

NORTH AMERICA ISSUE 2
NEW STRATEGIES FOR
ENHANCING POULTRY VALUE
AND PERFORMANCE

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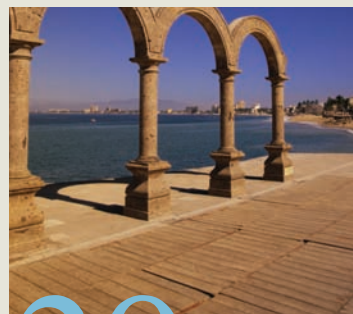


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The intestinal health of broilers dominated much of the technical program at the XXIII World's Poultry Congress, which included a well-attended panel discussion on vaccinal strategies for controlling necrotic enteritis.

special report

Western Poultry Disease Conference offered new insights for managing necrotic enteritis and coccidiosis.



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(on the cover)

Between the dense bird population and extremes in the weather on the Delmarva Peninsula, broiler managers at Allen Family Farms thought they had seen it all when it came to disease — at least until gangrenous dermatitis hit the operation. Fortunately, Dr. Gary Gladys had some innovative solutions (see article, page 19). *Photo by Lisa Helfert.*

opinion: “ Science Meets Skill ”

Few would argue that to achieve the best performance in poultry or any other species, health care must be based not just on science but on the skilled application of science that comes only with hands-on experience.

This issue of *Intestinal Health* is full of both science and skill. In the following pages, you'll read in down-to-earth language about careful research demonstrating that if chicks develop early immunity to coccidiosis, such as that achieved with coccidiosis vaccination, it can have a significant, positive impact on gut health.

You'll also learn why early development of immunity against coccidiosis is less costly than late immunity development. Other articles about coccidiosis in this issue are based on real-world experience and show how coccidiosis-control programs can be evaluated and house-management

practices adapted to get the best results. Science and skill.

An offshoot of the articles about coccidiosis is a story about Allen Family Farms of Seaford, Delaware, a producer that was surprised with an outbreak of gangrenous dermatitis. The disease has

Careful research demonstrates that if chicks develop early immunity to coccidiosis, it can have a significant, positive impact on gut health.

become a growing problem in the poultry industry, and it's been associated with coccidiosis. Even though Allen farms had good coccidiosis control, the disease still struck. Find out how the outbreak was quashed, in large part due to skillful experience and intuition.

More science-based articles are included about necrotic enteritis (NE), where researchers using state-of-the-art

technology are exploring the role that alpha-toxin plays in this devastating disease. Additional articles based on studies here and abroad reveal the results from trials where an alpha-toxin vaccine is being applied in the field to control NE. Again, science and skill.

Science and skill also characterize Intervet/Schering-Plough Animal Health. Our broad, innovative portfolio of products reflects our company's focus on science and our commitment to research and development. We bring skill to the field through our technical service team, which has unparalleled expertise in the field of intestinal health and is dedicated to helping poultry producers raise healthy, productive flocks as cost-effectively as possible. We hope you take advantage of all we have to offer.

Charlie Broussard, DVM

*Director, US Technical Services
Intervet/Schering-Plough Animal Health*

Phone: +1 706-224-2127

Email: charles.broussard@sp.intervet.com



Russia's capital

provided a

fascinating

backdrop for

intestinal health

topics.

Moscow



VIV Europe 2008 in Moscow provided an opportunity to incorporate the emerging Eastern European market into the biannual livestock and poultry trade show. Of the more than 5,500 visitors from 70 countries who attended, many were from the Ukraine and Belarus and most represented poultry farms.

In a talk on coccidiosis, Dr. Luciano Gobbi, an Italy-based veterinarian and technical service manager with Intervet/Schering-Plough Animal Health, explained how coccidiosis vaccination has evolved from a solitary control method into a broad management tool.

Anticoccidials remain the mainstay of coccidiosis control, yet coccidiosis remains

widespread, costing poultry producers more than any other disease, Gobbi said.

A primary problem with anticoccidials is coccidial resistance resulting from overuse of the products. However, Gobbi presented evidence demonstrating how rotating a coccidiosis vaccine with anticoccidials restores ionophore sensitivity and yields bird performance within normal range.

Coccidiosis vaccination has other advantages. It complements flock thinning — the practice of periodically removing some birds, usually females, leaving the whole house to grow larger males — because no withdrawal feed or withdrawal time is needed; producers can pull light or heavy birds as needed to meet market demand.

Coccidiosis vaccination also simplifies life at the feed mill; there's no need to clean equipment to prevent unwanted carryover of anticoccidials. Producers are also relieved of concerns about anticoccidial residues in broiler meat, he said.

innovation^s

New ideas, trends, products and technologies

! Multimedia tool helps producers ensure best broiler diet, growth

An interactive educational program designed to help broiler operators ensure optimal coccidiosis control and maximum growth potential has been developed by Intervet/Schering-Plough Animal Health.

The program is currently available as a multimedia presentation on CD format and will soon be accessible by logging onto Intervet/Schering-Plough websites.

An introductory video explains the "Quadrants of Performance," a concept that helps producers understand how coccidiosis-control programs work, their impact on coccidial cycling and how late coccidial cycling can affect other important diseases such as gangrenous dermatitis.

The program contains a section that covers the scientific background behind the Quadrants concept, including video interviews with prominent poultry researchers, as well as a close-up look at the real-life experiences of growers using strategies featured in the program.

Some of the specific issues covered in the program include how used-versus-

new litter affects the development of immunity, the impact of coccidiosis on performance and how to improve coccidiosis control during summer months and in smaller birds. The use of light to enhance the efficacy of coccidiosis vaccination, how to manage anticoccidial resistance and the importance of timing coccidiosis challenges in growing birds are other topics covered.

"We wanted to detail the latest findings in broiler nutrition, immunology and physiology, and how they relate to coccidiosis control. But we wanted to do that in a way that was comprehensive yet easy to grasp," says Marcelo Lang, global poultry marketing director at Intervet/Schering-Plough Animal Health. The program features easy-to-follow graphics and runs on virtually any PC. It is currently available in English only, but a Spanish version will be introduced in the future. For more information, contact your Intervet/Schering-Plough Animal Health representative.

! Flavored feed has potential

Flavored chicken feed might provide a way to improve management of broilers and layers, says B.L. Damron, of the Institute of Food and Agriculture Sciences, University of Florida.

Data demonstrating statistically significant benefits from flavoring chicken feed is lacking, but fairly consistent numerical improvement has been shown, which is why Damron says the notion of flavoring feed shouldn't be discarded.

Contrary to the notion that they lack a sense of taste, research shows that birds have well-defined taste mechanisms. When flavored drinking water is offered to birds, they detect compounds consistently and prefer unflavored water, Damron says, in an article from the Feed Industry Network's *Feed Formulator*.

Additional research with offensive flavors shows the ability to chemically regulate feed or fluid intake in birds, opening a number of possibilities for commercial poultry production.

Flavoring, for instance, might help improve palatability and performance. It might also prevent early "starve-outs" and keep birds on feed during times of disease or stress. Flavoring may also limit decreased feed consumption caused by ingredients such as blood meal, fish solubles and fermentation byproducts or dusty ground grains like wheat and milo, he says.

"An important possibility under hot weather conditions is the potential improvement of feed intake by hens and broilers..." Damron adds.

more on page 45



research watch



NE strains of *C. perfringens* are mighty gut warriors

Strains of *Clostridium perfringens* that cause necrotic enteritis (NE) replace *C. perfringens* strains that do not cause the disease — and also battle against one another in the chicken gut — indicates research that could eventually lead to improved understanding and better management of NE.

Investigators from the University of Arizona inoculated broiler chicks with mixtures of *C. perfringens* strains to explore the single-strain dominance that has been observed in natural cases of NE.

In the first of two studies, birds received one NE strain known as JGS4143, PFGE pattern 8, as well as four strains that do not cause NE. Lesions typical of NE developed after inoculation. However, only the NE strain could be recovered through the first post-inoculation day, despite “intense” efforts to recover the other strains. Thereafter, previously undetected PFGE strains were found, and JGS4143 was undetectable.

Findings in a second study were similar. Birds were inoculated with five NE strains, including JGS4143, and developed NE lesions. Initially, only JGS4143 was recovered, but birds began to be repopulated with other NE strains that were not used for inoculation.

“All NE strains inhibited growth of normal flora, but normal flora strains did not

inhibit any NE strain,” write Angelique J. Barbara and colleagues about their study, published in *Veterinary Microbiology* 126 (2008) 37-382. In addition, there were two NE strains that inhibited each other and normal flora strains, but normal flora strains did not inhibit each other, showed the study, supported in part by Intervet/Schering-Plough Animal Health.

The inhibition of one NE strain by another may be due completely, or in part, to factors other than bacteriocins — proteins produced by the bacteria of one strain against those of a closely related strain. Those other factors might include superior adhesion characteristics, more rapid multiplication and production of specific toxins, the investigators say.

Improved understanding of how NE strains displace non-NE strains in the chicken gut could, in time, bring about a better understanding about the pathogenesis of NE and provide targets for managing the disease, Barbara and colleagues write.

Surveys indicate increase in coccidiosis-infected flocks

Two extensive surveys conducted in Norway indicate the incidence of coccidia-infected broiler flocks has increased from 42% to 76% during a 3-year period.

In addition, three coccidia species predominated; one was relatively benign,

but the other two were extremely pathogenic and were found in 77% and 25% of flocks.

“Twenty years’ use of the same type of coccidiostat in the broiler industry may have contributed to the increased incidence of coccidiosis on Norwegian farms,” says Anita Haug, who performed the surveys as part of her doctoral thesis.

The studies, conducted on broilers receiving in-feed narasin during 2000 to 2004, were published in the June 2008 issue of *Avian Pathology* and were the focus of a recent article on thepoultrysite.com.

Although the total parasite load countrywide did not alter significantly during the study period, there were large regional differences in the numbers of infected flocks, the level of infection and the dominant coccidial species, Haug found.

Parasite load alone was not a good measure of the economic significance of coccidial infection, but reduced production occurred when there were over 50,000 parasites per gram of feces and the pathogenic strains dominated. A corresponding level of infection with more benign coccidial strains did not have the same effect on production.

The surveys were funded by the Research Council of Norway; Haug performed her work at the National Veterinary Institute in Oslo and the National Veterinary Institute at Uppsala, Sweden. She developed new test methods by simplifying traditional ones, and also developed a “robust, effective and sensitive” molecular-biological test.



Getting a grip

Why are researchers investigating the role of **alpha-toxin** in the development of **necrotic enteritis**? Two scientists share insights from studies that could impact the management of NE, a serious and costly disease.

SCHRADER: STRONG EVIDENCE DEMONSTRATES ALPHA-TOXIN'S ROLE IN NECROTIC ENTERITIS



Strong evidence that alpha-toxin plays a role in the development of necrotic enteritis (NE) has been demonstrated in studies utilizing an alpha-toxin test kit and immunohistochemistry, Dr. Joan Schrader said at the World's Poultry Congress.

