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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 sound science but on the skilled application of science that only comes with hands-on experience.

This issue of *Intestinal Health* has plenty of both science and skill. In the pages that follow, you'll read in down-to-earth language about researchers using state-of-the-art technology to explore the important role that alpha-toxin plays in necrotic enteritis (NE), a devastating disease that has become more prevalent as producers strive to meet market demands for poultry meat grown without antibiotics. Additional articles reveal results from trials conducted here and abroad with a new alpha-toxin vaccine called Netvax that is being applied in the field to control NE. It's administered to hens, which in turn convey immunity against NE to their progeny. Science and skill.

There are several articles in this issue about coccidiosis. One focuses on an intensive European study that demonstrates how attention to certain management practices

Attention to certain management practices can significantly boost performance in birds vaccinated with the coccidiosis vaccine Paracox-5.

can significantly boost performance in birds vaccinated with the coccidiosis vaccine Paracox-5. Another explains how coccidiosis vaccination has evolved simply from a means of controlling coccidiosis into a broad management tool with benefits including the restoration of anticoccidial sensitivity, enhanced marketing ability, reduced costs and improved food safety. There's an article about a field study that turned up some surprises about the impact of new and used litter on coccidiosis-control programs. Again, science and skill.

Science and skill also characterize Intervet/Schering-Plough Animal Health. Our broad, innovative portfolio of products reflects our company's emphasis 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.

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Puerto Vallarta

At the WPDC-ANECA conference, presentations underscored the importance of early coccidial cycling.

Veterinary professionals learned about the importance of early coccidial cycling during the 57th Western Poultry Disease Conference and XXXIII ANECA annual convention held in Puerto Vallarta, Mexico.

Dr. Matilde Alfonso, technical service veterinarian for Intervet/Schering-Plough Animal Health, presented results from a multi-state US study showing that coccidiosis vaccination induces earlier coccidial cycling and less prevalent and severe lesions than anticoccidials.

When cycling occurs before the bird's major growth spurt (after 4 weeks of age), it's less likely to interfere with growth at the time broilers eat the most feed, she said.

Further evidence that early cycling is preferable came from Dr. Robert Teeter, University of Oklahoma. His metabolic studies show that low-level coccidiosis is costly at any age, but much less so early in the bird's life since less overall energy is used.

Get as "close to lesion-free as possible" with coccidiosis control, he said, since even birds with lesion scores of only 2 have impaired daily gain.

Dr. Steve Fitz-Coy, a parasitologist with Intervet/Schering-Plough Animal Health technical services, encouraged monitoring for coccidiosis because it changes patterns. Citing cases from commercial poultry operations, he showed how monitoring can indicate when a coccidiosis-control program needs to be changed to improve disease control — and profits.

Dr. Linnea Newman, also of Intervet/Schering-Plough Animal Health technical services, advised coordinating coccidiosis control with management.

Coccidiosis-vaccinated flocks on reused litter in houses with higher bird density developed immunity earlier than vaccinated flocks on clean litter in a full house; early immunity is preferable because the adverse effects of subclinical coccidiosis on performance worsen with age, she said.

Editor's note: For a more detailed report on the WPDC/ANECA meeting, see the North American or Latin American edition of Intestinal Health *online at www.thepoultrysite.com/intestinalhealth.*

innovations

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 Animal Health 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.

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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.

more on page 41

Adding More Value

Nearly 3 years ago, Dr. Rae Fischer made what some people in the broiler industry might have called a very brave decision. Others might have said it was foolhardy.

The innovative CEO of Fisher Feeds[1], Listowel, Ontario, decided to stop using in-feed anticoccidials at his company's feed mill, which produces 100,000 tons of feed per year — about two-thirds for poultry.

About half of the poultry feed produced at the mill supplies his family's poultry operation, Bert Fisher Farms, and its contract growers.

His strategy, Fischer explained at the time, was to put more emphasis on nutrition, particularly in the early stages of chick development, and to keep coccidiosis in check, relying more on a live-oocyst vaccine.

Emphasis on nutrition

"This may sound funny coming from someone who owns and operates a feed mill, but I just hate putting chemicals and antibiotics in the feed," he told *CocciForum* magazine in 2005 (Issue 11). "I want the emphasis to be on good nutrition programs, with quality raw materials, ingredients and processing."

He added, "As a veterinarian, I want to see these decisions made on science, not emotion or propaganda. But either way, if [poultry raised without antibiotics] is what the consumer wants, somebody's going to have to produce it. We need to be ready for drug-free production, and that's one of the reasons we wanted to start vaccinating."

Fischer still separates broilers by sex, growing cockerels to 2.5 kg (5.53 lbs) in 38 days and pullets to 2.4 kg (5.30 lbs) in 40 days. After a few weeks of his new program, he knew he was onto something good when he saw that birds vaccinated for coccidiosis performed as well or better than birds that received an in-feed anticoccidial.

"We never saw the dramatic drop in performance that we were looking for," Fischer reported at the time, "probably because our nutrition program helped to condition the gut and help it tolerate the challenge from the vaccine."

All or nothing

So how's his new program working more than 2 years later? Apparently, very well.

Adding More Value

Encouraged by the strong performance of his flocks, Fischer decided to take the next big step and get rid of all in-feed antibiotics — a move he thought would allow his operation to focus even more on good management, nutrition and vaccination as means of promoting good intestinal health. He says he's not regretting the decision — and neither are his birds, which have not experienced any significant setbacks in health, performance or profitability.

Two factors drove Fischer's decision:

- **Consumer demand.** He was acutely aware of shifting consumer perceptions and the growing demand for birds raised without antibiotics.

- **Better science.** Fischer felt that dropping in-feed antibiotics was simply better science. With a degree in veterinary medicine, Fischer wasn't convinced that medicated feed was the best way to keep enteric disease in check. He was also concerned about reports from other markets about growing resistance, which reportedly developed after prolonged use of in-feed anticoccidials. Closer to home, he felt some feed medications were losing their effectiveness.

