst viability decreases over time. Not all originally counted infective oocysts will be viable by the expiration date on the vaccine."

Consequently, the Millsboro team makes sure that every vaccine formulation contains sufficient infective oocysts to allow for the decay of some infective live oocysts. "That way, the vaccine maintains potency through its stated shelf-life," he says.

Antigen production is complete once the harvest has been treated with a chemical sterilant and quality control has released the lot on sterility, purity and titer, he says.

Potency testing

To be absolutely certain every final vaccine serial (a blend of individual vaccine lots) is efficacious, technicians conduct potency testing in live birds, says Knight. "Vaccinated birds are challenged with each *Eimeria* line to make sure immunity has developed. Unvaccinated control birds also are challenged to ensure the validity of the test."

Besides making sure that every batch of vaccine contains enough viable, sporulated oocysts, the count also ensures that birds aren't exposed to too many, which could cause a stronger than needed immune response.

"Actually, the formulation ensures that fresh vaccine is not too potent," says Knight, "but as an extra precaution, we conduct safety tests in birds at an increased dose level.

"The nature of the potency test makes it both time- and resource-consuming. It also eats into the available expiration dating of the product. However, we still believe that a challenge potency test is the best way to demonstrate vaccine efficacy," he says.

Additional testing

Every batch of Coccivac also is tested for the presence of extraneous viable bacteria and fungi, according to USDA regulations and, for added measure, for

mycoplasma even though this testing is not a government requirement. "Although the potency test is critical because it demonstrates that all species are present in sufficient numbers to initiate the immunizing process, that alone is not enough to warrant release of the vaccine," he says. Further testing is carried out to demonstrate freedom from contaminating viral agents.

The effectiveness of Coccivac is ensured by a range of measures starting with the quality of raw materials and ending with shipment of the product, according to Knight.

"Every single stage of production, be it a quality-control test, an in-process check, an incubation temperature or storage time, is documented and reviewed for compliance prior to release of the product," he says.

Cocci FAQs continued from page 11

Q. HOW DOES STOCKING DENSITY AFFECT COCCIVAC VACCINATION?

A. You'll get more uniform results if the initial stocking density is 0.75 ft^2 to 1.0 ft^2 per bird. Higher stocking density could result in excessive litter moisture and a high litter oocyst density.

After vaccination, third-house and half-house brooding encourages proper Coccivac cycling for the first 7 to 14 days. Remember that for full immunity to develop, birds require not only the initial "dose" of live sporulated oocysts administered via the vaccine, but two or more life cycles of coccidia.

Q. WHAT'S THE BEST LITTER MOISTURE CONTENT FOR BIRDS THAT RECEIVE COCCIVAC?

A. A minimum litter moisture content of about 25% is needed to stimulate the coccidial life cycle, but too much moisture will lead to poor Coccivac results. If the litter is too wet, coccidial cycling may be heavy, causing too much reaction in birds and even overgrowth of the bacteria that causes necrotic enteritis. Too much litter moisture also can lead to transient immunosuppression due to "cold stress" as well as high ammonia, resulting in blindness and poor flock uniformity.

Q. WHAT TIPS DO YOU HAVE FOR GOOD MANAGEMENT OF LITTER MOISTURE?

A. One way to reduce litter moisture is by properly maintaining nipple drinkers, which reduce the amount of water spilled into the litter. Consider your ration formulation. Some rations reduce the amount of excreted moisture. Litter type and depth should accommodate the amount of moisture expected in the house. Ventilation also can be adjusted to control litter moisture. Bird density needs to be controlled to avoid the moisture concentration.

For more information on managing litter moisture, see the article by Mike Czarick on page 12.

Have more questions about coccidiosis vaccination? Send yours to the editor at JFeeks@prworks.net or by fax to 928-569-2491. You'll get a personal reply from a Schering-Plough Animal Health Corporation technical service representative and we may include it in our next issue of CocciForum.

Cocci Confidential continued from page 9

Table 2
Summary of Performance: Coccivac-B vs. Control

Treatment	Age (days)	No. started	% Liv	Gross lbs sold	Average weight	Feed conv	Cal conv	Cal conv adj 7.5lb	ADG	Cost per lb
Coccivac-B: Total or Average	59.8	156,600	97.33	1,161,909	7.623	2.096	3022	2994	0.128	.2088
Anticoccidial: Total or Average	59.8	156,600	97.47	1,148,824	7.527	2.112	3033	3027	0.126	.2105
Coccivac-B Advantages	N/A	N/A	-0.14	13,085	0.096	-0.016	-11	-33	.002	-.0017

previous flocks before their immunity is fully established, which explains why performance results may seem irregular with the first cycle of Coccivac-B.