Alpha-toxin is a toxic protein secreted by the bacterium *Clostridium perfringens*. It is also a component of *C. perfringens* type A toxoid, a conditionally licensed US vaccine that is administered to breeders for control of NE in progeny chicks. The vaccine was developed by Intervet/Schering-Plough Animal Health, said Schrader, a scientist with the company.

The recent availability of a commercial diagnostic test-strip kit designed to detect *C. perfringens* and alpha-toxin in feces

provided a new way to evaluate the role of alpha-toxin in the development of NE, she said. Schrader also conducted immunohistochemistry to physically demonstrate alpha-toxin at the lesion site.

The test utilizes monoclonal antibodies to both *C. perfringens* type A and alpha-toxin bound to a paper strip. When the strip is exposed to these antigens in solubilized chicken feces, one line develops color in the presence of *C. perfringens* type A, and a second line develops color in the presence of alpha-toxin.

Study details and results

For the study, 52 commercial, day-old broiler chicks were placed in floor pens at the company's R&D facility in Elkhorn, Nebraska. Thirty-five test chicks were housed in one hut, and the remaining

chicks were housed in another hut and were used as controls.

Chicks were fed a non-medicated starter ration for the first 5 days and were then switched to a high-protein diet for the remainder of the study. When the test chicks were 19, 20 and 21 days of age, a *C. perfringens* type A challenge was performed by oral gavage.

At 23 days of age, fecal material was collected from the caudal rectum/cloaca of each chicken and tested according to the kit instructions. Three strips were tested for each sample, Schrader said.

Chickens were also scored for NE lesions, which were used to determine the true prevalence of NE, and the ability of the test strips for detecting *C. perfringens* and alpha-toxin was determined, she said.

The overall prevalence of positive test, according to lesion score, for *C. perfringens* in birds was 33% for score 0 (6/18), 18% for score 1 (14/78), 19% for score 2 (9/48), 61% for score 3 (11/18) and 88% for score 4 (16/18). The incidence of positive test strips for *C. perfringens* was not different between birds positive or negative for NE (Table 1), Schrader said.

NE status	Challenged group		Negative control	
	CP	α-toxin	CP	α-toxin
Negative	0/7	0/7	6/15	0/15
Positive	8/17	7/17	NA	NA

Table 1. Incidence of positive test strips for *C. perfringens* was not different between birds positive or negative for NE.

CP = *Clostridium perfringens* Note: Alpha-toxin was only detected in chickens positive for NE

Alpha-toxin was not detected by the test strips until lesion scores reached 3 or 4: The test kit was able to detect alpha-toxin in 37% (7/18) of tests among chickens with lesion scores of 3 and in 71% (13/18) of tests among chickens with lesion scores of 4, she said.

The study showed a good correlation between lesion score and the detection of alpha-toxin, with higher lesion scores resulting in greater detection of alpha-toxin with the test kit, Schrader said.

In addition, the finding that high lesion scores correlated with positive test-strip results for *C. perfringens* and alpha-toxin at the site of NE lesions “supports the hypothesis that the severity of the gross

lesions is directly proportional to the number of *C. perfringens* present and amount of alpha-toxin produced,” she said.

Immunohistochemistry results

Schrader then performed immunohistochemistry on NE lesions, a technique that has been widely used to detect the presence of disease agents in tissues. To perform the test, a very thin-sliced tissue sample is fixed to a slide. An “anti-antibody” that has fluorescent or pigmented material is added to the slide and binds to the antibody in question if that antibody is present. In this case, “There was clearly a positive binding of antibodies,” Schrader explained (see Figure 1).

continued



The results of the study, Schrader concluded, “strongly demonstrate the involvement of alpha-toxin in the disease of necrotic enteritis.”

Although the test kit was useful for the purposes of her study, she said, it would not be particularly helpful in the field for producers trying to detect subtle, subclinical NE that can go unnoticed but eat away at performance. It would pick up birds with overt, clinical NE and high lesion scores, and “by then you’d already know the birds are sick.”

Figure 1

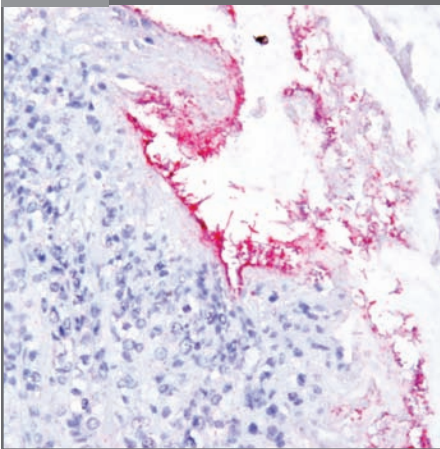


Figure 1. An “anti-antibody” with fluorescent or pigmented material binds to the antibody in question if that antibody is present.



US study suggests alpha-toxin plays role in cause of NE



Vaccination of broilers with recombinant alpha-toxin protected broilers against an experimental challenge with *Clostridium perfringens*, suggesting that alpha-toxin plays a role in the pathogenesis of necrotic enteritis (NE).

In the study, broiler chicks were vaccinated subcutaneously with recombinant alpha-toxin at 5 and 15 days of age, then 10 days later were challenged with *C. perfringens*, the cause of necrotic enteritis. The challenge was administered twice daily for 4 consecutive days by mixing *C. perfringens* cultures with feed.

Non-vaccinated birds challenged with *C. perfringens* developed NE at the rate of 87.8%, but only 54.9% of vaccinated birds developed lesions. In addition, non-vaccinated birds had lesion scores averaging 2.37, compared to 1.35 in vaccinated birds, write K. K. Cooper and colleagues at the University of Arizona, Tucson, in the June 2008 issue of *Veterinary Microbiology*.

Vaccination also produced an antibody response — post-vaccination anti-alpha-toxin titers in vaccinated birds were more than 5-fold greater than in non-vaccinated birds. After challenge, vaccinated birds had average IgG (IgY) titers >15-fold higher compared to those of non-vaccinated birds, the investigators say.

NE in poultry has re-emerged as a concern for poultry producers due in part to the ban on in-feed antimicrobial growth promoters, but the results of this study, say the investigators, suggest that alpha-toxin may serve as an effective immunogen and, as such, may play a role in pathogenesis of necrotic enteritis.



PRESCOTT: PROTEIN PROVIDES EXCELLENT PROTECTION AGAINST NECROTIC ENTERITIS



Independent research by Canadian investigators using state-of-the-art technology confirms that alpha-toxin, a secreted protein from *Clostridium perfringens*, plays a key role in the development of necrotic enteritis (NE) in broilers and that other proteins may also be involved in the pathogenesis of this complex disease.

Immunization with alpha-toxin provided almost total protection of broiler chickens against NE, while other secreted proteins produced by virulent *C. perfringens* yielded various degrees of immunity, Dr. John F. Prescott, of the University of Guelph, Ontario, said at a meeting of the Ontario Association of Poultry Practitioners held in 2008 in Guelph.

Necrotic enteritis has become an economically important disease for the broiler industry. Although the cause is known to be *C. perfringens*, exactly how this bacterium causes NE has been uncertain, Prescott explained.

The major culprit has been assumed to be alpha-toxin. Earlier this year, however, Australian researchers published an article about a novel *C. perfringens* toxin they

identified, called NetB. In widely publicized findings, they indicated that NetB was the main cause of NE and, on the basis of their carefully conducted research, discounted the role of alpha-toxin.

Canada study

Studies by Prescott and his colleagues, however, show that immunization with alpha-toxin provided the best protection against a severe *C. perfringens* challenge with a virulent strain that contains the NetB gene.

In their studies, which Prescott reviewed at the Ontario meeting, several proteins secreted by *C. perfringens* were evaluated for their ability to protect broilers against the virulent strain of the organism. The proteins were alpha-toxin, glyceraldehyde-3-phosphate dehydrogenase, pyruvate:ferredoxin oxidoreductase (PFOR), fructose 1,6-biphosphate aldolase, and a fifth one called hypothetical protein (HP).

Broilers were immunized two to three times with one of the proteins, then one week after their last immunization, they were challenged with the virulent strain,

which was administered in feed at 4 weeks of age.

The severity of the challenges differed; a mild challenge, for instance, involved feeding the virulent strain to birds three times daily for 3 days, and the most severe challenge involved feeding the virulent strain daily to birds continuously for 5 days. The severity of each challenge was confirmed by NE lesion scores in non-immunized but challenged control birds.

All the proteins significantly protected broilers against the relatively mild challenge. For the more severe challenge, alpha-toxin, PFOR and HP provided significant protection, Prescott said.

Alpha-toxin provided best protection

The greatest protection against severe challenge, however, occurred in birds that were primed twice with alpha-toxoid — a toxin that is altered so it is no longer toxic but still initiates immunity — and then boosted with active, purified toxin, Prescott and colleagues found in

their study, published in 2007 in the September issue of *Clinical and Vaccine Immunology*.

In addition, serum and intestinal washings from protected birds had high antigen-specific antibody titers for all proteins used in their study, the researchers found.

NetB may be marker

Prescott and associates also used polymerase chain reaction to test the virulent challenge strain and found it was positive for the NetB gene.

“The fact...that immunization with alpha-toxin strongly protected birds against experimental NE caused by a NetB-containing isolate suggests that alpha-toxin actually is critical to the development of NE, and perhaps that NetB may only initiate infection,” he said.

“I know that the Australian workers think that the success of antibody against alpha-toxin in protecting so well against NE is because it may interfere with the secretion of all proteins by this organism, including, for example, the secretion of NetB. It will

be hard to prove this, and actually may not matter if alpha-toxin immunization works so well,” he added.

In addition, unpublished observations from Ontario show that genetically unrelated isolates from sick birds in flocks with NE “were systematically NetB-positive, whereas isolates from healthy birds at slaughter were usually negative” for NetB, he said.

“Almost but not quite all [*C. perfringens*] isolates from birds with NE or from flocks experiencing NE have NetB, so it’s a good marker for a strain of *C. perfringens* that causes necrotic enteritis,” Prescott said.

Asked by *Intestinal Health* why the search continues for other secreted proteins when it has already been shown that alpha-toxin can protect broilers from NE, Prescott said, “It will help us understand NE better, though I agree that alpha-toxin should be the main focus. On the basis of the findings of protection of birds following immunization, alpha-toxin apparently has a central role in NE,” but there may be an advantage to using more than one protein.

Getting a grip

Proteins differ, he added, in their structure, in their activity, including toxicity, and in their targets.

Favors vaccine

Methods for controlling NE might include probiotics to provide bacterial competition for *C. perfringens* or killing *C. perfringens* with novel antibiotics, but Prescott favors immunization.

“I think a vaccine probably has the most promise because it should be the most reliable. I like the idea of an oral vaccine because it could also be used to deliver other antigens and products,” said Prescott, who has been experimenting with an orally administered, attenuated salmonella vaccine vector with *C. perfringens* antigens.