> "We're always looking to add more value — that's the key."
>
> DR. RAE FISCHER

"We've also been watching the regulatory climate, " he says. "There aren't any new in-feed anticoccidials being developed, and the trend in Europe — a market that's very similar to ours in Canada — has been to ban some drugs altogether. We don't have as many tools available to us as we did 5 or 10 years ago. And now, there's growing pressure from regulators and consumers to reduce and even eliminate drugs from the feed.

"We saw this situation as an opportunity to differentiate our product and add value," he explains. "We also wanted to prepare ourselves for a market that was coming up."

Focus on first 7 days

Fischer's feed formulations are proprietary, so he can't get into details about the changes he's made in his nutrition program to improve intestinal health.

"What I can tell you is that we have to be far more specific on our nutrition and more precise in our management," he says. "With antibiotics, you can have some insult to the livestock and still not have a visible disease problem. When you go drug-free, you have to remove all those stresses that antibiotics might protect you from."

continued

[1]Dr. Rae Fischer and his company, Fisher Feeds, have similar names, but they are spelled differently.

> "The first 7 days are extremely important in antibiotic-free production. You carry that thought process out a little farther, extend it for the life of the birds and do absolutely everything you can to minimize any insult that might be thrown in their way."

Fischer says the first 7 days of a bird's life are the most critical for developing immunity. Under optimum conditions, he says chicks can grow to five times their initial body weight in their first 7 days of life. Fischer also says that research shows that the weight of 5- to 7-week-old broilers has a direct linear relationship to the first week of rearing, regardless of breeder age or day-old chick weight (Pezeshkian, 2002). His primary genetics suppliers, Aviagen and Cobb-Vantress, have also told him that 1 extra gram of weight at 7 days means an extra 5 to 7 grams at market weight.

"Even with conventional production, focusing on the first 7 days will give you an advantage because it will broaden your protection and health status," Fischer says. "The first 7 days are extremely important in antibiotic-free production. You carry that thought process out a little farther, extend it for the life of the birds and do absolutely everything you can to minimize any insult that might be thrown in their way."

As a feed manufacturer, Fischer is in the perfect position to optimize the nutrition component of a vaccine-based intestinal health program, but he says that's not the only component.

"We've been far more precise on our environmental controls — temperature, air movement, humidity, lighting — to provide that optimal environment and less opportunity for a challenge," he explains.

Threat from NE

Another ever-present issue for poultry producers is necrotic enteritis, particularly in drug-free programs. "When you take out the effects of antibiotics or ionophores, you need to take steps both nutritionally and management-wise that would reduce any kind of clostridium effect," he says.

He is encouraged by preliminary results with Netvax, a new vaccine for necrotic enteritis also known as *Clostridium perfringens* type A toxoid. The vaccine, which is conditionally licensed in the US and, in Canada, is under restricted licensing while awaiting full approval, was developed by Intervet/Schering-Plough Animal Health and is the first product of its kind for poultry. "We've had some promising results with it, and certainly it will be one of the tools we would want to use,"

continued

Which vaccine is right for you?

Intervet/Schering-Plough Animal Health has licensed two lines of field-proven coccidiosis vaccines.

In Europe, Japan, Australia, South Africa and parts of the Middle East, the vaccine of choice for broilers is Paracox-5. In other markets, such as the Americas and Asia, the company has obtained licenses for Coccivac-B.

Ask your local Intervet/ Schering-Plough Animal Health representative about the coccidiosis vaccines available in your area.

Fischer says. "We're very optimistic on that."

This extra attention to detail required for a coccidiosis-vaccination program — nutrition and environmental management — has an inevitable impact on production costs. There are also processor costs associated with segregating and documenting birds from drug-free production. But Fischer does not see these costs as significant, especially in the context of cost increases for other inputs, most notably feed.

While the poultry industry strives for more efficiency, its efforts could be blindsided by the impact of ethanol demand on corn supply — a trend that's rippling through all livestock industries.

"We've looked at some of the byproducts and whether or not we can utilize them, but we've stayed away from them until this point because of inconsistencies and other issues. It's something we may have to look at again, and maybe that is another challenge we will have to face."

And while it's not a welcome development, one can't help but think that it's another challenge Fischer would be quite happy to take on.

Commanding premiums

While there will always be a segment of the market that is very price sensitive, Fischer says his company is focusing on areas it can control — like commanding a premium on a high percentage of his farm's production. Pleased with drug-free experiences so far, the restless innovator already has his eye on other opportunities to keep his operation at the head of the pack.

One possibility is the use of Omega 3-enriched rations to increase the availability of Omega 3 for consumers. It's an attractive goal but a tough nut to crack, Fischer admits. The challenge right now is that the Omega 3 accumulates in the birds' subcutaneous fat. This tends to be discarded along with the skin when chicken is eaten by health-conscious consumers — the same people who would be attracted by an Omega 3-enriched product in the first place. Consumers may well find a more convenient delivery system for Omega 3 in the future, but Fischer isn't ready to let go of the idea just yet.

"We're always looking to add more value — that's the key," he says.

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.

1

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 held recently in Brisbane, Australia.

Alpha-toxin is a toxic protein secreted by the bacterium *Clostridium perfringens*. It is also a component of Netvax, a *Clostridium perfringens* type A toxoid vaccine. Netvax, which is conditionally licensed in the US, 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

Table 1

	Challenged group		Negative control	
NE status	*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

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.

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.

...high lesion scores correlated with positive test-strip results for *C. perfringens* and alpha-toxin at the site of NE lesions...

continued

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).

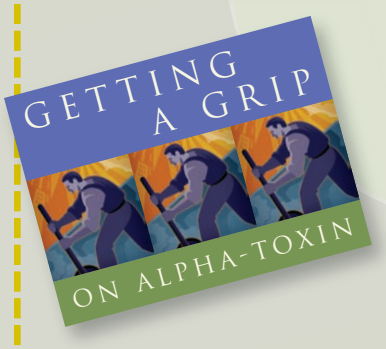


Figure 1

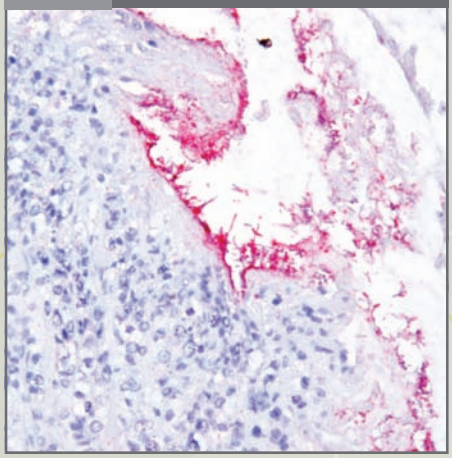


Figure 1. An "anti-antibody" with fluorescent or pigmented material binds to the antibody in question if that antibody is present.