In this rare multi-farm, paired-house study, Coccivac-B was successfully used for the long-term management of coccidiosis without sacrificing performance. In other words, Coccivac-B used in real-world conditions not only controlled coccidiosis, it improved broiler performance compared to ionophore anticoccidials.

Coccidiosis Vaccination in China continued from page 17

during the trial, including on-site visits at 7, 14 and 21 days post-vaccination. The trial enabled customers to realize the advantage of Coccivac-B and helped Schering-Plough Animal Health learn how to adapt Coccivac-B to local conditions.

"We have about 2,500 growers," adds Wang. "It is very difficult for us to teach all growers about using Coccivac-B in a short time."

As a result, Schering-Plough Animal Health's technical staff in China provided training to most of the growers, which are located in 75 villages within a 200 km radius. In addition, the company provided a concise and easily understood post-vaccination management list, which included information on feed, litter, moisture, necrotic enteritis and vaccination reaction control, he says.

The training, coupled with post-vaccination on-site visits and management tips, enabled growers to develop adequate skills and knowledge about Coccivac-B. "That eventually solved the problems of resistance and residues — both issues that perplexed our business before," Wang says.

Schering-Plough Animal Health's technical service specialist David Xuan agrees, adding, "The success of Coccivac-B depends on post-vaccination management, which must be customized to the local situation. Coccivac-B can become the best choice for coccidiosis control if a concrete and practical technical service plan is provided."

Making It Different Makes the Difference

Innovation is the key. According to business and marketing gurus, we must "think outside the box" or "break paradigms" if we are to win a competitive advantage. They say that in a business environment, the capacity to learn and adapt to new situations faster than the competitors is essential to survival.

While very important for any company, products, brands and processes do not guarantee its future. Research by Royal Dutch/Shell in the 1980s concluded that the average lifetime of the biggest company in the most important industries worldwide is 40 years – only half of the time most of us expect to live![1]

Does this apply to the poultry industry? Is it possible to innovate or we can be successful by focusing only on lower production costs? Depending on your company and its strategy, both answers can be correct.

But let's look at the whole industry, not individual companies. How well have we learned? How well have we understood customers' changing needs? Have we used this knowledge to gain a competitive advantage for our products?

The poultry industry has achieved incredible technical advances in the last 30 years. We produce much faster, cheaper, and with higher quality than any other primary industry. We have state-of-the-art products, processes and facilities. But has the industry been able to capitalize on this advantage and convert it into profits?

This raises more questions than answers. We are in a period of transition driven by food safety and animal welfare concerns, mainly from Europe. These concerns have arisen partly as a result of issues surrounding poultry meat's quality and safety.

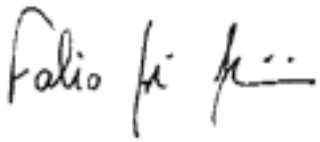
How have we responded to these challenges? How do consumers perceive poultry products? It's important to know, because the perception of value determines how much we are willing to pay for a given product. If we look at the evolution of poultry meat prices, we will see that every cent we saved in production costs was passed on to the consumer through lower prices. Have we been able to make customers aware of the real value of our products?

As debates about food safety continue, the industry has the opportunity to define its future: we can ignore the crisis; we can react to it or; we can be proactive by reading the customers' needs and anticipating situations. If we choose to be proactive we can start improving customers' perceptions of our products and planning a promising future for the industry.

We at Schering-Plough Animal Health have worked to develop efficient and safe technologies to manage coccidiosis and intestinal health – and provide poultry producers with the means to innovate with the customer in mind.

Our coccidiosis vaccines have proven their cost benefit advantages in different commercial situations and their use is continuing to grow. More and more companies are taking advantage of coccidiosis vaccination, either in rotation programs to maximize the drugs' efficacy or in continuous programs which give comparable performance, a residue-free product and greater flexibility.

That's our goal: to give the industry the tools to innovate… and to secure a competitive advantage for the future.



FabioPaganini
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[1] *The Fifth Discipline*, Peter Senge.

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In the future, you will not depend on drugs to control coccidiosis.

If you want to start your future now, talk to us.

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