Even though there is still much to be learned about NE, he predicts rapid advancement in the quest to conquer the disease, thanks to large-scale genome sequencing and other technologies. Due to these advances, “scientists working on NE around the world have made more strides in the last 3 to 4 years than in the previous 25 years,” he said.



NE is a
complicated
disease



spotlight

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Finding ways to prevent or control necrotic enteritis (NE) in broilers is challenging because *Clostridium perfringens*, the bacterium that causes the disease, has chameleon-like qualities, and other factors, such as management, may be involved.

At the World's Poultry Conference this summer in Brisbane, Dr. John Prescott, of the University of Guelph, called *C. perfringens* “an absolute thug.”

The bacterium is “exquisitely adapted as an environmental anaerobe to grow very rapidly in injured or dead animal tissue. Consider that *Escherichia coli* doubles every 20 minutes. In contrast, *C. perfringens* is the fastest growing organism known and, under optimal conditions, doubles every 8 to 10 minutes,” he said.

“It is superbly designed to take advantage of injured tissue,” he said. It secretes multiple toxins and enzymes that maximize the destruction of tissues.

Dr. Joan Schrader, a scientist with Intervet/Schering-Plough Animal Health who has researched NE and helped develop the company's *Clostridium perfringens* type A toxoid for broilers, agrees (see article, page 7).

“It's as though virulent *C. perfringens* has an arsenal of toxins it can produce, and depending on the environment the bacterium is in, it will use the toxins that are most advantageous for the circumstances. It's very much a multifactorial disease,” she says.

Schrader echoes Prescott's opinion, saying that while “alpha-toxin is a key player, other secreted proteins from *C. perfringens* may be involved in development of this complicated disease.”

In addition, secreted proteins may be only part of the story.

In his OAPP talk, Prescott pointed to published evidence that dietary components might adversely affect intestinal motility or damage intestinal mucosa, which in turn affect *C. perfringens* toxin production or the growth of *C. perfringens*. Coccidial infection can be a contributing factor too, he said.

“The interaction of [*C. perfringens*] with other intestinal microflora, including non-NE isolates, and the effect of other microflora on intestinal innate immunity” may be important, he said.

There's no question, he and Schrader say, that NE is a complex infection.



ALPHA-TOXIN GENE LINKED TO NECROTIC ENTERITIS IN INDIA



A study conducted on broilers from India confirmed that *Clostridium perfringens* type A was the cause of necrotic enteritis (NE) and that alpha-toxin may play a significant role in development of the disease, say Arunava Das of the Bannari Amman Institute of Technology, and associates.

After six broilers died at 2 to 3 weeks of age on a poultry farm in Meghalaya, India, investigators performed scanning electron microscopy (SEM) and evaluated intestinal contents and liver samples.

SEM revealed massive necrosis and complete destruction of the intestinal villi within the intestinal mucosa. Bacterial isolation confirmed that *C. perfringens* was the cause. Polymerase chain reaction (PCR) testing of 10 clinical isolates showed they all harbored the alpha-toxin

gene of *C. perfringens*; four were positive for the beta2 toxin gene; and none were positive for the beta, epsilon, iota or enterotoxin genes.

All isolates derived from NE belonged to *C. perfringens* type A and there was 97.6% to 100% homology among the *C. perfringens* isolates, they write in a recent issue of the *International Journal of Poultry Science* (7 (6): 601-609, 2008).

The study confirms that *C. perfringens* type A is the most predominant one associated with necrotic enteritis in broiler chickens in this region of India and that the alpha-toxin gene might play a significant role in the pathogenesis of the disease in broiler chickens, the investigators conclude.





WPC panel explores necrotic enteritis



More than 1,700 veterinarians, poultry industry representatives and researchers gathered in Brisbane, Australia, recently for the XXIII World's Poultry Congress to focus on some of today's biggest health issues.

vaccinal strategies for controlling necrotic enteritis.

Intestinal Health magazine's Phil Stewart attended the panel discussion and filed this report.

The intestinal health of broilers dominated much of the technical program, which included a well-attended panel discussion on

For more coverage on the WPC sessions, see the European edition of *Intestinal Health* at www.thepoultrysite.com/intestinalhealth.

continued

World's Poultry Congress

XX
III

World's Poultry Congress

NE vaccine
trials
in Europe

1

Two clinical field trials conducted in Europe demonstrated that vaccination of hens with a *Clostridium perfringens* type A toxoid, or necrotic enteritis (NE) vaccine, prevented development of NE in broiler progeny, reported Dr. Luciano Gobbi, Intervet/Schering-Plough Animal Health's technical manager for poultry, Italy.

The vaccine, which contains a toxoid of *C. perfringens* type A toxin, was administered to hens to convey immunity against NE to their progeny via passive transfer of maternal antibodies.

Investigators conducted trials on commercial farms in Italy and Germany as part of the application process for licensure of the vaccine in the EU.

They used mortality, evidence of *C. perfringens* type A-associated gut lesions, weight gain and feed-conversion ratio to evaluate vaccine performance. The investigators also monitored antibody levels in vaccinated breeder hens, their eggs and in hatched, 7-day-old progeny.

Vaccinating breeders induced a significant antibody response against *C. perfringens* alpha-toxin, Gobbi said. This response was found not only in breeders but also in their eggs and in serum from progeny chicks. The high antibody titers were reflected in the level of NE infection. None of the progeny of vaccinated breeders had *C. perfringens*-associated gut lesions, but control birds did.

Weight gain and feed-conversion ratios were satisfactory and within the standards of the two poultry companies hosting the trials. And with one exception caused by a yolk-sac infection rather than NE, mortality figures in the trials favored the vaccinated birds' progeny, compared to controls.

Investigators also measured clinical safety and reported no animal health or welfare issues due to the vaccination of breeders. Birds received intramuscular vaccination in the breast, although the toxoid vaccine can also be given subcutaneously.

Gobbi noted that although clinical NE can cause significant mortality, the underlying costs of subclinical disease — estimated at US \$0.05 per bird — should not be discounted. In addition, 95% of NE that occurs is subclinical.

Protection of broilers against the alpha-toxin through maternal antibodies lasted for about 3 weeks, Gobbi explained in an interview with *Intestinal Health*.

By the time antibody titers dropped, the birds' gut development was sufficiently advanced to establish their own immunity.

Gobbi, who was lead investigator for the trial in Italy, said that poultry companies involved in the trials are interested in using the vaccine, once registration is completed, probably in 2009.





A trial in the US designed to test the *Clostridium perfringens* type A toxoid vaccine under real-world conditions demonstrated that the vaccine lowers the risk for NE mortality, according to Dr. Charlie Broussard, a veterinarian with Intervet/Schering-Plough Animal Health.

The poultry company that took part in the trial was moving toward antibiotic-free (ABF) production and had an established vaccination program for coccidiosis control, Broussard said.

NE was a recurring problem, however, particularly during cold winter months,

and the company's poultry houses lacked ideal environmental control, he said.

The trial involved about 4.6 million control birds and 1.3 million broilers that were progeny of breeders vaccinated with the *C. perfringens* type A toxoid vaccine, which is conditionally licensed in the US and awaiting approval in other major poultry markets. The control group had some birds from hens vaccinated with the NE vaccine; this would be expected to reduce the gap between the two groups in terms of NE mortality, but there was still a significant difference in favor of the non-control group, Broussard said.

The trial was carried out between August and February (late summer through late winter). For each group of broilers grown, investigators recorded mortality for three consecutive age periods — 8 to 14 days, 15 to 21 days and 22 to 28 days.

NE deaths occurred in each of these periods, especially the 22-to-28-day period, Broussard said, but trend lines showed mortality was consistently lower for birds from hens vaccinated with the NE vaccine. Overall, mortality for both groups was higher during the cooler months, but among the group from the vaccinated hens, the trend line remained lower by a margin of 0.25% to nearly 0.5%. Birds in this group also had a far

smoother mortality pattern, with fewer spikes in the graph.

“Looking at it another way, the chance of mortality for the broilers of vaccinated hens was 47% lower than for the controls throughout the entire trial,” he said. “During cooler months, this advantage grew to 72%.”

In an interview with *Intestinal Health* after his presentation, Broussard said that the poultry company that hosted the field trials was very encouraged by the results and was continuing with the NE vaccine as part of its ABF production plans. “In fact, they are taking it one step further,” Broussard added. “As ABF production becomes more mainstream, they are moving toward a totally organic system. Sourcing good-quality organic feed will be key to their success in that market.”

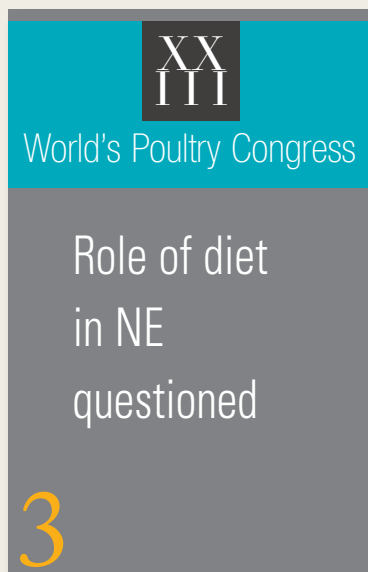
Looking ahead, Broussard said that the *C. perfringens* type A toxoid vaccine could eventually become integrated into the routine care of many breeders.

“*C. perfringens* type A toxoid vaccine is a standalone product now, but there are some exciting possibilities for synergies with other vaccines. This would streamline production and help address labor cost issues.”





Drs. Mathis, Schrader, Broussard and Gobbi listen to Dr. Scott's presentation.



During a lively interchange between panel members and the audience, session chairman Dr. Peter Scott said he has been surprised at the higher prevalence of NE in North America compared to Australia, where the diet for broilers is wheat-based. Wheat-based diets have been linked to a higher prevalence of NE.

“Since 1989 I can count the farms where I’ve seen necrotic enteritis,” said Scott, a senior research fellow at the University of Melbourne and managing director of avian and animal health consultancy, Scolexia. This was despite the infrequent use of growth-promoting antibiotics, which can be effective against NE, as well as deep litter, which can harbor clostridial organisms. “I really believe it comes down to how the feeds are formulated.”

Scott called for more attention on nutrition and gut health, such as fiber levels in feed. “It’s there in black and white: If you maintain adequate fiber levels in your feed, you’ll achieve better coccidiosis control and, by default, better necrotic enteritis control,” he argued.

With the drought in Australia, oats and barley have been less available and more

sorghum and soy is used, which has led to a deterioration of gut health. “I think nutrition needs to be looked at a lot more,” Scott added.

Intervet/Schering-Plough Animal Health’s Dr. Charlie Broussard said that, in the US, he has observed a reduction in the use of ionophores and growth promoters and a move toward non-medicated, less costly feed as soon as possible in the production cycle. This change has occurred for economic reasons but is probably one reason why NE has become an emerging problem in that country.

Reusing litter is not necessarily a negative, he commented. “It can assist with coccidial cycling, and you can achieve a better balance than you do with a total cleanout,” he added.