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."

With immunohistochemistry, "there was clearly a positive binding of antibodies."

2

PRESCOTT

Alpha-toxin provides excellent protection against NE; other proteins likely involved

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

alpha-toxin

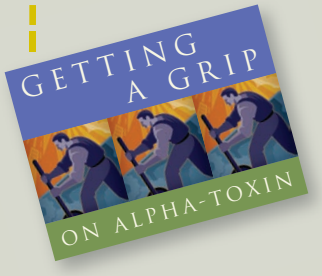
challenge was confirmed by NE lesion scores in non-immunized but challenged control birds.

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.



This was the first report demonstrating the immunizing ability of *C. perfringens*-secreted proteins in protecting broilers against NE, Prescott and associates wrote.

They concluded that there are certain secreted proteins, in addition to alpha-toxin, involved in immunity to NE in broiler chickens.

> "I agree that alpha-toxin should be the main focus," but there may be an advantage to using more than one protein.

NetB may be marker for NE strains

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,

PRESCOTT

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.

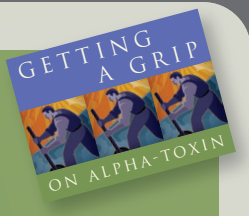
Proteins differ, he added, in their structure, in their activity, including toxicity, and in their targets.

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

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.

> "...*C. perfringens* is the fastest growing organism known and, under optimal conditions, doubles every 8 to 10 minutes."

"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 Netvax, the company's *Clostridium perfringens* type A toxoid for broilers, agrees (see article, page 11).

"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.

necrotic enteritis

Alpha-toxin gene linked to necrotic enteritis in India

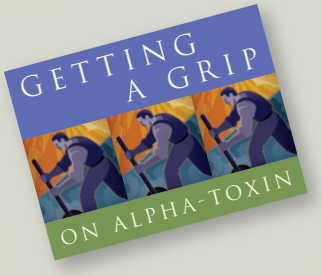
A study conducted on broilers from India confirmed that *Clostridium perfringens* type A was the cause of necrotic enteritis (NE) and that alpha-toxin may play a significant role in development of the disease, say Arunava Das of the Bannari Amman Institute of Technology, and associates.

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.



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 the pathogenesis of necrotic enteritis.

XXIII World's Poultry Congress

Brisbane, Australia

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

Maintaining the intestinal health of broilers while reducing dependence on in-feed medications dominated much of the technical program, which included a wide-ranging session on "hot topics in necrotic enteritis" and a well-attended panel discussion on vaccinal strategies for controlling the disease. Coccidiosis vaccination was also covered extensively.

Intestinal Health magazine's Phil Stewart attended the congress and filed this three-part report.

World's Poultry Congress

Part One: **Necrotic Enteritis**

A panel of experts provided a comprehensive overview of the potential for vaccination to control necrotic enteritis (NE).

XXIII World's Poultry Congress

Antibody recognizes antigen in NE lesions

A study using immunohistochemistry to demonstrate alpha-toxin in intestinal lesions of necrotic enteritis clearly showed that antibody raised against the alpha-toxoid in Netvax 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 the article on page 11 of this issue.

XXIII World's Poultry Congress

NE vaccine trials in Europe

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

Netvax, 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 Netvax 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 with Netvax 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.

XXIII World's Poultry Congress

Vaccine lowers NE risk in 'real-world' conditions

A trial in the US designed to test the *Clostridium perfringens* type A toxoid Netvax under real-world conditions demonstrated that the vaccine lowers the risk for NE mortality, according to Dr. Charles 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 Netvax, 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 Netvax; 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 to14 days, 15 to 21 days and 22 to 28 day.

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 Netvax. 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 Netvax 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 Netvax could eventually become integrated into the routine care of many breeders. "Netvax 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.

XXIII World's Poultry Congress

Role of diet in NE questioned

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.

Dr. Charles Broussard of Intervet/Schering-Plough Animal Health 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.

XXIII World's Poultry Congress

Alpha-toxin a 'primary player'

In a discussion on the mechanisms of protection conferred by the Netvax vaccine, which contains a toxoid derived from the alpha-toxin, Dr. Charles Broussard of Intervet/Schering-Plough Animal Health confirmed that there is more to be discovered about why and how the vaccine triggers immunity.

Dr. Scott: Classic virulence

Dr. John F. Prescott, University of Guelph, Ontario, who gave a presentation on NE and alpha-toxin during another session at the poultry conference (see article page 14), 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 Netvax, 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 Netvax 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.

"If you maintain adequate fiber levels in your feed, you'll achieve better coccidiosis control..."

XXI World's Poultry Congress

Toxoid vaccine key to NE control

A surge of interest in antibiotic-free broiler production throughout Canada's poultry industry, coupled with the eventual availability of an effective vaccine to prevent necrotic enteritis, could become an important catalyst for change in the world poultry industry.

This trend is clearly evident in Canada, where producers see commercial potential for antibiotic-free (ABF) production but need a way to thwart losses from necrotic enteritis (NE) that stymied ABF production in the past, reported Dr. Linnea Newman, a consulting veterinarian with Intervet/Schering Plough Animal Health.

Newman described the results of two, on-farm trials with Netvax, a novel *Clostridium perfringens* type A toxoid vaccine for NE that is conditionally licensed in the US and under restricted licensing in Canada while awaiting full approval. The vaccine is administered to hens, which pass on immunity against NE to broiler progeny. The trials were conducted in two separate regions of Canada, each with conditions associated with NE.