World's Poultry Congress

Alpha-toxin
a 'primary
player'

4

In a discussion on the mechanisms of protection conferred by the *Clostridium perfringens* type A toxoid vaccine, Broussard confirmed that there is more to be discovered about why and how the vaccine triggers immunity.

Dr. John F. Prescott, University of Guelph, Ontario, who gave a presentation on NE and alpha-toxin during another



Dr. Scott: Classic virulence

session at the poultry conference (see article, page 10), expressed surprise that Australian researchers, whose work was widely publicized, were able to induce NE using *C. perfringens* that lacked the alpha-toxin gene.

“When we vaccinate with just alpha-toxoid — not just secreted protein — we can get excellent protection, so the alpha-toxin has to be involved,” he reported.

Dr. Joan Schrader, the Intervet/Schering-Plough Animal Health researcher involved in the development of the *C. perfringens* type A toxoid vaccine, said she had no difficulty “giving other proteins their due,” but emphasized that she and many other researchers had identified alpha-toxin as a primary player. “I think *C. perfringens* type A toxoid vaccine is efficacious because of that,” she added.

Prescott reminded the audience that alpha-toxin is “the classic clostridial toxin — the First World War gas gangrene toxin. It’s how *C. perfringens* is recognized.”

A lot of secreted proteins have been discovered that are virulence-associated, but “certainly alpha-toxin is the classic virulence factor of *C. perfringens*,” he added.



World's Poultry Congress

Antibody
recognizes
antigen in
NE lesions

5

A study using immunohistochemistry to demonstrate alpha-toxin in intestinal lesions of necrotic enteritis clearly showed that antibody raised against the alpha-toxoid in a *Clostridium perfringens* type A toxoid vaccine recognized antigens in the NE lesions, according to Dr. Joan Schrader, a scientist with Intervet/Schering-Plough Animal Health.

The alpha-toxin was also detected using a commercially available monoclonal antibody test-strip kit.

For details on Schrader’s study, see article on page 7.





BANKING ON EXPERIENCE



Dr. Gladys: It was chronic.

No one ever said producing broilers in Sussex County, Delaware, would be easy. Between extremes in the weather and a dense bird population — over 200 million produced annually within 938 square miles, more than any other county in the nation — birds are highly susceptible to disease.

Gumboro disease and runting-stunting syndrome, for instance, are not uncommon in the area. What came as an unexpected development, though, was an outbreak of gangrenous dermatitis at Allen Family Farms in early 2007. And oddly enough, the solution for the costly skin disease was improved intestinal health.

“Dermatitis was affecting from eight to 10 birds per 1,000, and 30 to 40 houses a week were breaking with it. On some of the farms, it was chronic — flock after flock,” reports Gary Gladys, PhD, vice president of live operations for Allen, which is located in Seaford and is the 15th-largest poultry producer in the US.

Although gangrenous dermatitis was unique at Allen Family Farms, it is not a rare occurrence in the poultry industry. The disease has become a major health concern in broiler flocks throughout the US, often resulting in high mortality, carcass condemnations and trimmed parts. Another reason gangrenous dermatitis results in heavy economic losses is because most birds are affected late in their short lives — after 4 weeks

continued

Photos by Lisa Helfert.





of age — at a time they should be experiencing the fastest growth spurt and when feed consumption is highest.

Gangrenous dermatitis has numerous signs, including loss of appetite, gangrenous skin, cellulitis and mortality. One report published in *Poultry USA* estimated that economic losses from the disease run as high as \$1.31 per affected bird.¹

On Allen's farms, birds with dermatitis were treated with antibiotics and most recovered, despite losses in performance. Some birds died.

Gladys, who joined Allen about 3 years ago, had an idea about how to indirectly manage the onslaught of dermatitis based on past experience at another US poultry company: coccidiosis vaccination.

At the other company, Coccivac-B live-oocyst vaccine was used year-round for organic broilers and was rotated with anticoccidials for a large portion of the antibiotic-free broilers. For whatever reason, "We never would see dermatitis in the birds on Coccivac-B vaccine," Gladys says.

Although Gladys was eager to implement coccidiosis vaccination at Allen Family Farms, not all of Allen's broiler grow-out

facilities — about 500 independent operations, plus 28 company-owned farms — were ready to buy into the idea, especially since in-feed anticoccidials seemed to be working well.

A few of Allen's farms, however, took the plunge and started vaccinating with Coccivac-B starting in April 2007. As Gladys anticipated, the incidence of dermatitis dropped significantly. In fact, on most of the farms vaccinating, dermatitis disappeared altogether, he says.



Advances in administration methods help ensure that all chicks are vaccinated for coccidiosis.

Other than switching to a coccidiosis vaccine, no changes were made that could account for the mysterious but welcomed disappearance of dermatitis. Vaccinated birds, for instance, received the same diets as Allen's other birds, Gladys says.

USE OF VACCINE SOARS

The reduction of dermatitis on Allen farms was so significant that, by the next round of birds, "everyone was jumping on board," he says. Every week Allen vaccinated about 800,000 broilers with Coccivac-B — close to one-third of the 2.5 million birds that it processes weekly.

"We started out just suggesting use of the vaccine; by the middle of summer, all of Allen's service reps wanted their flocks on the vaccine," Gladys says.

Feed conversion in the vaccinated birds has been the same as conventionally raised birds, and by decreasing dermatitis, the high cost of production was improved, he says. In addition, routine posting sessions indicated good coccidiosis control.

Performance with the coccidiosis vaccine was consistent with Gladys's previous experience at the other poultry company. "There we had antibiotic-free and

¹Norton, R.A. et al. Gangrenous dermatitis reemerges in broilers. *Watt Poultry USA*. 2000.

conventional birds on the same farm but in different houses,” he says. “The performance of coccidiosis-vaccinated birds was the same as conventional birds. Antibiotic-free birds had slightly higher feed conversion, but I don’t think it had anything to do with the coccidiosis vaccine.”

Is coccidiosis vaccination cost-effective? “Yes, certainly more so than feeding salinomycin” if you look at the long-term results, Gladys says. There’s more to consider than the initial cost of vaccination.

Asked why he thinks dermatitis stopped after initiating coccidiosis vaccination, Gladys pauses and then freely admits that he doesn’t know for sure. He’s aware of one theory, however.

Dermatitis is thought to be caused by the bacterium *Clostridium perfringens*, an opportunistic infection also linked to necrotic enteritis. One supposition is that when in-feed anticoccidials begin to lose their effectiveness — even when it’s not noticeable — “leakage” of coccidiosis-causing *Eimeria* organisms occurs. That results in coccidiosis breaks and an unhealthy gut, setting the stage for *C. perfringens* to take over. The detrimental effect of anticoccidial leakage is worse when it occurs late in the growout cycle — after 28 days of age — which is the same time that gangrenous dermatitis usually strikes.



Chicks are showered with Coccivac-B in the hatchery to stimulate natural immunity against coccidiosis.

Coccidiosis vaccination stimulates the bird’s natural immune system to provide lifetime coccidiosis control and, by doing so, results in a healthier gut, so *C. perfringens* can’t get a foothold (see sidebar on page 23). Furthermore, because coccidiosis vaccination is applied at 1 day of age, birds are fully immune to coccidiosis by 4 weeks of age.

Gladys, however, says that before coccidiosis vaccination was implemented, Allen maintained excellent coccidiosis control by rotating in-feed anticoccidials. To keep a close eye on its program, the company turns to technical service representatives from various animal health companies who necropsy six birds from 35 flocks every 8 weeks and score them for lesions. The team includes Intervet/Schering-Plough Animal Health’s parasitologist Dr. Steve Fitz-Coy, who maintains a diagnostic laboratory near the company’s Coccivac-B manufacturing plant in nearby Millsboro, Delaware.

continued on page 24

“ We started out just suggesting use of the vaccine; by the middle of summer, all of Allen’s service reps wanted their flocks on the vaccine. ”





Late coccidial cycling, dermatitis linked

Gangrenous dermatitis, a subcutaneous infection in poultry that's often due to clostridial organisms, is topping the list of health problems at some US poultry companies.

When it strikes, the disease reportedly affects 10% to 25% of flocks, with mortality running about 2% to 4% — and sometimes higher, says Dr. Charles Broussard, US poultry technical service director, Intervet/Schering-Plough Animal Health.

It was once thought that gangrenous dermatitis started with a scratch that became infected with a bacterium, which proliferated when birds were immunosuppressed due to diseases such as infectious bursal disease (IBD) or chick anemia virus (CAV). But control of IBD and CAV has not resolved the problem for most producers, nor have management changes such as low lighting or special diets aimed at keeping birds calm to prevent scratching, he says.

Broussard points to research conducted by Dr. Steve Collett of the Poultry Diagnostic and Research Center, University of Georgia, Athens. Collett demonstrated that immunosuppression due to IBD and CAV did not increase the severity of gangrenous dermatitis lesions. The Georgia veterinarian also theorized that the skin and scratches are not always the way that clostridial organisms enter the body and cause dermatitis, and that an alternative route is most likely the gastrointestinal tract.

Indeed, field experience and trials suggest that late coccidial cycling predisposes birds to the development of gangrenous dermatitis, Broussard says. Dermatitis tends to be seen in flocks on chemical-to-ionophore and straight ionophore programs that allow late coccidial cycling. In contrast, flocks that are vaccinated against coccidiosis at 1 day of age tend not to develop dermatitis.

"It's possible that coccidiosis might have been a cause of dermatitis all along," he says. "Coccidiosis vaccination itself is not effective against gangrenous dermatitis, but it prevents late coccidial cycling. Reducing the severity of gut epithelial damage from coccidia, or shifting the time at which it occurs, could be an important means of preventing or at least reducing the prevalence of gangrenous dermatitis."



continued from page 22

Impressed by initial results, all of Allen's farms started using the vaccine this year and will continue to do so until December, when they will switch back to in-feed anticoccidials.

"We've been doing this for many years and we rotate anticoccidials based on findings in the posting sessions. We don't wait until our anticoccidials poop out before we make a change," Gladys says.

He agrees though that the disappearance of dermatitis after coccidiosis vaccination could simply have occurred because Coccivac-B vaccine contributes to a healthier gut.

Impressed by initial results, all of Allen's farms started using the vaccine this year and will continue to do so until December, when they will switch back to in-feed anticoccidials.

By the end of December, Gladys expects the houses to be "seeded down" with oocysts from Coccivac-B, which are highly sensitive to in-feed anticoccidials, he says. This allows Allen to rotate back to traditional in-feed anticoccidials in the winter months before returning to vaccination in the spring.

BROODING MANAGEMENT CRUCIAL

One important factor contributing to Allen's newfound enthusiasm for coccidiosis vaccination was a presentation provided by Dr. Matilde Alfonso, technical service veterinarian with Intervet/Schering-Plough Animal Health, about how coccidiosis vaccination works. "It was a real eye-opener," Gladys says.

A crucial point Alfonso emphasized was the need to provide the full house to



Dr. Gladys: Everyone was jumping on board.

coccidiosis-vaccinated birds in half-house brooding by 14 days of age. "It's one of those things that gets easily overlooked," he says (see sidebar on pages 25-26).

Despite great results with coccidiosis vaccination and although Gladys says that "I've seen coccidiosis vaccination work in organic birds year-round," Allen plans to keep rotating coccidiosis vaccination with anticoccidials.

Come winter, wind makes it difficult to keep birds warm. In addition, ventilation is less than ideal, and it's hard to keep litter dry. In birds vaccinated with Coccivac-B, about 25% litter moisture is needed to stimulate the coccidial life cycle, but excessive litter moisture promotes heavy cycling and too much coccidia reaction in birds. These problems make Allen more comfortable using in-feed anticoccidials during cold months.

TRANSITION TO VACCINE EASY

Introducing coccidiosis vaccination in the spring, however, was a breeze, Gladys says, adding that "it was a really nice transition" for the hatchery.

Day-old chicks are vaccinated with Coccivac-B vaccine via a Spraycox II unit, which has dual nozzles for more



uniform distribution. Red dye is included in the vaccine mix to encourage preening and ingestion of the vaccinal oocysts, which stimulate the chicks' immunity. Halogen lamps are used to further encourage preening and to help dry the birds and prevent them from getting chilled, he says.

Making the switch at the feed mill was not a problem, either. Allen's feed mill, located in nearby Delmar, Maryland, produces over 28 million pounds of feed (more than 12.7 million kilograms) per week, and a second feed mill is underway in Sussex County to provide feed for 5 million company broilers located within a 10-mile radius of the new mill.

To ensure that coccidiosis-vaccinated birds do not accidentally get feed with anticoccidials, feed for vaccinated chicks is colored red with iron oxide for easy identification.

"Red spots, red feed," Gladys quips, referring to the red dye sprayed on birds with vaccination at the hatchery and the colored feed.

Initiating coccidiosis vaccine requires preplanning, Gladys says, but "it just comes down to whether you want something to work or not."



Tips

Successful coccidiosis vaccination depends on good brooding management as well as proper administration of the vaccine in the hatchery at 1 day of age, says Dr. Matilde Alfonso, a technical service veterinarian with Intervet/Schering-Plough Animal Health.

Here are a few tips that can help ensure good results:

1

- Understand coccidial cycling.

After vaccination, coccidial parasites replicate in the birds, which then shed coccidial oocysts in feces at about 7 days of age. Birds ingest oocysts from litter, parasites replicate again and a second cycle of oocysts are shed at about 14 days of age. The process initiates the development of immunity.

for successful coccidiosis vaccination

2

- Use half-house brooding

from 1 day of age until 10 to 14 days of age to help ensure all birds ingest enough oocysts to achieve uniform coccidial cycling. Proper stocking density varies with bird size.

3

- Give vaccinated birds access

to the full house at 10 to 14 days of age — before the second coccidial cycling. If the second cycling occurs before the full house is used, birds still in the half-house area ingest too many oocysts, while those in the rest of the house ingest too few. Flock immunity won't be uniform.

4

- Guard against wet litter.

It promotes more coccidial cycling than necessary.

5

- Provide enough feeders.

Otherwise birds peck litter more and ingest more oocysts than needed.

6

- Don't panic at the first sight of gut lesions.

Some lesions in vaccinated birds are expected and normal. In fact, they're a sure sign that the vaccine is working to stimulate the birds' immune systems. Consult an Intervet/Schering-Plough Animal Health poultry sales or technical services representative to learn what level of post-vaccination reaction is to be expected.



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*References and data on file
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W P D C

Fine-tuning coccidiosis control to improve bird performance and save costs was the theme of several presentations at the **57th Western Poultry Disease Conference & XXXIII ANECA** annual convention in Puerto Vallarta, Mexico.

Experts explained that developing early immunity to intestinal diseases — before the bird’s major growth spurt and when birds eat the most — can have a significant impact on gut health, feed absorption, resistance management and bird performance.

Conference participants also learned why monitoring the pattern of coccidiosis in commercial flocks is essential if a coccidiosis-control program is to be effective and prevent losses.

Intestinal Health magazine attended the coccidiosis presentations and interviewed speakers for additional insights. Our report follows.

Puerto Vallarta, Mexico



57th Western Poultry Disease Conference

Early coccidial cycling could help control feed costs



Alfonso

Evaluation of coccidial-infection levels among flocks in the US shows that coccidiosis vaccination induces early and mild coccidial cycling — an especially relevant finding considering the soaring cost of feed, Dr. Matilde Alfonso said.

“The results of the study demonstrate that poultry producers can use coccidiosis vaccination to change the timing, prevalence and severity of coccidiosis challenge and also to reduce feed costs,” she said.

Alfonso, a technical service veterinarian with Intervet/Schering-Plough Animal Health, said the study encompassed over 2,500 broilers from four different US commercial operations in different states. Birds ranging from 14 days to market age were examined for coccidial lesions during necropsy. The prevalence and severity of the lesions were considered, as well as the time they occurred.

The necropsies were conducted by technical service veterinarians from Intervet/Schering-Plough Animal Health, and primarily by Alfonso.

Alfonso used the well-known Johnson and Reid method to score gross lesions due to the common coccidial species *Eimeria acervulina*, *E. maxima* and *E. tenella*. There are some limitations when using lesion scores alone to assess flock performance or the efficacy of an anticoccidial program, Alfonso added, but it is a widely used method in the field for monitoring coccidiosis in commercial broiler operations. When performed consistently, lesion scoring allows comparisons over time and between different coccidiosis-control programs.

In addition to gross-lesion scoring, Alfonso and her colleagues microscopically examined mucosal scrapings from the mid-intestine to assess the presence of *E. maxima* oocysts. While other *Eimeria* species such as *E. acervulina* and *E. tenella* can usually be diagnosed by observing gross lesions with the naked eye, *E. maxima* is different, Alfonso explained.

“*E. maxima* can be there in high numbers, damaging the intestinal lining and negatively affecting bird performance

and XXXIII ANECA annual convention

Early coccidial cycling could help control feed costs

Alfonso

without causing visible lesions. That’s why we always determine the presence of *E. maxima* with the microscope, as well as by gross-macroscopic inspection. We score the findings based on how many oocysts we see per microscopic field,” she said.

The most obvious result in the study was that the prevalence and severity of coccidial lesions and the time they occurred varied with the control program used — early in vaccinated birds and later in birds receiving in-feed anticoccidials, the veterinarian said.

At a major poultry company in South Carolina, where a traditional in-feed anticoccidial shuttle program was rotated with coccidiosis vaccination, birds on the shuttle program experienced late coccidial cycling from 5 to 7 weeks (35 to 49 days) of age. But after coccidiosis vaccination was initiated, cycling occurred at 3 to 4 weeks (21 to 28 days) of age, Alfonso said (see Figure 1).

continued on page 33

Shuttle vs. Coccidiosis Vaccine

Shifting coccidial cycling from 5-7 weeks to 3-4 weeks

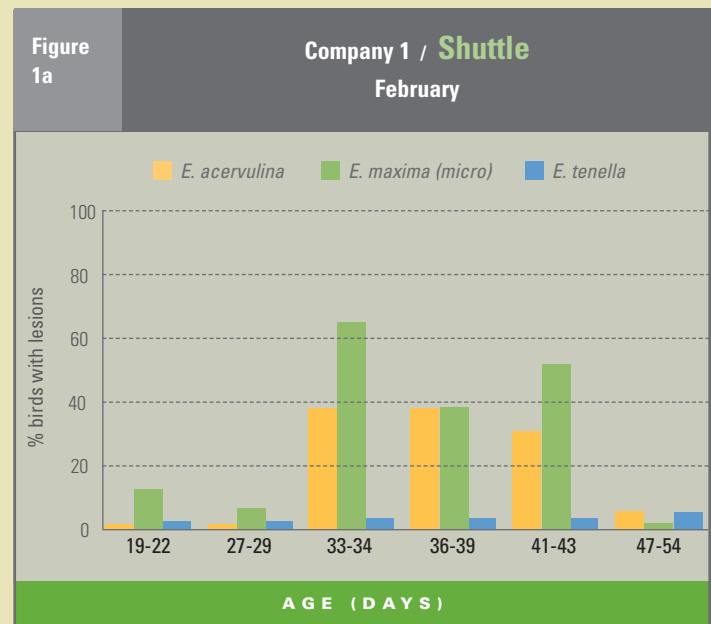
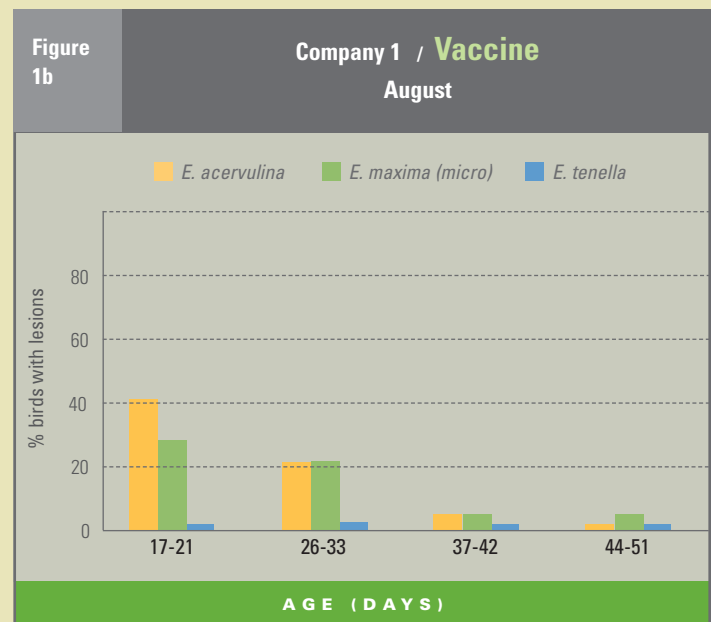


Figure 1 a/b. Coccidiosis vaccination initiated after use of a traditional in-feed anticoccidial shuttle program resulted in earlier coccidial cycling.