The Ontario trial was conducted in a region with high pH soils and a history of NE outbreaks even on farms using in-feed anticoccidials with full-level antibiotic growth promoters. High pH soil appears to be a major risk factor for NE, she said.

Seventeen flocks from hens vaccinated with Netvax were fed ionophores without antibiotic growth promoters (AGPs). Performance was compared to flocks using traditional in-feed medication, including AGPs placed at the same time and processed at the same age.

Mortality followed normal patterns, and the average daily gains for the Netvax progeny grown without AGPs actually exceeded those of the flocks using a full in-feed medication program.

'COMPELLING' OUTCOME

Because the trial was small, the results could be interpreted only as trends and were not statistically significant, but the outcome was compelling, nonetheless, she said.

A separate trial in western Canada was carried out on a farm that had previously tried to introduce a coccidiosis-control program using Coccivac-B live vaccine. Despite the use of in-feed, growth-promoting antibiotics, the farm experienced heavy losses due to NE in six successive flocks.

NE is a major challenge for producers in this region, Newman noted, largely because of wheat-based diets. However, after wheat was removed, NE breaks still occurred and hit each flock twice. "It was almost as though nothing we did on this farm could prevent NE," she added.

The trial used imported progeny of birds vaccinated with the *C. perfringens* type A toxoid, but this time, the birds were vaccinated with the live coccidiosis vaccine Coccivac-B. Initially, in-feed antibiotics were also used, resulting in two flocks that were successfully raised without NE. The producers then took it a step further and removed the in-feed antibiotic. The result: Two more flocks were raised on a full ABF program with no NE breaks.

The Ontario trial was conducted in a region with high pH soils and a history of NE outbreaks even on farms using in-feed anticoccidials with full-level antibiotic growth promoters.

Newman said the producer has now introduced a small, ongoing ABF program, using progeny from local breeders vaccinated with Netvax and feeding them an all-vegetable ration.

In an interview with *Intestinal Health*, Newman said that several Canadian producers tried to introduce ABF programs 5 to 10 years ago but had disastrous results due to NE. Now, interest in ABF production has renewed, judging in part by requests that Newman has received to speak in Canada on the topic.

The keen interest in ABF production is being driven by intense competition in the Canadian poultry industry, she said.

VACCINATION JUST ONE TOOL

Newman cautioned that although Netvax represents an important tool for controlling NE, it's not the only one that producers will need.

"Growers must have all the other good management factors lined up," she said. "Birds must get onto their feed straight away. That means good access, good lighting, good temperature control and supplemental feed.

"If they're using an all-vegetable ration," she continued, "this must be done right if they are to avoid too much wet litter — which only encourages *C. perfringens* and NE. Getting it right can mean some difficult juggling with ingredients and cost. Too much soy and you start getting too much indigestible feed. Use more synthetic amino acids, and you start getting cost issues."

Want more information? For a proceedings booklet summarizing the intestinal health presentations at WPC, contact your Intervet/ Schering-Plough Animal Health representative or download a copy at **www.NetvaxforPoultry.com.**

Feed additives and NE

NE is the most common and financially devastating bacterial disease in broilers, and the subclinical form is by far the most damaging for producers.

Acute necrotic enteritis (NE) may come and go like a storm, but it's ongoing subclinical NE that does the real economic damage — and why finding dietary alternatives to control the disease would be a boon to poultry producers, said Professor Mingan Choct, chief executive officer of the Australian Poultry Cooperative Research Center.

NE is the most common and financially devastating bacterial disease in broilers, and the subclinical form is by far the most damaging for producers, Choct said.

There are numerous alternative feed additives available that claim to promote gut health and suppress harmful organisms in the absence of in-feed antibiotic growth promoters (AGPs). However, "for alternatives to have a place in the post-AGP era... they must be able to prevent the occurrence or reduce the severity of NE," he said.

Choct and colleagues have looked at up to 30 feed additives that are supposed to improve gut health, some more promising than others. Feed additives, he added, should not be compared to AGPs but instead evaluated in production systems where antibiotics are not used.

The professor described recent work carried out at the University of New England, Australia, where broiler feed was changed on day 17 from a sorghum-based diet to a wheat-based diet, which is thought to contribute to NE.

The trial birds were split into two groups, with one receiving an enzyme-based additive. In the non-enzyme group, there was noticeably higher proliferation of *Clostridium perfringens* — the bacterium that causes NE.

Once in the intestinal tract, enzymes undergo complex interactions with proteins, starches and other constituents, but there is evidence that certain enzymes can cleave molecules into smaller, less convoluted parts. "This reduces viscosity so the gut becomes more fluid. Anaerobic bacteria then have less time in the gut, and the amount of oxygen is increased," said Choct, who believes it eventually may be possible to use a combination of feed additives to successfully control NE.

It is well understood how diets based on coarse grains — wheat, rye, barley or oats, to name a few — may contribute to NE by altering the gut wall and creating a favorable environment for *C. perfringens*, he continued. The water-soluble non-starch polysaccharides make the gut content "as thick as honey." Birds may also be predisposed to *C. perfringens* and NE due to coccidiosis, which damages the intestinal mucosa.

AGPs, he explained, effectively control NE by selectively modifying gut flora, suppressing bacterial catabolism and reducing bacterial fermentation — all of which lead to increased nutrient availability and enhanced growth performance. In a 1997 review of more than 12,000 scientific papers on antibiotic use in animal feed, it was reported that in most cases antibiotics improved feed-conversion efficiency by 2% to 3%.

"That represents a lot of money for producers at today's feed prices," Choct said.

XXIII World's Poultry Congress

Coccidiosis field research yields surprising results

Part Two: **Coccidiosis**

Although coccidiosis is found on virtually every broiler farm, researchers continue to solve new mysteries about this omnipresent, costly disease.

Editor's Note: This presentation from the World's Poultry Congress is based on the presenter's experience in North America. While reused litter has been shown to be an effective way of building immunity to coccidiosis, the practice must be weighed against other disease-management practices in Europe, as well as local regulations, which might require replacement of litter with every flock. For more on coccidiosis management, see articles beginning on pages **7**, **31** and **39**.