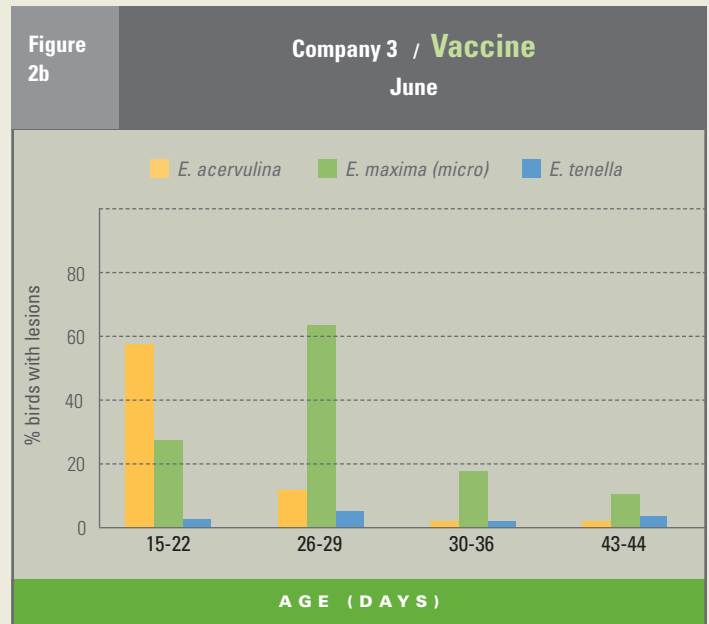
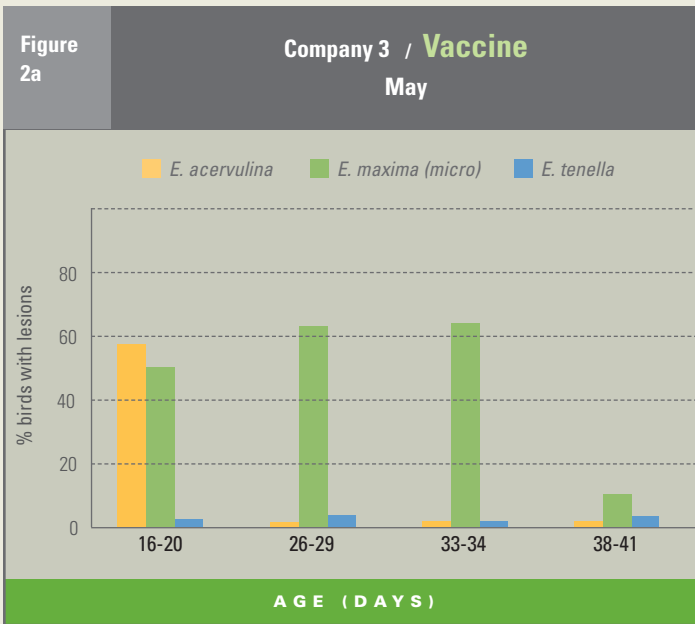


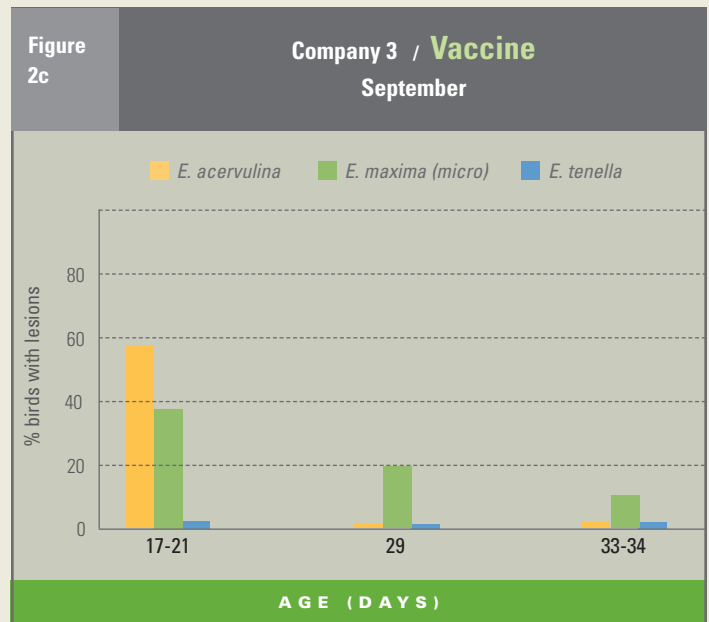
Figure 2 a/b/c. Continuous coccidiosis vaccination implemented after a traditional drug program decreased the occurrence and severity of lesions and yielded an early cycling pattern and consistent immunity.

Coccidiosis Vaccine

April-September

Challenge due to previous drug program. Decreased occurrence and severity of lesions with extended use.

Early cycling at 3-4 weeks. Consistent immunity at 5 weeks.





Early coccidial cycling could help control feed costs

Alfonso

The importance of early coccidial cycling is underscored by studies conducted at Oklahoma State University by Dr. Robert Teeter and associates (see article, page 39). Those results show that the later in life birds experience coccidial cycling, the greater the impact on performance — and on the producer's bottom line, Alfonso said.

In other words, if coccidial cycling occurs before the bird's major growth spurt, which occurs after 4 weeks (28 days) of age, it is less likely to interfere with growth at a time when birds are consuming the most feed, she said.

At the same South Carolina company, vaccinating with Coccivac-B — a live vaccine that provides a controlled, balanced dose of coccidial oocysts to stimulate immunity — restored sensitivity to ionophore anticoccidials by seeding the poultry houses with drug-sensitive strains, Alfonso noted.

Tracking at the South Carolina company further demonstrated that inconsistent

coccidial cycling occurs when ionophore anticoccidials alone are used, Alfonso said.

Alfonso also discussed a poultry company in Mississippi, which rotated in-feed anticoccidials with coccidiosis vaccination. Over a 5-year period beginning in 2001, extended vaccine use led to a low occurrence of *E. acervulina* gross lesions and decreased the severity of lesions. The occurrence and severity of *E. maxima* microscopic lesions also declined, Alfonso said.

E. maxima can be there in high numbers, damaging the intestinal lining and negatively affecting bird performance without causing visible lesions.

In Alabama, where a poultry company used coccidiosis vaccine continuously following a traditional control program with in-feed medications, the occurrence and severity of lesions declined with time.

Coccidial cycling occurred earlier and the birds consistently had immunity by 5 weeks (35 days) of age (see Figure 2, page 32), Alfonso said.

The impact of coccidiosis vaccination following an ionophore program was also evident at a company in Arkansas. Four months after initiating vaccination, the number of birds with coccidial lesions declined, coccidial cycling occurred at 3 to 4 weeks (21 to 28 days) of age, and birds had consistent immunity by 5 weeks (35 days) of age, she said.

Similar results were achieved at the Arkansas company when coccidiosis vaccination in antibiotic-free birds was compared to the continuous use of anticoccidial drugs in conventional birds. Vaccinated birds had earlier cycling and immunity plus fewer lesions compared to birds on the drug program, Alfonso said.



Synchronized coccidiosis control, management yield better broiler results

Newman



Performance in broiler flocks can be improved by coordinating the coccidiosis-control program with management practices, Dr. Linnea Newman said.

Broilers perform better if they develop immunity against coccidiosis early rather than late into the production cycle. However, exactly when immunity develops varies with different anticoccidial programs and management practices, she said.

“For instance, flocks that are on reused litter in houses with higher bird density develop immunity earlier than flocks started on clean litter in a full house,” said Newman, of Intervet/Schering-Plough Animal Health technical services.

“Coccidiosis control in broilers depends upon immunity — regardless of whether in-feed medication or vaccination is used,” she said.

Early immunity against coccidiosis is preferable because the adverse effects of subclinical coccidiosis on performance worsen with age, she said.

Newman cited work at Oklahoma State University (see article, page 39), which demonstrated that the later in life broilers develop immunity to coccidiosis, the greater the negative impact on performance and the greater the cost.

This work is important, she explained, because “many coccidiosis-control programs, especially ionophores, shift the immunity-building process to the last 2 weeks of a broiler’s life — when the most significant weight is to be gained, the greatest amount of feed will be consumed and there is the greatest potential for a loss in performance.”

Oocyst counting and weight analysis on commercial broiler farms further confirm the link between late development of immunity and worsening performance — as well as the role of the anticoccidial and management programs used, Newman said.

Clean litter can delay immunity

“Surprisingly, cleaning out houses works against the immunity-building process, especially with in-feed anticoccidial



Synchronized coccidiosis control, management yield better broiler results

Newman

programs, causing an even greater impact late in the life of a broiler,” Newman said. On anticoccidial programs, coccidial populations may explode after 28 days, with higher peaks and a greater potential for an adverse impact on broiler weight gain. In contrast, used litter exposes birds early in life to coccidia, which initiate the gradual development of immunity.

Actual oocyst counts from clean litter obtained in British Columbia and Ontario demonstrate the pattern of *Eimeria spp.*-oocyst shedding in full-house brooders where birds receiving various in-feed anticoccidials were started on clean litter with a mandatory 2-week (or greater) down time.

When the farm in Ontario collected oocysts and determined daily weight for one of its broiler flocks, the impact of a late subclinical coccidial challenge was evident, with weight diverging from the Ross 308 published breed standard (see Figure 1). “This is a real-world example of the model developed by the Oklahoma State University researchers,” Newman added.

Half-house brooding on reused litter produced different patterns. Higher bird density (<0.80 ft²/bird) and heavily reused litter from a farm located in the Delmarva Peninsula of the US produced an early oocyst peak at 19 days, regardless of whether the program was Nicarb-narasin or salinomycin, she said.

Coccidiosis control with in-feed anticoccidials no longer provides complete “control” as it did 25 years ago when many of the products were introduced.

In contrast, birds that received salinomycin and were in houses with low bird density (1.0 ft²/bird), half-house brooding and reused litter produced a later pattern after two flocks; the pattern was similar to that found in clean houses.

Birds that are vaccinated against coccidiosis demonstrate some variability in the development of immunity, but the pattern is usually earlier than natural immunity programs utilizing anticoccidials, Newman said.

Vaccinated flocks started with half-house brooding on reused litter peaked at 19 days (see Figure 2). In contrast, vaccinated birds started in a full house on clean litter had a delayed peak at 25 days.

Coccidiosis control with in-feed anticoccidials, Newman said, no longer provides complete “control” as it did 25 years ago when many of the products were introduced. “Most coccidiosis control in broilers today depends on immunity — either natural immunity moderated by anticoccidial drugs, or immunity developed through vaccination,” she added.

The successful development of immunity is moderated by management style: clean vs. reused litter, stocking density and house management, which all impact when and how immunity develops, she continued.

The development of immunity to coccidiosis requires exposure to multiple life cycles of *Eimeria spp.* This necessary exposure has an adverse impact on weight gain and feed conversion, but the impact will be a different magnitude depending upon where it occurs on the growth curve, Newman explained.



“If you want to maximize the genetic-performance potential of your broilers,” she said, “analyze your coccidiosis-control program to determine when immunity is completed on the growth curve and the consistency of that timing over the full production year.

“Coordinate your broiler-house management and coccidiosis-control programs to induce earlier immunity,” Newman concluded.

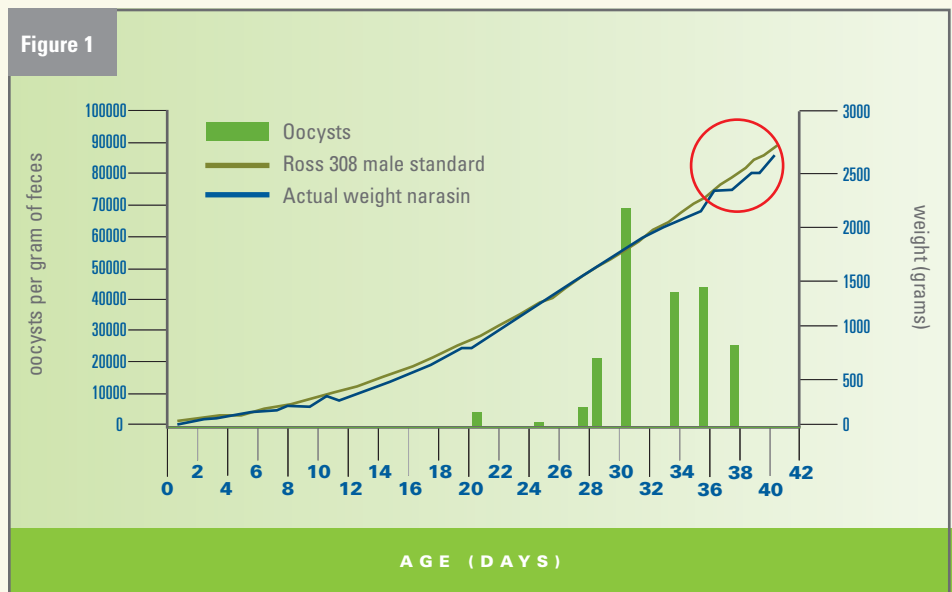
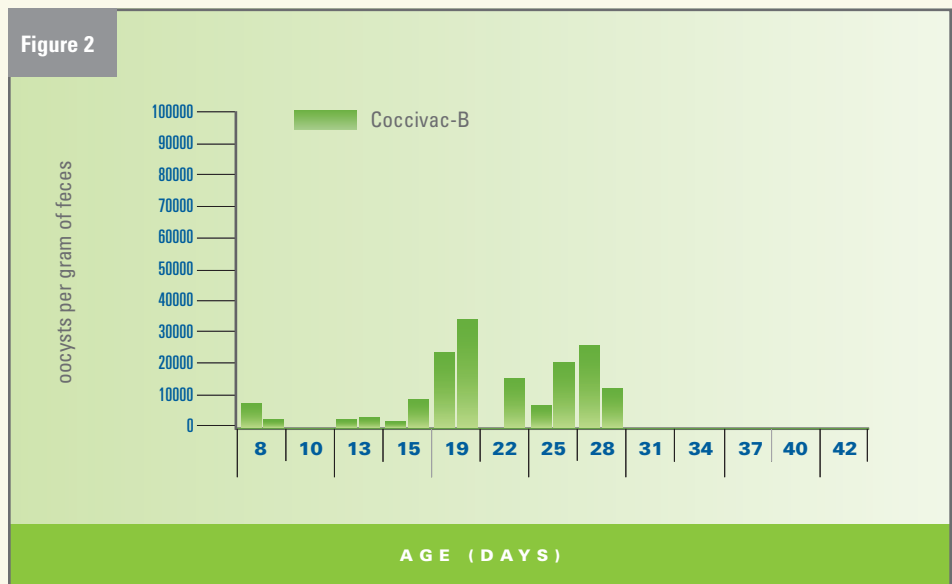


Figure 1.
Oocyst counts from clean litter versus the actual growth curve show divergence from the Ross 308 male 2007 breed standard. These birds received narasin.

Figure 2.
The oocyst peak in flocks vaccinated against coccidiosis with Coccivac-B and started in half-house brooding on reused litter was an early 19 days. Density was 0.80 ft²/bird.





Immunity develops earlier in turkeys vaccinated for coccidiosis



Radu

Development of immunity against coccidiosis in turkeys varies markedly with the type of control program and appears to start earliest in birds that are vaccinated against the disease, reported Dr. John Radu, a technical service veterinarian with Intervet/Schering-Plough Animal Health.

Early immunity is desirable because it is less likely to interfere with performance. With in-feed anticoccidials, immunity against coccidiosis develops later into the production cycle, when birds experience a major growth spurt and consume more feed, he said.

To reach this conclusion, Radu reviewed the coccidial-shedding patterns of commercial turkey flocks in the US. Determining the shedding rate of coccidial oocysts is a good indicator of immunity development. It is also helpful for diagnosing coccidiosis, which tends to present as subclinical disease with no overt signs of infection.

Exposure to infective oocysts, he explained, occurs either when poults pick at litter or receive a controlled dose of live

oocysts administered via live vaccine at 1 day of age. Seven species of *Eimeria* affect turkeys, and four of them are considered significant: *Eimeria adenoids*, *E. gallopavonis*, *E. meleagrimitis* and *E. dispersa*.

For the oocyst analysis, the lead investigator, Dr. Greg Mathis of Southern Poultry Research in Athens, Georgia, collected feces sequentially throughout the first 13 weeks of life and then counted the number of oocysts per gram.

Included in the analysis were flocks that received either diclazuril, the chemical anticoccidial; a combination of ionophore anticoccidials; or a live coccidiosis vaccine, which is becoming a more common method of coccidiosis control in US flocks. Vaccinated birds received Coccivac-T, a live-oocyst vaccine administered at day 1 that contains a controlled dose of the four *Eimeria* species that cause coccidiosis in turkeys.

Flocks receiving diclazuril developed immunity at 50 days of age (about 7 weeks of age). Birds on ionophore anticoccidials developed immunity earlier — by day 35 (5 weeks of age) — but did



Subclinical coccidiosis can not only lead to losses in performance, it provides an opportunity for secondary intestinal diseases such as necrotic enteritis.

not appear to have complete immunity to *E. meleagridis*. Immunity against this coccidial species did not occur until later in the sampling period, from 56 to 84 days of age (8 to 12 weeks). Radu said it's possible that *E. meleagridis* was less competitive with other coccidial species and cycled enough to initiate immunity

only after the other species declined, he said.

Birds that received Coccivac-T produced an oocyst-shedding pattern similar to that seen in birds treated with ionophores, but immunity occurred 1 week earlier, at 28 days or 4 weeks of age (see Table 1); in addition, oocyst counts were not as high as some of the counts found in birds that received ionophores, and immunity to *E. meleagridis* appeared to be more complete. There was no late cycling in the 8-to-12-week window, Radu said.

The results not only demonstrated that coccidiosis is present on turkey farms, they showed that different coccidiosis-control programs in commercial turkey flocks

produce markedly different coccidiosis-immunity patterns, Radu said.

"With vaccination," Radu said in an interview following his presentation, "oocyst shedding occurs earlier, which allows immunity to develop at a time when the birds aren't growing so fast. Overall performance is not affected much.

"With other coccidial-control methods such as ionophores or chemicals, we definitely see a pattern of oocyst shedding later in life. Immunity development is delayed and, when it does occur, it coincides with that spike in bird growth," he said.



Table 1		
Number of flocks	Coccidiosis-control program	Age that immunity was developed
4	Diclazuril (chemical)	7 weeks
12	Ionophore	5 weeks*
4	Coccivac-T	4 weeks

Table 1.
Development of immunity
in turkey flocks on various
coccidiosis-control
programs.

*Immunity against *E. meleagridis* was incomplete until 8 to 12 weeks of age.



Nutritionist: Aim for lowest coccidiosis-lesion score

Poultry producers should aim for the lowest possible coccidiosis-lesion score in their broilers to maintain optimum performance, according to Dr. Robert Teeter, a nutritionist at Oklahoma State University.

“Some producers would view a lesion score of 2 as being nominal, but in our studies, we saw the average daily gain in birds with this lesion score drop to zero,” Teeter said. “Growers really need to pay attention to their coccidiosis program and get it as close to lesion-free as possible,” he said in an interview after his presentation.

Teeter and colleagues evaluated the metabolic impact of coccidiosis at different points on the broiler growth curve. Teeter has presented results from the studies before, but since then he has more closely examined the data and influence of coccidiosis on the energy costs in broilers.

Further analysis confirms that low-level coccidiosis produces a measurable cost at any age, but the cost is much less if it occurs early in the bird’s life, when there is less overall impact on energy utilization, he said.

In his presentation, Teeter explained how he and his colleagues used metabolic chambers in studies designed to mimic coccidiosis challenge in a commercial production environment. Over 1,000 Cobb X Cobb broilers were evaluated five times over a 48-day period.

Teeter’s team challenged birds with an orally administered mixture of *E. maxima*, *E. acervulina* and *E. tenella* — three common pathogenic species of the protozoan parasite that causes coccidiosis — at days 14, 21, 28, 35 and 42. Control birds received only a sterile saline solution.

After each challenge, investigators placed birds in metabolic chambers, where they were evaluated for a variety of parameters such as live weight, body composition, heat production, retained energy and excretion. After 6 days in the chamber, they necropsied the birds and scored the coccidial lesions.

Teeter



Lesions, poor performance correlated

As expected, control birds had no coccidial lesions, but the challenged birds did. Compared to controls, birds with lesions had lower live weight and weight gain, and poorer feed efficiency. They also had less energy consumption, and their ability to retain energy worsened with age (see Table 1), Teeter said.

The metabolic chambers, Teeter noted in the interview, are “very conservative and the costs determined in this study would be less than in the field.”

For instance, at 20 days of age, energy retained by birds with a gross-lesion score of 2 was 100 Kcal/day, at a time when they were eating 300 Kcal/day. By 48 days, energy retained daily by the same birds fell to 0 Kcal, even though the birds were consuming 482 Kcal/day, he said.

Even in birds with a gross lesion score of only 0.5, the results demonstrated the impact of a coccidiosis challenge. At 20 days of age, retained energy was 170 Kcal,

VARIABLE		LESION SCORE ²				
		0.0	0.5	1.0	1.5	2.0
Average daily gain						
Age (days)	Initial live wt. (g)					
14-20	904	76.5	70.2	60.6	48.7	40.7
28-34	2096	92.6	72.2	54.3	38.2	27.3
42-48	3398	97.3	61.0	32.7	10.0	-7.0
Maintenance cost (Kcal/day)						
Age (days)	Initial live wt. (g)					
14-20	904	124	148	151	154	281
28-34	2096	187	215	218	222	308
42-48	3398	281	311	308	264	315
Metabolic energy (ME_n) consumption/day (Kcal)						
Age (days)	Initial live wt. (g)					
14-20	904	386	364	342	318	300
28-34	2096	562	516	477	444	420
42-48	3398	701	628	570	522	482
Added excreta (Kcal/day)						
Age (days)	Initial live wt. (g)					
14-20	904	16	5	22	37	35
28-34	2096	24	30	57	81	86
42-48	3398	38	57	94	122	130
Retained energy (Kcal/day)						
Age (days)	Initial live wt. (g)					
14-20	904	188	170	149	121	100
28-34	2096	274	210	162	119	87
42-48	3398	305	191	110	49	-0.9
Gain/feed						
Age (days)	Initial live wt. (g)					
14-20	904	0.64	-	0.60	-	0.38
28-34	2096	0.54	-	0.37	-	-0.04
42-48	3398	0.43	-	0.10	-	-0.49

Table 1. Coccidiosis-mediated gross-lesion-score effects on production and energetic criteria at standardized weights.¹

¹ Values created using predictive models ($R^2 > .95$) and standardized initial weights.