Field research has yielded unexpected findings about the impact of clean or used litter on coccidiosis control and underscores the importance of initiating coccidiosis immunity early in broiler flocks.

"Poultry producers, pay attention!" challenged Dr. Linnea Newman, a consulting veterinarian with Intervet/Schering Plough Animal Health. "Birds eat half their total feed in their final 2 weeks. With corn prices so high, you don't want to give up feed-conversion efficiency and weight gain because of coccidiosis-linked production losses."

The research that prompted these comments from Newman involved the collection of fecal samples from multiple broiler farms that were using

Coccidiosis field research yields surprising results

continued

a variety of coccidiosis-control programs. The sampling, which was done over time at farms in Canada and various US locations, has opened an interesting window into the dynamics of coccidiosis in commercial broiler operations.

Canadian broiler houses are required to do a complete cleanout between flocks to help manage viral challenges from diseases such as Newcastle, Marek's and infectious bursal disease, Newman explained. In stark contrast, US broiler houses reuse litter. Broiler houses on the Delmarva Peninsula have litter that may be used for up to five years without cleanout, in part because environmental constraints prohibit dumping used litter.

The coccidiosis-management programs used on the various farms studied included both in-feed anticoccidials and vaccination programs, Newman said.

Contrary to expectations, the patterns of oocyst shedding on the Canadian farms showed a high and late coccidial challenge and even more so in lower-density flocks.

Contrary to expectations, the patterns of oocyst shedding on the Canadian farms showed a high and late coccidial challenge and even more so in lower-density flocks. Similar results have been seen on US farms that use clean litter, and it's an unwelcome pattern when birds are changed to unmedicated withdrawal feed, she said.

In contrast, broilers on Delmarva farms that were raised at higher densities and on heavily used litter showed earlier and lower oocyst-shedding peaks.

Flocks in the US vaccinated year-round for coccidiosis produced an earlier oocyst peak on used litter.

Vaccinated flocks in Canada that were raised on clean litter produced oocyst peaks a week later than the vaccinated birds on used litter, but these peaks were still earlier and lower than what was seen in flocks using in-feed anticoccidials, Newman said.

Anticoccidials losing effectiveness

In an interview with *Intestinal Health*, Newman said the contrasting patterns she's observed highlight two important realities about coccidiosis: In-feed anticoccidials are continuing to lose their effectiveness, and immunity plays a key role in controlling the timing and severity of oocyst peaks.

It was unlikely that Canadian producers were aware that coccidiosis was sapping productivity to the extent that it was. "This was a wake-up call for them," Newman said.

The smartest thing a poultry producer can do to minimize late intestinal damage and maximize genetic potential is to transfer to a coccidiosis-vaccination program — either a year-round program or rotate a vaccine with anticoccidials to renew their

anticoccidials

sensitivity to the *Eimeria* strains that cause coccidiosis, Newman advised.

"Both strategies work, but year-round vaccination is probably a better option. It's a paradigm shift that's needed," she said. "The only problem you can get with a rotation is that every time you rotate, you shift the pattern back to the late coccidial challenge that you get with in-feed anticoccidials."

The high and late oocyst peaks of medicated broilers that are in houses cleaned out between flocks have exposed the growing ineffectiveness of anticoccidials used alone, Newman continued. "If you re-use litter, you get some natural vaccination from the oocyst populations shed by the previous flock, which helps stimulate immunity; if you have clean litter, you are wholly dependent on anticoccidials for control."

She predicts the effectiveness of in-feed anticoccidials will continue to wane. Commenting on the recent reintroduction of the chemical anticoccidial clopidol in the US after a 15-year absence, Newman said it has been providing excellent coccidiosis control, but the resistance that originally forced it off the market will likely return.

"Some people are increasing doses of anticoccidials to extend the useful life of the product, but that strategy won't last," she warned. "Another approach is to use anticoccidials in the withdrawal feed to combat the late peaks. Again, this is likely to cause increasing resistance."

"Some people are increasing doses of anticoccidials to extend the useful life of the product, but that strategy won't last."

Another way to extend the life of in-feed anticoccidials might be to follow the example of one large integrator in the US. "The company uses very low levels of anticoccidial and lets natural immunity through field exposure do the rest," she explained. "The only problem with this approach is that we don't know the nature of the incoming oocyst challenge. Is it more or less pathogenic? You never know."

The ideal strategy — and this is the nub of Newman's take-home message to growers — is to stimulate an early coccidial challenge in a controlled way, preferably through vaccination.

"Producers need to find out what their normal oocyst-shedding patterns are and also understand seasonal variations. One sample alone won't do it — that's just a snapshot. They need to see what happens during different seasons and during the different phases of a rotation program. I can guarantee you that most farm managers won't be aware of these patterns," she said.

Consistency, Newman concluded, is the name of the game. "Consistent feed conversion and weight gain performance, early immunity, avoiding late gut lesions — that's what it's about," she added.

consistency

XXIII World's Poultry Congress

Management practices that boost performance

An intensive European study has pinpointed management factors that can yield the best performance results in broilers vaccinated against coccidiosis.

The attenuated coccidiosis vaccine Paracox-5 has proved to be an effective way to protect broilers from coccidiosis since its 2000 introduction in Europe and especially since regulators prohibited the use of in-feed antibiotic growth promoters, said Italy's Dr. Luciano Gobbi, a technical manager for Intervet/Schering-Plough Animal Health.

However, the results of a study conducted on an integrator's commercial farms in northern Italy demonstrate that attention to certain management practices can significantly boost performance in coccidiosis-vaccinated birds, the veterinarian said.

The study involved some 2 million broilers vaccinated at day 1 with Paracox-5, which was administered with a specially designed hatchery spray cabinet, Gobbi said.

The efficacy of the vaccine was based on both clinical observations, such as mortality and coccidiosis breaks, and performance results, specifically final live weight and feed conversion. Performance parameters were then correlated against four sets of factors:

1. **Effectiveness of vaccine distribution in the hatchery spray cabinet**
2. **Quality of bird-management practices during full grow-out**
3. **Influence of the genetic line on susceptibility to necrotic enteritis**
4. **Use of tactical antibiotic chemoprophylaxis**

Accurate vaccination essential

The accuracy of vaccine administration made a clear impact, the study showed. More accurate spray administration alone resulted in a 30-gram advantage in final live weight, a feed-conversion ratio (FCR) advantage of 0.045 and mortality was lower by 0.7%, Gobbi reported.