² Mixed lesion scores were utilized for all variables except gain/feed, where homogenous arrays of 0, 1, 2, 3 and 4 were applied.



Nutritionist: Aim for lowest coccidiosis-lesion score

Teeter

compared to 188 for controls. By 40 days of age, it was 191 Kcal, compared to 305 for controls.

Higher maintenance energy cost

Lesions in challenged birds correlated with maintenance energy cost. In other words, the cost for birds challenged with *Eimeria* increased with the lesion score and became higher later into the broiler-growth curve, he said.

Lesion scores were also positively correlated with excreta energy loss, Teeter said. “With a coccidiosis challenge, you can see an additional 75 to 125 Kcals of energy lost in excreta. Now that’s a pretty big proportion of the bird’s daily appetite,” he observed. “In addition, we saw the maintenance requirements go up and an additional number of K-calories lost as heat and carbon dioxide, which goes into the atmosphere.”

He pointed out that the consequences of coccidiosis challenge were directionally similar for birds with low and high lesion scores. Feed-efficiency responses paralleled energy responses, with the consequences of coccidiosis challenge becoming more profound late in the growth curve.

“Some producers would view a lesion score of 2 as being nominal, but in our studies... average daily gain in birds with this lesion score [dropped] to zero.”

“The consequence of coccidiosis challenge is much more severe when you get up in the second half of the growth curve, when the bird is consuming most of the feed it will eat during its lifetime,” and at a time when feed costs are soaring, Teeter said. “You absolutely don’t want that bird developing immunity during that time period, because it will be extremely costly and result in a tremendous loss for producers.”

It’s true that birds can bounce back and make up the performance loss that occurs after a coccidiosis challenge. But when that challenge occurs in the second half of the growth curve, there’s not enough time to close the gap. “Once a calorie is lost in excreta, it’s gone. Those calories would just be a direct write-off to the bottom line in terms of profitability,” he said.

The same findings probably apply to any kind of immunity, not just immunity against coccidiosis, Teeter supposes. “A bird that has developed immunity early appears to handle energy more efficiently later on,” he added.



Monitoring coccidiosis patterns can improve bird performance, profits

Producers looking for ways to improve bird performance and their bottom lines were advised to put more emphasis on routine monitoring of coccidial patterns in their flocks.

“Sometimes what’s not seen can hurt,” said parasitologist Dr. Steve Fitz-Coy. Without monitoring, coccidiosis could be taking a serious but unrecognized toll.

As it is now, many poultry producers put coccidiosis control on the back burner — until it becomes a problem. In the meantime, performance may suffer and dollars are lost, said Fitz-Coy, of Intervet/Schering-Plough Animal Health.

In addition to monitoring coccidiosis patterns, he encouraged producers to avoid choosing the least expensive coccidiosis-control program. It’s understandable, considering the rising costs of production, but what might initially seem less costly may not be the most cost-effective over the long-term.

Coccidiosis, Fitz-Coy emphasized, is a dynamic disease and the patterns may change. That’s why monitoring is important. It can reveal trends within a

poultry operation that tell producers whether a coccidiosis-control program is working or that it needs to be modified.

Coccidia, which are present in virtually all commercial poultry houses, can cause tremendous destruction of bird tissues, which leads to impaired food intake, digestion and absorption. The result is weight loss, poor feed utilization, poor pigmentation and even mortality, he said.

The damage caused by coccidial organisms is directly related to the number and species of *Eimeria* ingested, he said.

Controlling coccidiosis requires maintaining a low parasite burden, which is accomplished by keeping the coccidial intake and replication rate low, which in turn results in minimal cell destruction and less impact on bird performance, Fitz-Coy said.

Complicating the issue is the development of drug tolerance or drug resistance to the in-feed anticoccidials that producers have relied upon for so long, he continued.

“Rotation of drugs alone to combat drug tolerance or resistance is futile,” Fitz-Coy



Fitz-Coy



Monitoring coccidiosis patterns can improve bird performance, profits

Fitz-Coy

said. Successful coccidiosis control may require rotating multiple in-feed anticoccidials in conjunction with live, effective vaccines. He also recommends routine necropsy sessions, regular testing for drug responsiveness with anticoccidial-sensitivity testing (AST) and conducting regular oocyst counts from litter or droppings, he said.

Fitz-Coy noted that several monitoring options are available and each has its own positives and negatives. Using a combination of methods consistently will provide useful and reliable data.

He cited examples from three different commercial poultry operations in different regions of the US to underscore his point that monitoring the pattern of coccidia and coccidiosis change is crucial.

In one complex, for example, necropsy data over several years showed that the prevalence of *Eimeria acervulina*, *E. maxima* and *E. tenella* was 33%, 45% and 9%, respectively.

During the early years, *E. acervulina* was more prevalent than *E. maxima* (see Figure 1). But with the use of certain anticoccidials during summer months, the prevalence of *E. maxima* increased and remained fairly high.

Many poultry producers put coccidiosis on the back burner...performance may suffer and dollars are lost.

In the winter months, the prevalence of *E. acervulina* trended downward and appeared to be associated with the use of a specific in-feed anticoccidial. The AST data were in agreement with the findings from necropsy data, leading to changes in the coccidiosis program and improved coccidiosis control.

Which species is it?

Identifying which coccidial species is affecting flocks is an important part of the coccidial-monitoring process, Fitz-Coy

emphasized in a second presentation at WPDC. He compared coccidia to zebras, which appear the same at first glance, even though each species is significantly different from the other.

“It’s the same with coccidia. Each species is unique, has unique pathology and pathogenicity and even drug responsiveness,” he said. Each species also must be treated as unique to achieve good coccidiosis control.

Features that differ among various *Eimeria* include fecundity — the ability to produce oocysts (eggs) — the region of intestine that each species parasitizes and the depth of parasitic development in the mucosa. Different species of *Eimeria* also vary in the size of their coccidial-endogenous (developmental) stage. The pathogenicity is influenced by these various characteristics, Fitz-Coy explained.

Eimeria praecox has relatively high fecundity but is a shallow invader of the mucosa and produces relatively small endogenous stages, he continued.





Figure 1. Necropsy data show that in early years, *E. acervulina* was more prevalent than *E. maxima*, which increased and remained high with the use of a specific anticoccidial.

E. tenella has moderately high fecundity, is a deep invader of the mucosa and the endogenous stages are large.

E. necatrix is a poor oocyst producer but is a deep invader with relatively large endogenous stages.

Because of these traits, *E. praecox* is considered non-pathogenic, while *E. tenella* and *E. necatrix* are highly pathogenic to chickens, Fitz-Coy said.

E. brunetti, *E. maxima* and *E. mivati* also develop deep into the tissues. Mature stages damage blood vessels, which results in bleeding into the intestinal lumen, he said.

Coccidial species that have a longer pre-patent period tend to be more pathogenic than those with shorter periods. The pre-patent time is how long it takes for the first appearance of oocysts in the feces of chickens after they are infected with *Eimeria*, and this pre-patent time can be used to help

differentiate various species of *Eimeria*, he said. *E. praecox* has the shortest pre-patent period — 84 hours — compared to 138 hours for *E. necatrix*.

Another way to differentiate *Eimeria* species is by their size and shape when viewed under a microscope, although it can be challenging and requires someone skilled at the art of oocyst identification.

The oocysts of *E. mitis*, for instance, are almost round, and the oocysts of *E. maxima* are large and have a tint of color. The size and shape of the non-pathogenic *E. praecox* can be hard to tell apart from *E. tenella*, *E. necatrix* and even *E. brunetti*.

Advanced procedures such as polymerase chain reaction (PCR) can be useful but may not be able to identify all *Eimeria* species, Fitz-Coy said.

“The best approach for differentiating various species of coccidia may be using multiple methods of identification, such as parasite biology as well as PCR,” he said.



innovation^s

! Vaccine project targets *E. coli*, salmonella

The old adage that “a bird in the hand is worth two in the bush” may apply to a new vaccine project underway at Arizona State University, reports thepoultrysite.com.

Research scientist Melha Mellata is leading a USDA-funded project to identify targets that could lead to development of a vaccine against avian pathogenic *Escherichia coli* (APEC), as well as salmonella.

E. coli infections are a serious problem in the poultry industry, causing significant economic losses; there is concern in the scientific community that APEC strains could become an emergent food pathogen in people. Salmonella is harmless to chickens but is a major cause of food-borne illness in people.

“What if you could get one vaccine to fight against a group of bacteria?” Mellata said. “We came up with a project where we would protect chickens, not only from *E. coli* infection but also salmonella, and in doing so, improve human health.”

For the USDA project, Mellata and her team will ultimately attempt to shuttle APEC genes into salmonella bacteria in the hopes of triggering a protective immune response against both *E. coli* and salmonella. The project is due to be completed in 2010.

research watch

Performance influenced by diet in coccidiosis-vaccinated broiler breeders

Integrators rearing replacement broiler breeders should take into account the finding that early flock performance can be influenced by dietary composition, depending on the genetic line and gender, Leslee A. Oden, of Texas A&M University, said at the American Association of Avian Pathologists annual meeting held last July.

Oden and associates conducted a study to determine the effect of diet on oocyst output, gross lesion development and performance in replacement broiler breeders from two genetic lines that received Coccivac-D, a live-oocyst coccidiosis vaccine, at 1 day of age.

There were two lines of breeders in the study — Line A and Line B — and in each group there were 240 males and 576 females. After delivery from the hatchery, birds received either the integrator diet or a diet recommended by the primary breeder of each line, Oden said. The protein concentration was higher in the breeder-recommended diets than in the integrator diets.

Oocyst shedding was evaluated and gross lesion scores were determined, as well as average bodyweight and flock uniformity, she said.

In Line A, oocyst peaks varied with gender and the dietary program. Breeders fed the

integrator diet had significantly higher lesion scores in the upper region of the small intestine when compared to breeders fed the breeder-recommended diet. There were no differences in bodyweight during the course of the trial, but on days 28 and 42, males fed the integrator diet had improved uniformity, she said.

In Line B, breeders fed both diets had oocyst peaks between days 14 and 18; the peaks varied with gender and the dietary program.

Males on the integrator diet had an increased lesion score in the mid-intestine compared to birds on the breeder-recommended diet. Females fed the breeder-recommended diet had increased bodyweight from days 21 to 42 and improved uniformity on days 28 and 42 compared to birds on the integrator diet, Oden said.

Lesion development and oocyst output are related and tend to be at their highest levels around day 17 when rearing replacement broiler breeders on fresh pine shavings, Oden concluded. The magnitude of oocyst output and number of identifiable peaks are influenced by genetic line, gender and dietary composition.



worth repeating



When the company came to me and said it wanted all of our birds to be totally antibiotic-free, I told them we could do it with the next cycle of birds. Five years ago, I probably would have told them we needed 15 years.

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DRAPER VALLEY FARMS
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ONTARIO, CANADA



We'll never know all there is to know about coccidia. These parasites are just too clever for us.

DR. RALPH MARSHALL
VETERINARY LABORATORIES AGENCIES
ENGLAND



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