When not uniformly sprayed, some birds do not receive initial exposure to vaccine oocysts. Instead, they are exposed to oocysts for the first time in 4 or 5 days, when the first coccidial cycling occurs in birds that received the vaccine. This delays establishment of immunity, resulting in poorer performance and an extra half-day to reach market weight, he explained.

Best and worst management practices

The analysis of management practices was revealing, Gobbi continued. Farms were classified into groups of the "best 10" and "worst 10" regarding management level. Good management was found to minimize the impact of necrotic enteritis, a multifactorial disease.

PARACOX-5

The best management groups also had a final live weight that was nearly 100 grams more and an FCR better by 0.077 compared to the 10 worst groups. In addition, mortality was 1.5% higher in the worst groups compared to the 10 best groups, he said.

In an interview with *Intestinal Health*, Gobbi said that good management covers everything from the hours before the day-old chicks are placed until they go to slaughter.

"Something as simple as temperature control is very important to getting birds off to the right start," he said. "If the temperature is as little as 2 degrees lower than it was in the hatchery and during transport, chicks will gather around heat lamps and pile onto each other. That stops them from eating and drinking."

In addition to careful temperature management, farm managers must look after basics such as ensuring birds have good access to food plates and properly functioning water nipples, Gobbi said.

"The first 10 days are critical, and up to 80% of the investment in management should be during this brooding phase," he said.

Considering the physiology of the birds, the physical state of the starter or pre-starter rations can have a big impact, he added. It is important that pelleted feed rations are "not too hard and not too soft" when crushed — otherwise more robust birds select granules out of the ration, leaving some birds to get only part of the formulation.

Genetic line matters

The influence of the vaccinated bird's genetic line on performance was also clear in the study, Gobbi said.

Genetic selection for muscle development can divert available amino acids from the immune system. "Although it hasn't been proven scientifically, there are some perceptions that certain genetic lines are more susceptible to lecithinase-C, which is produced by the alpha-toxin of *Clostridium perfringens* type A," he reported.

Some genetic lines were also less tolerant of sudden feed changes. For instance, when birds from certain lines were switched from a grower to finisher ration, they would sulk 1 to 2 days and pick at litter, thus ingesting feces and exposing themselves to greater gut-health challenges, he said.

The level of response to the presence of feed mycotoxins, which are an immunosuppressant, appeared to be genetically linked, with some lines more likely to refuse feed containing the toxins, Gobbi noted.

When other factors such as the hatchery source were equalized, there were clear performance differences between two breed lines identified in the Italian study. The relative advantages were consistent for liveweight, FCR and mortality, he said.

The final parameter evaluated — therapeutic antibiotic therapy — revealed that treated broilers fared slightly better than the birds that did not receive therapeutic antibiotics.

Gobbi concluded that the study highlighted the importance of field management in achieving successful coccidiosis vaccination. "The integrator involved in our study has standard operating procedures, but the human factor comes into play when applying these procedures," he said. "When birds are vaccinated and managed well, intestinal health problems in all flocks can be prevented or minimized in the long term."

XXIII World's Poultry Congress

Vaccine is valuable tool in high-end broiler market

SOUTH AFRICAN EXPERIENCE

Paracox-5, an attenuated coccidiosis vaccine, is playing an important role in the valuable free-range and organic broiler markets of South Africa.

Avian medicine consultant Dr. Scott Elliott of Skeerpoort told *Intestinal Health* that there are three distinct market segments in the Republic: "green" birds that are completely organic, green birds that are free range or "country reared," and conventionally reared broilers.

While organic broiler production requires organically grown feed crops, country-reared/free-range birds can receive non-organic feed and are allowed limited use of antibiotic growth promotants. Some alternative feed additives are also used in conjunction with vaccination in organic and country-reared/free-range production, Elliott said.

It costs about €1.29/kg (US $1.80/kg) to produce a conventional broiler and about €1.57/kg (US $2.20/kg) to produce a country-reared/free-range bird. Producers receive about €2.29/kg (US $3.20/kg) for an organic bird, which retails for about €3.01/kg (US $4.20/kg), he said, adding that the organic market in South Africa is crowded and competitive.

Paracox-5, an attenuated live vaccine for preventing coccidiosis in broilers, has been used in the organic and free-range segments since it became available.

Paracox-5, an attenuated live vaccine for preventing coccidiosis in broilers, has been used in the organic and free-range segments since it became available, and has enabled producers supplying these valuable markets to stop or minimize the use of in-feed anticoccidials. Its sister product, Paracox-8, is widely used in layers and breeders in South Africa, he said.

Paracox vaccines have two competitors in South Africa, but those products do not contain mild *Eimeria* strains and can cause lesions, Elliott said.

"Birds treated with [one of the other vaccines] can cycle too much, and if they are kept enclosed, you can end up with clinical coccidiosis at the 14- to 28-day stage. That causes a lack of uniformity, which is significant at peak production in layers and breeders. This damage could also trigger necrotic enteritis," he said.

Necrotic enteritis is not generally a big issue in South Africa, where poultry diets are based on corn and soy, he noted, and antibiotic growth promoters play a large role in controlling the disease.

Elliott's clients sometimes run "green" and conventional broiler operations side-by-side. "Sometimes, the free-range, country-reared birds do better than the conventionally raised broilers," he commented.

Because infectious bursal disease is endemic in South Africa, total cleanout of poultry houses in between flocks is a common practice. However, a chronic shortage of wood for making litter, compounded by an absence of many viable alternative materials other than sunflower husks, is forcing producers to rethink this practice. "We are looking at using deep litter for the first time in 30 years," Elliott said.

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Vaccine development — where to from here?

Two points of view

Part Three: **Looking to the future**

Scientists weighed the pros and cons of various vaccine technologies aimed at boosting protection.

Vaccines are emerging as a tool for managing an increasingly broad range of poultry diseases even though the application of new vaccine technology is slow coming to the poultry industry, according to two speakers.

The "backbone" of today's poultry-immunization programs remains developments that occurred during the postwar period from the 1950s through the 1970s, when the industry became intensified and new disease challenges emerged, said Dr. John Glisson, poultry veterinarian and professor at the University of Georgia, Athens.

The application of "high science" and new-generation vaccine technologies in the poultry industry has been hindered by a variety of factors. One is economics. Big poultry customers have used their

Vaccine development – where to from here?

continued

buying power to drive prices down until some vaccines are "almost free," he said.

This apparent lack of profitability with poultry vaccines has diverted research and development efforts toward potentially more lucrative areas such as companion animal or horse health. In addition, government funding for R&D in the poultry industry has fallen, prompting universities to spend less effort in this area, Glisson said.

Among other constraints to new poultry vaccine development are labor costs, he continued. "A lot of vaccines have to be injected, which makes them viable only for breeders or layers," he said. "For broilers, labor costs generally preclude anything except mass vaccination at the hatchery or in the field. In the US, every time you pick up a bird, the labor cost is as much as 10 cents per bird."

Vaccine opportunities

Despite obstacles to the development of new poultry vaccines, Glisson says there are opportunities. One of several examples he cited is in the area of food safety.

A combination of high-energy diets and the withdrawal of antibiotics is allowing a number of diseases to blossom... presenting opportunities for new vaccines.

"Consumers aren't interested in Newcastle disease or Marek's, but they do know about campylobacter and salmonella," he noted. "There's an opportunity to do more to help producers reduce the risk to humans from these pathogens."

He sees potential for *in ovo* vaccines, too. They have their limitations because some viruses may be harmful to the embryo and maternal antibody interference can be an issue, but they also present great opportunities and may provide a route for economically viable mass vaccination.

A combination of high-energy diets and the withdrawal of antibiotics is allowing a number of diseases to blossom, such as clostridial enteritis, colibaccilosis and coccidiosis — again, presenting opportunities for new vaccines, Glisson said.

Mapping of the chicken genome is significant. "But don't get too excited," he warned. "Development will be incremental and, when it comes to using genetics, disease control will always take a back seat to feed conversion and growth rates."

Existing vaccines 'extremely good'

A similar message came in a separate presentation. The UK's Dr. Ian Tarpey, R&D manager for virology at Intervet/Schering-Plough Animal Health, predicted that there will be progress in new poultry vaccines, but it will not be revolutionary.

Many of today's poultry vaccines have been used for years, in part because many of them are still "extremely good," he said. He also said that change in the field of poultry vaccines may seem slow because

DR. JOHN GLISSON

expectations, which are often fueled by breakthroughs in human vaccines, are simply too high.

Economics is a constraint to vaccine development, Tarpey agreed with Glisson. "This is a low-cost business, so we can't use high technology if no one will pay for it." New vaccines will have to provide the same or better economic performance as current vaccines. They will need to be broad-spectrum and in the right combinations for different regions.

"Add to that issues about poultry welfare, regulations, unique geographical needs, consumer fear of genetically modified organisms and politics, and there are many other factors constraining new vaccine development," Tarpey said.

Nonetheless, he is optimistic about poultry vaccine development and outlined up-and-coming technology that may yield new vaccines to fill industry needs.

Improve adjuvant design

The technology used now to stimulate an immune response is about 40 years old and could be improved upon, Tarpey said. Adjuvant design is one area where this could occur.

Another approach known as immunomodulation could focus on changing the bird's ability to respond to a vaccine.

"Chickens have many of the same chemical messengers that mammals have — the information flow between cells needed in an immune response," he said. This work has already been pursued by several groups, though improvements to immune responses demonstrated in the laboratory experiments have yet to be translated into new vaccines.

In the future, some recombinant vaccines may well be based on ribonucleic acid (RNA) viruses.

The importance of cell-mediated immunity should not be underestimated, Tarpey added. Mucosal immunity, which is important in the gut, would be another area with potential for improved vaccine performance.

Molecular biology

Molecular biology is an area of science that has held promise for poultry vaccines for the past 20 years. Most progress has been made on DNA viruses, which are relatively easy to manipulate. A number of recombinant vaccines are now licensed in the US and doing well.

In the future, some recombinant vaccines may well be based on ribonucleic acid (RNA) viruses. These could have a number of advantages including mass applicability, a fast response and limitation of virus shedding.

Another area of focus is improved knowledge of microorganisms. "We are starting to understand how microorganisms survive and replicate in the host. Reverse engineering — identifying what the organism does to survive — could be used to either augment this process in the live vaccine or to engineer it out of the virulent organism to attenuate it," he said.

XXIII World's Poultry Congress

Life after antibiotics: Vaccines part of holistic solution

The poultry industry can produce birds without the benefit of growth promoters, but it will take a well-thought-out holistic approach to make it work, cautioned Dr. Peter Scott.

In a talk on poultry production without antibiotics, Scott had harsh words for anyone who muddies the waters of perception about the use of antibiotics in livestock. In the minds of consumers, the term "growth promoters" is often mistakenly confused with "growth hormones,'" said Scott, a senior research fellow at the University of Melbourne and managing director of avian and animal health consultancy, Scolexia.

The issue of antibiotic resistance is complex and often misconstrued. "A lot of people are interested, and for different reasons," he said.

Genetic selection for resistance is well understood; pathogens are constantly evolving in response to antibiotics from natural sources, not just as a result of antibiotic use, he pointed out. Resistance in pathogens affecting livestock does not necessarily translate into problems for human health, Scott asserted, but he did concede that pathogens carried by poultry such as salmonella and *Campylobacter* spp. were of more direct concern.

During two decades working in the Australian poultry industry, Scott has seen an enormous drop in therapeutic antibiotic use, achieved largely by the use of vaccines, improved husbandry, phytosanitary procedures and biosecurity.

"Alternative treatments are not a complete replacement for growth promoters or a panacea, but they can be useful and effective."

There is a mixed understanding in the industry of the importance of gut microflora in young birds, he said. Livestock managers are often anxious to use therapeutic antibiotics within the first week to reduce mortality if they detect sick birds, but this causes disruption to natural gut flora and could lead to more disease-control problems later on. "I tell them it's not warranted and shouldn't be done," he added.

DR. PETER SCOTT

Forecast for alternative systems

Alternative systems, he predicted, "are going to be much harder to run if you're going to avoid disease problems and the use of therapeutic antibiotics."

Withdrawing antibiotics in the form of in-feed growth promoters, Scott said, could lead to a drop-off in feed conversion efficiency if other measures are not taken, which has been the case in countries where they are no longer permitted. He urged producers to take a "multifactorial" approach; this involves improved husbandry practices, nutrition, use of enzymes to prevent accumulation of non-polysaccharide starches in the gut, improved immunity through vaccination and the use of alternative treatments. "Alternative treatments are not a complete replacement for growth promoters or a panacea, but they can be useful and effective," he noted.

Removing in-feed growth promoters is a big challenge in these tough economic times when labor and feed costs are rising, and Scott says he's seen some husbandry practices deteriorating as a result of financial pressures. Access to therapeutic antibiotic treatments must be maintained for controlled and regulated use in specific situations, such as managing risk from pathogens that threaten food safety.

"The poultry industry can accommodate the strategic removal of growth promoters and limitations on the use of therapeutics through a holistic approach and the strategic use of alternative products and biologicals combined with improved husbandry," he concluded.

Fringe benefits

Coccidiosis vaccination used to be viewed solely as a method for controlling coccidiosis, but years of experience have transformed it into a broad management tool with multiple benefits for poultry producers.

Besides keeping the disease in check, coccidiosis-vaccination programs can help producers restore the sensitivity of in-feed anticoccidials; they can also complement the practice of thinning flocks and enhance marketing, reduce costs, simplify work at the feed mill and improve food safety, Dr. Luciano Gobbi, a veterinarian and technical service manager with Intervet/Schering-Plough Animal Health, said at VIV Europe 2008 — Moscow Edition.

The primary means of controlling coccidiosis has been in-feed anticoccidials, which accounted for 87% of coccidiosis control in the EU, according to a 2007 report. Yet coccidiosis remains widespread in the EU and elsewhere, reportedly costing producers €2 billion (US $2.9 billion) annually — more than any other disease — primarily due to lost performance that results from subclinical coccidiosis, said the Italy-based Gobbi.

One of the main problems with anticoccidials is the development of coccidial resistance, which has resulted from the overuse of the products, he said. Gobbi cited studies from the US, South America, Germany and the Netherlands documenting resistance. In the Dutch study, for instance, testing of 15 *Eimeria* isolates showed that 78% were resistant to diclazuril, 72% were resistant to monensin, 68% were resistant to nicarbazin and 64% were resistant to salinomycin.

Vaccine benefits

A major benefit of coccidiosis vaccination, he said, is the restoration of *Eimeria* sensitivity, which can be accomplished by rotating a vaccine with anticoccidials. Gobbi showed an example demonstrating that in flocks where ionophore sensitivity had declined, the use of the coccidiosis vaccine Paracox-5 for three consecutive flocks restored ionophore sensitivity.

"One hypothesis is that vaccinal *Eimeria* lines conjugate with wild lines and render *Eimeria* strains less pathogenic and more sensitive to anticoccidials. Conjugation means an exchange of genetic material, leading to less pathogenicity and a restoration of sensitivity to anticoccidial drugs," he explained.

In trials designed to assess bird performance when a coccidiosis vaccine was rotated with anticoccidials, performance was within the normal

range, whether the birds were light or heavy, although the best results were achieved in heavy birds raised to 2.0 kg or above, Gobbi said.

Compensatory growth occurred by day 30 onwards and performance improved with continuous vaccination of successive flocks because the challenge of natural coccidiosis declined. In addition, good results were achieved when flocks returned to in-feed anticoccidials, he said.

Enhances thinning

Continuous use of a coccidiosis vaccine not only provides complete control of the disease, it also can enhance the practice of flock thinning, Gobbi said. With thinning, male and female birds in one house are divided by a barrier. For the first thinning, females are usually removed and the barrier is taken away, leaving the whole house to grow larger males. Thinning also makes it easier to grow heavy birds in hot summer months.

Coccidioisis vaccination complements thinning because there is no anticoccidial drug in the feed and, consequently, no withdrawal time, which enables producers to pull light or heavy birds as needed to meet market demand, he said.

Coccidiosis remains widespread in the EU and elsewhere, reportedly costing producers €2 billion (US $ 2.9 billion) annually — more than any other disease.

DR. LUCIANO GOBBI

"Vaccination makes it easier to thin flocks, which in turn helps increase meat production per square meter," Gobbi explained.

With more birds raised in the same house, costs are reduced for transporting feed and for moving chicks to the house and transporting them to market. In fact, transportation costs can decrease by as much as 20% to 30%, he said.

Since integrators pay farmers on the basis of kg of meat per square meter, which is improved with coccidiosis vaccination, farmers have reduced fixed costs, while integrators have a reduction in variable costs. "Hence, the integrator has reduced remuneration to farmers per kg of meat produced by each square meter," Gobbi said.

Life at the feed mill is simplified, too, with coccidiosis vaccination, he said. There is no need for cleaning the manufacturing lines and equipment to reduce or prevent the unwanted carryover of anticoccidials, Gobbi said.

Another important benefit of coccidiosis vaccination is the reduced risk for drug residues in broiler meat that can result from the use of anticoccidials or therapeutics administered when coccidiosis outbreaks occur.

"Consequently, finished-meat products are from a traceable, clean production chain and can be guaranteed as safer for consumers," he said. "This is particularly important for producers growing drug-free birds who want to differentiate their product."

Continued from pages 4 and 5

innovations

! 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 recent annual meeting of the American Association of Avian Pathologists.

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 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

"The trend toward growing broilers with fewer or no in-feed antibiotics is forcing producers to consider an array of dietary approaches that may or may not improve intestinal health..."

DR. ROB TEN DOESCHATE
ABNATECH GLOBAL
PETERBOROUGH, ENGLAND

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DR. RALPH MARSHALL
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